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TOXICOLOGICAL REVIEW

OF

CARBON TETRACHLORIDE

(CAS No. 56-23-5)

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

May 2008

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U.S. Environmental Protection Agency
Washington, DC

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LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ACSL	Advanced Continuous Simulation Language
AIC	Akaike's Information Criterion
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
BCF	Bioconcentration factor
BMD	Benchmark dose
BMDL	Benchmark dose, 95% lower bound
BMDS	Benchmark dose software
BMR	Benchmark response
BrdU	5-Bromo-2'-deoxyuridine
BUN	Blood urea nitrogen
BW	Body weight
CASRN	Chemical Abstracts Service Registry Number
CBZ	N-benzyloxycarbonyl-valine-phenylalanine methyl ester
CCl4	Carbon tetrachloride
CHO	Chinese hamster ovary
CI	Confidence interval
CITI	Chemicals Inspection and Testing Institute
C_{max}	Maximum tissue concentration
CPN	Chronic progressive nephropathy
CPK	Creatine phosphokinase
CYP450	Cytochrome P450
DMSO	Dimethyl sulfoxide
EPA	Environmental Protection Agency
FEL	Frank effect level
G6Pase	Glucose-6-phosphatase
GCL	γ -Glutamylcysteine ligase
GD	Gestational day
GDH	Glutamate dehydrogenase
GGT	γ -Glutamyl transferase
GI	Gastrointestinal
GSH	Glutathione (reduced)
HA	Hemagglutinin
HEC	Human equivalent concentration
4-HNE	4-Hydroxynonenal
IFN- γ	Interferon- γ
Ig	Immunoglobulin
i.p.	Intraperitoneal
IRIS	Integrated Risk Information System

JBRC	Japan Bioassay Research Center
K_m	Michaelis-Menten constant
LAP	Leucine aminopeptidase
LDH	Lactate dehydrogenase
LOAEL	Lowest-observed-adverse-effect level
MCA	mean arterial concentration
MCL	mean liver concentration
MDA	Malondialdehyde
MRAMKL	Mean rate of metabolism in the liver
MN	Micronucleus
MW	Molecular weight
NCI	National Cancer Institute
NHL	Non-Hodgkin's lymphoma
NK	Natural killer
NLM	National Library of Medicine
NOAEL	No-observed-adverse-effect level
NRC	National Research Council
OCT	Ornithine carbamoyl transferase
8-OHdG	8-hydroxy-2'-deoxyguanosine
OR	Odds ratio
PBPD	Physiologically based pharmacodynamic
PBPK	Physiologically based pharmacokinetic
PFC	Plaque-forming cell
PNMT	Phenylethanolamine-N-methyltransferase
PND	Postnatal day
POD	Point of departure
RfC	Reference concentration
RfD	Reference dose
SAM	S-adenosylmethionine
SCE	Sister chromatid exchange
SD	Standard deviation
SDH	Sorbitol dehydrogenase
SMR	Standardized mortality ratio
SOS	Inducible DNA repair system
SRC	Syracuse Research Corporation
$t_{1/2}$	Half-life
TBA	Total bile acids
TBARS	Thiobarbituric acid-reactive substances
TGF	Tumor growth factor
T_{max}	Time at which the maximum occurred
TNF- α	Tumor necrosis factor α
UF	Uncertainty factor
USD	Unscheduled DNA synthesis
V_{max}	Maximum velocity of enzyme reaction

FOREWORD

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

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 the data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are provided in Appendix A.

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 carbon tetrachloride. 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 effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

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

Development of these hazard identification and dose-response assessments for carbon tetrachloride 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, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and*

Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996a), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 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, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through December 2007.

2. CHEMICAL AND PHYSICAL INFORMATION

Carbon tetrachloride is a colorless liquid with a sweetish odor (NLM, 2003; Lewis, 1997). Synonyms include tetrachloromethane and perchloromethane (NLM, 2003; O'Neil and Smith, 2001). The chemical structure of carbon tetrachloride is shown in Figure 2-1. Selected chemical and physical properties of carbon tetrachloride are listed below.

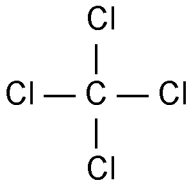


Figure 2-1. Carbon tetrachloride.

CAS number:	56-23-5 (Lide, 2000)
Molecular weight (MW):	153.82 (O'Neil and Smith, 2001)
Chemical formula:	CCl_4 (O'Neil and Smith, 2001)
Boiling point:	76.8 °C (NLM, 2003; Lide, 2000)
Melting point:	–23 °C (NLM, 2003; Lide, 2000)
Vapor pressure:	1.15×10^2 mm Hg at 25 °C (NLM, 2003)
Density:	1.5940 g/mL at 20 °C (NLM, 2003; Lide, 2000)
Vapor density (air=1):	5.32 (NLM, 2003; U.S. Coast Guard, 1999); 5.41 (O'Neil and Smith, 2001)
Water solubility:	7.93×10^2 mg/L at 25 °C (NLM, 2003; Horvath, 1982)
Other solubility:	Miscible with alcohol, benzene, chloroform, ether, carbon disulfide, petroleum ether, oils (NLM, 2003; O'Neil and Smith, 2001)
Partition coefficient:	$\log K_{ow} = 2.83$ (NLM, 2003; Hansch et al., 1995)
Flash point:	Not flammable (NLM, 2003; U.S. Coast Guard, 1999)
Autoignition temperature:	>1000 °C (Holbrook, 1993)
Latent heat of vaporization:	1.959×10^5 J/kg (U.S. Coast Guard, 1999)
Heat of fusion:	5.09 cal/g (NLM, 2003; U.S. Coast Guard, 1999)
Critical temperature:	556.35 °C (Daubert and Danner, 1995)
Critical pressure:	4.56×10^6 Pa (Daubert and Danner, 1995)
Viscosity:	0.922 cp at 24 °C (U.S. Coast Guard, 1999)
Surface tension:	0.027 N/m at 20 °C (U.S. Coast Guard, 1999)
Henry's law constant:	2.76×10^{-2} atm m^3/mol at 25 °C (NLM, 2003; Leighton and Calo, 1981)
OH reaction rate constant:	1.20×10^{-16} cm^3/molecule second at 25 °C (NLM,

	2003; Atkinson, 1989)
K_{oc}:	71 (NLM, 2003)
Bioconcentration factor (BCF):	3.2–7.4 (NLM, 2003; CITI, 1992)
Conversion factors:	1 mg/m^3 = 0.16 ppm (25 °C)
	1 ppm = 6.29 mg/m^3 (25 °C)

In the United States, carbon tetrachloride is most commonly prepared by chlorinating methane or by a chlorinating cleavage reaction with less than or equal to C_3 hydrocarbons or chlorinated hydrocarbons (Rossberg, 2002). Prior to the late 1950s, carbon tetrachloride was produced primarily by carbon disulfide chlorination (NLM, 2003; Rossberg, 2002).

Carbon tetrachloride has been used as a dry-cleaning agent, fabric-spotting fluid, solvent, reagent in chemical synthesis, fire extinguisher fluid, and grain fumigant (NLM, 2003; Holbrook, 1993), but its primary use was in chlorofluorocarbon (CFC) production (NLM, 2003; Rossberg, 2002). Since the mid-1970s, annual use and production has generally declined. The Consumer Product Safety Commission banned the use of carbon tetrachloride in consumer products in the 1970s. Decline in the use of carbon tetrachloride also accompanied EPA's increased regulation of the use of CFCs in propellants (a ban on CFCs in aerosol products went into effect in 1978), and the adoption of the Montreal Protocol, an international agreement to reduce environmental concentrations of ozone-depleting chemicals, which was implemented in the U.S. via Title VI of the Clean Air Act Amendments of 1990 (ATSDR, 2003; Doherty, 2000; Holbrook, 1993). The ban on production and import of carbon tetrachloride in developed countries, including the U.S., took effect on January 1, 1996. Excluded from the production and import ban is the manufacture of a controlled substance that is subsequently transformed or destroyed and small amounts exempted for essential laboratory and analytical uses (40 CFR Part 82; 72 Fed Reg 52332, Sept 13, 2007).

Production figures for carbon tetrachloride since the 1970s reflect the regulatory history of the chemical. Carbon tetrachloride production peaked in the early 1970s, with annual U.S. production exceeding one billion pounds. Production in the early 1990s had declined to approximately 300 million pounds (Doherty, 2000). According to ATSDR (2005), manufacture of carbon tetrachloride in the U.S. in the early 2000s was limited to one company (Vulcan Materials Company) at two plants with a combined 130 million pound capacity; however, these capacities were considered flexible because other chlorinated solvents are made using the same equipment.

Historically, carbon tetrachloride was released into the environment predominantly through direct emissions to air, with lower amounts discharged to soil and water (ATSDR, 2003). Carbon tetrachloride released to soil or water is expected to volatilize to air based on its vapor pressure and Henry's Law constant (NLM, 2003). In air, carbon tetrachloride will exist as a vapor, as indicated by its vapor pressure (NLM, 2003). The behavior of carbon tetrachloride in

the atmosphere is the most important aspect of this chemical's environmental fate. Carbon tetrachloride does not undergo photodegradation (Holbrook, 1993) or absorb light at wavelengths found in the troposphere and hence does not undergo direct photolysis in that region of the atmosphere (NLM, 2003). Carbon tetrachloride that remains in the troposphere eventually rises into the stratosphere, where it is photolyzed by the shorter wavelength light (Molina and Rowland, 1974). When carbon tetrachloride photolyzes in the stratosphere, the chlorine radicals responsible for the destruction of atmospheric ozone are released.

In soil, carbon tetrachloride is expected to be highly mobile based on its K_{oc} and is expected to leach to lower soil horizons and groundwater (NLM, 2003). BCF values indicate that carbon tetrachloride will not bioconcentrate appreciably in aquatic or marine organisms (NLM, 2003). Carbon tetrachloride may biodegrade in soil or water under anaerobic conditions; however, biodegradation of carbon tetrachloride under aerobic conditions does not occur readily (NLM, 2003; U.S. EPA, 1996b; Semprini, 1995).

3. TOXICOKINETICS

Carbon tetrachloride is rapidly absorbed by any route of exposure in humans and animals. Once absorbed, it is widely distributed among tissues, especially those with high lipid content, reaching peak concentrations in less than 1–6 hours, depending on exposure concentration or dose. It is metabolized by the liver, lung, and other tissues. Carbon tetrachloride is rapidly excreted, primarily in exhaled breath.

3.1. ABSORPTION

3.1.1. Oral Exposure

Carbon tetrachloride is readily absorbed through the gastrointestinal tract in humans and animals. There is evidence of gastrointestinal absorption in humans based on reports of toxicity following poisoning incidents (Ruprah et al., 1985; Gosselin et al., 1976; von Oettingen, 1964; Stewart et al., 1963; Umiker and Pearce, 1953). In male Sprague-Dawley rats receiving gavage bolus doses of approximately 18 or 180 mg/kg, peak concentrations of carbon tetrachloride were detected in the liver within 1 minute and in the blood within 10 minutes (Sanzgiri et al., 1995; Bruckner et al., 1990). Total absorption was reduced by 37–56% when the same doses were administered by infusion over a 2-hour period. An oral dose of about 3200 mg/kg attained a peak blood concentration in about 2 hours in rats (Marchand et al., 1970). After radiolabeled carbon tetrachloride was injected into the duodenum of rats, at least 82% was absorbed based on recoveries of label in exhaled air (Paul and Rubinstein, 1963).

Administration of carbon tetrachloride in a vehicle changes the rate and percentage of gastrointestinal absorption. Peak blood concentrations were achieved within 3.5–6.0 minutes after oral exposure in male Sprague-Dawley rats dosed with 25 mg/kg of neat (i.e., undiluted) carbon tetrachloride (Kim et al., 1990a, b; Gillespie et al., 1990). Relative to the neat compound, the initial rate of gastrointestinal absorption of 25 mg/kg of carbon tetrachloride was faster with administration as a saturated solution in water or 0.25% aqueous Emulphor[a] emulsion but slower when administered in corn oil. Although the initial rate of absorption in the presence of corn oil was relatively slow, the total percentage absorbed over 9 hours when administered in corn oil (83.1%) exceeded the percent absorption for the neat compound (62.8%) and was comparable to that for the 0.25% aqueous emulsion (85.4%). The highest percent absorption was obtained from a water vehicle (91.9%). Pharmacokinetic data suggested that corn oil vehicle resulted in slower absorption from the gastrointestinal tract and subsequently lower peak blood concentrations and

[a] Emulphor is a polyethoxylated vegetable oil used to incorporate volatile organic compounds (VOCs) and other lipophilic compounds into aqueous solutions.

delayed removal from the blood stream (Kim et al., 1990a).

3.1.2. Inhalation Exposure

Data from humans and animals suggest that carbon tetrachloride is rapidly absorbed through the lungs, which is inferred from the rapid onset of symptoms of toxicity or detection of carbon tetrachloride in blood or in exhaled air. In volunteers exposed to 10 ppm for 180 minutes, carbon tetrachloride was detectable in exhaled air within 15 minutes (Stewart et al., 1961). Human subjects exposed to 60 mg/L (9600 ppm) or higher reported symptoms of toxicity within the first minute of exposure; symptoms appeared after 3 minutes in subjects exposed to 30 mg/L (4800 ppm) (Lehmann and Schmidt-Kehl, 1936). After male Sprague-Dawley rats were exposed at 100 or 1000 ppm, carbon tetrachloride was detected in arterial blood in the initial 5-minute samples (Sanzgiri et al., 1995; Bruckner et al., 1990); blood levels rose during the 2-hour exposure period to a near steady-state level. In dogs exposed to 5000 ppm of carbon tetrachloride, blood levels reached a near steady-state level within 2 hours (von Oettingen et al., 1950).

Lehmann and Schmidt-Kehl (1936) estimated that approximately 63% of inhaled carbon tetrachloride vapor was absorbed by the lungs in human subjects exposed to "a few mg per liter." In monkeys exposed to carbon tetrachloride at 46 ppm for periods between 2 and 5 hours, an average of 30% of the total amount inhaled was absorbed, and the rate of absorption averaged 0.022 mg/kg-minute (McCollister et al., 1951). Rats that were exposed at 4000 ppm for 6 hours had initial body burdens of approximately 14 mg of carbon tetrachloride and 257 μg of its metabolite chloroform (Dambrauskas and Cornish, 1970). Initial body burdens in rats, mice, and hamsters that were exposed to 20 ppm of carbon tetrachloride vapor for 4 hours were 7.7, 10.6, and 4.0 mg/kg, respectively (Benson and Springer, 1999). In vitro experiments of carbon tetrachloride indicated blood/air partition coefficients of 2.73–4.20 for human blood (Fisher et al., 1997; Gargas et al., 1989) and 4.52 for rat blood (Gargas et al., 1986).

3.1.3. Dermal Exposure

Carbon tetrachloride is absorbed rapidly through the skin. The chemical was detected in alveolar air within 10 minutes in human subjects who immersed their thumbs in neat liquid (Stewart and Dodd, 1964). Animal studies have found similar results. Carbon tetrachloride was detected in blood within 5 minutes of dermal application of neat liquid in guinea pigs (Jakobson et al., 1982). The percutaneous absorption rate for carbon tetrachloride applied neat to the abdominal skin of male ICR mice was estimated as 53.6 ± 9.3 nmoles/minute/cm^2 (Tsuruta, 1975). Morgan et al. (1991) compared dermal absorption of carbon tetrachloride in rats when applied neat or in aqueous solution. With neat application, maximum blood levels were reached within 30 minutes, and approximately one quarter of the applied volume (0.54 mL) was absorbed in a 24-hour period. With application in saturated aqueous solution, absorption was slower (peak

blood levels were not attained until 10 hours after exposure), and a somewhat lower amount (0.39 mL) was absorbed in 24 hours.

Dermal absorption of radiolabeled carbon tetrachloride vapor was low in monkeys exposed to 485 or 1150 ppm for about 4 hours (McCollister et al., 1951). Blood concentrations at the end of exposure were approximately equivalent to 0.012–0.03 mg carbon tetrachloride/100 g blood but were undetectable after 48 hours; concentrations in exhaled air were equivalent to 0.0008–0.003 mg carbon tetrachloride/L but were undetectable 120 hours later. The authors concluded that, for whole-body exposures to carbon tetrachloride vapor, the dermally absorbed fraction would be negligible.

3.2. DISTRIBUTION

3.2.1. Oral Exposure

No data are available for the distribution of carbon tetrachloride in humans. Animal studies indicate that the largest fraction of an absorbed oral dose of carbon tetrachloride is initially distributed to fat. After administration of about 3200 mg/kg to rats, peak levels of radiolabeled carbon tetrachloride were observed after about 2 hours in blood, muscle, liver, and brain and after 5.5 hours in fat (Marchand et al., 1970). Peak tissue levels of carbon tetrachloride were similar in blood and muscle but were twice as high in the brain, 5 times higher in liver, and 50 times higher in fat. Similar results were obtained in rabbits treated with a low dose of carbon tetrachloride (Fowler, 1969). Six hours after an oral dose of 1.6 mg/kg, recoveries of parent compound totaled 787 μg/g in fat, 96 μg/g in liver, 20 μg/g in kidney, and 21 μg/g in muscle; distributions of the carbon tetrachloride metabolites chloroform and hexachloroethane were highest in fat and liver but were below 5 μg/g. Forty-eight hours after dosing, tissue concentrations of the parent compound were 45 μg/g in fat, 3.8 μg/g in liver, and <1 μg/g in the other tissues; chloroform was present at <1 μg/g in the four tissues, whereas hexachloroethane was present at 6.8 μg/g in fat, 1 μg/g in liver, and <1 μg/g in other tissues.

3.2.2. Inhalation Exposure

A similar pattern of distribution has been found in animals exposed to carbon tetrachloride by inhalation. Rats exposed to 4000 ppm for 6 hours showed the largest concentrations of carbon tetrachloride in the fat (1674 μg/g), followed by the brain (407 μg/g), kidney (233 μg/g), liver (136 μg/g), and blood (64 μg/g) (Dambrauskas and Cornish, 1970). The liver also contained 10 μg/g of chloroform (as a carbon tetrachloride metabolite). Monkeys exposed to 46 ppm of radiolabeled carbon tetrachloride vapor for 5 hours had the highest concentration of label in fat, with decreasing amounts in the liver, bone marrow, blood, brain, kidney, heart, spleen, muscle, lung, and bone (McCollister et al., 1951). The concentrations in fat and liver were eightfold and threefold higher, respectively, than concentrations in blood.

Bergman (1983) followed the distribution of radiolabeled carbon tetrachloride by whole-

body autoradiography in mice exposed by inhalation for 10 minutes and sacrificed at time points up to 24 hours; sections were either processed at low temperatures to retain volatile radioactivity (primarily parent compound), evaporated to retain only nonvolatile radioactivity (metabolites), or evaporated and then extracted to retain only protein- and nucleic acid-bound radioactivity (metabolites covalently bound to protein and nucleic acids). Immediately after exposure by inhalation, high levels of volatile radioactivity were detectable in fat, bone marrow, and nervous tissues (spinal cord and white matter of the brain). Nonvolatile and partly nonextractable radioactivity was detected in the liver, kidney cortex, lung, bronchi, gastrointestinal mucosa (especially in the glandular stomach, colon, and rectum), nasal mucosa, salivary glands, vaginal and uterine mucosa, and, interstitially, in the testis; nonvolatile radioactivity was also detected in urine and bile. The distribution pattern of volatile carbon tetrachloride and its nonvolatile metabolites was similar 30 minutes after exposure. Volatile radioactivity was detectable at relatively high levels in the nervous system at 4 hours and in fat at 8 hours but not at 24 hours. The pattern of labeling in the liver demonstrated a centrilobular concentration. Bergman (1983) reported a good correlation between nonextractable radioactivity and published tissue concentrations of cytochrome (CYP) P450.

Sanzgiri et al. (1997) compared the tissue distribution of carbon tetrachloride administered by inhalation (1000 ppm for 2 hours) and the equivalent oral dose (179 mg/kg) given as a single bolus dose or gastric infusion over 2 hours. Table 3-1 shows area under the curve (AUC) for the 24-hour monitoring period, the maximum tissue concentrations (C_{max}), and the times (T_{max}) at which the maxima occurred. Maximal tissue concentrations were reached quickest by gavage dosing, followed by inhalation and then gastric infusion. By all routes, attainment of maximal levels was slower in fat than in other tissues. Maximal levels in fat were considerably in excess of the maximal levels in other tissues, regardless of route of exposure. Among tissues other than fat, distribution kinetics of carbon tetrachloride were generally similar for the different tissues, except that maximal levels were higher and attained more quickly in the liver than in other tissues following bolus oral administration.

Table 3-1. AUC, C_{max}, and T_{max} in rat tissues following administration of 179 mg/kg carbon tetrachloride by inhalation (1000 ppm for 2 hours), oral bolus dosing, or gastric infusion over 2 hours

Tissue	Inhalation			Oral bolus			Gastric infusion		
	AUC (µg×minute/ mL)	C_{max} (µg/g)	T_{max} (min)	AUC (µg×minute/ mL)	C_{max} (µg/g)	T_{max} (min)	AUC (µg×minute/ mL)	C_{max} (µg/g)	T_{max} (min)
Liver	2823	20	30	1023	58	1	149	0.5	120
Kidney	3064	25	30	3029	14	5	800	4	120
Lung	2952	24	30	2908	10	15	2842	6	180
Brain	3255	28	30	4223	15	15	2683	10	150
Fat	230,699	1506	240	235,471	246	120	165,983	179	360
Heart	2571	18	30	2747	10	5	1900	8	120
Muscle	3248	18	30	4117	7	60	2164	10	150
Spleen	2035	13	30	4096	12	5	1660	6	150

Source: Sanzgiri et al., 1997.

Benson et al. (2001) compared the initial and delayed tissue distribution of inhaled carbon tetrachloride in rats, mice, and hamsters exposed to 20 ppm of radiolabeled carbon tetrachloride for 4 hours. Immediately after exposure, the percentage of the initial body burden present in major tissues was 30% in rats and hamsters and 40% in mice; the highest proportion at that time was in the liver of mice and hamsters and in the fat in rats. Two days later, the liver contained the highest amount in all three species. The results in rats reflect the initial lipophilic distribution of carbon tetrachloride and the subsequent accumulation in the liver.

3.2.3. Dermal Exposure

Few data are available regarding tissue concentrations of carbon tetrachloride following dermal exposure. One study of guinea pigs given topical application of carbon tetrachloride found that blood concentrations of the chemical increased during the first half hour of exposure but then declined to about 25% of peak levels despite continued exposure over a 6-hour period (Jakobson et al., 1982).

3.2.4. Lactational Transfer

Fisher et al. (1997) experimentally derived a human milk/blood partition coefficient of 3.26 for carbon tetrachloride, which would suggest a potential sensitive subpopulation of nursing infants based on the possibility of lactational transfer.

3.3. METABOLISM

Carbon tetrachloride is metabolized in the body, primarily by the liver, but also in the kidney, lung, and other tissues containing CYP450. The percent of a given dose that is metabolized varies with dose, as discussed in Section 3.4.

The metabolism of carbon tetrachloride has been extensively studied in in vivo and in vitro mammalian systems. Based on available data, a proposed metabolic scheme for carbon tetrachloride is illustrated in Figure 3-1. There is considerable evidence that the initial step in biotransformation of carbon tetrachloride is reductive dehalogenation: reductive cleavage of one carbon-chlorine bond to yield chloride ion and the trichloromethyl radical (Reinke and Janzen, 1991; Tomasi et al., 1987; McCay et al., 1984; Mico and Pohl, 1983; Slater, 1982; Poyer et al., 1980, 1978; Lai et al., 1979).

The initial reaction step is catalyzed by an NADPH-dependent CYP450 that is inducible by phenobarbital or ethanol (Castillo et al., 1992; Noguchi et al., 1982a; Sipes et al., 1977). In humans and animals, CYP2E1 is the primary enzyme involved with carbon tetrachloride bioactivation, while CYP3A may be involved under high exposure conditions (Zangar et al., 2000; Raucy et al., 1993). As demonstrated in studies with CYP2E1 genetic knockout mice, this enzyme is required for the development of hepatotoxicity (as measured by elevated liver enzymes and liver histopathology) in mice exposed to carbon tetrachloride (Wong et al., 1998).

The fate of the trichloromethyl radical is dependent on the availability of oxygen and includes several alternative pathways for anaerobic or aerobic conditions. Anaerobically, the trichloromethyl radical may dimerize to form hexachloroethane, which has been detected in animal tissues (Uehleke et al., 1973; Fowler, 1969). Addition of a proton and an electron to the radical results in the formation of chloroform ($CHCl_3$), which has been detected in exposed rats and rabbits (Reynolds et al., 1984; Ahr et al., 1980; Glende et al., 1976; Uehleke et al., 1973; Dambrauskas and Cornish, 1970; Fowler, 1969). The trichloromethyl radical can undergo further reductive dehalogenation catalyzed by CYP450 to form dichlorocarbene ($:CCl_2$), which can bind irreversibly to tissue components or react with water to form formyl chloride (HCOCl), which decomposes to carbon monoxide (Galelli and Castro, 1998; Pohl et al., 1984; Ahr et al., 1980; Wolf et al., 1977). The trichloromethyl radical can bind directly to microsomal lipids and proteins (Fanelli and Castro, 1995; Ansari et al., 1982; Villarruel et al., 1977), as well as the heme portion of CYP450.

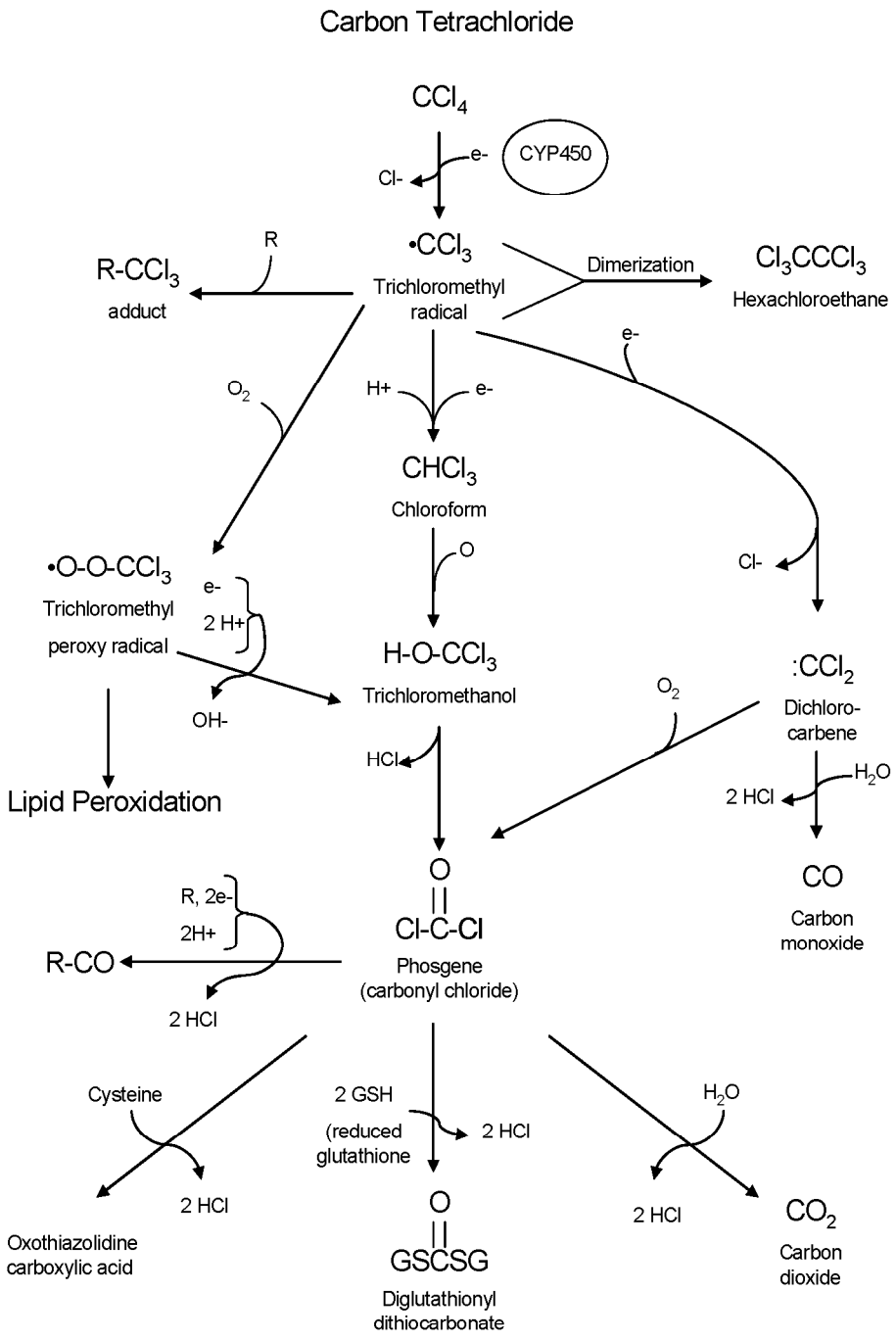


Figure 3-1. Metabolic scheme for carbon tetrachloride.

CYP450, usually CYP2E1, but also CYP3A; R = acceptor molecule, such as protein or lipid.

Source: ACGIH, 2001.

Aerobically, the trichloromethyl radical can be trapped by oxygen to form the trichloromethyl peroxy radical, which can bind to tissue proteins (Galelli and Castro, 1998; Packer et al., 1978) or decompose to form phosgene ($COCl_2$) (Pohl et al., 1984) and an electrophilic form of chlorine (Pohl et al., 1984). The trichloromethyl peroxy radical is the primary initiator of lipid peroxidation that occurs from exposure to carbon tetrachloride (Boll et al., 2001a; McCay et al., 1984; Rao and Recknagel, 1969). Carbon dioxide is generated by the hydrolytic cleavage of phosgene (Shah et al., 1979). Phosgene may also be conjugated to reduced glutathione (GSH) to form diglutathionyl dithiocarbonate or to cysteine to form oxothiazolidine carboxylic acid (U.S. EPA, 2001a).

Continued exposure to carbon tetrachloride has been shown to temporarily reduce its initial toxicity in rat studies (Glende, 1972). This phenomenon is related to the loss of CYP450 content (suicide inactivation), which has also been observed in treated rats (de Toranzo et al., 1978), resulting from the formation of reactive intermediates, such as the trichloromethyl radical (Fernández et al., 1982; Noguchi et al., 1982b; de Groot and Haas, 1981; Glende, 1972). Under anaerobic conditions, heme tetrapyrrolic structures of the human or rat CYP450 enzymes are destroyed in a process that follows pseudo first-order kinetics (Manno et al., 1992, 1988). Although the fast and slow half-lives for the two species are similar (3.2 and 28.9 minutes for the rat and 4.0 and 29.8 minutes for the human), inactivation is more severe in the rat, with 1 molecule of rat CYP450 enzyme lost for every 26 molecules of substrate metabolized, compared with a loss of 1 molecule of human enzyme for every 196 molecules of substrate processed (Manno et al., 1992, 1988). Based on the studies by Manno et al. (1992, 1988), deactivation of the CYP450 enzyme is reduced more in rats than in humans. Accordingly, enzyme deactivation is less significant in humans than in rats.

As demonstrated qualitatively by the distribution of nonvolatile radioactivity (metabolites) in the autoradiography study by Bergman (1983) and quantitatively in other in vivo assays (see Section 3.2), carbon tetrachloride is metabolized in many tissues throughout the body but most significantly in the liver. The amount of carbon tetrachloride metabolized in a given tissue is related to the CYP450 content of the tissue (Bergman, 1983; Villarruel et al., 1977). In the liver, the greatest accumulation of carbon tetrachloride metabolites occurs in the centrilobular region, which has high CYP450 levels (Bergman, 1983).

Zangar et al. (2000) measured carbon tetrachloride metabolic rate constants for human and animal hepatic microsomal preparations in vitro (Table 3-2). Results suggest that the metabolic rate in humans is more similar to the rate in rats than in other rodent species.

Table 3-2. Metabolic rate constants for hepatic microsomes in vitro

Species	K_m[a] (μM)	V_{max}[b] (nmol/minute/mg protein)
Human	56.8	2.26
Rat	59.1	3.1
Mouse	29.3	2.86
Hamster	30.2	4.1

[a] K_m= Michaelis-Menten constant.
[b] V_{max} = Maximum velocity of enzyme reaction.

Source: Zangar et al., 2000.

Metabolism of carbon tetrachloride can be induced by chemicals that increase the expression of CYP2E1 or CYP3A (see Section 4.8.6. for further discussion).

3.4. ELIMINATION

In humans and animals exposed to carbon tetrachloride by any route, the unmetabolized parent compound is excreted in exhaled air. Additionally, animal studies show that volatile metabolites are released in exhaled air, whereas nonvolatile metabolites are excreted in feces and to a lesser degree in urine.

Six hours after an attempted suicide by ingestion of an unknown amount of carbon tetrachloride in a mixture with methanol, the concentration of carbon tetrachloride in expired air was ~2500 μg/L and declined to ~120 μg/L after 1 day and to ~1 μg/L after 20 days (Stewart et al., 1963). In a worker acutely exposed to mixed solvent vapors, the concentration of carbon tetrachloride in alveolar air declined from an initial value of ~4000 ppm to ~0.003 ppm after 15 days (Stewart et al., 1965). Human subjects (n=6) who inhaled carbon tetrachloride vapor at 10 ppm for 3 hours had concentrations in expired air of 1 ppm 15 minutes postexposure and about 0.28 ppm 5 hours postexposure (Stewart et al., 1961). Approximately 33% of the absorbed dose was excreted in exhaled air within 1 hour in human subjects who inhaled radiochlorine-labeled carbon tetrachloride in a single breath (Morgan et al., 1970). Following dermal exposure to neat carbon tetrachloride, excretion into alveolar air was detectable within 10 minutes in three human subjects (Stewart and Dodd, 1964). Concentrations in alveolar air ranged from 0.11–0.83 ppm by the end of a 30-minute exposure, peaking 30 minutes postexposure and beginning to decline 1 hour postexposure; after 5 hours, the concentrations were 0.12–0.14 ppm. Using a physiological four compartment model, Sato and Nakajima (1987) calculated that 93% of inhaled carbon tetrachloride vapor was removed unchanged via the lungs (assuming an alveolar ventilation rate

of 336 L/hour), while 7% was cleared metabolically in humans.

Animal studies evaluated elimination of carbon tetrachloride following oral or inhalation exposures. In rats receiving equivalent doses by inhalation or bolus gavage, terminal elimination half-lives ($t_{1/2}$) were about 4 hours (Bruckner et al., 1990).

Reynolds et al. (1984) evaluated elimination parameters during a 24-hour period in rats exposed by gavage to [^{14}C]-carbon tetrachloride at doses ranging from 15 to 4000 mg/kg. At the low dose of 15 mg/kg, 19% of the administered dose was eliminated in exhaled air as the parent compound, 28% as CO_2 (accounting for 83% of metabolites), and 0.11% as chloroform (0.3% of metabolites); 2.9% of metabolites remained bound in the liver, while 2.7% were excreted in urine and 11% in feces. At doses ≥600 mg/kg, ≥76% of the administered dose was exhaled as parent compound, <2% was exhaled as CO_2 (accounting for 50–60% of metabolites), and <0.40% as chloroform (11–19% of metabolites); 2–4% of metabolites remained bound in the liver, while 3–9% of metabolites were excreted in urine and 7–30% in feces. At 15 mg/kg, peak exhalation rates were 11, 2.6, and 0.02 μmoles/hour per kg for CO_2, parent compound, and chloroform, respectively; the timing of the peak rates occurred in 15–45 minutes, within 2 hours, and slightly after 2 hours for CO_2, parent compound, and chloroform, respectively. At 4000 mg/kg, peak exhalation rates were 88, 1550, and 3.40 μmoles/hour per kg for CO_2, parent compound, and chloroform, respectively; compared with the lower doses, peak rates were achieved more quickly for CO_2 than for parent compound and chloroform.

In monkeys exposed by inhalation to radiolabeled carbon tetrachloride at 46 ppm for 5.75 hours, 21% of the total absorbed dose was eliminated during the initial 18 hours as carbon dioxide and parent compound or volatile metabolite (McCollister et al., 1951). Within 75 days following the end of exposure, 11% was eliminated as carbon dioxide and 40% as parent compound or volatile metabolite in exhaled breath. The majority of urinary and fecal excretion occurred in the 5 days following exposure; a small amount of label was detectable in feces after 12 days and in urine after 15 days.

In rats exposed to radiolabeled carbon tetrachloride vapor by inhalation at 100 or 1000 ppm for 8 hours for 1–5 days, no fecal elimination was detected (Page and Carlson, 1994); in comparison, intravenous administration resulted in biliary and nonbiliary fecal elimination that was less than 1% of the administered dose.

Sanzgiri et al. (1997) measured the elimination of carbon tetrachloride from tissues in rats exposed to 1000 ppm via inhalation for 2 hours or the equivalent oral dose of 179 mg/kg administered as a single bolus dose or by intragastric infusion over 2 hours. The half-lives of elimination from various tissues are given in Table 3-3. Elimination half-lives were slowest for fat, which is poorly perfused, but similar for the other tissues.

Table 3-3. Elimination half-life ($t_{1/2}$) and apparent clearance of carbon tetrachloride from rat tissues following administration of 179 mg/kg (1000 ppm, 2 hours) by inhalation, oral bolus dosing, or gastric infusion over 2 hours

Tissue	Inhalation		Oral bolus		Gastric infusion	
	$t_{1/2}$ (minutes)	Clearance (mL/minute/kg)	$t_{1/2}$ (minutes)	Clearance (mL/minute/kg)	$t_{1/2}$ (minutes)	Clearance (mL/minute/kg)
Liver	249	63	323	175	269	1198
Kidney	204	58	278	59	190	224
Lung	226	61	442	62	249	72
Brain	248	55	313	42	250	67
Fat	665	0.8	780	0.8	358	1
Heart	274	70	490	65	216	94
Muscle	218	55	649	43	262	83
Spleen	273	88	472	44	208	108

Source: Sanzgiri et al., 1997.

Benson et al. (2001) compared elimination parameters in rats, mice, and hamsters exposed to 20 ppm of [^{14}C]-labeled carbon tetrachloride for 4 hours. In the 48 hours following exposure, approximately 65–83% of the initial body burdens were eliminated as volatile organic compounds or CO_2 in exhaled air. Elimination half-times were 7.4, 8.8, and 5.3 hours for CO_2 and 4.3, 0.8, and 3.6 hours for the volatile organic compounds for rats, mice, and hamsters, respectively. Elimination in the urine and feces combined constituted less than 10% of the initial body burden in rats and less than 20% in mice and hamsters.

Paustenbach et al. (1986a, b) and Veng-Pedersen et al. (1987) compared the pharmacokinetics of carbon tetrachloride in rats exposed to 100 ppm of carbon tetrachloride vapor in scenarios that mirror human work schedules: 8 hours/day for 5 days or 11.5 hours/day for 4 days. Additional groups were exposed on a 2-week schedule for 5 or 3 additional days, respectively. Following 2 weeks of exposure at 8 hours/day, 45% of the label was eliminated in exhaled air (~97.5% as parent compound) and 48% in feces. Exposure at 11.5 hours/day for 2 weeks resulted in elimination of 32% in exhaled air and 62% in feces. On either schedule, less than 8% was excreted in urine and less than 2% was exhaled as CO_2. The elimination profiles for exhaled air were biphasic. For the 2-week 8 hours/day and 11.5 hours/day schedules, elimination of the parent compound in breath had half-lives for the fast and slow phases of 96 and 455 minutes and 89 and 568 minutes, respectively. Similarly, half-lives for the fast and slow phases of elimination of CO_2 were 305 and 829 minutes on the 8-hour schedule and 455 and 1824 minutes on the 11.5-hour schedule. The authors concluded that the longer daily exposure

placed more of the absorbed dose into the poorly-perfused fat compartment. The half-lives of elimination in urine and feces for the 2-week exposures were 1066 and 3700 minutes for the 8-hour schedule and 944 and 6700 minutes for the 11.5-hour schedule.

Rats or gerbils intraperitoneally injected with carbon tetrachloride at a dose of 128–159 mg/kg eliminated 80–90% in exhaled air as carbon tetrachloride and less than 1% as CO_2 (Young and Mehendale, 1989).

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Physiologically based pharmacokinetic (PBPK) models are available for carbon tetrachloride for exposures by the inhalation route (Yoon et al., 2007; Fisher et al., 2004; Thrall et al., 2000; Benson and Springer, 1999; Evans et al., 1994; Paustenbach et al., 1988, 1987; Gargas et al., 1986) and the oral route (Fisher et al., 2004; Semino et al., 1997; Gallo et al., 1993). The models are based primarily on experimental data from rodents. However, Thrall et al. (2000) derived in vivo metabolic rate constants for humans based on human in vitro metabolic constants and in vivo/in vitro ratios for metabolic rate constants derived from animals (also reported in Benson and Springer, 1999).

Gargas et al. (1986)

Gargas et al. (1986) used the PBPK model framework developed by Ramsey and Andersen (1984) for styrene, together with experimentally derived tissue partition coefficients and gas uptake data for carbon tetrachloride, to estimate in vivo metabolic rate constants for carbon tetrachloride in rats. The model comprises a series of differential equations describing the rate of carbon tetrachloride entry into and exit from a series of body compartments, including liver, fat, muscle, and viscera (richly perfused organs), as well as arterial and venous blood. Gas-uptake data were obtained in a closed recirculated exposure system. Partition coefficients were experimentally derived in a series of in vitro studies using the tissues of interest. The researchers found that the uptake kinetics of carbon tetrachloride were adequately described by modeling metabolism of the compound as a single saturable process with V_{max} of 0.92 μmol/hour (0.14 mg/hour) and K_m of 1.62 μmol/L (0.25 mg/L).

Paustenbach et al. (1988, 1987)

Paustenbach et al. (1988, 1987) developed a four-compartment PBPK model (similar in structure to Gargas et al., 1986) to describe the disposition of carbon tetrachloride absorbed during inhalation, based on the framework developed by Ramsey and Andersen (1984) and the parameter values reported by Gargas et al. (1986). Metabolism, assumed to occur only in the liver compartment, was modeled as a single, saturable pathway. Metabolites were apportioned into three separate storage compartments, leading to elimination in the exhaled breath, urine, and feces, respectively. In order to accommodate the observed biphasic elimination of CO_2,

equations were included to allow for the interconversion from the urinary or fecal pools to production of CO_2. The model also included a time delay of 23.5 hours for fecal excretion to account for the observed delay in appearance of radioactivity in the feces. Parameter values needed to run the model included partition coefficients (determined experimentally by vial equilibration), biochemical constants for carbon tetrachloride metabolism (determined experimentally by gas uptake studies), and physiological parameters (estimated from the literature, from previous pharmacokinetic studies, and from the process of fitting the carbon tetrachloride data during model development). Selection of the optimal parameters for fat compartment volume, blood flow, V_{max}, and K_m were determined by the quality of the visual fit of the model predictions with laboratory data; sensitivity analysis indicated that changes to other parameters had little effect on the simulation and were thus not subject to optimization. Model parameters are presented in Table 3-4. Calibration of the rat model was done using data for Sprague-Dawley rats exposed to 100 ppm of carbon tetrachloride for 4, 5, 7, or 10 exposures as reported in Paustenbach et al. (1986a, b). The model reliably predicted values for the following experimental parameters: concentration of [^{14}C] activity in adipose tissue, concentration of [^{14}C]-carbon tetrachloride in the expired breath, concentration of $^{14}CO_2$ in the expired breath, activity of ^{14}C in the urine, and activity of [^{14}C] in the feces.

Table 3-4. Physiological parameters for the rat, monkey, and human PBPK models for carbon tetrachloride

Parameter	Rat (0.42 kg)	Monkey (4.6 kg)	Human (70 kg)
Cardiac output (liters blood/hour)	8.15	41.2[a]	256[a]
Alveolar ventilation (liters air/hour)	7.91	43.9[a]	254[a]
Tissue volumes (percent of total)			
Liver	4	4	4
Fat	8	10	20[c]
Muscle	74	72	62
Richly perfused organs	5	5	5
Blood flow (percent of total)			
Liver	25	25	25
Fat	4	4	6
Muscle	20	20	18
Richly perfused organs	51	51	51
Metabolism			
V_{max} (mg/hour)	0.35	1.91[a]	12.72[a]
K_m (mg/liter)	0.25	0.25[b]	0.25[b]

[a] Allometrically scaled from the rat data using (body weight)$^{0.75}$.
[b] Assumed to be the same as in rats.
[c] Tissue volume for fat in humans is shown in Table 2 of Paustenbach et al. (1998) as 10%; however, the text of this paper states that the rat model was scaled up to humans using a fat compartment of 20% of body weight. The 20% value was determined to be correct.

Source: Paustenbach et al., 1988.

In order to extend the model to monkeys and humans, the rat model was scaled up, resulting in models for monkeys and humans that were used to predict the concentration of carbon tetrachloride in expired air. For both the monkey model and the human model, cardiac output, alveolar ventilation, and V_{max} were estimated using (body weight)$^{0.75}$, and the K_m was assumed to be the same as for the rat. The rat model was scaled to monkeys, using a body weight (BW) of 4.6 kg, a body fat estimate of 10%, and fat perfusion of 4% of cardiac output; other parameters were assumed to be the same as in the rat. The monkey model was calibrated by using the data of McCollister et al. (1951), which measured the concentration of expired carbon tetrachloride after a 370-minute exposure to 50 ppm. The time course was accurately predicted, except for long periods (>240 hours) after exposure in which the model predicted lower concentrations than were demonstrated experimentally. The study authors suggested that small amounts (0.4%) of carbon tetrachloride may have been converted into C_2Cl_6, which has a much longer half-life in adipose tissue and would account for the slow elimination of small

amounts of radiolabel. The rat model was scaled up to humans by using an experimentally measured human blood:air partition coefficient, a body weight of 70 kg, and a fat compartment of 20% BW. Model simulations of concentration of carbon tetrachloride in expired air over time were compared with the data of Stewart et al. (1961), who exposed human volunteers to 49 ppm of carbon tetrachloride for 70 minutes or to 10 ppm of carbon tetrachloride for 180 minutes; there was good agreement between the model simulation and the measured results. The model predicted that at concentrations up to 100 ppm, the rat, monkey, and human metabolize carbon tetrachloride in a similar manner. Because of physiological differences, the models predicted species differences in carbon tetrachloride accumulation in fat. The rat PBPK model accurately described carbon tetrachloride concentrations in adipose tissue where no significant day-to-day accumulation in fat or blood was observed following repeated exposure to 100 ppm for 8 or 11.5 hours/day, whereas the human model predicted day-to-day increases in carbon tetrachloride in fat following inhalation exposure to 5 ppm for 8 hours/day.

Thrall et al. (2000); Benson and Springer (1999)

Thrall et al. (2000) and Benson and Springer (1999) expanded the rat PBPK model of Paustenbach et al. (1988) to include parameters for the mouse and the hamster. The mouse and hamster models consist of five compartments identical to the rat model (lung, liver, fat, muscle, and richly perfused tissues). Metabolism is still assumed to occur only in the liver and is modeled by a single, saturable pathway that results in products that may be eliminated in the expired air, urine, or feces. For the mouse, tissue:air partition coefficients were assumed to be equal to those for the rat, with the exception of the blood:air coefficient, which was measured with the vial equilibration technique. Tissue:blood partition coefficients were then calculated by dividing the tissue:air coefficients by the blood:air coefficients. Metabolic rate constants (i.e., V_{max} and K_m) were measured in whole animals by using gas uptake studies with a closed recirculating chamber; in comparison to the rat, the mouse has a slightly higher capacity (higher in vivo V_{max}) and lower affinity (higher in vivo K_m) for metabolizing carbon tetrachloride. Physiological parameters for the mouse model were based on published values in the literature (Andersen et al., 1987). Model predictions for initial body burden, exhaled carbon tetrachloride, and exhaled CO_2 were compared with data collected over a 48-hour period following a 4-hour inhalation exposure to 20 ppm of [^{14}C]-carbon tetrachloride (data from a personal communication and not presented in the manuscript); ratios of predicted/observed concentrations ranged from 1.1 to 1.4, indicating very good agreement among observed and predicted values. For the hamster, coefficients for blood:air, muscle:air, liver:air, and fat:air were determined by the vial equilibration technique. Hamster tissue:air partition coefficients did not differ significantly from those of the rat. Tissue:blood partition coefficients were then calculated by dividing the tissue:air coefficients by the blood:air coefficients. Metabolic rate constants (i.e., V_{max} and K_m) were measured in whole animals by using gas uptake studies with a closed

recirculating chamber; in comparison to the rat, the hamster has a higher capacity (higher in vivo V_{max}) and lower affinity (higher in vivo K_m) for metabolizing carbon tetrachloride. Physiological parameters for the hamster model were those used in the rat model. The hamster model tended to overpredict uptake from exposure at low concentrations and underpredict the uptake from exposure at high concentrations (1800 ppm exposure). Model predictions for initial body burden, exhaled carbon tetrachloride, and exhaled CO_2 were compared with data collected over a 48-hour period following a 4-hour inhalation exposure to 20 ppm of [^{14}C]-carbon tetrachloride (data from a personal communication and not presented in the manuscript); ratios of predicted/observed concentrations ranged from 0.6 to 2.1 for all three species, and from 0.6 to 1.4 for rats and mice (see Appendix C for a comparison of model predictions and experimentally-derived data).

Thrall et al. (2000) and Benson and Springer (1999) used in vitro data on metabolism of carbon tetrachloride by human liver microsomes (Zangar et al., 2000), together with in vitro and in vivo rodent data, to estimate the in vivo human metabolic rate constants. The calculation is presented in Table 3-5. Briefly, in vivo V_{max}/K_m ratios were obtained for the rodent species after V_{max} was normalized for milligrams of liver protein. The corresponding in vitro V_{max}/K_m ratios were calculated in the same manner, and the in vivo/in vitro ratios were calculated, giving values of 1.40, 1.01, and 1.70 for the rat, mouse, and hamster, respectively. As these values were very similar, a human in vivo V_{max}/K_m ratio of 1.37 was estimated as the mean of the rat, mouse, and hamster ratios. Because the human K_m in vitro is similar to that of the rat, the in vivo human K_m was assumed to be the same as that of the rat, allowing for the calculation of a human in vivo V_{max} of 29.15 mg/hour. The researchers used the new value for V_{max} in the human PBPK model of Paustenbach et al. (1988), with other parameters remaining as previously described, and compared it with the human data of Stewart et al. (1961). The model simulation of expired carbon tetrachloride levels provided good agreement with the experimental data, particularly at longer periods postexposure (see Appendix C for a comparison of model predictions and experimentally-derived data).

Table 3-5. Comparison of metabolism from in vitro and in vivo studies

	Rat	Mouse	Hamster	Human
BW (kg)	0.25	0.025	0.15	70
Liver weight (g)[a]	10	1	6	2800
mg protein/g liver[b]	13.8	21.9	17.8	12.8
In vivo V_{max} (mg/hour/kg BW)[c]	0.4	0.79	6.39	1.49
In vivo V_{max} (mg/hour)[d]	0.15	5.97×10^{-2}	1.69	29.15
In vivo V_{max} (mg/hour/mg protein)	1.1×10^{-3}	2.7×10^{-3}	0.016[f]	8.1×10^{-4}
In vivo K_m (mg/L)[c]	0.25	0.46	1.14	0.25[h]
In vivo V_{max}/K_m	4.4×10^{-3}	5.9×10^{-3}	0.014[g]	3.2×10^{-3}
In vitro V_{max} (µmol/hour/mg protein)[e]	0.186	0.1712	0.246	0.135
In vitro K_m (µmol/L)[e]	59.1	29.3	30.2	56.8
In vitro V_{max}/K_m (L/hour/mg protein)	3.15×10^{-3}	5.86×10^{-3}	8.14×10^{-3}	2.38×10^{-3}
Ratio (in vivo/in vitro)	1.4	1.01	1.7	1.37[i]

[a] Calculated as 4% of body weight.
[b] From Reitz et al. (1996), except hamster, which was estimated as the mean of mouse and rat.
[c] Rodents: experimentally measured; humans: calculated (see text).
[d] Rodents: calculated from in vivo V_{max} (mg/hour/kg BW) using $BW^{0.7}$ (personal communication; email dated 9/5/2006, from Dr. Karla Thrall, Pacific Northwest National Laboratory, to Susan Rieth, U.S. EPA); humans: calculated (see text).
[e] Data from Zangar et al. (2000).
[f] Corrected from value of 0.16 in Table 5 of Thrall et al. (2000) (personal communication; email dated 9/5/2006, from Dr. Karla Thrall, Pacific Northwest National Laboratory, to Susan Rieth, U.S. EPA).
[g] Corrected from value of 0.14 in Table 5 of Thrall et al. (2000) (personal communication; email dated 9/5/2006, from Dr. Karla Thrall, Pacific Northwest National Laboratory, to Susan Rieth, U.S. EPA).
[h] Assumed to be equal to the rat based on in vitro K_m comparisons.
[i] Calculated as the average of the rat, mouse, and hamster in vivo/in vitro ratios.

Source: Thrall et al., 2000.

Other Extensions of the Paustenbach et al. (1988) Model

Several other models have been developed as extensions of the Paustenbach et al. (1988) model. Semino et al. (1997) added a gastrointestinal compartment to the inhalation model of Paustenbach et al. (1988) to describe uptake of carbon tetrachloride administered by a single gavage dose at levels of 25 or 50 mg/kg in corn oil or at a dose of 17.25 mg/kg in 0.25% aqueous Emulphor to male F344 rats. The gastrointestinal compartment was divided into a series of sequential absorption subcompartments, each characterized by three parameters: emptying time, absorption rate constant (describing input to the portal circulation), and bioavailability. These parameters were optimized against the experimental results for concentrations of parent carbon tetrachloride in arterial blood or exhaled air. The number of subcompartments was also varied; nine subcompartments were needed to obtain a good fit of this data set for delivery by corn oil gavage, whereas only six or seven subcompartments were needed for aqueous Emulphor. The

model simulated the higher rapid initial uptake with the aqueous vehicle and the more pulsatile absorption profile observed from corn oil delivery following a single exposure. The subcompartments were not intended to correspond to actual anatomic segments of the gastrointestinal tract, and the values generated for oral uptake parameters were not intended to represent true physiological measurements.

Thrall and Kenny (1996) adapted the PBPK model of Paustenbach et al. (1988) to simulate an intravenous route of exposure in the male F344 rat. The model added equations to simulate the introduction of carbon tetrachloride into the mixed venous blood pool. Physiological parameters were adjusted to account for the smaller body size of F344 rats compared with Sprague-Dawley rats, using data from Arms and Travis (1988). The model was used to predict the concentration of carbon tetrachloride in the expired air after a single intravenous exposure and was compared with real-time monitoring data from rats given a single injection of carbon tetrachloride at 0.6 or 1.5 mg/kg BW. With the exception of underestimation of the initial peak in exhalation, the model predictions were in good agreement with the measured data.

El-Masri et al. (1996) modified the PBPK rat model of Paustenbach et al. (1988) to include a linked physiologically based pharmacodynamic (PBPD) model for hepatocellular injury and animal death. First-order rate constants governed simulated cell mitosis and birth, injury (due to carbon tetrachloride-induced vacuolation and incidental injury), repair, delay of mitosis and repair, cell death, and phagocytosis by macrophages. Animal death was simulated to occur when ≥50% of hepatocytes died. The data of Lockard et al. (1983) was used to visually optimize the PBPD model rate constants.

Other models of carbon tetrachloride disposition were developed independent of Thrall et al. (2000) or Paustenbach et al. (1988) and are discussed further below.

Gallo et al. (1993)

Gallo et al. (1993) developed a physiological and systems analysis hybrid pharmacokinetic model for blood concentration-time data obtained during intravenous or oral administration. The systems analysis procedure was based on a disposition-decomposition method for deriving an absorption input function for each regimen. Equations were derived, representing input into the blood, distribution to and from the blood to the peripheral tissues, and elimination from the blood, allowing for the estimation of arterial and venous blood concentrations but not concentrations in target tissues. Experimental data were collected for male Sprague-Dawley rats given a single oral dose of 25 mg/kg in one of four ways (undiluted, in corn oil, as an emulsion in 0.25% Emulphor, or in water) and from other rats receiving the same dose in aqueous polyethylene glycol 400 as an intravenous bolus injection. A hybrid model that combined model parameters available in the literature with the absorption input functions obtained by systems analysis adequately described the observed blood concentration-

time data. The same model using conventional first-order absorption inputs provided less accurate fits to the data. Both the standard model and the hybrid model overestimated the initial concentration in blood for the oral or intravenous routes.

Evans et al. (1994)

Evans et al. (1994) developed a PBPK model for carbon tetrachloride in rats based on the Ramsey and Andersen (1984) model for styrene. Flow-limited compartments for liver, fat, and rapidly and slowly perfused tissues were connected by arterial and venous blood. The investigators derived partition coefficients from blood, liver, fat, and muscle samples of naïve male Fischer-344 rats. Physiological parameter values were taken from the literature. Metabolism of carbon tetrachloride was constrained to the liver and described by Michaelis-Menten kinetics. V_{max} and K_m were estimated by optimizing the model to closed-chamber gas uptake data, generated by the study authors, for adult male Fischer-344 rats exposed to 25, 100, 250, or 1000 ppm carbon tetrachloride for 6 hours. The resulting V_{maxC} and K_m values were 0.37 mg/hr/kg and 1.3 mg/L, respectively. The predicted decreases in chamber carbon tetrachloride concentrations were very similar to observations for all exposure levels and time points. A sensitivity analysis was performed on all of the model parameters. For the low exposure (25 ppm), the blood:air partition coefficient (5.49), followed by the fat:blood partition coefficient (51.3) and fat tissue volume (8%), had the greatest effects on simulated chamber concentration. However, the fat:blood partition coefficient and fat tissue volume dominated the decrease in chamber concentration in the 1000-ppm exposure.

The model of Evans et al. (1994) was applied to examine the effect of methanol pretreatment of rats (10,000 ppm for 6 hours) at 24 and 48 hours prior to 6-hour closed-chamber carbon tetrachloride exposures of 25, 100, 250, or 1000 ppm (Evans and Simmons, 1996). V_{maxC} was optimized against the gas uptake data from all exposure levels. A V_{maxC} value of 0.48 mg/hr/kg for the 24-hour methanol pretreatment group resulted in very good agreement of the predicted and observed chamber concentrations at all exposure levels, indicating that induction of carbon tetrachloride metabolism could be adequately simulated. Good agreement was also achieved between predicted and observed chamber concentrations at all exposure levels for the 48-hour methanol pretreatment group. The estimated V_{maxC} value of 0.18 mg/hr/kg, which was very close to the carbon tetrachloride-only value of 0.11 mg/hr/kg (from Evans et al., 1994), indicated that the effect of methanol induction of carbon tetrachloride metabolism had practically ceased by this time.

Yoshida et al. (1999)

Yoshida et al. (1999) used a classical compartment pharmacokinetic model to derive rates of absorption of carbon tetrachloride in rats exposed at low concentrations in a closed chamber system. Experimentally, rats were exposed at initial concentrations between 10 and 1000 ppb,

and the changes in chamber concentrations were measured over 6 hours. The model, like the experimental system, had three compartments: a tank containing barium chloride to capture the compound, the exposure chamber into which the compound was injected, and the rat. The model consisted of three differential equations describing the apparent volumes of distribution for the three compartments. The model included single rate constants for inhalation, exhalation, and metabolic elimination processes in the rat. The rate constant for exhalation was determined to be higher than that for elimination. Metabolic elimination of carbon tetrachloride was estimated as 0.53 µmol/hour/kg at 10 ppm.

Andersen et al. (1996) developed a model to describe the anaerobic in vitro metabolism of carbon tetrachloride in a two-phase, closed-chamber headspace vial. Data were generated from hepatic microsomal preparations from fed or fasting adult male F344 rats. Partition coefficients were experimentally derived for phosphate buffer to air and microsomal suspension to air. In addition to the Michaelis-Menten kinetic constants, a first-order loss-rate constant was required for accurate fitting of the model. The model described the kinetics of anaerobic transformation of carbon tetrachloride to chloroform.

Fisher et al. (2004)

Fisher et al. (2004) developed a PBPK model for simultaneous exposures to carbon tetrachloride and tetrachloroethylene in mice. The model contained a 4-compartment structure (liver, fat, and richly and slowly perfused tissues) for carbon tetrachloride based on the Ramsey and Andersen (1984) model and tetrachloroethylene based on a modified form of the Gearhart et al. (1993) model. Absorption from the gastrointestinal tract was simulated as a 2-compartment, 3-parameter model (Figure 3-2). Rate coefficients were estimated by visually fitting these parameters to blood data following single oral gavage doses of carbon tetrachloride (20, 50, or 100 mg/kg carbon tetrachloride alone, 10 or 100 mg/kg tetrachloroethylene alone, and 1, 5, 20, 50, or 100 mg/kg carbon tetrachloride followed 1 hour later by 10 or 100 mg/kg tetrachloroethylene; all oral bolus doses were administered in aqueous emulsion vehicle). Metabolism for both chemicals was represented as a saturable Michaelis-Menten pathway in the liver only. Carbon tetrachloride-induced suicide inhibition was modeled with a second-order inhibition constant, K_D, which was used to calculate the loss of metabolic capacity (V_{maxC}) for both carbon tetrachloride and tetrachloroethylene. A submodel for trichloroacetic acid, the sole metabolite of tetrachloroethylene oxidation, was included in which the rate of trichloroacetic acid production in the liver was equal to the rate of tetrachloroethylene metabolism. Four compartments for trichloroacetic acid were included: liver, kidney, and rapidly and slowly perfused tissues.

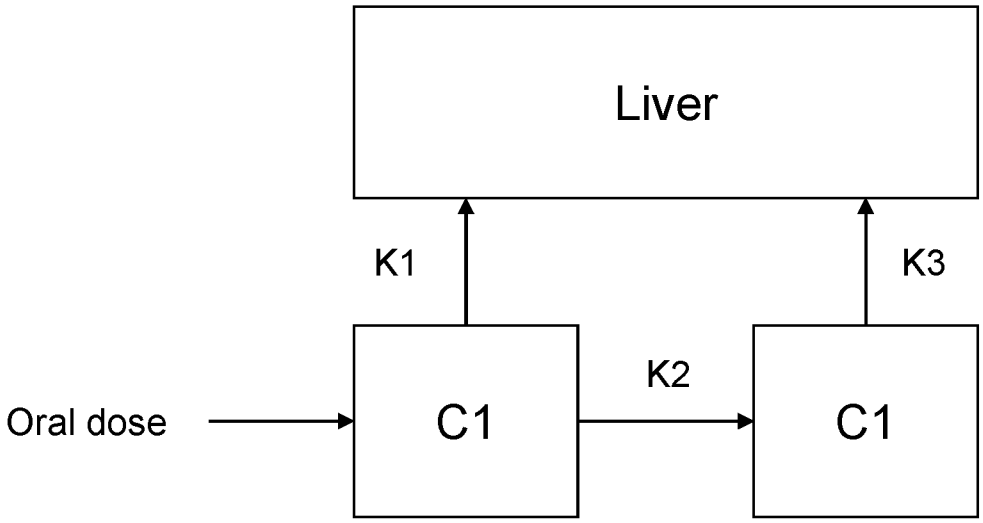


Figure 3-2. Two-compartment model for simulating gastrointestinal absorption of carbon tetrachloride administered to mice as a single gavage dose in Emulphor (Fisher et al., 2004).

Values for rate coefficients were derived by visual fit of model predictions to observed blood carbon tetrachloride kinetics in mice. The value for K1 was dose dependent (0.4 hr^{-1} for 20 mg/kg dose and 10 hr^{-1} for 50 and 100 mg/kg doses). Values for K2 and K3 were 2 and 0.05 hr^{-1}, respectively.

Carbon tetrachloride partition coefficients for blood, liver, fat, and muscle (representing slowly perfused tissue) were determined by the study authors (Fisher et al., 2004) using the vial equilibration method of Gargas et al. (1989). Partition coefficients for tetrachloroethylene and trichloroacetic acid were taken from Gearhart et al. (1993) and Abbas and Fisher (1997), respectively. Physiological constants for mice were taken from the compendium of Brown et al. (1997). Data for carbon tetrachloride gas uptake exposures of 130 ppm (Thrall et al., 2000) and 50, 450, or 1250 ppm (Fisher et al., 2004) in male B6C3F1 mice were used to optimize V_{maxC} and K_m, resulting in values of 1 mg/hr/$kg^{0.75}$ and 0.3 mg/L, respectively. For tetrachloroethylene, gas uptake-derived V_{maxC} and K_m values of 6 mg/hr/$kg^{0.75}$ and 3 mg/L, respectively, were taken from Gearhart et al. (1993). Oral absorption rate constants for carbon tetrachloride and tetrachloroethylene were visually fitted from the blood concentration data for each chemical. The value for K_D was estimated by optimization of the model to blood trichloroacetic acid concentrations following co-exposures of tetrachloroethylene and carbon tetrachloride via oral bolus dosing. See Appendix C for a summary of parameter values used in the Fisher et al. (2004) model.

Yoon et al. (2007)

Yoon et al. (2007) explored the effect of extrahepatic carbon tetrachloride metabolism in rats and humans on estimates of hepatic V_{max} and K_m. The investigators developed an 8-

compartment, flow-limited PBPK model, including compartments for lung, liver, brain, kidney, fat, rapidly and slowly perfused tissues, and the gastrointestinal tract. Physiological parameter values were taken from the literature (Delp et al., 1991; U.S. EPA, 2000e; Brown et al., 1997). Tissue partition coefficients for the rat were taken from Evans et al. (1994). Gas uptake data from closed-chamber experiments (Evans et al., 1994) were used to estimate values of V_{max} (0.13 mg/kr/kg$^{0.75}$) and K_m (1.10 mg/L) in the liver. Data for estimation of extrahepatic metabolism were generated from in vitro CYP2E1-mediated microsomal metabolism of carbon tetrachloride in liver, brain, skin, kidney, lung, and fat. No metabolic activity was detected in the fat, brain, or skin. Estimates of extrahepatic in vivo metabolism in the lung and kidney were modeled as the liver V_{max} adjusted by the tissue volume-normalized ratio of $V_{max,\ in\ vitro\ tissue}$ / $V_{max,\ in\ vitro\ liver}$. Simulations of open-chamber inhalation exposures (ATSDR, 2003) were used to compare the effect of the presence or absence of extrahepatic metabolism on the following dose metrics: carbon tetrachloride blood C_{max}, AUC for carbon tetrachloride in blood over a 24-hour period, total carbon tetrachloride metabolized in the body, and carbon tetrachloride metabolized in the liver (normalized for liver volume). The presence or absence of extrahepatic metabolism did not affect either the estimation of hepatic V_{max} and K_m or the predicted dose metrics. The proportion of liver metabolism estimated for the lung and kidney was quite small, 0.79 and 0.93%, respectively, based on the microsomal studies. This resulted in identical values for V_{max} and all of the examined dose metrics, and similar values for K_m (1.10 and 1.14 mg/L without and with extrahepatic metabolism, respectively).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Oral Exposure

4.1.1.1. *Human Poisoning Incidents*

Case reports reveal that individuals acutely poisoned with carbon tetrachloride can exhibit gastrointestinal toxicity (nausea, vomiting, diarrhea, and abdominal pain) and neurotoxicity (drowsiness, coma, or seizures) (Ruprah et al., 1985; Stewart et al., 1963; New et al., 1962). Hepatic involvement has been demonstrated by liver enlargement and significant elevations in serum enzyme (>100-fold increases in alanine aminotransferase [ALT] or aspartate aminotransferase [AST]) and bilirubin levels (Ruprah et al., 1985; Stewart et al., 1963). One of two individuals who received one 5 mL dose of carbon tetrachloride as an antihelmintic exhibited microscopic pathology in the liver (granular degeneration); a third person who received a second dose 2 weeks later had fatty degeneration of the liver, as well as swelling of the proximal tubules of the kidney (Docherty and Nicholls, 1923; Docherty and Burgess, 1922). Renal effects (oliguria and increases in blood urea nitrogen [BUN]) may occur within 1–8 days of acute exposure (New et al., 1962). Umiker and Pearce (1953) noted that, after ingestion of fatal doses of carbon tetrachloride, the primary cause of death during the first week was hepatic injury and afterwards was renal insufficiency. Pulmonary lesions (lung congestion, edema, bronchopneumonia, fibrinous exudate, alveolar epithelial proliferation) appear about 8 days after exposure and have been considered to be secondary effects of renal failure (Umiker and Pearce, 1953). Human fatalities from ingestion of carbon tetrachloride may occur with ingestion of amounts as low as 2–3 mL (45–68 mg/kg, based on the reference adult BW of 70 kg) (Ruprah et al., 1985; Gosselin et al., 1976).

4.1.1.2. *Epidemiology Studies*

Epidemiological studies have investigated possible associations between oral exposure to carbon tetrachloride and a variety of adverse birth outcomes (Croen et al., 1997; Bove et al., 1995, 1992a, b); however, because of multiple chemical exposures and insufficient power, these studies are considered limited and insufficient to determine whether there is an association between carbon tetrachloride exposure and adverse birth outcomes.

Bove et al. (1995, 1992a,b)

Bove et al. (1995, 1992a,b) evaluated the relationship between contamination of public drinking water with organic compounds (including carbon tetrachloride) and adverse birth outcomes in a cross-sectional study of births in four counties in northern New Jersey. The study

population consisted of registered live births and fetal deaths occurring from January 1, 1985, to December 31, 1988, in 75 towns (selected from a total of 146 in the four counties), where most residents were served by public water systems and most births occurred in the state. After exclusion of plural births and fetal deaths from therapeutic abortions or chromosomal anomalies, the subjects totaled 80,938 live births and 594 fetal deaths. Fetal death certificates available for all fetal deaths with gestational age greater than 20 weeks and the New Jersey Birth Defects Registry were used to gather data on a selection of adverse birth outcomes. A comparison group of 52,334 births that had no adverse outcomes was included in the study to evaluate categorical outcomes. Exposure to organic compounds was estimated from the monthly records of the 49 water companies serving the study population (water samples were collected at the tap). In addition to carbon tetrachloride, other contaminants in the drinking water included trihalomethanes (primarily chloroform), 1,2-dichloroethane, dichloroethylenes, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and benzene. Levels of all of these compounds, other than benzene, were higher than carbon tetrachloride; levels of trihalomethanes were 20- to 40-fold higher. For carbon tetrachloride, the exposed population was defined in one of two ways: those with exposure to >1 ppb in the drinking water or those with any detectable amount in the drinking water. In either case, the size of the comparison group with exposure to carbon tetrachloride was small: 357 births where levels >1 ppb were detected and 1993 births where any carbon tetrachloride was detected.

Carbon tetrachloride and the other contaminants were evaluated for effects on 13 selected birth outcomes (birth weight among term births, term low birth weight, small for gestational age, preterm birth, very low birth weight, fetal death, central nervous system defects, neural tube defects, oral clefts, major cardiac defects, ventricular septal defects, all cardiac defects, and all surveillance defects). Odds ratios (ORs) for an association between each outcome and carbon tetrachloride were calculated as the ratio of the risk of the outcome in the population with the specified exposure (either > nd or >1 ppb) to the risk in the population without the specified exposure. ORs were adjusted for maternal age, race, education, parity, adequacy of prenatal care, and sex of the child. Positive associations were found between exposure to carbon tetrachloride in drinking water at concentrations above 1 ppb and certain adverse outcomes: low birth weight (<2.5 kg) among term births (OR = 2.26, 95% confidence interval [CI]: 1.41–3.60) and small (at or below their race-, sex- and gestation week-specific tenth percentile weight) for gestational age (OR = 1.34, 95% CI: 1.02–1.80). These same effects, however, were also significantly associated with exposure to trihalomethanes, which were present in much higher levels and were much more prevalent in the drinking water supply (i.e., had a much larger exposed population and number of cases). While there was a statistically positive association between exposure to >1 ppb carbon tetrachloride and occurrence of neural tube defects (OR = 5.39, 95% CI: 1.31–22.2), it was based on only two cases in the exposed population. Using a criterion of OR ≥1.5 without consideration of CIs, the authors also reported positive relationships

between carbon tetrachloride and several of the other adverse outcomes tested. However, the reliability of these purported relationships is suspect without statistical support. Maternal interviews were conducted for a sample of the study population to collect more detailed information about potential confounders, such as maternal occupational exposures, smoking, medical histories, height, and gestational weight gain. Adjustment for these additional risk factors had no appreciable effect on the results for carbon tetrachloride. Interpretation of the study results is hindered by simultaneous exposure to multiple chemicals in the drinking water, the relatively small number of people exposed to carbon tetrachloride and the low levels to which they were exposed, and the limited characterization of exposure to carbon tetrachloride (and the other chemicals tested).

Croen et al., 1997

Croen et al. (1997) used data from two population-based case-control studies to determine whether maternal residential proximity to hazardous waste sites increased the risk for certain birth defects in California. Residential histories were obtained by interviews with mothers of infants with specific birth defects (neural tube defects [507 cases] in one study; heart defects [201 cases] and oral cleft defects [439 cases] in the other) and mothers of controls in the two studies (517 for the neural tube study and 455 for the other two defects). Information was collected on 764 inactive waste sites as well as 105 National Priority List sites. Multivariate analysis was used to control for potential confounding effects, such as maternal race/ethnicity, income, and education. The study found no increased risk of heart defects or oral cleft defects among offspring of mothers living near a waste site containing carbon tetrachloride, but this study had little power to detect effects. Odds ratios for neural tube defects associated with carbon tetrachloride were not provided.

4.1.2. Inhalation Exposure

4.1.2.1. Acute Exposure Incidents

The initial acute effects of carbon tetrachloride in humans exposed by inhalation are similar to effects reported from humans exposed orally (Stewart et al., 1965; New et al., 1962; Norwood et al., 1950); these effects include gastrointestinal symptoms (nausea and vomiting, diarrhea, abdominal pain), hepatic effects (elevated serum AST, mild jaundice, and, in fatal cases, necrosis of the liver), and neurological effects (headache, dizziness, weakness). As with acute oral exposure, inhalation exposure causes renal effects (oliguria, elevated BUN) that appear 1–8 days after exposure, with an average delay of 4 days (New et al., 1962). Renal histopathological effects in fatal cases include nephrosis, degeneration, and interstitial inflammation of the kidney (Norwood et al., 1950). Pulmonary edema is a secondary consequence of renal insufficiency (Umiker and Pearce, 1953; Norwood et al., 1950). Some case reports noted that a high intake of alcohol, which can enhance carbon tetrachloride toxicity, was

common among the patients intoxicated by inhaled carbon tetrachloride (New et al., 1962; Norwood et al., 1950).

Lehmann and Schmidt-Kehl (1936) described the neurological symptoms in humans exposed briefly to carbon tetrachloride vapor at concentrations of 20 mg/L (3200 ppm) and above. No effect was observed following exposure at 20 mg/L for 5 minutes. Exposure at 30 mg/L (4800 ppm) for 2.5 minutes resulted in slight drowsiness after 5 minutes. Exposures at 40 mg/L (6400 ppm) for 3 minutes resulted in tremor and drowsiness, followed by staggering. The highest tested exposure, 89 mg/L (14,100 ppm) for 0.8 minutes, resulted in loss of consciousness. Stewart et al. (1961) reported no adverse effects (such as nausea or dizziness) in male volunteers exposed to carbon tetrachloride vapor at 49 ppm for 70 minutes or 10–11 ppm for 180 minutes.

4.1.2.2. *Epidemiology Studies*

Occupational exposure to unknown concentrations of carbon tetrachloride vapor for periods between 6 weeks and 3 months resulted in gastrointestinal effects (nausea, vomiting, abdominal pain, anorexia), hepatic effects (jaundice), and neurological effects (headache, dizziness) (Norwood et al., 1950). Kazantzis and Bomford (1960) described symptoms in 17 workers exposed to carbon tetrachloride vapor at concentrations between 45 and 97 ppm without adequate ventilation. Symptoms in 15/17 workers included anorexia and nausea and, in more than half of the workers, vomiting, epigastric discomfort or distension, depression, irritability, headache, or giddiness. Symptoms typically developed in the latter half of the workweek and cleared over the weekend. One of the workers, who reported having symptoms for 2 years, previously had an increased serum AST level, but levels were normal for this individual and seven others examined by the authors for this study. Similarly, Elkins (1942) reported results of industrial hygiene evaluations in 11 plants in which workers were exposed to carbon tetrachloride vapor. At concentrations between 5 and <85 ppm, nausea was the most common symptom, but vomiting, headache, and body weight loss were also observed.

Tomenson et al., 1995

Tomenson et al. (1995) conducted a cross-sectional study of hepatic function in 135 carbon tetrachloride-exposed workers in three chemical plants in northwest England and in a control group of 276 unexposed workers. The latter came from two sites, including one of the plants that provided workers for the exposed group and a plant nearby where carbon tetrachloride was not used. Controls had not held jobs with potential exposure to carbon tetrachloride or other known hepatotoxins during the previous 5 years. Subjects were administered a questionnaire that collected information on medical history, alcohol consumption, and length of service in a job exposed to carbon tetrachloride. Blood samples were obtained from subjects after a 12-hour fast that included abstinence from alcohol; samples were collected for about 60 subjects over 2 weeks

in November 1986 and for the remaining subjects over 8 weeks starting in February 1987. Blood samples were analyzed for ALT, AST, alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), glutamate dehydrogenase (GDH), 5'-nucleotidase, total bile acids, cholesterol, triglycerides, and hematological variables.

The exposure assessment was based on historical personal monitoring data for various jobs at the three plants. Subjects were placed into one of three exposure categories (low, medium, or high), according to their current jobs. When objective monitoring data were not available for a particular combination of job and location (as was the case for 23 of 40 in the low exposure group, 35 of 54 in the medium exposure group, and 2 of the 61 in the high exposure group), an industrial hygienist classified the exposure qualitatively based on comparison with similar groups. The quantitative exposure levels nominally associated with each of these categories were: ≤1 ppm for "low," 1.1–3.9 ppm for "medium," and 4 ppm–11.9 ppm for "high." Exposed workers were also categorized according to length of time in job (<1 year, 1–5 years, and >5 years).

Study and control groups were found to be well matched for age, height, weight, work patterns, and, generally, alcohol consumption. Almost all (97–98%) control and exposed workers were current drinkers, and the proportions of low, medium, and high alcohol drinkers were roughly similar in the two groups ($p = 0.30$ for Chi-square comparison of 4 levels of alcohol use between exposed and non-exposed). However, there was a slightly higher proportion of very high drinkers (5–7 units every day or > 8 units at least 3-4 times per week) in the exposed group (27%) than in controls (20%) ($p = 0.20$ for Chi-square comparison of high alcohol use between exposed and non-exposed). Serum levels of GGT, bile acids, and triglycerides were significantly increased in the high and/or very high alcohol consumption groups. In addition, serum levels of GGT, cholesterol, triglycerides, AST, and 5'-nucleotidase were found to be significantly related to age. Ages of workers in both control and exposed groups were approximately normally distributed, with similar means and ranges.

Analysis of variance was used to investigate the relationship between carbon tetrachloride exposure and serum chemistry and hematology variables, while controlling for age, sampling time, and alcohol consumption. Initial analyses also included an interaction term between carbon tetrachloride and alcohol consumption, but no evidence for any interaction was found and the term was dropped from subsequent analyses. No analyses based on length of time on job (i.e., duration of exposure) are presented in the published paper.

Multivariate analysis, based on simultaneous consideration of ALT, AST, ALP, and GGT as dependent variables, revealed a statistically significant ($p<0.05$) difference between exposed and unexposed workers. There was no evidence, however, of a dose-response across the levels of exposure. In univariate analyses, in which each dependent variable was assessed separately, there were no significant differences between the carbon tetrachloride-exposed group and the control group for any of the serum chemistry variables. However, there was evidence of

increased levels of ALP and GGT in the medium and high exposure groups, with the differences between the medium exposure group and controls being statistically significant ($p < 0.05$) (see Table 4-1). GDH was significantly increased in the medium-exposure group but declined in the high-exposure group to the level seen in controls (see Table 4-1). There was little difference in the mean adjusted serum ALT, AST, bile acids, and 5'-nucleotidase levels across exposure categories.

Table 4-1. Mean of selected serum chemistry and hematology variables in relation to carbon tetrachloride exposure in British chemical workers

Variable[a]	Control	Exposure group		
		Low	Medium	High
ALT (mU/mL)[b]	20.54 (1.03)	20.35 (1.08)	20.82 (1.05)	19.39 (1.06)
AST (mU/mL)[b]	16.48 (1.02)	15.25 (1.05)	15.88 (1.04)	15.62 (1.04)
ALP (mU/mL)[b]	125.79 (1.02)	122.2 (1.05)	137.10[c] (1.04)	135.1 (1.04)
GGT (mU/mL)[b]	26.89 (1.05)	26.89 (1.11)	33.17[c] (1.08)	31.5 (1.08)
GDH (mU/mL)[b]	3 (1.05)	3.26 (1.10)	3.57[c] (1.07)	2.98 (1.07)
Total bile acids (μmol/L)[b]	1.06 (1.06)	1 (1.00)	1.25 (1.25)	1.28 (1.28)
5'-Nucleotidase (mU/mL)	5.89 (1.03)	6.54 (1.08)	6.25 (1.06)	5.75 (1.06)
Hemoglobin (g/dL)	15.97 (0.08)	15.6 (0.19)	15.39[c] (0.14)	15.71 (0.14)
Packed cell volume (%)	48.54 (0.23)	47.32[c] (0.54)	47.32[c] (0.39)	48.05 (0,41)
Red blood cell count ($\times 10^{12}$/L)	5.61 (0.03)	5.5 (0.08)	5.47[c] (0.06)	5.5 (0.06)

[a]Results are presented as least square means, adjusted for age, sampling time, and alcohol consumption.
[b]Analyzed after logarithmic transformation; values are geometric means with standard error of the mean (SEM).
[c]$p<0.05$ (pairwise comparison).
Source: Tomenson et al., 1995.

Statistically significant changes were found for some of the hematological variables (decreased red blood cell count, hemoglobin, and packed cell volume) in the univariate analyses but without a dose response. Compared with the unexposed controls, there were very slight (2.5–3.5%) statistically significant decreases in all three of these variables in the medium exposure group and in packed cell volume in the low-exposure group (Table 4-1). Values for all three hematological variables were similar to controls in the high-exposure group.

In an alternative analysis, a normal range was determined for each serum chemistry and hematology variable based on the 2.5 and 97.5% quantiles in the control group. The proportion of exposed workers exceeding the normal range was significantly elevated for ALT (8%) and GGT (11%) but not for the other serum chemistry or hematology variables. This analysis did not include any adjustment for alcohol intake or other potential confounders. The researchers noted

that, for the serum chemistry variables, the upper normal limits defined based on the control group were notably higher than the upper limits of the reference ranges for these tests supplied by the manufacturers, indicating a difference between the control group and the population used to derive the reference values, which are often hospital or university employees. This may have been related to high alcohol consumption in the study controls, whose alcohol intake was similar to the exposed group.

Individuals with one or more test results in excess of three standard deviations (SDs) outside the control group mean were examined by a gastroenterologist. One exposed worker had clinically detectable liver disease, but this could not be related to exposure to carbon tetrachloride. The only other clinical findings were non-Hodgkin's lymphoma (NHL) in an exposed worker and hemochromatosis in a control worker.

The observed decreases in hemoglobin, packed cell volume, and red blood cell count were not considered to indicate a biologically significant effect of carbon tetrachloride, as the observed changes were minimal and not clearly related to level of carbon tetrachloride exposure. The results were generally suggestive of an effect on the liver, but were not consistent across the liver variables or exposure levels. The overall difference seen in the multivariate analyses of the four enzymes (ALT, AST, ALP, GGT) seemed to be driven by the increase in GGT, and to a lesser extent in ALP, in the medium and high exposure groups. For GGT, the levels in the medium and high carbon tetrachloride exposure groups were similar to the levels seen in the high and very high alcohol use categories (geometric mean 30.04 and 32.32 mU/mL, respectively, in these two alcohol use groups compared with 24.6 mU/mL in the low alcohol use groups). There was little difference between the low carbon tetrachloride exposure group (≤1 ppm estimated exposure levels) and the no exposure group on any of the liver enzymes.

It is unclear to what extent the observed changes in serum enzyme levels reflect clinically significant changes. The researchers suggest that their results show some enzyme leakage from cells but without a measurable deficit in liver function (as assessed by total bile acid levels), and they note that no effects of clinical significance were observed. Increased serum levels of ALT, AST, ALP and GGT are indicators of liver damage (with ALP and GGT increased in exposed workers), but none are specific for liver disease. Elevated ALP is used in the diagnosis of hepatobiliary disease and bone disease, and elevated GGT in the diagnosis of liver disease. The measurement of serum GTT levels can be used to ascertain whether observed elevations of ALP are due to skeletal disease of reflect the presence of a hepatobiliary condition (Tietz, 1976).

One limitation of the study is the lack of information pertaining to the reliability (e.g., coefficient of variation, comparison with known standards) of the enzyme measures. The investigators noted that a follow-up study conducted at one site 3 years later revealed clear evidence of differences in laboratory procedures between the laboratories that had performed the testing of blood samples in the cross sectional and follow-up studies. In addition, it was noted that differences in the hematological variables (i.e., hemoglobin, packed cell volume, and red

blood count) were observed between the samples collected in November 1986 and those collected in February and March of 1987.

Overall, this study provides suggestive evidence of an effect from occupational carbon tetrachloride exposure on hepatic serum enzymes, indicative of effects on the human liver. Specifically, serum enzyme results suggested an exposure-related effect in the medium and high exposure categories (>1–3.9 ppm [>6.3–24.5 mg/m^3] and 4–11.9 ppm [25.2–75 mg/m^3]). ALP and GGT were elevated to a similar degree in both medium and high exposure categories (although the difference was statistically significant only in the medium exposure category), and enzyme levels in these exposure groups were comparable to the levels of ALP and GGT seen in very high alcohol consumers. Confidence in the exposure monitoring for the medium exposure group is relatively low, where exposures were estimated for over half (35/54) of the workers. Confidence in the exposure monitoring for the high exposure group, where exposures were measured for 59/61 workers, is higher. Because enzyme levels in these two groups were comparable, an average concentration of the medium and high exposure groups (weighted by number of subjects within specific exposure ranges) of 5.5 ppm (35 mg/m^3) was considered to be an estimate of the lowest-observed-adverse-effect level (LOAEL).[b] No effects on serum enzyme levels were seen in the low exposure category (i.e., ≤1 ppm [≤6.3 mg/m^3]). Because exposures were estimated for more than half (23/40) of the workers in this exposure category and because this category covers exposures less than 1 ppm, a NOAEL could not be determined.

Seidler et al., 1999

Seidler et al. (1999) evaluated the association between maternal occupational exposure to

[b] An average exposure concentration for medium and high exposure categories (weighted by number of subjects within specific exposure ranges) was calculated as follows using data in the appendix to Tomensen et al. (1995):

Exposure category	Exposure conc. (ppm) [mid-point of range]	Number of subjects	Product of conc. × number of subjects (ppm-subject)
Medium	1.5	4	6
	2.5	10	25
	3.5	5	17.5
	2.5 (estimated)*	35	87.5
High	5	14	70
	7	14	98
	9	16	144
	11	15	165
	8 (estimated)*	2	16
Sum		115	629
Average conc. for medium and high exposure categories (ppm)	5.5**		

* Estimated exposures were assumed to be the mid-point of the exposure category.
** Average calculated as the sum of the product of exposure concentration × number subjects for the individual exposure ranges in the medium and high exposure categories divided by the total number of subjects, or 629 ppm-subject ÷ 115 subjects = 5.5 ppm.

chemicals and the risk of infants small for gestational age in singleton births in a prospective cohort study of 3946 pregnant women in West Germany from 1987 to 1988. The final group of 1865 women included those who completed a questionnaire on sociodemographic, psychosocial, nutritional, environmental, and occupational factors, for whom pregnancy outcomes were known and who were working at the time of the interview. Women with stillbirths, multiple births, and incompletely recorded outcomes were excluded. A semiquantitative job-exposure matrix, incorporating consideration of likelihood of exposure, intensity of exposure, and proportion of time at work, was used to classify occupational exposure to eight chemicals or chemical groups, including carbon tetrachloride. ORs were calculated, adjusting for age, smoking status, alcohol consumption, body mass index, number of former births, and income as potential confounders. The study found no association between occupational exposure to carbon tetrachloride and the risk of infants small for gestational age. The power of this study was limited. Of the 1865 births, only 64 mothers had potential exposures to carbon tetrachloride characterized as "low" or "moderate."

Cancer studies

Several epidemiological studies have investigated potential associations between cancers of various types and exposure to carbon tetrachloride. The subjects of all of these studies experienced multiple chemical exposures, and the exposures were estimated qualitatively based on historical information. These studies, therefore, can provide only suggestive evidence for such associations.

Exposure to carbon tetrachloride was not found to be associated with cancer risk in case-control studies for astrocytic brain cancer in white males (300 cases and 320 controls) from three areas of the U.S. where a high proportion of the workforce is employed in petroleum refining and chemical manufacture (after adjustment for several potential confounders) (Heineman et al., 1994), for lung cancer in male employees (308 cases and 588 controls) of a Texas chemical plant (Bond et al., 1986), for pancreatic cancer in residents (63,097 cases and 252,386 controls) from 24 U.S. states (Kernan et al., 1999), for renal cell carcinoma in Minnesota residents (438 cases and 687 controls) (Dosemeci et al., 1999), for rectal cancer in Montreal residents (257 cases and 533 controls) (Dumas et al., 2000), or for lymphoma in a population (age 18-80 years) recruited from six study regions in Germany. In the general population-based case-control studies (Seidler et al., 2007; Kernan et al., 1999: Dosemeci et al., 1999; Dumas et al., 2000), occupation/industry information obtained from questionnaires, interviews or death certificates in combination with a job exposure matrix was used to characterize chemical exposures. There was evidence for a weak association between exposure to carbon tetrachloride and excess risk for breast cancer among white female residents of 24 U.S. states; the OR was 1.21 (95% CI: 1.1–1.3) for those thought to have had the highest intensity of exposure to carbon tetrachloride [based on occupation listed on death certificates] (Cantor et al., 1995). Among white male workers at a

rubber manufacturing plant in Akron, Ohio, there was a significant age-adjusted association between exposure to carbon tetrachloride and death from lymphosarcoma (6 exposed out of 9 cases, OR = 4.2, $p<0.5$) and lymphocytic leukemia (8 exposed out of 10 cases, OR = 15.3, $p<0.001$) (Wilcosky et al., 1984; Checkoway et al., 1984). Kubale et al. (2005) reported that exposure to solvents (including carbon tetrachloride and benzene) was significantly associated with leukemia mortality in civilian workers at the Portsmouth Naval Shipyard in Kittery, Maine (OR = 1.03, 95% CI: 1.01-1.06). The findings with respect to carbon tetrachloride are uncertain, however, because solvent exposures cannot be separated, exposure misclassification was considered likely, and the phase out of carbon tetrachloride began in 1948, whereas the cohort considered deaths between 1952 and 1996. No case-control studies were identified that looked for an association between carbon tetrachloride and liver tumors or adrenal gland tumors (the tumor types found in laboratory bioassays with carbon tetrachloride).

Spirtas et al. (1991) conducted a retrospective cohort study of 14,457 aircraft maintenance workers at Hill Air Force Base in Utah to evaluate mortality associated with workplace exposures, particularly trichloroethylene. Carbon tetrachloride was one of more than 20 chemicals include in the study. Spirtas et al. found increased mortality for NHL in white female workers who had been exposed to carbon tetrachloride, in comparison with the Utah population (Spirtas et al., 1991). However, in a follow-up study of the same cohort (Blair et al., 1998) that extended the follow-up of worker mortality from 1982 to 1990, the relative risk (calculated as the ratio of the rate of NHL mortality in the exposed and unexposed portions of the cohort, adjusted for date of birth, calendar year of death, and sex) of NHL mortality was not significantly increased in the female cohort (relative risk = 3.3, 95% CI: 0.9–12.7). A cohort of dry cleaners in St. Louis, Missouri, showed slight significant excesses for deaths from all cancers (standardized mortality ratio [SMR] = 1.2, 95% CI: 1.0–1.3), esophageal cancer (SMR = 2.1, 95% CI: 1.1–3.6), and cervical cancer (SMR = 1.7, 95% CI: 1.0–2.0) (Blair et al., 1990, 1979). Risk of esophageal cancer was increased specifically in workers with the highest cumulative exposure (SMR = 0.9, 0.3, and 2.8 in the low, medium, and high cumulative exposure categories). There also appeared to be an increase in the risk of lymphatic and hematopoietic cancers in the high-exposure group (SMR = 4.0), although this apparent increase was based on only five cases. While some of these workers are likely to have been exposed to carbon tetrachloride, no separate analysis was conducted for those exposed to carbon tetrachloride or any other individual chemical. A cohort of Finnish laboratory workers exposed to carbon tetrachloride and other chemicals showed no increased risk of cancer of any type, although the average follow-up time of 15.7 years for the cohort may have been too short to reveal risks for rare cancers with longer latency periods (Kauppinen et al., 2003).

An association between inhalation of carbon tetrachloride and liver cancer in humans was suggested by two case reports (Tracey and Sherlock, 1968; Johnstone, 1948). Johnstone (1948) reported the death of a 30-year-old female from liver cancer after 2–3 years of occupational

exposure (assistant to a metallurgist) to carbon tetrachloride at levels that produced signs of central nervous system toxicity, fatigue, and jaundice. Carbon tetrachloride exposure levels were not assessed. Prior to carbon tetrachloride exposure, the woman had a history of "biliary colic" and jaundice and had been studied for "gall bladder disease." A 66-year-old man died of hepatocellular carcinoma 7 years after acute inhalation exposure from carpets that had been cleaned with carbon tetrachloride (Tracey and Sherlock, 1968). The man was asymptomatic for 5 days after exposure but then developed vomiting, diarrhea, anuria, and jaundice. Although the patient had no prior history of liver disease, he reported daily consumption of "several alcoholic drinks"; the duration of alcohol consumption was not given. At the time of death, the liver tumor was extensive, with very little normal tissue remaining. The potential contribution of alcohol consumption to liver disease in this patient could not be ruled out. Because of complicating factors (e.g., alcohol consumption, previous history of liver disease), small number of individuals involved, single exposure in one case, and relatively short time spans between exposure and tumor appearance, a causal relationship between carbon tetrachloride and liver tumors cannot be established from these case reports.

4.1.3. Dermal Exposure

There is evidence from one case report of health effects from exposure to carbon tetrachloride that can at least partially be attributed to absorption across the skin (Farrell and Senseman, 1944). The worker was exposed 8 hours/day by using a fine spray of carbon tetrachloride to saturate a cloth wrapped around the fingers. Although some exposure is likely to have occurred by inhalation, the authors considered absorption through the skin of the hands to be the primary route of exposure. After an unspecified period of time at this job, the worker developed polyneuritis. Symptoms included weakness, pain in the limbs, and loss or reduction of certain reflexes. The patient, whose body weight was not reported, lost 8 pounds in the month between onset of illness and hospitalization. The signs and symptoms of neurotoxicity reversed after several months without exposure.

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

Consistent with human data, toxicity assays in animals exposed orally or by inhalation identify the liver to be the major target organ, with oral NOAELs between 0.71 and 0.86 mg/kg and oral LOAELs between 7.1 and 17.8 mg/kg. Hepatic carcinogenicity has also been reported in rats and mice exposed orally or by inhalation to carbon tetrachloride. While the liver appears to be the primary target organ for both oral and inhalation studies, the kidney is also a sensitive target organ for carbon tetrachloride exposure. Nephritis and nephrosis are very common effects following inhalation exposure to carbon tetrachloride.

4.2.1. Oral Exposure

4.2.1.1. *Subchronic Toxicity*

Litchfield and Gartland, 1974

Litchfield and Gartland (1974) conducted a series of assays evaluating hepatic effects in beagle dogs treated with carbon tetrachloride in gelatin capsules prior to their daily food intake. In one experiment, groups of six male and six female young adult dogs were dosed with 797 mg/kg-day for up to 28 days. Blood samples taken before treatment and at 7-day intervals were evaluated for serum ALT, AST, ALP, ornithine carbamoyl transferase (OCT), and creatine kinase. At termination, livers were examined for histopathology. In a second experiment, three female dogs were given 32 mg/kg-day for 8 weeks. Blood was sampled before treatment and at 2, 3, 5, 6, 7, and 8 weeks. Livers were examined for histopathology after sacrifice. Control values were obtained from untreated dogs. No clinical signs of toxicity were observed. In dogs treated at 797 mg/kg-day, increases in serum ALT levels (2- to 34-fold in 4/6 males and 6/6 females) and OCT (2- to 20-fold in 3/6 males and 6/6 females) were observed after 14–28 days. All dogs exhibited hepatic histopathology (minimal to moderately severe centrilobular fatty vacuolization, sometimes accompanied by single cell necrosis), the severity of which correlated with the level of serum ALT and OCT in individual dogs. Dogs that showed no enzyme level effect or a twofold increase only in ALT had minimal vacuolization with very occasional necrosis. Dogs that had two- to eightfold increases in ALT and two- to threefold increases in OCT had minimal to moderate vacuolization with occasional necrosis. Dogs with 8- to 11-fold increases in ALT and 4- to 7-fold increases in OCT had moderate vacuolation with single cell necrosis, and those with 18- to 34-fold increases in ALT and 20-fold increases in OCT had moderately severe vacuolation with single cell necrosis. The female dogs given 32 mg/kg-day for 8 weeks showed no change in serum enzyme levels and no histopathology of the liver. In this study, 797 mg/kg-day was a LOAEL based on reported hepatic effects in six male and six female dogs, and 32 mg/kg-day was a NOAEL based on no hepatic effects reported in three female dogs. Given the wide dose spacing in this study, there is considerable uncertainty about the assigned value of the NOAEL and LOAEL.

Bruckner et al., 1986

Groups of 15–16 adult male Sprague-Dawley rats were given doses of 0, 1, 10, or 33 mg/kg of analytical-grade carbon tetrachloride by gavage in corn oil 5 days/week for 12 weeks (time-weighted average doses of 0, 0.71, 7.1, or 23.6 mg/kg-day). Body weight was measured twice weekly. Blood samples were taken from five rats from each group at 2-week intervals (each individual animal served as a blood donor twice, at 6-week intervals). After 12 weeks, 7–9 animals from each group were sacrificed. The remaining animals were maintained without carbon tetrachloride treatment for an additional 2 weeks and then sacrificed. Following sacrifice, a terminal blood sample was taken by cardiac puncture. The liver and kidneys were removed,

weighed, and processed for histopathological examination. Blood samples were used for determination of serum ALT, OCT, and sorbitol dehydrogenase (SDH), all of which are indicators of liver injury, and BUN, an indicator of kidney damage. At the end of the exposure period, substantial toxicity was evident in rats exposed to 23.6 mg/kg-day. Body weight gain in this group was significantly reduced by about 6% after 30 days and 17% after 90 days. Liver toxicity in this group was manifested by significantly elevated ALT (up to 34 times control levels), SDH (up to 50 times control levels), and OCT (up to 8 times control levels) from week 2 through the end of exposure, significantly increased liver:body weight ratio, and extensive occurrence of degenerative lesions. Observed liver lesions included lipid vacuolization, nuclear and cellular polymorphism, bile duct hyperplasia, and periportal fibrosis. Severe degenerative changes, such as Councilman-like bodies (single-cell necrosis), deeply eosinophilic cytoplasm, and pyknotic nuclei, were occasionally noted as well. No evidence of nephrotoxicity was observed. Only moderate effects were seen in animals exposed to 7.1 mg/kg-day. Body weight gain was similar to controls, and liver toxicity was shown only by a significant (two- to threefold) elevation of SDH during the second half of the exposure period and the presence of mild centrilobular vacuolization in the liver. During the 2-week recovery period, serum ALT and SDH levels returned towards control levels in both mid- and high-dose rats. Hepatic lesions were still present in both groups, but severity was reduced for lesions other than fibrosis and bile duct hyperplasia, the severity of which did not change. No effects were observed in rats exposed to 0.71 mg/kg-day. This study identified a NOAEL of 0.71 mg/kg-day and a LOAEL of 7.1 mg/kg-day for carbon tetrachloride-induced liver toxicity.

Allis et al., 1990

Allis et al. (1990) conducted a study to investigate the ability of rats to recover from toxicity induced by subchronic exposure to carbon tetrachloride. Groups of 48 60-day-old male F344 rats were given 0, 20, or 40 mg/kg of carbon tetrachloride 5 days/week for 12 weeks (average daily doses of 0, 14.3, or 28.6 mg/kg-day) by gavage in corn oil. Food consumption by cage was measured throughout the study. Rats were weighed several times during the first week and once a week thereafter. After 12 weeks, treatment with carbon tetrachloride was stopped. Six animals from each group were sacrificed 1, 3, 8, and 15 days after exposure termination. Upon sacrifice, a terminal blood sample was taken for determination of total bilirubin, triglycerides, cholesterol, ALT, AST, ALP, and lactate dehydrogenase (LDH). The liver was weighed, and samples were taken for light microscopic examination and determination of protein and CYP450. The remaining 24 animals were used to determine liver uptake relative to the spleen for a sulfur colloid labeled with technetium-99m and for tritiated 2-deoxyglucose[c]. Rats

[c] Relative efficiency of liver uptake of the labeled sulfur colloid is a diagnostic test for human cirrhosis and considered by investigators to be an indirect measure of hepatocyte function. Hepatic uptake of 2-deoxyglucose is an indicator of hepatic glucose utilization.

used for this purpose were maintained as long as 22 days postexposure. The only toxicity endpoint measured in these "remaining" animals was liver weight. Both doses of carbon tetrachloride were hepatotoxic, although the high dose produced significantly greater toxicity than the low dose. One day after the end of exposure, significant dose-related changes were found for liver:body weight ratio and serum ALT, AST, and LDH (all increased) and liver CYP450 (decreased) in both dose groups. In addition, serum ALP and cholesterol were increased in the high-dose group. Histopathological examination of the liver revealed, among low-dose rats, cirrhosis in 2/6 and vacuolar degeneration and hepatocellular necrosis in 6/6 and, among high dose rats, cirrhosis (as well as degeneration and necrosis) in 6/6. Serum enzyme levels and CYP450 returned to control levels within 8 days of the end of exposure. Severity of microscopic lesions declined during the postexposure period, but cirrhosis persisted in the high-dose group through the end of the experiment. Relative liver weight decreased during the postexposure period but did not reach control levels in the high-dose group even after 22 days. Neither of the radiolabeled tracer techniques detected a decreased functional capacity in cirrhotic livers, a finding that could not be explained by the investigators. The low dose of 14.3 mg/kg-day was a LOAEL for hepatic toxicity in this study.

Koporec et al., 1995

Koporec et al. (1995) evaluated the effect of different dosing vehicles on the subchronic oral toxicity of carbon tetrachloride in the rat. Groups of 11 male Sprague-Dawley rats were treated with carbon tetrachloride by gavage at doses of 0, 25, or 100 mg/kg, 5 days/week for 13 weeks (average daily doses of 0, 17.8, or 71.4 mg/kg-day). The compound was administered in corn oil or as an aqueous emulsion in 1% Emulphor. An untreated control group was followed in addition to vehicle controls. Blood samples were taken from 4–5 rats/group after weeks 4 and 8 for analysis of SDH and ALT. All surviving rats were sacrificed at the end of exposure at which time additional blood samples were collected and the liver was weighed and sampled for histopathology and biochemical studies (triglyceride, microsomal protein, CYP450, and glucose-6-phosphatase [G6Pase]).

Mortality was found in all treated groups. The number of deaths was higher for rats treated with the Emulphor vehicle than with corn oil and increased with dose for both vehicles. Mortality was about 75% and 25% in the high- and low-dose Emulphor groups and about 45% and 10% in the high- and low-dose corn oil groups. No deaths occurred in any of the control groups. Body weight decreased in a dose-related fashion throughout the study to a comparable extent in rats treated with either vehicle. Terminal body weights were reduced about 25% (statistically significant) in the high-dose groups (both vehicles) and about 6% in the low-dose groups (both vehicles). Serum chemistry analyses showed statistically significant dose-related increases in SDH and ALT at both dose levels after 4–13 weeks of treatment with either vehicle. Increases in SDH were as high as 10-fold in the low-dose groups and 100-fold in the high-dose

groups, while increases in ALT were about twofold in the low-dose groups and 25-fold in the high-dose groups. The results were similar for rats treated in either vehicle. Liver microsomal enzyme activities (CYP450 and G6Pase) were significantly reduced only in the high-dose groups, and, again, the magnitudes of the effects were similar for rats treated in either vehicle. Absolute and relative liver weights were slightly but significantly increased in the high-dose rats treated in Emulphor but in no other groups. The researchers noted that the livers were perfused with saline to facilitate collection of biochemical data and suggested that this procedure may have influenced the liver weight results. Liver histopathology findings were similar in rats treated in either vehicle. In the low-dose groups, lesions, seen in almost all animals, consisted primarily of minimal-to-slight vacuolation and minimal fibrosis. In the high-dose groups, vacuolation and fibrosis were moderate-to-moderately severe (all animals), and other lesions were also seen in all animals, including minimal-to-slight necrosis and moderate-to-moderately severe cytomegaly, nodular hyperplasia, oval-cell hyperplasia, and bile-duct hyperplasia. The low dose of 17.8 mg/kg-day, which produced hepatic effects in rats with either the corn oil or the Emulphor vehicle, was considered a frank effect level (FEL) by the U.S. EPA because of the increased mortality at this dose level. Vehicle did not influence hepatotoxicity in this study, but lethality appeared to be enhanced by dosing in Emulphor.

Condie et al., 1986

A study comparing the effects of two different gavage vehicles on subchronic toxicity of carbon tetrachloride was also performed in mice. CD-1 mice (12/sex/group) were treated with 0, 1.2, 12, or 120 mg/kg of carbon tetrachloride (98.2% pure) by gavage in either corn oil or 1% Tween-60 aqueous emulsion 5 days/week for 12 weeks (average daily doses of 0, 0.86, 8.6, or 86 mg/kg-day) (Condie et al., 1986). The mice were caged in groups of six and provided with food and water ad libitum. Food and water consumption and body weights were measured twice weekly. At terminal sacrifice, blood samples were drawn for determination of serum ALT, AST, and LDH. The livers were examined grossly, weighed, and processed for histopathological examination. Fifteen deaths occurred during the study, half of which were attributed to gavage error; the others were not dose related. These early deaths were scattered over dose groups and did not appear to influence the study outcome. Body weight was not affected by treatment in any exposure group. Hepatotoxicity was indicated in the high-dose group (86 mg/kg-day) by significantly elevated liver weight and liver:body weight ratio; significantly elevated ALT (77–89 times control levels in corn oil and 10–19 times control levels in Tween-60), AST (14–15 times control levels in corn oil and 3–4 times control levels in Tween-60), and LDH (12–15 times control levels in corn oil and 2–3 times control levels in Tween-60); and increased incidence and severity of hepatic lesions, such as hepatocellular vacuolization, inflammation, hepatocytomegaly, necrosis, and portal bridging fibrosis. At this dose, the only difference between gavage vehicles was a greater incidence and severity of necrosis in mice given carbon

tetrachloride in corn oil. The difference between vehicles was more apparent at the middle dose of 8.6 mg/kg-day. This dose produced significantly elevated ALT and mild-to-moderate liver lesions in mice gavaged with corn oil but was identified as a NOAEL for mice gavaged with Tween-60. The low dose of 0.86 mg/kg-day was identified as the NOAEL for mice gavaged with corn oil. In general, both sexes responded similarly, with severity of histopathologic changes in males slightly greater than females.

Hayes et al., 1986

Another study in mice was conducted at higher doses. CD-1 mice (20/sex/group) were gavaged daily with 0, 12, 120, 540, or 1200 mg/kg-day of carbon tetrachloride (high performance liquid chromatography grade, purity >99%) in corn oil for 90 days (Hayes et al., 1986). An untreated control group of 20 male and 20 female mice was maintained as well. The mice were observed for clinical signs of toxicity twice daily and weighed weekly. At termination of exposure, the mice were sacrificed, blood was collected by cardiac puncture, and gross necropsy was performed. Organ weights were determined for brain, liver, spleen, lungs, thymus, kidneys, and testes, and samples were taken from the liver and kidney for histopathological examination. The blood samples were used for comprehensive hematological and clinical chemistry analyses. Urinalysis was also performed, although collection of urine was not described. Determination of effect was made by comparing test groups to the vehicle controls. Untreated controls were also compared with the vehicle controls. Observed effects were reported in mice of both sexes at all dose levels and generally appeared to be dose-related. These effects included increases in serum LDH, ALT, AST, ALP, and 5'-nucleotidase and a decrease in serum glucose. Absolute and relative liver, spleen, and thymus weights were increased. A variety of treatment-related lesions were observed in the liver, including fatty change, hepatocytomegaly, karyomegaly, bile duct hyperplasia, necrosis, and chronic hepatitis. No treatment-related lesions were observed in the kidney. No changes were found in urinalysis or hematology parameters. It should be noted that, compared with untreated controls, vehicle controls themselves had significantly elevated serum LDH and ALT, altered organ weights, and increased incidence of liver lesions (e.g., necrosis in 5/19 versus 0/20 in untreated controls and 20/20 in 12 mg/kg-day group). This study failed to identify a NOAEL; the low dose of 12 mg/kg-day was a LOAEL for hepatic effects.

4.2.1.2. *Chronic Toxicity and Carcinogenicity*

4.2.1.2.1. *Early National Cancer Institute studies*

Edwards, 1941

Researchers at the National Cancer Institute (NCI) performed a series of early experiments on the tumorigenicity of orally ingested carbon tetrachloride in mice. In the first of these experiments, groups of 143 male strain C3H mice (2–3.5 months old) were treated with 0.1

mL of a 40% solution of carbon tetrachloride in olive oil (0.04 mL or 64 mg of carbon tetrachloride) by gavage two or three times/week for a total of 23–58 doses per mouse over a period of 8–16 weeks (Edwards, 1941). [Because body weights were not provided, doses in mg/kg-day could not be estimated.] This dose produced parenchymal necrosis of the liver but no renal damage and was not lethal with repeated administration. Necropsies performed 2–147 days after the last feeding, when the animals were between 6 and 10 months of age, found hepatomas in 126/143 mice (88%). Tumors were typically multiple and were similar in appearance to spontaneous hepatoma. No metastases were found. As in spontaneous hepatoma, the tumor cells were morphologically similar to hepatic parenchymal cells. An olive oil control group consisted of 23 male C3H mice given 39–50 gavage doses of 0.1 mL of olive oil (two or three per week) and autopsied between 9 and 11 months of age. Only 1 of the 23 mice in this group (4%) had a hepatoma. In untreated male C3H mice from the same stock, autopsies performed on 17 animals at 8.5–9 months of age found no hepatic tumors, while the incidence was 10% in animals autopsied at 11 months of age and 26% in 341 animals autopsied at 11–19 months of age.

Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941

Similar experiments performed by the same researchers in other strains of mice with lower spontaneous incidence of hepatoma than C3H mice (strains A, C, Y, and L) produced similar results (Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941). A lower, but still hepatotoxic (based on histopathologically observed cirrhosis), dose was administered in one experiment. A group of 58 strain A female mice 2.5 months of age were treated with 0.1 mL of 5% carbon tetrachloride in olive oil (0.005 mL or 8 mg of carbon tetrachloride) three times weekly for 25–29 doses over a 2-month period (Edwards and Dalton, 1942). [Because body weights were not provided, doses in mg/kg-day could not be estimated.] The mice were autopsied from 2 days to 4.5 months after the last dosing. The incidence of hepatoma was 71%. The tumors were morphologically similar to those seen in mice treated with the higher dose. In a related experiment by the same investigators, doses ranging from 0.005 mL (8 mg) to 0.04 mL (64 mg) did not produce any hepatomas in 2-month-old mice treated only one to three times and autopsied 2–12 months later. The livers of mice in this latter experiment showed complete regeneration, with only limited evidence of the earlier damage caused by dosing. These studies, and a subsequent one designed specifically to investigate the possibility of a sex-related difference in susceptibility to carbon tetrachloride tumorigenicity in C3H mice (Andervont, 1958), found no evidence of any such difference between the sexes.

Eschenbrenner and Miller, 1946

A study with multiple dose levels was conducted by Eschenbrenner and Miller (1946) in order to investigate the relationship between necrotic damage and regenerative processes in the

liver and induction of hepatoma. Strain A mice (five/sex/group) were treated by gavage with 0, 0.125, 0.25, 0.5, or 1% of carbon tetrachloride in olive oil, receiving either 30 doses of 0.02 mL/g BW at 4-day intervals or 120 doses of 0.005 mL/g BW daily. Doses of carbon tetrachloride, then, were 0, 10, 20, 40, or 80 mg/kg-day daily or 0, 40, 80, or 160 mg/kg-day every 4 days for 120 days. The mice were 3 months old at the start of treatment and 7 months old at the end of treatment. Mice were maintained for one month without treatment. One additional dose was given 24 hours before sacrifice (at 8 months of age). Mice were examined for presence of hepatomas and necrotic lesions in the liver. No necrosis or hepatoma was found in control animals. No necrosis was observed in mice treated with either 0.005 or 0.02 mL/g of 0.125% solution (i.e., 120 doses of 10 mg/kg-day or 30 doses of 40 mg/kg-day). Although no hepatomas were found by gross examination, two mice in the group that received 30 intermittent 40 mg/kg-day doses were found to have very small tumors (hepatomas) by microscopic examination. Necrosis was produced only with 30 intermittent doses of 80 and 160 mg/kg-day. Hepatomas were produced with 30 intermittent doses of 80 and 160 mg/kg-day as well as 120 continuous doses of 20, 40, or 80 mg/kg-day. The investigators observed, based on results of separate experiments involving 1 or 2 doses, that all dose levels under both dosing regimens (except 120 daily doses of 10 mg/kg-day) were expected to have produced initial liver necrosis, although it was not observed at terminal sacrifice.

Della Porta et al., 1961

An oral cancer bioassay for carbon tetrachloride in hamsters was also conducted. Della Porta et al. (1961) treated Syrian golden hamsters (10/sex) with carbon tetrachloride by gavage weekly for 30 weeks. For the first 7 weeks, 0.25 mL of 5% carbon tetrachloride in corn oil (12.5 μL or 20 mg of carbon tetrachloride) was administered; this dose was halved for the remainder of the exposure period. [Because body weight was not provided, doses in mg/kg-day could not be estimated.] Animals were observed for an additional 25 weeks prior to sacrifice. Four females and five males died during the treatment period, and three more females died during the observation period. The remaining three females and five males were sacrificed at the end of the 55th week. Cirrhotic changes in the liver were seen in the animals that died during treatment and to a lesser extent in the other animals as well. Of the 10 hamsters (five males and five females) that died or were killed between weeks 43 and 55, all had liver-cell carcinomas, typically multiple, and one had metastasized to the mesenteric and cervical lymph nodes. No liver-cell tumors were observed in an untreated group of 109 male and 145 female hamsters from the same breeder or in another group of 50 males and 30 females given 0.5 mL of corn oil by gavage twice weekly for 45 weeks.

4.2.1.2.2. ***NCI bioassay***. NCI (1977, 1976a, b; Weisburger, 1977) used carbon tetrachloride as a positive control in cancer assays for chloroform, trichloroethylene, and 1,1,1-trichloroethane in

rats and mice, and findings are reported in appendices to the bioassay reports for these other chlorinated solvents. Neoplastic and nonneoplastic incidence data were also available through the National Toxicology Program database search application (NTP, 2007).[d] Groups of Osborne-Mendel rats (50/sex/group) were administered carbon tetrachloride by corn oil gavage at time-weighted average doses of 47 or 94 mg/kg for males and 80 or 159 mg/kg for females, 5 days/week for 78 weeks. Rats were maintained without treatment for an additional 32 weeks. Only 7/50 (14%) males and 14/50 (28%) females in the high-dose group and 14/50 (28%) males and 26/50 (52%) females in the low-dose group survived to 110 weeks. In the pooled negative control group, 26/100 (26%) males and 51/100 (51%) females survived to the end of the study. Both doses of carbon tetrachloride resulted in marked heptotoxicity (including fatty changes), with resultant fibrosis, cirrhosis, bile duct proliferation, and regeneration. Based on the NTP database of neoplastic and nonneoplastic incidences (NTP, 2007), all other major organ systems were examined for histopathological changes; however, no treatment-related effects other than those in the liver were reported. The incidence of liver tumors was low in all groups. Hepatocellular carcinoma was recorded in 1/99 pooled control, 2/49 low-dose, and 2/50 high-dose males and in 0/98 pooled control, 4/49 low-dose, and 2/49 high-dose females. Neoplastic nodules in the liver were seen in 0/99 pooled controls and 2/50 low-dose and 1/50 high-dose males, and in 2/98 pooled controls and 2/49 low-dose and 3/49 high-dose females. The increase in carcinomas was statistically significant in low-dose females in relation to pooled controls. High early mortality, particularly in the high-dose group, may have affected the power of this study to detect a carcinogenic effect.

In the same study, groups of male and female B6C3F1 mice received gavage doses of 1250 or 2500 mg/kg, 5 days/week for 78 weeks, and were maintained without treatment for 32 additional weeks. Mortality was markedly increased in treated mice. Survival was about 20% in low-dose groups and <10% in high-dose groups at 78 weeks (versus 70% in control males and 90% in control females), and only one treated mouse survived to study termination at 92 weeks (versus 50% in control males and 80% in control females). Liver toxicity (cirrhosis, bile duct proliferation, toxic hepatitis, and fatty liver) was reported in only a few treated mice. According to the NTP database of neoplastic and nonneoplastic incidences (NTP, 2007), the only other nonneoplastic lesions in mice that appeared to be increased in a dose-related fashion was chronic murine pneumonia in the lungs. Almost all treated mice, even those that died early, had hepatocellular carcinomas (49/49 low-dose males, 47/48 high-dose males, 40/41 low-dose females, and 43/45 high-dose females). In pooled controls, incidence was only 5/77 (6%) in males and 1/80 (1%) in females. The incidence of adrenal adenoma and pheochromocytoma was also increased in male mice (concurrent control: 0/18, low-dose: 28/49, high-dose: 27/48) and

[d] In a few instances, the tumor incidence values differed slightly between the NCI bioassay reports where carbon tetrachloride was included as a positive control, the Weisburger (1977) review, and the NTP database. In those instances, the incidence value included in the Toxicological Review was taken from the NTP database.

female mice (concurrent control: 0/18, low-dose: 15/41, high-dose: 10/45) (NTP, 2007; Weisburger, 1977).

4.2.2. Inhalation Exposure

4.2.2.1. *Subchronic Toxicity*

Smyth et al., 1936

Smyth et al. (1936) exposed groups of 24 guinea pigs (strain not specified) and 24 Wistar-derived rats (mixed sexes of both species) to 50, 100, 200, or 400 ppm (315, 630, 1260, or 2520 mg/m^3) of carbon tetrachloride vapor (>99% pure), 8 hours/day, 5 days/week for up to 10.5 months. The guinea pigs in this study received a purely vegetarian diet, but, because the authors felt that low calcium in this diet may have affected the toxicity results, additional groups of 16 guinea pigs fed diets supplemented with calcium were tested at concentrations of 25 ppm (157 mg/m^3), as well as 50, 100, and 200 ppm. In addition to the rats and guinea pigs, groups of four monkeys (species and sex not specified) were exposed to 50 or 200 ppm using the same protocol. Use of controls was not described, although controls apparently were included in the study. All animals were weighed weekly. Blood counts (all species) and urinalysis (guinea pigs and monkeys) were performed monthly. The fertility of rats and guinea pigs, which were housed in mixed-sex groups and produced litters during the study, was monitored. All animals that survived to scheduled sacrifice (including some animals that were sacrificed only after recovery periods of varying durations) and most of those dying during the study were examined for gross pathology. Tissue samples for histopathological examination were taken from the liver, kidney, adrenal gland, spleen, heart, sciatic and optic nerves, and ocular muscle. Serum chemistry analyses were performed on some animals as well. No statistical tests were conducted.

Guinea pigs of all exposure groups, including those that received diets supplemented with calcium, suffered substantial mortality (≥25–80% among "uninfected" guinea pigs). Mortality in controls was not reported. In contrast, mortality among "uninfected" rats was limited to two animals exposed to 400 ppm. No monkeys died during the study. Body weight gain was reported to be markedly reduced among survivors in all groups of guinea pigs, compared with that in controls. Body weight gain was also reduced by about 30% among rats exposed to 400 ppm. Too few litters were born to guinea pigs during the study to determine if exposure had any effect, but, in rats, fertility was reduced in the 200 and 400 ppm groups. In guinea pigs, fatty changes in the liver were seen at all dose levels, and cirrhosis developed at ≥50 ppm. In rats, fatty changes were seen at ≥50 ppm and cirrhosis at ≥100 ppm. In monkeys, mild fatty degeneration of the liver was found at both 50 and 200 ppm. Other pathological changes in animals exposed to these concentrations included renal tubular degeneration, degeneration of the adrenal glands (with necrosis in guinea pigs), and damage to the sciatic nerve. This study did not include concentrations low enough to identify a NOAEL for any of the three species tested. For guinea pigs, the low concentration of 25 ppm was a frank effect level that produced substantial

mortality. For rats and monkeys, the low concentration of 50 ppm was a LOAEL that produced fatty changes in the liver. This study provides evidence of the progression of toxic liver effects from fatty changes in the liver at lower doses to liver cirrhosis at higher doses. Because of the age of the study, knowledge that bacterial and viral infections were a common problem at that time, and the confounding that pregnancy (or lack of pregnancy) could have had on body weights, the findings from this study much be interpreted with caution.

Adams et al., 1952

Adams et al. (1952) conducted studies in which Wistar-derived rats (15–25/sex), outbred guinea pigs (5–9/sex), outbred rabbits (1–2/sex), and rhesus monkeys (1–2 of either sex) were exposed to carbon tetrachloride vapor (>99% pure), 7 hours/day, 5 days/week for 6 months at concentrations of 5, 10, 25, 50, 100, 200, or 400 ppm (31, 63, 157, 315, 630, 1260, or 2520 mg/m^3). Matched control groups, both unexposed and air exposed, were included in these experiments. Animals were observed frequently for appearance and general behavior and weighed twice weekly. Selected animals were used for hematological analyses periodically throughout the study. Moribund animals and those surviving to scheduled sacrifice were necropsied. The lungs, heart, liver, kidneys, spleen, and testes were weighed, and sections from these and 10 other tissues were prepared for histopathological examination. In many cases, terminal blood samples were collected and used for serum chemistry analyses, and part of the liver was frozen and used for lipid analyses.

In this study, the primary target of carbon tetrachloride in all species was the liver. In guinea pigs, liver effects progressed from a slight, statistical increase in relative liver weight in females, but not males, at 5 ppm (not considered adverse by itself) to include slight-to-moderate fatty degeneration and increases in liver total lipid, neutral fat, and esterified cholesterol at 10 ppm, and cirrhosis at 25 ppm. Liver effects became progressively more severe at higher concentrations. Growth retardation was first observed at 25 ppm and progressed to rapid loss of weight at 200 ppm. In the kidney, slight tubular degeneration was first observed at 200 ppm and increased kidney weight at 400 ppm. Mortality was increased at ≥100 ppm. A similar progression of effects was seen in rats, with no effects at 5 ppm, mild liver changes at 10 ppm, cirrhosis at 50 ppm, and liver necrosis, kidney effects, testicular atrophy, growth depression, and mortality at ≥200 ppm. In rabbits, 10 ppm was without effect, 25 ppm produced mild liver changes, 50 ppm produced moderate liver changes, and 100 ppm produced growth depression. Monkeys were the most resistant species tested, with evidence of adverse effects (mild liver lesions and increased liver lipid) only at 100 ppm, the highest concentration tested. This study identified NOAEL and LOAEL values, respectively, of 5 and 10 ppm in rats and guinea pigs, 10 and 25 ppm in rabbits, and 50 and 100 ppm in monkeys, all based on hepatotoxic effects.

Prendergast et al., 1967

Prendergast et al. (1967) exposed groups of 15 Sprague-Dawley or Long-Evans rats, 15 Hartley guinea pigs, three New Zealand rabbits, two beagle dogs, and three squirrel monkeys (sex not specified) to carbon tetrachloride vapor ("highest purity available") either by continuous exposure to 1 or 10 ppm (6.1 or 61 mg/m^3) for 90 days or intermittent exposure (8 hours/day, 5 days/week) to 82 ppm (515 mg/m^3) for 6 weeks. The control group consisted of 304 rats, 314 guinea pigs, 48 rabbits, 34 dogs, and 57 monkeys. In order to generate the 1 ppm concentration, the researchers found it necessary to dilute the carbon tetrachloride in 10 ppm of n-octane. Therefore, a vehicle control group exposed to 10 ppm of n-octane was included in this study. Animals were observed routinely for signs of toxicity and weighed monthly. Blood samples for hematological analysis were taken at the end of the exposure period. Following sacrifice, animals were necropsied and sections of the heart, lung, liver, spleen, and kidney were taken for histopathological examination. Serum chemistry and liver lipid analyses were performed on some animals. No statistical tests were conducted.

Intermittent exposure to 82 ppm resulted in the death of 3/15 guinea pigs and 1/3 monkeys. [This compares to mortality in the control groups of 7/304 (2.3%) rats, 2/314 (0.64%) guinea pigs, 2/48 (4.2%) rabbits, 0/34 dogs, and 1/57 (1.7%) monkeys.] Body weight gain was reduced in all species relative to the controls, and all species except rats actually lost weight during the study. Mottled livers were seen in all species except dogs. Histopathological examination of the liver revealed fatty changes that decreased in severity from guinea pigs to rats to rabbits to dogs to monkeys. Liver lipid content of guinea pigs was increased about threefold compared with controls. The only other effect noted was interstitial inflammation in the lungs of all species. Continuous exposure to 10 ppm resulted in the deaths of 3/15 guinea pigs. Body weight gain was depressed in all species relative to the controls, and monkeys appeared visibly emaciated. Gross examination showed the presence of enlarged/discolored livers in all species except dogs. Microscopic examination revealed fatty changes in the liver that were most prominent in rats and guinea pigs but were present in the other species as well. Lung effects were not reported in this group. Continuous exposure to 1 ppm produced no mortality or clinical signs of toxicity. Weight gain relative to the controls was reduced in guinea pigs, rabbits, dogs, and monkeys but not in rats. The only histopathological findings were nonspecific inflammatory changes in the liver, kidney, heart, and lungs. No effects were noted in the n-octane control group. The results of this study suggest a NOAEL of 1 ppm (6.1 mg/m^3) and a LOAEL of 10 ppm (61 mg/m^3) for rats, guinea pigs, rabbits, dogs, and monkeys based on hepatotoxicity. Effects on growth were reported at both exposure levels, but the data are difficult to interpret, as only starting body weights and percent change are reported, the changes did not occur in a dose-related manner in all species, and no statistical comparisons were performed. It is unclear whether inflammatory changes observed in the lungs of some exposed animals occurred in controls as well.

Nagano et al., 2007a [Japan Bioassay Research Center (JBRC), 1998]

Groups of F344/DuCrj rats (10/sex/group) were exposed (whole body) to 0, 10, 30, 90, 270, or 810 ppm (0, 63, 189, 566, 1700, or 5094 mg/m^3) of carbon tetrachloride (99.8% pure) vapor for 6 hours/day, 5 days/week for 13 weeks (Nagano et al., 2007a). [This study was previously available as an unpublished study by the Japan Bioassay Research Center (JBRC, 1998).] Rats were observed once a day for clinical signs, behavioral changes, and mortality and were weighed weekly. Urinalysis (pH, protein, occult blood, glucose, ketone body, bilirubin, and urobilinogen) was performed at the end of the dosing period. Blood for hematological (erythrocytes, hemoglobin, hematocrit, platelets, and leukocyte differential) and serum chemistry analyses (AST, ALT, LDH, ALP, total bilirubin, creatine phosphokinase, urea nitrogen, creatinine, total protein, albumin, albumin/globulin ratio, glucose, total cholesterol, phospholipid, sodium, potassium, chloride, calcium, and inorganic phosphorus) was taken during euthanization at the scheduled sacrifice after overnight fasting. All organs and tissues were examined for gross lesions, and organ weights were recorded for the thymus, adrenal gland, ovary, testis, heart, lung, kidney, spleen, liver and brain. Tissues (not specified) were fixed for histopathological analysis; lesions were presented for selected tissues (liver and kidney). Additionally, livers of control and 810-ppm male rats were sectioned for examination of hepatic altered cell foci, a preneoplastic lesion, by immunohistochemical staining with anti-GST-P using an avidin-biotin-peroxidase complex method.

No deaths occurred in any group. Body weight in the 810 ppm males was lower than in controls throughout the study. At termination, the decrease was about 20% ($p<0.01$). Body weight was consistently lower than controls in the 810 ppm females as well, but the difference at termination was slight (4%) and not statistically significant. Statistically significant, dose-related decreases in hemoglobin and hematocrit were observed at 90 ppm in both males and females. At 810 ppm, red blood cell count was also significantly decreased in both sexes. Serum chemistry changes included large, statistically significant and dose-related increases in ALT, AST, LDH, ALP, and LAP (leucine aminopeptidase) in males at 270 ppm and females at 90 ppm. Total bilirubin was significantly increased in male rats at 810 ppm and female rats at ≥270 ppm. Serum levels of creatine phosphokinase (CPK) were statistically increased in females at 30 ppm and above, but there was little change as exposure level increased from 90 to 810 ppm. CPK levels in males were not statistically different from those in controls. In the urine, protein levels were increased in males at 270 ppm and in females at 90 ppm. Urinary pH was decreased and the presence of occult blood was noted in males and females at 810 ppm. Relative liver weights were significantly increased in a dose-related fashion in male rats (≥10 ppm) and female rats (≥30 ppm). Significant, dose-related increases in absolute and relative weights were also recorded for the kidneys, spleen, heart, and lungs in both males and females, primarily at 90 ppm and above. Females at 810 ppm also had significant reductions in absolute and relative ovary weights. Males at 270 or 810 ppm had significantly reduced absolute testes weights, but relative

weights were similar to those in controls. Dose-related increases in the incidence and severity of histopathological lesions of the liver were observed at 10 ppm in both sexes. At the low level of 10 ppm, treatment-related lesions included slight fatty change, cytological alteration, and granulation. Additional lesions at higher levels included ceroid deposits, fibrosis, pleomorphism, proliferation of bile ducts, and cirrhosis. Altered cell foci were observed in male rats at ≥270 ppm and in female rats at ≥90 ppm (based on H&E-stained sections). The altered cell foci in 810-ppm male rats also stained positively with the anti-GST-P antibody. Renal lesions (localized glomerulosclerosis) were seen in the 810 ppm males and females. The low concentration of 10 ppm was a LOAEL for hepatic effects in rats (increased liver weight and histopathology). A NOAEL was not identified.

These researchers conducted a similar study in mice. Groups of Crj:BDF1 mice (10/sex/group) were exposed (whole body) to 0, 10, 30, 90, 270, or 810 ppm (0, 63, 189, 566, 1700, or 5094 mg/m3) of carbon tetrachloride (99.8% pure) vapor for 6 hours/day, 5 days/week for 13 weeks. Endpoints monitored were the same as described above for the 13-week rat study. No treatment-related deaths occurred. Body weights were lower than in controls for most of the study in males at 30 ppm; at termination, the decreases in these groups ranged from 8% to 15% and were statistically significant. Body weights in treated females were similar to those in controls throughout the study. Hematology findings included slight, significant decreases in red blood cell count and hemoglobin at 270 ppm and hematocrit at 810 ppm in females and in hemoglobin at 810 ppm in males. Serum chemistry changes of note included significant increases in ALT and LAP in males and females at 90 ppm (and ALP in males at ≥30 ppm), slight significant increases in total protein and/or albumin in males and females at 270 ppm, and a significant increase in AST in males at 810 ppm. Urinalysis revealed no treatment-related changes in males but a significant decrease in the pH of urine in females at 810 ppm. Organ weight changes in treated mice included significant increases in absolute and/or relative weights of the liver, kidney, and spleen in males and females, primarily at 90 ppm and above. Organ weight changes in males were confounded by body weight decreases in most treated male groups. Histopathological changes in mice were found only in the liver. In both sexes, the hepatic lesions exhibited dose-related increases in incidence and severity. The only effect at the low level of 10 ppm was an increase in incidence of slight cytoplasmic globular and fatty change (large droplets) in males. Additional liver lesions noted in the higher exposure groups were: nuclear enlargement with atypia and altered cell foci (≥270 ppm) and collapse (presumably resulting from the necrotic loss of hepatocytes) (≥30 ppm). Altered cell foci included acidophilic, basophilic, clear cell and mixed cell foci. The lowest exposure level of 10 ppm is a minimal LOAEL for hepatic effects (slight cytological alterations) in male mice.

Benson and Springer, 1999

Groups of F344 rats, B6C3F1 mice, and Syrian hamsters (10 males/species) were

exposed by inhalation to carbon tetrachloride vapor at concentrations of 0, 5, 20, or 100 ppm (31.5, 126, or 630 mg/m^3) for 6 hours/day, 5 days/week for 12 weeks (Benson and Springer, 1999; Nikula et al., 1998). An indicator of DNA replication, 5-bromo-2'-deoxyuridine (BrdU), was administered to animals of all species several days prior to sacrifice. Additional satellite groups of 5–6 animals/species were sacrificed after 1 and 4 weeks. At sacrifice, blood was collected for ALT and SDH determinations, and liver sections were collected for histopathological examination (quantitative evaluation of necrosis in the hepatic parenchyma) and BrdU detection. Serum levels of ALT and SDH were significantly increased in mice at ∃20 ppm and in rats and hamsters at 100 ppm. The increases in mice and hamsters were larger than those in rats. The actual magnitude of the changes could not be assessed from the graphical presentation of the data. The volume percent of the hepatic parenchyma that was necrotic also was significantly increased in mice at ≥20 ppm and in rats and hamsters at 100 ppm. No necrosis was seen in controls or 5 ppm animals of any species. After 12 weeks, the volume percent of necrosis in the liver of the groups showing statistically significant increases ranged from approximately 5–10% in all species. More precise measures of necrosis could not be determined from the graphical presentation of the data. BrdU labeling indices were also significantly increased in mice at ≥20 ppm and hamsters at 100 ppm but were not increased in rats at any concentration tested (except for a small nonsignificant increase at 100 ppm). In mice, the percent of BrdU positive hepatocytes at 12 weeks was about 20% at 20 ppm and 60% at 100 ppm. In hamsters at 100 ppm, the percent of BrdU positive hepatocytes at 12 weeks was about 40%. In controls, the percent of BrdU positive hepatocytes at 12 weeks was approximately 2%. These results show the occurrence of hepatocellular proliferation only at doses that also produced necrotic damage. The study identified 5 ppm as a NOAEL and 20 ppm as a LOAEL for hepatotoxicity in mice. Hamsters and rats were less sensitive than mice, with NOAEL values of 20 ppm and LOAEL values of 100 ppm in these species.

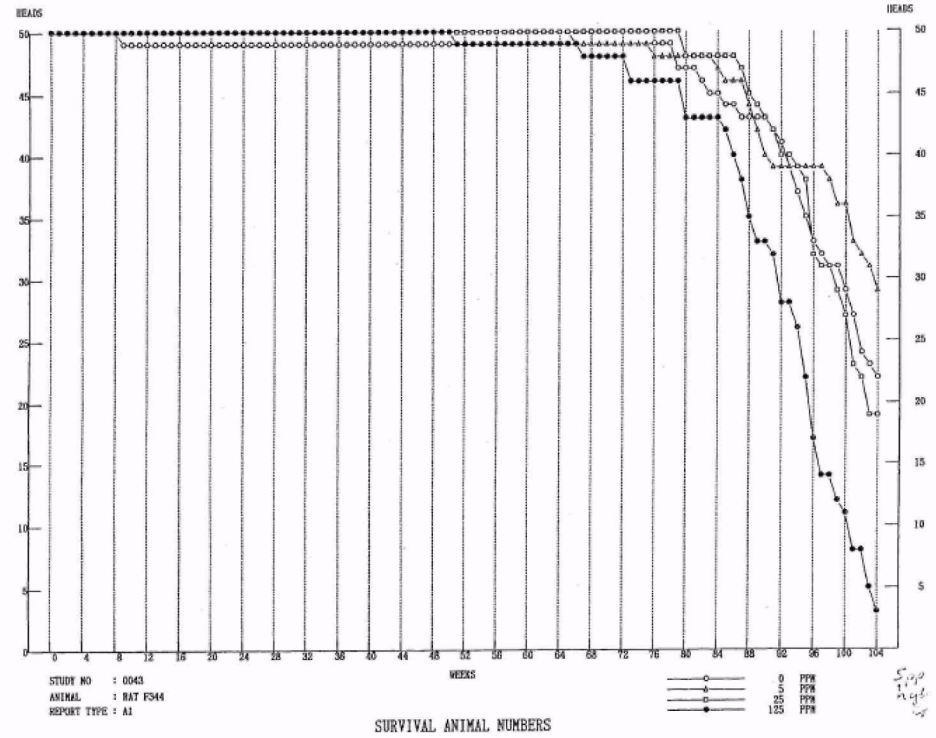
4.2.2.2. *Chronic Toxicity and Carcinogenicity*

Nagano et al., 2007b [Japan Bioassay Research Center (JBRC), 1998]

Groups of F344/DuCrj rats (50/sex/group) were exposed (whole body) to 0, 5, 25, or 125 ppm (0, 31.5, 157, or 786 mg/m^3) of carbon tetrachloride (99.8% pure) vapor for 6 hours/day, 5 days/week for 104 weeks (Nagano et al., 2007b). [This study was previously available as an unpublished study by the Japan Bioassay Research Center (JBRC, 1998).] Animals were observed daily for clinical signs, behavioral changes, and mortality. Body weights were measured once a week for the first 14 weeks and every 2 weeks thereafter. Urinalysis, hematology, and clinical chemistry tests were conducted at study termination as described above for the 13-week rat study, except that GGT was added to the list of serum enzymes monitored. All organs and tissues were examined for gross lesions and organ weights were recorded for the adrenal gland, testis, ovary, heart, lung, kidney, spleen, liver and brain. All major tissues were examined for histopathologic changes.

Survival curves are for the male and female rat are shown in Figure 4-1. Survival was high in all groups through week 64. After week 64, survival declined precipitously in the 125-ppm males and females. Only three males and one female from this group survived to 104 weeks. Liver tumors and chronic progressive nephropathy were the main causes of death. Survival in the other treated groups (19–28/50 in males and 39–43/50 in females) was similar to controls and adequate for evaluation of late developing tumors. Body weights were reduced throughout most of the study in 125 ppm males (reduced 22% at termination) and after week 84 in 25 ppm males (reduced approximately 10% at termination). In females, body weight was reduced during the second year of the study in both the 125 ppm (reduced 45% at termination) and 25 ppm (reduced approximately 10% at termination) groups. The body weight decreases in the 25 ppm males and females at termination were statistically significant. Low survival of rats in the 125 ppm group limited statistical comparison of this group with controls.

Male rat



Female rat

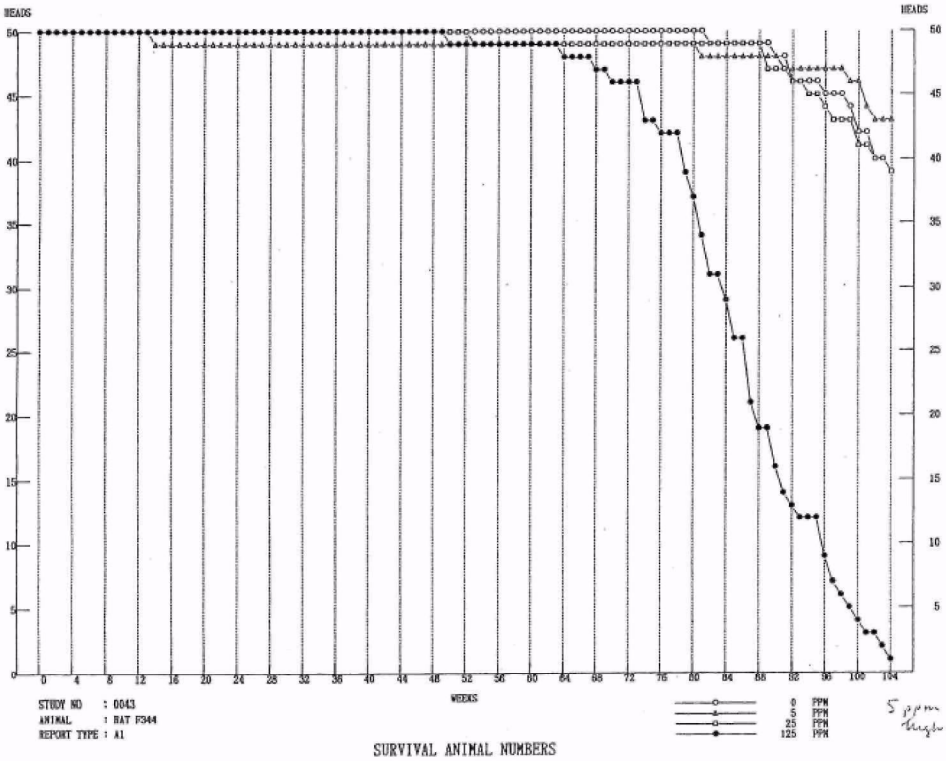


Figure 4-1. Survival curves for male and female rats

Source: JBRC (1998)

Hematology analyses showed trends for decreased red blood cell count, hemoglobin, and hematocrit in males and females at 25 and 125 ppm, although only the decreases for hemoglobin and hematocrit in 25 ppm females were statistically significant (there was no statistical evaluation for the 125 ppm group). Serum chemistry changes included statistically significant increases in AST (males), ALT (males and females), LDH (females), and GPT (females) at 25 ppm; the increases over control in individual serum chemistry parameters at 25 ppm ranged from 1.2- to twofold. There were also significant increases in BUN in both males and females at 25 ppm (25 to 63% over controls). At 125 ppm, BUN, creatinine, and inorganic phosphate were increased by two- to threefold over the control (but were untestable statistically because of the small number the surviving animals at 125 ppm). Consistent with the subchronic rat study, there was a significant increase in CPK in 25 ppm females but not males. An increase was reported in the number of male and female rats with high levels of proteinuria in the 5 and 25 ppm groups (too few data to test in the 125 ppm group) (Table 4-2).

Table 4-2. Urinalysis results in rats after 2-year exposure to carbon tetrachloride

Concentration (ppm)[b]	Protein content of urine[a]			
	+	2+	3+	4+
Male				
0	0/22 (0%)	2/22 (9%)	20/22 (91%)	0/22 (0%)
5[c]	0/31 (0%)	2/31 (6%)	5/31 (16%)	24/31 (77%)
25[c]	0/19 (0%)	1/19 (5%)	3/19 (16%)	15/19 (79%)
125	0/3 (0%)	0/3 (0%)	3/3 (100%)	0/3 (0%)
Female				
0	1/39 (3%)	2/39 (5%)	35/39 (90%)	1/39 (3%)
5[c]	0/43 (0%)	2/43 (5%)	15/43 (35%)	26/43 (60%)
25[c]	0/40 (0%)	0/40 (0%)	3/40 (8%)	37/40 (92%)
125	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)

[a] Urine protein concentrations were measured with a semi-quantitative dipstick test. Equivalent concentrations are: +: 30 mg/dl; 2+: 100 mg/dl; 3+: 300 mg/dl; 4+: 1000 mg/dl (letter dated March 8, 2004, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA).
[b] The exposure concentrations adjusted to continuous exposure (i.e., multiplied by 5/7 x 6/24) = 0.9, 4.5, and 22.3 ppm.
[c] The study report indicated that urine protein results in male and female rats in the 5- and 25-ppm groups were statistically elevated ($p \leq 0.01$) based on a χ^2 test. Whether the statistical test represented a trend test or pairwise comparison of the graded responses was unclear from the study report.

Source: JBRC, 1998.

Organ weight changes were generally unremarkable and limited to the 25 and 125 ppm groups, where they were confounded by body weight decreases in both males and females. Clear increases in the incidence and severity of nonneoplastic liver lesions (fatty change, fibrosis, cirrhosis) were seen at 25 and 125 ppm in both males and females (Table 4-3). Liver lesions (e.g., fatty liver, granulation) in the 5 ppm group were of similar type, incidence, and severity as controls. In the kidney, there was a dose-related increase in the severity of chronic nephropathy (progressive glomerulonephrosis[e]) at 25 and 125 ppm in both males and females (Table 4-3). Nephropathy was characterized as severe in most members of the 125 ppm group. Other dose-related histopathological changes were increased severity of eosinophilic change (eosinophilic globules in cytoplasm) in the nasal cavity at ≥25 ppm in males and ≥5 ppm in females and increased incidence and severity of granulation in the lymph nodes at 125 ppm in both sexes (Table 4-3).

[e] Chronic nephropathy (progressive glomerulonephrosis) is another term for the progressive renal disease in aging rats more recently referred to as chronic progressive nephropathy (CPN) (Peter et al., 1986).

Table 4-3. Incidence of selected nonneoplastic lesions in F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)[a]

Lesion	Male				Female			
	0 ppm	5 ppm	25 ppm	125 ppm	0 ppm	5 ppm	25 ppm	125 ppm
Liver								
Fatty change								
+		7/50	30/50	27/50	5/50	3/50	18/50	17/50
2+	3/50		9/50	22/50	1/50	4/50	27/50	29/50
3+	1/50						4/50	
Fibrosis								
+			43/50				34/50	
2+				2/50			11/50	
Cirrhosis								
+			1/50	14/50			1/50	23/50
2+				26/50			1/50	27/50
Kidney								
Chronic nephropathy								
+	16/50	8/50	9/50	8/50[b]	31/50	37/50	19/50	5/50
2+	26/50	32/50	23/50	9/50[b]	13/50	7/50	25/50	7/50
3+	7/50	9/50	18/50	33/50[b]		1/50	5/50	38/50
Nasal cavity								
Eosinophilic change								
+	43/50	47/50	25/50	13/50	39/50	33/50	25/50	4/50
2+			25/50	34/50		16/50	25/50	46/50
Lymph nodes								
Granulation								
+	4/50	9/50	11/50	6/50	3/50	5/50	11/50	12/50
2+		1/50	1/50	27/50			2/50	28/50

[a] A blank cell indicates that the incidence of the histopathologic finding at that severity level was zero. The exposure concentrations adjusted to continuous exposure (i.e., multiplied by 5/7 x 6/24) = 0.9, 4.5, and 22.3 ppm.
[b] The published paper of the JBRC bioassay shows an incidence (all scores combined) of 49/50 125-ppm male rats. The study report shows a total incidence of 50/50.

Source: Nagano et al., 2007b; JBRC, 1998.

The low exposure level of 5 ppm was associated with an increase in the severity of proteinuria in male and female rats at this concentration; however, there was no effect on the incidence of proteinuria at any exposure level. Histopathological examination revealed clear evidence of treatment-related glomerular damage (increased severity of glomerulonephrosis) in

male and female rats exposed to 25 or 125 ppm. Increases in BUN (at ≥25 ppm) and serum creatinine and inorganic phosphorus (primarily at 125 ppm) show impairment of glomerular function (i.e., decrease in glomerular filtration rate) at the same concentrations as the observed lesions. The increased proteinuria at 5 and 25 ppm could be related to the glomerular changes indicated by histopathology and serum chemistry results at 25 and 125 ppm. For reasons discussed more fully in Section 4.6.2., interpretation of the observed proteinuria in the F344 rat, a strain with a high spontaneous incidence of renal lesions, is problematic. Therefore, 5 ppm was considered a NOAEL and 25 ppm a LOAEL for effects on the liver and kidney.

Tumor incidence data for rats are presented in Table 4-4. The incidence of hepatocellular adenomas and carcinomas was statistically significantly increased in male and female rats at 125 ppm. The incidence of hepatocellular carcinomas in female 25-ppm rats (6%) was not statistically elevated compared with the concurrent control, but did exceed the historical control range for female rats from JBRC (0-2%). The increase in liver carcinoma over historical control (2/1797) was statistically significant (based on Fisher's exact test; two-tailed p-value = 0.0002). No other tumors occurred with an increased incidence in treated rats. Incidences of hepatic altered cell foci (preneoplastic lesions of the liver), including clear, acidophilic, basophilic, and mixed cell foci, were significantly increased in the 25-ppm female rats; in males, only the incidence of basophilic cell foci was increased at 125 ppm.

Table 4-4. Incidence of liver tumors in F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)[a]

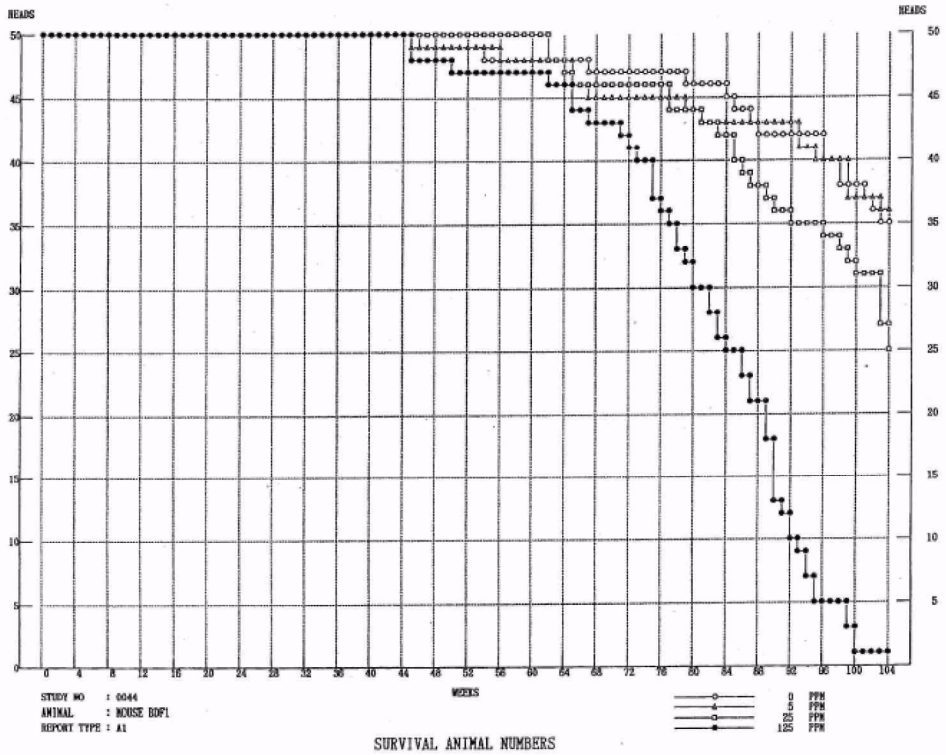
Tumor	Male				Female			
	0 ppm	5 ppm	25 ppm	125 ppm	0 ppm	5 ppm	25 ppm	125 ppm
Hepatocellular adenoma	0/50[b]	1/50	1/50	21/50[c]	0/50[b]	0/50	0/50	40/50[c]
Hepatocellular carcinoma	1/50[b]	0/50	0/50	32/50[c]	0/50[b]	0/50	3/50[d]	15/50[c]
Hepatocellular adenoma or carcinoma	1/50[b]	1/50	1/50	40/50[c]	0/50[b]	0/50	3/50[d]	44/50[c]

[a] The exposure concentrations adjusted to continuous exposure (i.e., multiplied by 5/7 x 6/24) = 0.9, 4.5, and 22.3 ppm.
[b] Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[c] Tumor incidence significantly elevated compared with that in controls by Fisher Exact test ($p \leq 0.01$).
[d] Statistically significant ($p \leq 0.001$ by Fisher Exact test) in comparison to the historical control incidence (2/1797).
Note: The historical control incidence of liver tumors in F344/DuCrj rats in JBRC studies was 1.7% (0–8%) in males and 1.2% (0–6%) in females for hepatocellular adenoma and 0.3% (0–2%) in males and 0.1% (0–2%) in females for hepatocellular carcinoma (based on data from 36-39 carcinogenicity studies carried out by JBRC; email dated April 5, 2007, from Kasuke Nagano, JBRC, to Susan Rieth, U.S. EPA).

Source: Nagano et al., 2007b; JBRC, 1998.

These researchers also conducted a 2-year study using Crj:BDF1 mice. Groups of Crj:BDF1 mice (50/sex/group) were whole-body exposed to 0, 5, 25, or 125 ppm (0, 31.5, 157, or 786 mg/m^3) of carbon tetrachloride (99% pure) vapor for 6 hours/day, 5 days/week for 104 weeks. Endpoints monitored were the same as described above for the 2-year rat study. Survival was high until week 64 of the study in all groups (see survival curves in Figure 4-2). Survival decreased rapidly in 125 ppm males and females, starting at week 64, and in 25 ppm males and females, starting at week 84. The decreases in survival were statistically significant in both sexes at both concentrations. At 104 weeks, only one male and one female survived in the 125 ppm group and 25 males and 10 females in the 25 ppm group (versus 35 males and 26 females in the control group). Investigators reported that liver tumors were the main cause of death at 125 ppm. At 25 ppm, deaths prior to study termination were also largely attributable to the presence of tumors (with liver adenomas or carcinomas present in 33/39 female mice and 22/23 male mice that died or were sacrificed prior to study termination). Body weights were markedly depressed throughout the study in 25 and 125 ppm males and females (22 to 39% reduction at termination).

Male mouse



Female mouse

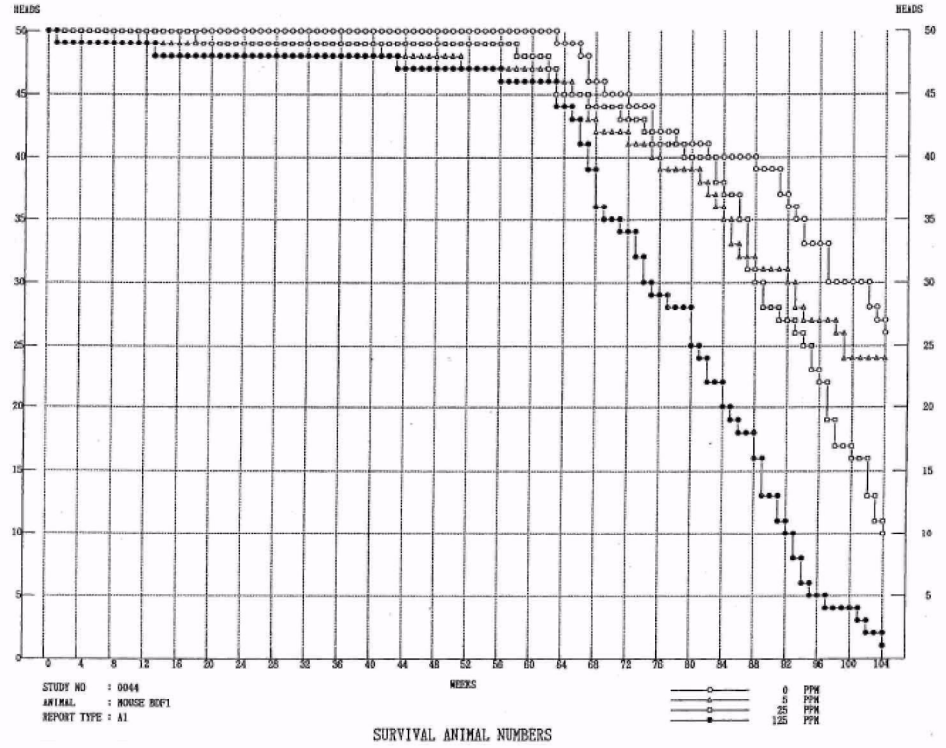


Figure 4-2. Survival curves for male and female mice

Source: JBRC (1998)

The survival of only one mouse of each sex at 125 ppm prevented statistical comparisons involving this group. Statistically significant increases in red blood cell count, hemoglobin, and hematocrit were found in 25 ppm females. Values for these variables were also higher than in controls (but not statistically increased) in the 25 ppm males and in the 125 ppm male and female. This is in contrast with the significant decreases in these variables seen in the subchronic mouse study and the rat studies.

Serum chemistry changes of interest were large, statistically significant increases in ALT, AST, LDH, ALP, protein, total bilirubin, and BUN in males and females at 25 ppm (increases over control ranged from 1.3- to 18-fold) and, for most of these variables, still larger increases in the 125 ppm male and female (based on one surviving mouse/sex at terminal sacrifice). Statistically significant decreases in ALT, AST, LDH, and CPK in 5 ppm males were not considered to be biologically significant by the researchers (letter dated March 8, 2004, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA). The decreases were inconsistent with the large increases seen at higher doses in males or the results in females and appeared to reflect unusually high serum levels of these enzymes in male controls rather than reduced levels in the 5 ppm males. Levels of these enzymes in control males exceeded historical control values for male Crj:BDF1 mice in 2-year studies from the same laboratory by 1.5- to 2.5-fold; this is in contrast to the results in females, where control values for all of these variables were within 10% of historical control values (historical control data provided in a letter dated March 9, 2004, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA). Urinary pH was significantly decreased in males and females at 25 ppm. The only organ weight changes of note were large significant increases in absolute (≈2.5-fold) and relative (≈three- to fourfold) liver weight in 25 ppm males and females. Liver weight data in the surviving 125 ppm male and female were consistent with these results as well. Treatment-related nonneoplastic lesions occurred in the 25 and 125 ppm males and females; these included increased incidence and/or severity of degeneration, cyst formation, and deposit of ceroid in the liver, protein casts in the kidney, and extra medullary hematopoiesis in the spleen (Table 4-5). The 25 ppm concentration was a LOAEL in this study for effects on the liver (increased weight, serum chemistry changes indicative of damage, and lesions), kidney (serum chemistry changes and lesions), and spleen (lesions); decreased growth; and reduced survival. The 5 ppm level was a NOAEL.

Table 4-5. Incidence of selected nonneoplastic lesions in BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)[a]

Lesion	Male				Female			
	0 ppm	5 ppm	25 ppm	125 ppm	0 ppm	5 ppm	25 ppm	125 ppm
Liver								
Degeneration								
+			4/50	7/50	1/50		4/50	6/50
2+	1/50		3/50	2/50			9/50	6/50
3+			1/50					
Cyst formation								
+	1/50	3/50	10/50	5/50	3/50	2/49	10/50	3/50
2+			1/50	3/50	1/50		2/50	3/50
Deposition of ceroid								
+	2/50		28/50	22/50			22/50	22/50
2+		1/50	8/50	14/50			6/50	13/50
Bile duct proliferation	0/50	0/50	19/50	22/50	0/50	0/49	5/50	9/50
Centrilobular hydropic change	1/50	0/50	8/50	9/50	1/50	0/49	13/50	12/50
Kidney								
Protein casts								
+	1/50		1/50	6/50				9/50
2+			5/50	1/50			2/50	3/50
Spleen								
Extramedullary hematopoiesis								
+	15/50	15/50	14/50	5/50	8/50	11/49	11/50	4/50
2+	12/50	8/50	25/50	26/50	7/50	4/49	18/50	30/50
3+	1/50	2/50	5/50	12/50	3/50	5/49	7/50	9/50

[a] A blank cell indicates that the incidence of the histopathologic finding at that severity level was zero. The exposure concentrations adjusted to continuous exposure (i.e., multiplied by 5/7 x 6/24) = 0.9, 4.5, and 22.3 ppm.

Source: Nagano et al., 2007b; JBRC, 1998.

Tumor incidence data in mice are presented in Table 4-6. The incidences of liver tumors in control mice (18% in males and 4% in females for hepatocellular adenomas and 34% in males and 4% in females for hepatocellular carcinomas) were similar to historical control data for liver

tumors in Crj:BDF1 mice in 20 studies at JBRC (see Table 4-6 for historical control liver tumor incidence). The gender differences in unexposed mice are thought to be related to inhibition of liver tumor formation by female estrogen levels. The incidences of hepatocellular adenomas and carcinomas were significantly elevated in both sexes at ≥25 ppm. At 5 ppm, the incidence of liver adenomas in female mice (8/49 or16%) was not statistically significantly elevated compared to the concurrent control, but did exceed the historical control range (2-10%).

Table 4-6. Incidence of liver and adrenal tumors in BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)[a]

Tumor	Male				Female			
	0 ppm	5 ppm	25 ppm	125 ppm	0 ppm	5 ppm	25 ppm	125 ppm
Hepatocellular adenoma	9/50[b]	10/50	27/50[c]	16/50	2/50[b]	8/49[d]	17/50[c]	5/49
Hepatocellular carcinoma	17/50[b]	12/50	44/50[c]	47/50[c]	2/50[b]	1/49	33/50[c]	48/49[c]
Hepatocellular adenoma or carcinoma	24/50[b]	20/50	49/50[c]	49/50[c]	4/50[b]	9/49	44/50[c]	48/49[c]
Adrenal pheochromocytoma[e]	0/50[b]	0/50	16/50[c]	32/50[c]	0/50[b]	0/49	0/50	22/49[c]

[a] The exposure concentrations adjusted to continuous exposure (i.e., multiplied by 5/7 x 6/24) = 0.9, 4.5, and 22.3 ppm.
[b] Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[c] Tumor incidence significantly elevated compared with controls by Fisher Exact test ($p \leq 0.01$).
[d] Tumor incidence significantly elevated compared with controls by Fisher Exact test ($p \leq 0.05$).
[e] All pheochromocytomas in the mouse were benign with the exception of one malignant pheochromocytoma in the 125-ppm male mouse group.
Note: Liver historical control data in Crj:BDF1 mice in 20 studies at JBRC: 17.1% (4–34%) in males and 5.2% (2–10%) in females for hepatocellular adenoma and 20.1% (2–42%) in males and 2.4% (0–8%) in females for hepatocellular carcinoma (letter dated March 8, 2004 and email dated March 9, 2004, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA).
Pheochromocytoma historical control data in Crj:BDF1 mice in 32 studies at JBRC: 0.3% (range: 0 to 2%) in both males and females (email dated October 15, 2005, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA).

Source: Nagano et al., 2007b; JBRC, 1998.

The incidence of adrenal pheochromocytoma was significantly increased in males at ≥25 ppm and in females at 125 ppm. This incidence exceeded the historical control incidence of pheochromocytomas in Crj:BDF1 mice in JBRC studies of 0.3% (range: 0 to 2%) in both males and females (email dated October 15, 2005, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

No adequate reproductive toxicity studies have been conducted in animals exposed by the oral route. Teratogenicity has not been observed in the offspring of rats orally exposed to carbon tetrachloride. However, total litter loss has been described at maternally toxic doses that are much higher than those associated with liver and kidney toxicity.

Alumot et al., 1976

Reproductive performance was monitored in an oral study in which rats of an unspecified strain (18/sex/group) were fed for up to 2 years on experimental diets that had been fumigated with carbon tetrachloride for 48 hours (Alumot et al., 1976). Doses could not reliably be estimated. Serial matings were performed throughout the study. Rats fed fumigated food showed no effects on reproduction (male and female fertility, litter size, and pup mortality and body weight at birth and weaning). There was widespread occurrence of chronic respiratory disease in animals from all groups after 14 months, but this probably did not affect the reproductive outcomes because most reproductive activity took place during the first year of the study (only seven successful matings occurred during the second year). Treatment-related parental toxicity was not reported, but only parental body weight was monitored concurrently with the reproductive part of the study. No evidence of liver toxicity was found by serum analyses or biochemical tests at the end of the study. This study found no evidence of reproductive or maternal effects, but doses received by the experimental animals are unknown.

Wilson, 1954

Wilson (1954) administered daily doses of 478 mg of carbon tetrachloride by gavage in corn oil to 29 pregnant rats (strain not specified) on 1 or 2 successive days of gestation beginning between gestational days (GDs) 7 and 11. The experiment was terminated on GD 20 at which time surviving dams were sacrificed, uteri were examined for resorptions, and litters were examined for external malformations. Fifty-nine percent of the dams failed to produce offspring; this included 6 of 29 dams (21%) that died (a rate less than the 50% mortality for nonpregnant rats given the same dose) and 11 of 29 dams (38%) that had total litter loss from early resorption. For the 12 of 29 dams (41%) that produced offspring, the resorption rate was within normal limits (9.1%), no fetuses were malformed, and only one litter contained fetuses with retarded growth. Because the single dose level of carbon tetrachloride used in this study caused 21% mortality in the dams, it is difficult to determine whether the observation of total litter loss was a direct effect of carbon tetrachloride or was secondary to maternal toxicity.

Narotsky and Kavlock, 1995; Narotsky et al., 1997a, b, 1995

Narotsky and Kavlock (1995) reported the results of a developmental toxicity screening

study in rats. Groups of 16–21 timed pregnant F344 rats were treated with 0, 112.5, or 150 mg/kg-day of carbon tetrachloride by gavage in corn oil on days 6–19 of gestation. Maternal body weight was monitored periodically throughout gestation. The dams were allowed to litter. Pups were examined on postnatal days (PNDs) 1, 3, and 6 and weighed on PNDs 1 and 6. Pups found dead without gross external malformations were dissected and examined for visceral malformations. After the final examination of their litters, dams were sacrificed and their uteri examined for implantation sites. Dams that did not litter by presumed day 24 of gestation were sacrificed for uterine examination. Ammonium sulfide stain was used as needed to detect full-litter resorption. No dams died during the study. The number of females actually pregnant in each group was 13, 9, and 14 in the control-, low-, and high-dose groups, respectively. Both doses of carbon tetrachloride caused maternal weight loss (4–8%) early in the treatment period and reduced extrauterine weight gain (35–45% lower than controls) over the treatment period as a whole. The incidence of full-litter resorption was markedly increased in both dose groups: 4/9 (44%) and 10/14 (71%) in the 112.5 and 150 mg/kg-day groups, respectively (versus 0/13 in controls). As a result, prenatal loss (reported as percent loss per litter) was significantly increased in both dose groups. Implantation sites of the resorbed litters were not grossly visible in most cases, requiring ammonium sulfide stain to find them. This suggested to the researchers that the resorptions occurred early in pregnancy. Among dams that maintained their pregnancies, resorptions were not increased nor were postnatal losses. Pup body weight was not markedly affected by treatment. No malformations were associated with carbon tetrachloride exposure. Reduced maternal weight gain and full-litter resorption were found at the low dose of 112.5 mg/kg-day in this study. In follow-up investigations, the researchers suggested that the all-or-none nature of the observed resorptions points to a maternally mediated response and produced evidence that the response is associated with reduced levels of progesterone and luteinizing hormone in the dams (Narotsky et al., 1997a, 1995).

Narotsky et al. (1997b) compared the developmental toxicity of carbon tetrachloride administered to rats by gavage in corn oil or an aqueous emulsion (10% Emulphor). Groups of 12–14 timed pregnant F344 rats received carbon tetrachloride at doses of 0, 25, 50, or 75 mg/kg-day in either vehicle on GDs 6–15. Maternal body weights were determined on GDs 5, 6, 8, 10, 13, 16, and 20. All dams were examined for clinical signs of toxicity and the day of parturition was recorded. Pups were examined for viability and body weight on PND 1 and 6. Pups that died without gross malformations were examined macroscopically for soft tissue alterations. Dams were sacrificed on PND 6 and uterine implantation sites were counted. The uteri of females that did not deliver were stained with 10% ammonium sulfide to detect sites of early resorption. There was no maternal mortality. Dose-related piloerection was observed in dams at ≥50 mg/kg-day for both vehicles but was seen in more animals and for longer periods in the corn oil groups. Dams exposed to 75 mg/kg-day in corn oil also exhibited kyphosis (rounded upper back) and marked weight loss. Dams exposed to 50 and 75 mg/kg-day in water showed only

significantly reduced body weight gain. Full-litter resorption occurred with an incidence of 0/13, 0/13, 5/12 (42%), and 8/12 (67%) in the control through high-dose corn oil groups and 0/12, 0/12, 2/14 (14%), and 1/12 (8%) in the respective aqueous groups. The difference between vehicles was statistically significant at the high dose. Among the surviving litters, there were no effects on gestation length, prenatal or postnatal survival, or pup weight or morphology. The 25 mg/kg-day dose was a NOAEL and the 50 mg/kg-day dose a LOAEL for full-litter resorption and maternal toxicity (piloerection) with either corn oil or aqueous vehicle, although these effects were more pronounced with the corn oil vehicle.

Hamlin et al., 1993

Hamlin et al. (1993) treated pregnant female B6D2F1 mice with 0, 82.6, or 826 mg/kg of carbon tetrachloride by gavage in corn oil on days 1–5 of gestation. In this strain, days 1–5 of gestation are characterized by sequential cleavage of the fertilized oocyte to generate a hatched blastocyte, with implantation occurring on day 5 and organogenesis occurring subsequently. Therefore, dosing in this study was limited to the preimplantation period. A total of 31 pregnant females were included in the experiment, with a minimum of 8 in each dose group (actual group sizes were not reported). Dams were allowed to give birth; litter size was recorded; and neonates were weighed, measured for crown-rump length, and checked for obvious birth defects. During lactation, the pups were weighed and measured for crown-rump length weekly. Lower incisor eruption and eye opening were assessed in all pups on postpartum days 11 and 15, respectively. Pups were weaned on postpartum day 22 and sacrificed. Dams were weighed weekly during pregnancy and on postpartum day 22 just prior to sacrifice. The liver and kidneys from the dams were removed and weighed. Liver and kidney tissue samples were collected for possible histopathological examination at a later date but were not examined for this report. Treatment with carbon tetrachloride had no effect on dam body weight during pregnancy or on absolute or relative liver or kidney weight at sacrifice. Treatment also had no effect on litter size, pup size at birth, the timing of developmental milestones (incisor eruption and eye opening), or pup growth through weaning (a statistically significant difference in body weight between high-dose pups and controls on day 15 postpartum was not considered to be biologically significant by the researchers because crown-rump length was not affected and no other body weight differences were found). No stillbirths or malformations were observed. The study report included only a limited presentation of the results and no data were shown.

4.3.2. Inhalation Exposure

The potential for reproductive toxicity of carbon tetrachloride in animals is suggested by Bergman's (1983) finding of partly nonextractable radiolabel in the interstitial testis of mice exposed by inhalation to [^{14}C]-carbon tetrachloride vapor. In the subchronic inhalation study by Adams et al. (1952), testicular atrophy was observed in rats exposed to 200 or 400 ppm (1260 or

2520 mg/m^3) of carbon tetrachloride vapor 7 hours/day, 5 days/week for 6 months. Testicular degeneration has also been reported in rats following repeated intraperitoneal (i.p.) doses of 1.5 mL/kg (Kalla and Bansal, 1975; Chatterjee, 1966). Smyth et al. (1936) found that fertility was reduced in rats exposed to 200 or 400 ppm (1260 or 2520 mg/m^3) of carbon tetrachloride vapor 8 hours/day, 5 days/week for up to 10.5 months.

The most detailed inhalation exposure study (Schwetz et al., 1974) suggests that developmental effects of carbon tetrachloride occur at concentrations toxic to the mother and at exposure concentrations higher than those associated with liver and kidney toxicity.

Gilman, 1971

As described in an abstract of an unpublished doctoral dissertation, Gilman (1971) exposed groups of pregnant albino Sprague-Dawley rats to ambient air or 250 ppm (1575 mg/m^3) of carbon tetrachloride vapor for 8 hours/day on GDs 10–15. There were no adverse effects on maternal body weight, litter size, the ratio of live to still births, or the incidence of skeletal abnormalities.

Schwetz et al., 1974

Groups of 22–23 pregnant female Sprague-Dawley rats were exposed by inhalation to carbon tetrachloride vapor at concentrations of 0, 334, or 1004 ppm (0, 2101, or 6316 mg/m^3) for 7 hours/day on GDs 6–15 (Schwetz et al., 1974). Exposures to the two different dose levels were not performed concurrently, so two separate control groups were used. Data from the two control groups were combined except where they differed significantly (e.g., incidence of delayed ossification of sternebrae). The rats were observed daily throughout pregnancy. Food intake was monitored every other day during the experiment, and body weight was determined on days 6, 13, and 21 of gestation. Following sacrifice on GD 21, the number and uterine position of live, dead, and resorbed fetuses were recorded. The fetuses were weighed, measured, and examined for external anomalies. Half of the fetuses in each litter were prepared so as to enable detection of soft tissue anomalies upon subsequent examination, and the remainder were prepared and examined for skeletal abnormalities. The litter was considered the unit of treatment and observation when comparing the results from the different exposure groups. Nonpregnant female rats were exposed simultaneously with the pregnant rats in order to monitor effects on the liver. Serum ALT was determined in these rats throughout exposure, and some were sacrificed for gross examination of the liver at the end of the exposure period. The remainder were sacrificed 6 days later (corresponding to the end of gestation in the pregnant rats) for ALT analysis, gross examination of the liver, and determination of liver weight. In the 334- and 1004-ppm groups, significant reductions in fetal body weight (7% and 14%, respectively) and crown-rump length (3.5% and 4.5%, respectively) were found. The incidence of delayed ossification of the sternebrae was significantly elevated in the high-dose group (13%) compared with the

concurrent control (2%) but not compared with the low-dose group or its concurrent control. No other effects attributable to carbon tetrachloride exposure were found. No anomalies were seen upon gross examination. A significant increase in subcutaneous edema was observed at 334 ppm but not at 1004 ppm. No other increases in individual soft tissue or skeletal anomalies were reported. Maternal toxicity was also observed in both dose groups. Food consumption and body weight were significantly reduced compared with controls, and hepatotoxicity was indicated by significantly elevated serum ALT (fourfold increase over control), gross changes in liver appearance, and significantly increased liver weight (26% at 334 ppm and 44% at 1004 ppm). This study, therefore, detected both maternal and developmental toxicity at a LOAEL of 334 ppm.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute and Short-term Toxicity Data

4.4.1.1. *Oral Exposure*

In animals acutely exposed to carbon tetrachloride by gavage, the liver appears to be the primary target organ; damage to the kidney appears to occur at slightly higher doses (Blair et al., 1991; Kim et al., 1990a, b; Bruckner et al., 1986; Hayes et al., 1986; Nakata et al., 1975; Litchfield and Gartland, 1974; Korsrud et al., 1972; Gardner et al., 1925). Lung effects have also been noted (Boyd et al., 1980; Gould and Smuckler, 1971). Hepatic toxicity is frequently measured by significant increases in serum enzyme activities that peak between 24 and 48 hours after dosing: ALT, AST, SDH, and OCT. The serum enzyme changes represent leakage from damaged hepatocytes. Korsrud et al. (1972) indicated that overt hepatic necrosis was unnecessary for detectable increases in serum enzymes. Reductions in the levels of microsomal protein, microsomal enzymes (G6Pase), and CYP450 levels also occur after carbon tetrachloride dosing (Kim et al., 1990a, b). Histopathological effects in the liver include centrilobular fatty vacuolization, degeneration, necrosis, and inflammation.

Wang et al., 1997

Wang et al. (1997) monitored the time course of hepatic injury in Wistar rats treated with 3188 mg/kg of carbon tetrachloride by gavage in corn oil. There were immediate steep declines in the hepatic microsomal protein and CYP450 content, so that metabolic rates declined by 50% or more, as measured in microsomal CYP content. Plasma levels of AST and ALT increased 100-fold by 24 hours. Immediate histopathological lesions of the liver included hepatocellular degeneration, necrosis, and hydropic swelling. Inflammatory cell infiltration was detectable within 3 hours, and proliferation of mesenchymal cells began after 24 hours.

Lee et al., 1998

Lee et al. (1998) examined the time course and distribution of toxicity and repair in the livers of male Sprague-Dawley rats 24, 36, and 48 hours after receiving 40 or 400 mg/kg carbon tetrachloride by gavage in corn oil. Cell proliferation was monitored by pulse-labeling with BrdU 1 hour before sacrifice. The high dose caused extensive damage in the perivenous-to-midlobular zones. Administration of 40 mg/kg induced regenerative hepatocyte proliferation, as indicated by a significant elevation in BrdU-positive cells in the periportal zone (the site of necrosis) at 24 hours, increasing at 36 hours and plateauing at 48 hours. BrdU-positive cells were close to the portal tract at 24 hours and then increasingly in the outer periportal and midlobular zones at later times. A few hepatocytes in the perivenous zone adjacent to the area of cell damage were labeled at all time points.

Steup et al., 1993

Steup et al. (1993) also found significantly elevated serum ALT and SDH levels in male F344 rats 3–72 hours after they received a single dose of 80 mg/kg carbon tetrachloride by gavage in 10% Emulphor; peak enzyme levels were at 24 hours. Hepatic GSH concentrations were significantly elevated in treated rats at 48 hours after dosing. Six hours after treatment, hepatocytes near terminal venules (zone 3) showed some depletion of glycogen and ballooning. Small collections of lymphocytes were adjacent to focal necrosis of single hepatocytes. More extensive injury involved confluent areas of necrotic cells. Hepatocellular lysis was evident by 48 hours and a mononuclear cell infiltrate concentrated around terminal hepatic venules. Mitotic figures predominated in the cells of the surrounding tissue. By 72 hours, recovery was evident with only a mild infiltrate of mononuclear cells at the site of injury.

Evidence of regeneration of livers in animals treated with carbon tetrachloride appears within 48 hours of dosing. In strain A mice dosed with 2550 mg/kg of carbon tetrachloride in olive oil, necrosis was detectable in half the hepatocytes at 24 hours, and mitotic activity appeared 48 hours after dosing (Eschenbrenner and Miller, 1946). Wistar rats treated with 7970 mg/kg had peak ALT levels at 24 hours, peak AST levels at 48 hours, and significantly elevated levels for activities of DNA-synthesizing enzymes thymidine kinase and thymidylate synthetase at 48 and 72 hours (Nakata et al., 1975); activity levels for DNA-synthesizing enzymes were reduced at 96 hours. Doolittle et al. (1987) found that, in male CD-1 mice administered a single oral gavage dose or multiple (1, 7, or 14) daily doses of carbon tetrachloride in corn oil (up to 100 mg/kg-day), dose levels high enough to elicit significant increases in serum ALT and AST also significantly increased the number of hepatocytes in S-phase, beginning 24 hours after dosing. Multiple doses tended to lower the concentration required to induce hepatotoxicity and increased the number of hepatocytes in S-phase (DNA-synthesizing phase of the cell-replication cycle).

The effect of dosing vehicle on carbon tetrachloride-induced hepatic toxicity has been investigated in several studies. Kim et al. (1990a, b) reported that administration in a corn oil vehicle resulted in lower acute hepatotoxicity (as measured by serum SDH and ALT levels over a 72-hour period) compared with administration in an aqueous emulsion or as undiluted carbon tetrachloride. Raymond and Plaa (1997) reported no consistent difference in serum ALT levels measured 48 hours after dosing in male Sprague-Dawley rats given carbon tetrachloride (5.2 to 25.8 mmol/kg) in corn oil, 5% aqueous Emulphor emulsion, or Tween-85 (undiluted carbon tetrachloride was not tested).

Damage to the lung has been noted in rodents exposed to carbon tetrachloride by gavage. After male Sprague-Dawley rats received a single dose of 4000 mg/kg in mineral oil, pulmonary histopathological effects included perivascular edema and mononuclear infiltration after 4 hours and atelectasis (collapsed lung) and intraalveolar hemorrhages after 8 hours (Gould and Smuckler, 1971). In male Swiss mice or Sprague-Dawley rats, there were significant reductions in pulmonary CYP450 levels and the activity of the microsomal enzyme benzphetamine demethylase 16 hours after receiving a single dose of 4000 mg/kg of carbon tetrachloride in 50% sesame oil (Boyd et al., 1980). Clara cells showed histopathological changes (swelling and necrosis with pyknotic nuclei), whereas the adjacent ciliated bronchiolar cells had normal histology.

4.4.1.2. *Inhalation Exposure*

The central nervous system and the liver are the primary targets in acute toxicity studies in animals exposed by inhalation. Suppression of the central nervous system occurs at relatively high concentrations and is an immediate effect. In Wistar rats exposed for 7 hours, stupor was observed at 4600 ppm, incoordination at 7300 ppm, and unconsciousness at 12,000 ppm (Adams et al., 1952); 16–24 hours after exposure, these rats exhibited increased liver weights and centrilobular fatty degeneration of the liver. Significant elevations in serum enzymes (ALT, AST, SDH, and GDH) have been observed within 24 hours of acute inhalation exposures (Paustenbach et al., 1986a, b; Siegers et al. 1985; Brondeau et al., 1983; Jaeger et al., 1975). In addition, hepatic histopathology within 24 hours of a 4-hour exposure showed centrilobular hydropic or necrotic parenchymal cell damage (Magos et al., 1982).

Hepatotoxicity, and to a lesser extent nephrotoxicity, appear to be the primary effects of short-term duration inhalation exposures. Exposures of male Sprague-Dawley rats at 100 ppm, 8 or 11.5 hours/day for 5 or more days resulted in fatty changes in the liver (Paustenbach et al., 1986a, b); nephrosis (degenerative changes in the kidney) was characterized as minor in rats exposed for 8 hours/day but was more significant in rats exposed for 11.5 hours/day.

Plummer et al. (1990) conducted a 4-week inhalation toxicity study in male Wistar rats exposed to carbon tetrachloride vapor continuously at 16 ppm (100 mg/m^3) for 24 hours/day, 7 days/week except for 1.5-hour periods on Mondays and Fridays, or discontinuously at 87 ppm

(50 mg/m^3) for 6 hours/day, 5 days/week. The total time-weighted average exposures (concentration H time) were the same: 10,507 ppm-hours for the continuous regimen and 10,458 ppm-hours for the discontinuous regimen. Liver histopathology (fibrosis and cirrhosis) was indistinguishable between the two groups, suggesting that inhalation toxicity from carbon tetrachloride is proportional to the product of concentration × time. In another 4-week study, Bogers et al. (1987) exposed groups of Wistar rats to 6-hour daily exposures of carbon tetrachloride vapor at 63 or 80 ppm, either uninterrupted or in 2-hour sessions with an interruption of 1.5 hours; peak loads were added for some groups. At 80 ppm, serum enzyme levels were slightly but significantly increased in the interrupted-exposure groups compared with the uninterrupted-exposure groups (the 63 ppm groups were not compared).

4.4.1.3. *Acute Studies Comparing Oral and Inhalation Exposures*

The effect of route of administration on the hepatic toxicity of carbon tetrachloride has been evaluated in rats (Sanzgiri et al., 1997; Bruckner et al., 1990). In both studies, male Sprague-Dawley rats were exposed (nose only) to carbon tetrachloride vapor at 100 or 1000 ppm (630 or 6300 mg/m^3) for 2 hours. The systemically absorbed doses were calculated from measurements of minute volume and differences between concentrations in inhaled and exhaled air over time; the doses were calculated as 18.9 and 186 mg/kg by Bruckner et al. (1990) and as 17.5 and 179 mg/kg by Sanzgiri et al. (1997). Subsequently, groups of four to nine rats were exposed by inhalation for 2 hours or given the same doses by gavage as a bolus delivery or as a gastric infusion over 2 hours. Hepatotoxicity was measured by activities of SDH and ALT in serum samples taken 24 hours after dosing, and the concentration of CYP450 and activity of G6Pase per mg of hepatic microsomal protein. The results of the two studies are similar; those for Sanzgiri et al. (1997) are presented in Table 4-7. SDH and ALT values were not significantly affected by inhalation exposure at 100 ppm or gastric infusion at 17.5 mg/kg but were significantly elevated at 1000 ppm or 179 mg/kg. In comparison, oral bolus dosing caused more severe elevations at both dose levels. CYP450 levels were significantly reduced in all treated groups, with more severe effects for the gastric routes at 17.5 mg/kg and the oral bolus route at 179 mg/kg. Suppression of microsomal G6Pase activity was most severe for gastric infusion at both doses, followed by bolus delivery at both doses. Inhalation exposure at 100 ppm slightly decreased G6Pase activity, but exposure at 1000 ppm was not significantly different from the control. Overall, the results indicate more severe hepatic toxicity when carbon tetrachloride is administered as a single bolus, compared with the same dose administered by inhalation or gastric infusion over a longer period of time.

Table 4-7. Hepatic toxicity in rats exposed to carbon tetrachloride by inhalation or by equivalent oral dosing as bolus or 2-hour gastric infusion

Exposure	Dose (mg/kg)	SDH (mU/mL)	ALT (mU/mL)	P450 (nmol/mg protein)	G6Pase (µmol/hour/mg protein)
Control[a]	0	5.2 ± 1.0[c]	24.4 ± 2.2[c]	0.81 ± 0.02[c]	14.5 ± 0.7[c]
Inhalation[b]	17.5	11.3 ± 3.7[c]	19.3 ± 1.7[c]	0.65 ± 0.05[d]	10.9 ± 0.5[d]
Gastric infusion	17.5	6.0 ± 1.6[c]	15.9 ± 2.3[c]	0.46 ± 0.04[e]	7.3 ± 0.7[e]
Oral bolus	17.5	64.6 ± 12.5[d]	55.5 ± 9.9[d]	0.49 ± 0.06[e]	12.5 ± 0.1[d]
Inhalation[b]	179	87.6 ± 25.7[d]	53.3 ± 14.7[d]	0.61 ± 0.04[d]	14.3 ± 0.9[c]
Gastric infusion	179	96.9 ± 18.0[d]	81.0 ± 8.2[d]	0.63 ± 0.05[d]	7.8 ± 0.7[e]
Oral bolus	179	269.0 ± 44.7[e]	176.5 ± 17.4[e]	0.47 ± 0.04[e]	8.9 ± 0.3[d]

[a]Controls were treated with corn oil by gavage.
[b]100 or 1000 ppm for 2 hours.
[c-e]Means of each parameter that are statistically equivalent share the same superscript.

Source: Sanzgiri et al., 1997.

Magos et al. (1982) compared the isotoxic oral and 4-hour inhalation concentrations of carbon tetrachloride in Porton-Wistar or Fischer rats. For exposures by either route, Fischer rats were twice as sensitive to hepatotoxic effects (based on SGPT and extent of liver centrilobular damage) of carbon tetrachloride as the Porton-Wistar rats. Fischer rats required an inhalation concentration 1.5 times lower and an oral dose 3.3 times lower than Porton-Wistar rats to produce a 10-fold increase in serum ALT levels, measured 20 hours after exposure.

4.4.2. Genotoxicity Studies

The results of genotoxicity studies of carbon tetrachloride are summarized in Tables 4-8 to 4-11. These tables are not intended to provide an exhaustive list of genotoxicity studies for carbon tetrachloride, but rather represent a reasonably comprehensive summary of the available genotoxicity literature.

Table 4-8. Genotoxicity studies of carbon tetrachloride in prokaryotic organisms

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Salmonella typhimurium TA100, TA1535	Reverse mutation	Plate incorporation assay	–	–	10,000 µg/plate	McCann et al., 1975
Salmonella typhimurium his G46, TA1950	Reverse mutation	Spot test	–	–	4000 µg/plate	Braun and Schoneich, 1975
Salmonella typhimurium his G46, TA1950	Reverse mutation	Host-mediated assay in male NMRI mice	NA	–	6400 mg/kg	Braun and Schoneich, 1975
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Plate incorporation assay	– (T)	– (T)	10,000 µg/plate in DMSO[d]	De Flora, 1981
S. typhimurium TA97, TA98, TA100	Reverse mutation	Plate incorporation assay	–	–	1000 µg/plate in DMSO[d]	Brams et al., 1987
S. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation assay	+[d]	[d]	2460 µg/plate in methanol	Varma et al., 1988
S. typhimurium TA1535, TA1538	Reverse mutation	Preincubation assay using capped tubes	–	–	1230 µg/mL	Uehleke et al., 1977
S. typhimurium TA97, TA98, TA100, TA1535, TA1537	Reverse mutation	Preincubation assay using capped tubes	–[+]	–	3333 µg/plate in DMSO	Zeiger et al., 1988
S. typhimurium TA97, TA98, TA100, TA1535	Reverse mutation	Preincubation assay using capped tubes	–	–	3333 µg/plate in DMSO	Zeiger et al., 1988
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Gas phase exposure in dessicator for 7–10 hours	–	–	ND	Simmon et al., 1977
S. typhimurium TA100, TA1535	Reverse mutation	Gas phase exposure in dessicator for 7–8 hours	–	–	ND	Simmon and Tardiff, 1978
S. typhimurium TA98, TA100, TA1535	Reverse mutation	Gas phase exposure in closed incubation system for 48 hours	–	–	2830 µg/plate	Barber et al., 1981
S. typhimurium TA100, TA1535, TA1537	Reverse mutation	Gas phase exposure in a gas sampling bag for 24 hours	– (T)	– (T)	50,000 ppm	Araki et al., 2004
S. typhimurium TA98	Reverse mutation	Gas phase exposure in a gas sampling bag for 24 hours	±	–	10,000 ppm	Araki et al., 2004

Table 4-8. Genotoxicity studies of carbon tetrachloride in prokaryotic organisms

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Escherichia coli WP2*uvr*A/pKM101	Reverse mutation	Gas phase exposure in a gas sampling bag for 24 hours	±	±	10,000 ppm	Araki et al., 2004
E. coli WP2/pKM101	Reverse mutation	Gas phase exposure in a gas sampling bag for 24 hours	+	+[e]	5000 ppm	Araki et al., 2004
E. coli WP2*uvr*A	Reverse mutation	Gas phase exposure in a desiccator	ND	±	25,000 ppm	Norpoth et al., 1980
S. typhimurium BA13 and BAL13	Forward mutation	Preincubation assay for L-arabinose resistance (Ara^R test)	–	–	1230 µg/plate in DMSO[d]	Roldan-Arjona et al., 1991
S. typhimurium BA13 and BAL13	Forward mutation	Preincubation assay for L-arabinose resistance (Ara^R test)	±	–	384 µg/plate in DMSO[d]	Roldan-Arjona and Pueyo, 1993
S. typhimurium TA1535/pSK1002	DNA repair	SOS response indicated by *umu* gene expression	–	–	5300 µg/mL	Nakamura et al., 1987
E. coli PQ37	DNA repair	SOS chromotest	–	–	1540 µg/mL in DMSO	Brams et al., 1987
E. coli WP2, WP67, CM871	Differential DNA repair	Liquid micromethod using sealed plates	+	+	12.5 µg	De Flora et al., 1984
E. coli WP2, WP67, CM871	Differential DNA repair	Preincubation assay in sealed tubes	+	ND	ND	De Flora et al., 1984
E. coli WP2, WP67, CM871	Differential DNA repair	Spot test	–	ND	ND	De Flora et al., 1984
E. coli K-12 343/636, K-12 343/591	Differential DNA repair	Preincubation assay	–	–	15,400 µg/mL	Hellmer and Bolcsfoldi, 1992

Table 4-8. Genotoxicity studies of carbon tetrachloride in prokaryotic organisms

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		

[a] + = positive, ± = equivocal or weakly positive, – = negative, (T) = toxicity, ND = no data.
[b] Exogenous metabolic activation used, typically induced rat liver S9.
[c] Lowest effective dose for positive results, highest dose tested for negative results, ND = no data, NA = not applicable.
[d] Increase in revertants not dose-related and cytotoxicity not discussed.
[e] Results similar with or without glutathione added to the S9 mix. Positive response is based on the magnitude of response as statistical analyses were not performed.

Table 4-9. Genotoxicity studies of carbon tetrachloride in non-mammalian eukaryotic organisms

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Saccharomyces cerevisiae D7	Gene conversion	Preincubation assay in capped tubes	+ (T)	ND	5230 µg/mL	Callen et al., 1980
S. cerevisiae D7	Mitotic recombination	Preincubation assay in capped tubes	+ (T)	ND	5230 µg/mL	Callen et al., 1980
S. cerevisiae D7	Reverse mutation	Preincubation assay in capped tubes	+ (T)	ND	5230 µg/mL	Callen et al., 1980
S. cerevisiae RS112	Intrachromosomal recombination	Preincubation assay	+ (T)	ND	2000 µg/mL	Brennan and Schiestl, 1998
S. cerevisiae RS112	Intrachromosomal recombination	Preincubation assay	+ (T)	+ (T)	4000 µg/mL	Schiestl et al., 1989; Galli and Schiestl, 1998
S. cerevisiae RS112	Interchromosomal recombination	Preincubation assay	+ (T)	+ (T)	4000 µg/mL	Galli and Schiestl, 1998
S. cerevisiae RS112 (arrested in S phase)	Intrachromosomal recombination	Preincubation assay	–	ND	8000 µg/mL	Galli and Schiestl, 1998
S. cerevisiae RS112 (arrested in S phase)	Interchromosomal recombination	Preincubation assay	–	ND	8000 µg/mL	Galli and Schiestl, 1998
S. cerevisiae RS112 (arrested in G1 phase)	Intrachromosomal recombination	Preincubation assay	+ (T)	ND	5000 µg/mL	Galli and Schiestl, 1996; Galli and Schiestl, 1998
S. cerevisiae RS112 (arrested in G1 phase)	Interchromosomal recombination	Preincubation assay	+ (T)	ND	5000 µg/mL	Galli and Schiestl, 1996; Galli and Schiestl, 1998
S. cerevisiae AGY3 (arrested in G2 phase or growing normally)	Intrachromosomal recombination	Preincubation assay	+ (T)	ND	8000 µg/mL	Galli and Schiestl, 1995
S. cerevisiae D61.M	Aneuploidy	Standard 16-hour incubation or cold-interruption regimen	–	ND	6400 µg/mL	Whittaker et al., 1989

Table 4-9. Genotoxicity studies of carbon tetrachloride in non-mammalian eukaryotic organisms

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Aspergillus nidulans P1	Somatic segregation due to cross over and aneuploidy	Plate incorporation assay	+ (T)	ND	0.5%	Gualandi, 1984
A. nidulans 35	Forward mutation	Plate incorporation and growth-mediated assays	± (T)	ND	0.5%	Gualandi, 1984
A. nidulans P1	Somatic segregation (positive for aneuploidy; negative for cross over)	Mitotic segregation assay	+(T)	ND	0.04%	Crebelli et al., 1988
A. nidulans P1	Somatic segregation (positive for aneuploidy; negative for cross over)	Mitotic segregation assay	+ (T)	ND	0.0275%	Benigni et al., 1993
Drosophila melanogaster	Mutation	Sex-linked recessive lethal assay	–	NA	25,000 ppm in feed or 2000 ppm injection	Foureman et al., 1994

[a] + = positive, ± = equivocal or weakly positive, – = negative, (T) = toxicity, ND = no data.
[b] Exogenous metabolic activation not used for most tests because fungi have metabolic capabilities.
[c] Lowest effective dose for positive results, highest dose tested for negative results, ND = no data, NA = not applicable.

Table 4-10. Genotoxicity studies of carbon tetrachloride in mammalian cells in vitro

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Human peripheral lymphocytes G_0	Chromosomal aberrations	30 Minute incubation in sealed tubes	– (T)	– (T)	76 µg/mL	Garry et al., 1990
Human peripheral lymphocytes G_0	Sister chromatid exchange	30 Minute incubation in sealed tubes	– (T)	– (T)	48 µg/mL	Garry et al., 1990
Human lymphocytes from 2 donors	Micronucleus formation	Test conducted in capped tubes	– (2–)[d]	± (1 –)[d]	1540 µg/mL	Tafazoli et al., 1998
Human lymphocytes	DNA breaks	Comet assay	–	–	3080 µg/mL	Tafazoli et al., 1998
Human lymphocytes	Unscheduled DNA synthesis	4-Hour culture, autoradiography	–	–	16,000 µg/mL	Perocco and Prodi, 1981
Lamb peripheral lymphocytes	Chromosomal aberrations	48-Hour incubation	–	ND	16 µg/mL	Sivikova et al., 2001
Lamb peripheral lymphocytes	Micronucleus formation	48-Hour incubation	+	+	8 µg/mL (w/out activation) 16 µg/mL (w/activation)	Sivikova et al., 2001
Lamb peripheral lymphocytes	Sister chromatid exchange	48-Hour incubation	+	±	4 µg/mL	Sivikova et al., 2001
h2E1 cell line (cDNA for CYP2E1)	Micronucleus formation	Immunofluorescent labeling of kinetochore proteins	+[e] (T)	ND	308 µg/mL	Doherty et al., 1996
MCL-5 cell line (cDNA for CYPs 1A2, 2A6, 3A4, and 2E1, and epoxide hydrolase)	Micronucleus formation	Immunofluorescent labeling of kinetochore proteins	+[e] (T)	ND	308 µg/mL	Doherty et al., 1996
AHH-1 cell line (expresses CYP1A1)	Micronucleus formation	Immunofluorescent labeling of kinetochore proteins	–	ND	1540 µg/mL	Doherty et al., 1996
Chinese hamster ovary cells	Chromosomal aberrations	Assay conducted in sealed flasks	–	–	3000 µg/mL in DMSO[f]	Loveday et al., 1990

Table 4-10. Genotoxicity studies of carbon tetrachloride in mammalian cells in vitro

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Chinese hamster ovary cells	Sister chromatid exchange	Assay conducted in sealed flasks	– (T)	–	1490 µg/mL (w/out activation) 2930 µg/mL(w/ activation) note: both in DMSO[f]	Loveday et al., 1990
Chinese hamster ovary cells	Lagging chromosomes and multipolar spindles	Anaphase analysis	+	ND	8000 µg/mL	Coutino, 1979
V79 Chinese hamster lung cell line	Aneuploidy	3-Hour incubation	+	ND	246 µg/mL	Onfelt, 1987
V79 Chinese hamster lung cell line	c-Mitosis (spindle disturbance)	30-Minute incubation	± (T)	ND	492 µg/ml	Onfelt, 1987
Syrian hamster embryo cells	Morphological transformation	Clonal assay	±[g]	ND	3 µg/mL	Amacher and Zelljadt, 1983
Mouse lymphoma L5178Y cells	Mutation at tk locus	4-Hour incubation	ND	– (T)	635 µg/mL	Wangenheim and Bolcsfoldi, 1988
Mouse lymphoma L5178Y cells	DNA strand breaks	Alkaline elution	ND	+(T)	1007 µg/mL	Garberg et al., 1988
RL_1 cultured cell line derived from rat liver	Chromosomal aberrations	Assay conducted in sealed flasks	–	ND	0.02 µg/mL in DMSO[d]	Dean and Hodson-Walker, 1979
RL_1 cultured cell line derived from rat liver	Sister chromatid exchange	Assay conducted in sealed flasks	–	ND	0.02 µg/mL in DMSO[d]	Dean and Hodson-Walker, 1979
Hepatocytes- primary cultures from 4 human donors	Unscheduled DNA synthesis	21.5 to 24 hr incubation periods	ND	– (4-)[d]	154 µg/mL	Butterworth et al., 1989
Hepatocytes isolated from male Sprague-Dawley rats	Unscheduled DNA synthesis	Autoradiography and flow cytometric assays	–	ND	154 µg/mL	Selden et al., 1994
Hepatocytes isolated from rats	DNA single strand breaks	Alkaline elution	± (T)	ND	461 µg/mL	Sina et al., 1983

Table 4-10. Genotoxicity studies of carbon tetrachloride in mammalian cells in vitro

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Hepatocytes isolated from female Wistar rats	DNA single strand breaks	Comet assay	±	ND	154 µg/mL	Beddowes et al., 2003
Hepatocytes isolated from female Wistar rats	DNA adduct formation	M_1dG adducts formed secondary to lipid peroxidation	±	ND	154 µg/mL	Beddowes et al., 2003
Hepatocytes isolated from female Wistar rats	DNA adduct formation	8oxodG adducts formed secondary to lipid peroxidation	± (T)	ND	615 µg/mL	Beddowes et al., 2003
Calf thymus DNA	DNA binding of radiolabeled chemical	30 min incubation with rat and mouse microsomes	+	+	5.6 µg/mL	Rocchi et al., 1973
Calf thymus DNA	DNA binding of radiolabeled chemical	60 min incubation under a N2 atmosphere	ND	+	154 µg/ml	DiRenzo et al., 1982
Mouse liver chromatin	DNA binding	2 and 4 hr incubation with binding measured in DNase I-sensitive and -resistant chromatin DNA	ND	+	192 µg/mL	Oruambo and Van Duuren, 1987
Hepatocytes isolated from Sprague-Dawley rats	DNA binding	Measured as radioactivity bound to DNA after a 1 hr incubation with microsomes	±	±	31 µg/mL	Castro et al., 1989
Hepatocytes isolated from C3H mice	DNA binding	Measured as radioactivity bound to DNA after a 1 hr incubation with microsomes	±	±	31 µg/mL	Castro et al., 1989
Hepatocytes isolated from Syrian golden hamsters	DNA binding	Measured as radioactivity bound to DNA after a 1 hr incubation with microsomes	±	±	31 µg/mL	Castro et al., 1989

Table 4-10. Genotoxicity studies of carbon tetrachloride in mammalian cells in vitro

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		

[a] + = positive, ± = equivocal or weakly positive, – = negative, (T) = toxicity, ND = no data.
[b] Exogenous metabolic activation used, typically induced rat liver S9.
[c] Lowest effective dose for positive results, highest dose tested for negative results, ND = no data, NA = not applicable.
[d] Results for the individual donors are presented.
[e] Increase mostly in kinetochore-positive (aneugenic) micronuclei which occurred at the lower (308 µg/ml) concentration, and some increase in kinetochore-negative (clastogenic) micronuclei which was significantly increased at the highest (1538 µg/ml) test concentration.
[f] DMSO = dimethyl sulfoxide
[g] Although declared positive by the authors, the induced frequency is well within the currently accepted control range.

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Mouse (101/H, male)	Chromosomal aberrations in bone marrow	Metaphase analysis of samples collected 6 to 48 hr after dosing	– (T)	NA	8000 mg/kg injected i.m.	Lil'p, 1982
Rat (Sprague-Dawley, male)	Chromosomal aberrations in bone marrow	Metaphase analyses from animals sacrificed 24 hr after dosing	–	NA	1600 mg/ml by gavage	Rossi et al., 1988
Mouse (BDF1, male)	Micronucleus formation in bone marrow	Analyzed polychromatic erythrocytes from specimens prepared 24 hours after dosing	– (T)	NA	2000 mg/kg by gavage (2×)	Morita et al., 1997; Suzuki et al., 1997
Mouse (BDF1, male)	Micronucleus formation in bone marrow	Analyzed polychromatic erythrocytes from specimens prepared 24 hours after dosing	– (T)	NA	2000 mg/kg by gavage	Morita et al., 1997; Suzuki et al., 1997
Mouse (BDF1, male)	Micronucleus formation in peripheral blood	Analyzed reticulocytes from specimens prepared 24–72 hours after dosing	–	NA	3000 mg/kg by i.p. injection	Suzuki et al., 1997
Mouse (CD-1, male)	Micronucleus formation in peripheral blood	Analyzed reticulocytes from specimens prepared 24–72 hours after dosing	–[d]	NA	2000 mg/kg by gavage in olive oil	Morita et al., 1997
Mouse (CD-1, male and female)	Micronucleus formation in bone marrow	Analyzed polychromatic erythrocytes from femur bone marrow of mice killed 24 or 48 hours after dosing	– (T)	NA	3000 mg/kg i.p. in olive oil	Crebelli et al., 1999
Mouse (CD-1, male)	DNA damage in stomach, kidney, bladder, lung, brain, and bone marrow	Comet assay on stomach, kidney, bladder, lung, brain, and bone marrow obtained 0, 3, or 24 hours after dosing	–	NA	2000 mg/kg by gavage	Sasaki et al., 1998
Rat (F344, male)	DNA breakage	Comet assay on peripheral blood cells	± (T)	NA	120 mg/kg by i.p. injection	Kadiiska et al., 2005

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Mouse (NMRI, male and female)	DNA strand breaks in liver	Alkaline elution of sample collected 4 hr after dosing	–	NA	4000 mg/kg by gavage	Schwarz et al., 1979
Rat (Wistar, female, partially hepatectomized)	DNA damage in liver	Caffeine elution 4 or 24 hours after dosing	–	NA	800 mg/kg by gavage in corn oil	Stewart, 1981
Rat (F-344, male)	DNA strand breaks in liver	Alkaline elution on primary hepatocytes isolated from rats sacrificed 2–48 hours after dosing	–	NA	400 mg/kg by corn oil gavage	Bermudez et al., 1982
Rat (strain and sex not specified)	DNA breaks in liver	Alkaline elution on liver nuclei obtained 1 hr after dosing	–	NA	4 mg/kg by i.p. injection	Kitta et al., 1982
Rat (BD-VI, male)	DNA strand breaks in liver	Alkaline elution on primary hepatocytes isolated from rats sacrificed 4 hours after dosing	– (T)	NA	4000 mg/kg by i.p. injection	Barbin et al., 1983
Rat (Sprague-Dawley, male)	DNA damage in liver	Viscometric assay on rats sacrificed 2 hours after dosing	–	NA	200 mg/kg by i.p. injection	Brambilla et al., 1983
Mouse (CD-1, male)	DNA strand breaks in liver	Alkaline elution	+ (T)	NA	80 mg/kg by corn oil gavage	Gans and Korson, 1984
Rat (Sprague-Dawley CD stain, female)	DNA strand breaks in liver	Alkaline elution on primary hepatocytes isolated from rats dosed 21 and 4 hr before sacrifice	–	NA	1050 mg/kg by oral gavage in corn oil (2x)	Kitchin and Brown, 1989
Rat (Sprague-Dawley, male)	DNA strand breaks in liver	DNA strand breaks in hepatocytes were measured by a fluorometric assay for DNA unwinding 1 hr after dosing	–	NA	160 mg/kg in corn oil by i.p.	Ikegwuonu and Mehendale, 1991
Rat (Wistar, male)	DNA strand breaks in liver	Breaks in DNA of non-parenchymal cells identified by in situ nick translation 12 to 96 hr after dosing.	± (T)[e]	NA	1600 mg/kg i.p. in olive oil	Nakamura and Hotchi, 1992

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Rat (Wistar, male)	DNA strand breaks in liver	Breaks in DNA of non-parenchymal cells identified by in situ nick translation after dosing twice a week until week 12 with sacrifices at 3, 6, 9, 12, 15, and 18 weeks.	± (T)[e]	NA	2000 mg/kg (24x)	Nakamura and Hotchi, 1992
Mouse (CD-1, male)	DNA damage in liver	Comet assay on liver obtained 0, 3, or 24 hours after dosing	+ (T)	NA	1000 mg/kg by gavage	Sasaki et al., 1998
Rat (Wistar, male)	DNA fragmentation in liver	TUNEL[f] assay on rats sacrificed 1 day after the second dose	+ (T)	NA	800 mg/kg by ip; (2x)	Cabre et al., 1999
Rat (Wistar, male)	DNA fragmentation in liver	TUNEL[f] assay on rats sacrificed at 10, 15, 20, 25 and 30 hr after dosing	+ (T)	NA	240 mg/kg in corn oil by ip	Yasuda et al., 2000
Rat (Wistar, female)	Unscheduled DNA synthesis in liver	Animals injected with hydroxyurea (to stop de novo DNA synthesis) and then [^{3}H]-thymidine 2 hours after dosing	–	NA	4000 mg/kg by gavage in liquid paraffin	Craddock and Henderson, 1978
Rat (Wistar, female)	Unscheduled DNA synthesis in liver	Animals injected with hydroxyurea (to stop de novo DNA synthesis) and then [^{3}H]-thymidine 17 hours after dosing	+ (T)	NA	4000 mg/kg by gavage in liquid paraffin	Craddock and Henderson, 1978
Rat (F-344, male)	Unscheduled DNA synthesis in liver	Rats sacrificed 2 hr after dosing; primary hepatocytes isolated by liver perfusion and cultured with [^{3}H]-thymidine	–	NA	100 mg/kg by corn oil gavage	Mirsalis and Butterworth, 1980

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Rat (F-344, male)	Unscheduled DNA synthesis in liver	Rats sacrificed 2–48 hours after dosing; primary hepatocytes isolated by liver perfusion and cultured with [^{3}H]-thymidine	– (T)	NA	400 mg/kg by corn oil gavage	Mirsalis et al., 1982
Mouse (B6C3F1, male)	Unscheduled DNA synthesis in liver	Rats sacrificed 12 hr after dosing; primary hepatocytes isolated by liver perfusion and cultured with [^{3}H]-thymidine	– (T)	NA	100 mg/kg by oral gavage	Mirsalis, 1987; Madle et al., 1994
Mouse (B6C3F1, female)	Unscheduled DNA synthesis in liver	Rats sacrificed 12 hr after dosing; primary hepatocytes isolated by liver perfusion and cultured with [^{3}H]-thymidine	– (T)	NA	100 mg/kg by oral gavage	Mirsalis, 1987; Madle et al., 1994
Mouse (CD-1, male)	Unscheduled DNA synthesis in liver	Mice sacrificed 3–48 hours after dosing; liver cells isolated and analyzed by autoradiography	– (T)	NA	100 mg/kg by corn oil gavage	Doolittle et al., 1987
Rat (Sprague-Dawley, male)	Unscheduled DNA synthesis	Unscheduled DNA synthesis by labeling of DNA in hydroxyurea-treated animals 1 hr after dosing	±	NA	160 mg/kg in corn oil by i.p.	Ikegwuonu and Mehendale, 1991
Mouse (DC-1, male)	Chromosomal fragments and bridges in liver	Anaphase analysis of squash preparations prepared 72 hr after dosing	–	NA	8000 mg/kg	Curtis and Tilley, 1968
Rat (F-344, male)	Chromosomal aberrations in liver	Analyzed primary hepatocytes cultured for 48 hr from rats sacrificed 0–72 hours after dosing	–	NA	1600 mg/kg by corn oil gavage	Sawada et al., 1991
Rat (F-344, male)	Sister chromatid exchange in liver	Analyzed primary hepatocytes cultured for 48 hr from rats sacrificed 0–72 hours after dosing	–	NA	1600 mg/kg by corn oil gavage	Sawada et al., 1991

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Rat (F-344, male)	Micronucleus formation in liver	Analyzed primary hepatocytes cultured for 48 hr from rats sacrificed 0–72 hours after dosing	–	NA	1600 mg/kg by corn oil gavage	Sawada et al., 1991
Rat (Wistar, male)	Micronucleus formation in liver	Analyzed primary hepatocytes harvested 72 hr after dosing, an optimal time to detect micronuclei.	± (T)	NA	3200 mg/kg by gavage in corn oil	Van Goethem et al., 1993
Rat (Wistar, male)	Micronucleus formation in liver	Analyzed primary hepatocytes harvested 72 hr after dosing, an optimal time to detect micronuclei.	+ (T)[g]	NA	3200 mg/kg by gavage in corn oil	Van Goethem et al., 1995
Mouse (CBAxC575BL/6, male)	Micronucleus formation and ploidy levels in liver	Analyzed primary hepatocytes from rats sacrificed 5 days after dosing and compared with a partially hepatectomized control.	–	NA	15-Minute inhalation at 0.05-0.1 mL/5 L	Uryvaeva and Delone, 1995
Mouse (B6C3F1, *lacI* transgenic; Big Blue™, male)	Mutations in *lacI* transgene in liver	The target *lacI* gene is recovered from genomic DNA after 5 daily doses and the animals sacrificed 7 days after the first dose	– (T)	NA	35 mg/kg-day (5x)	Mirsalis et al., 1994
Mouse (CD2F1 *lacz* transgenic, Mutamouse™, male)	Mutations in the *lacz* transgene in liver	The target *lacz* gene is recovered from genomic DNA after a single dose with the animals being sacrificed 14 days later	– (T)	NA	80 mg/kg by gavage in corn oil	Tombolan et al., 1999; Lambert et al., 2005
Mouse (CD2F1 *lacz* transgenic, Mutamouse™, male)	Mutations in the *lacz* transgene in liver	The target *lacz* gene is recovered from genomic DNA after dosing with the animals being sacrificed 7, 14 or 28 days later	– (T)[i]	NA	1400 mg/kg by gavage	Hachiya and Motohashi, 2000; Lambert et al., 2005

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Rat (Wistar, male)	DNA binding in liver	DNA extracted from liver of rats (with or without methylcholanthrene pretreatment) sacrificed 12 hours after dosing	–	NA	56 mg/kg i.p.	Rocchi et al., 1973
Mouse (Swiss, male)	DNA binding in liver	DNA extracted from liver of mice (some pretreated with methylcholanthrene) sacrificed 12 hours after dosing	+[h]	NA	56 mg/kg i.p.	Rocchi et al., 1973
Rat (Sprague-Dawley, male)	DNA binding in liver	DNA isolated from liver slices of rats sacrificed 6 hours after dosing	±	NA	1.4 mg/kg i.p. in olive oil	Diaz Gomez and Castro, 1980a
Mouse (A/J, male)	DNA binding in liver	DNA isolated from liver slices of mice sacrificed 6 hours after dosing	±	NA	1.4 mg/kg i.p. in olive oil	Diaz Gomez and Castro, 1980a
Mouse (A/J, male)	DNA binding in liver	DNA isolated from liver slices of mice sacrificed 6 hours after dosing	+ (T)	NA	3200 mg/kg i.p. in olive oil	Diaz Gomez and Castro, 1980a
Rat (Sprague Dawley, male)	DNA binding to mitochondria and nucleus	Mitochondrial DNA isolated from the livers at 5 and 24 hr after dosing	+ (T)	NA	3.2 mg/kg in corn oil	Levy and Brabec, 1984
Rat (Sprague-Dawley, male)	DNA binding in liver	DNA isolated from liver slices of rats sacrificed 6 hours after dosing	±	NA	1200 mg/kg i.p. in olive oil	Castro et al., 1989
Mouse (C3H, male)	DNA binding in liver	DNA isolated from liver slices of mice sacrificed 6 hours after dosing	±	NA	1200 mg/kg i.p. in olive oil	Castro et al., 1989
Hamster (Syrian golden, male)	DNA binding in liver	DNA isolated from liver slices of hamsters sacrificed 6 hours after dosing	±	NA	1200 mg/kg i.p. in olive oil	Castro et al., 1989

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Rat (strain and sex not specified)	DNA adducts in liver	Deoxyguanosine-malondialdehyde adducts measured 48 hr after dosing	+ (T)	NA	1600 mg/kg by gavage	Hadley and Draper, 1990
Rat (Sprague-Dawley, sex not specified)	DNA adducts in liver	M_1dG adducts formed secondary to lipid peroxidation measured 4 days after dosing	+ (T)	NA	0.1 mg/kg by corn oil gavage	Chaudhary et al., 1994
Rat (strain and sex not specified)	DNA adducts in liver	Deoxyguanosine-malondialdehyde adducts measured 48 hr after dosing	–	NA	160 mg/kg by oral gavage	Draper et al., 1995
Hamster (Syrian golden, female)	DNA adducts in liver and kidney	13-HPO and malondialdehyde-derived adducts formed secondary to lipid peroxidation detected by ^{32}P-postlabelling analysis 4 hr after treatment	± (T)	NA	160 mg/kg by corn oil gavage	Wang and Liehr, 1995
Rat (F-344, male)	DNA adducts in liver	HNE-dG adducts formed secondary to lipid peroxidation	+ (T)	NA	3200 mg/kg i.p. in olive oil	Chung et al., 2000
Rat (F-344, female)	DNA adducts in liver, kidney, lung, colon, and forestomach	HNE-dG adducts formed secondary to lipid peroxidation. Samples collected 4, 8, 16 or 24 hr after final dose.	+ (T)	NA	500 mg/kg i.p. (1 or 4×)	Wacker et al., 2001
Rat (Fischer, male)	DNA adducts in liver	8-OHdG adducts were measured by immunohistochemistry and electrochemical detection at times from 6 hr to 7 days	+ (T)	NA	3200 mg/kg by gavage in olive oil	Takahashi et al., 1998
Rat (F-344, male)	DNA adducts in liver	8-OHdG adducts measured at the end of week 1 after dosage on days 1 and 4	± (T)	NA	400 mg/kg by s.c. injection (2x)	Iwai et al., 2002

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
Rat (F344, male)	DNA adducts in urine	8-OHdG adducts measured in the urine 7 and 16 hr after a single dose	+ (T)	NA	120 mg/kg by i.p. injection	Kadiiska et al., 2005
Mouse (CD-1, female)	DNA binding in liver	8-oxodG measured in the livers of 2 month and 14 month animals dosed for 3 days and sacrificed on day 4.	+	NA	43 mg/kg i.p. in mineral oil	Lopez-Diazguerrero et al., 2005
Mouse (ICR, male)	DNA binding in liver	^{32}P -Postlabeling was used to identify indigenous adducts present 24 hr after a single injection	+ (T)	NA	1200 mg/kg by i.p. in corn oil	Nath et al., 1990
Mouse (ICR, male)	DNA binding in liver	^{32}P -Postlabeling was used to identify indigenous and exogenous adducts present 1, 4, and 8 weeks after two injections given a week part.	– (T)	NA	1200 mg/kg by i.p. in corn oil	Nath et al., 1990
Rat (F-344, male)	DNA methylation in liver	Hydrolyzed DNA was analyzed for aberrant methylation as increases in 7-methylguanine and O^6-methylguanine, 12 hr after dosing	+ (T)	NA	1000 mg/kg in corn oil	Barrows and Shank, 1981
Rat (Wistar, male)	DNA hypo-methylation in liver	The *in vitro* incorporation of ^{3}H-methyl groups into isolated hepatic DNA was increased indicating that the DNA was hypomethylated.	+	NA	800 mg/kg by i.p. injection 2X per week for 3 weeks	Varela-Moreiras et al., 1995

Table 4-11. Genotoxicity studies of carbon tetrachloride in mammalian systems in vivo

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		

[a] + = positive, ± = equivocal or weakly positive, – = negative, (T) = toxicity, ND = no data.
[b] Exogenous metabolic activation not applicable (NA) for these *in vivo* studies.
[c] Lowest effective dose for positive results, highest dose tested for negative results, ND = no data, NA = not applicable.
[d] The small statistically significant increase detected was considered biologically insignificant by the authors (and other reviewers).
[e] At this dose a roughly 3 fold increase in micronucleus formation was seen along with a decrease in binucleated cells (about 35-50%) indicating a cytostatic and cytotoxic effect.
[f] TUNEL – terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling
[g] Increase was in both centromere-lacking (5.5-fold) and centromere-containing (3.6-fold) micronuclei.
[h] With methylcholanthrene pretreatment only.
Note 1: The data in the paper by Sarkar and associates (Sarkar *et al.*, 1999) was judged to be insufficiently reliable to be included in the table.

4.4.2.1. *Genotoxicity Studies: Prokaryotic Organisms*

Studies of bacterial reverse mutation assays (see Table 4-8) indicate that, in most cases, carbon tetrachloride is not directly genotoxic at concentrations below cytotoxic levels. Carbon tetrachloride was negative in most standard plate incorporation assays for reverse mutation in *Salmonella typhimurium*, with or without addition of a mammalian metabolic activation system (Brams et al., 1987; de Flora, 1981; McCann et al., 1975). Increases in reversion frequency were reported by Varma et al. (1988), but the changes were not dose-related. The positive responses were reported at lower doses and negative responses at higher doses. Varma et al. (1988) did not present data for the positive controls nor discuss cytotoxicity, making it unclear how to interpret these data. Some *S. typhimurium* reversion studies used modified testing techniques in order to account for the volatile nature of carbon tetrachloride. Preincubation assays conducted in capped tubes were performed by Uehleke et al. (1977) and Zeiger et al. (1988). Both of these research groups obtained negative results. Gas-phase exposure studies have been conducted in various closed systems (Araki et al., 2004; Barber et al., 1981; Simmon and Tardiff, 1978; Simmon et al., 1977). Results were negative in most of these studies, although Araki et al. (2004) found a small increase in reversion frequency in TA98 at concentrations of 1% (10,000 ppm) and above, when tested without activation. It should be noted that the average control frequency of 13 revertants per plate in this study is unusually low, and even the elevated response of 31 revertants per plate seen at the 50,000 ppm concentration is well within the range of spontaneous revertants typically seen in TA98 controls (30-50 revertants per plate) (Maron and Ames, 1983).

In other studies using *S. typhimurium*, negative or equivocal results were reported for carbon tetrachloride in a preincubation forward mutation assay using strains BA13 and BAL13 with and without metabolic activation (Roldan-Arjona and Pueyo, 1993; Roldan-Arjona et al., 1991), and in an SOS induction assay using strain TA1535/pSK1002 (Nakamura et al., 1987). More varied results were seen in experiments using *E. coli*. Carbon tetrachloride was negative in a SOS chromotest assay (Brams et al., 1987), in a spot test (De Flora et al., 1984), and a preincubation assay when evaluated for differential DNA repair (Hellmer and Bolcsfoldi, 1992). In contrast, using strains that are more sensitive to oxidative mutagens, increases in DNA repair were reported by De Flora et al. (1984) and increases in reverse mutation were reported by Araki et al. (2004) and Norpoth et al. (1980). In the DeFlora et al. (1984) study, carbon tetrachloride was more toxic to the *E. coli* strain CM871 (*uvrA- recA- lexA-*) than it was to the isogenic repair-proficient WP2 strain or WP67 (*uvrA- polA-*). Although a similar pattern was seen in the presence of metabolic activation, carbon tetrachloride was more active in the absence of activation. The differential toxicity was seen initially using the liquid micromethod, and then confirmed using a 2-hour pre-incubation assay. In the report of Araki et al. (2004), carbon tetrachloride produced a modest 2.5-fold increase in mutations in the WP2uvrA/pKM101 strain of *E. coli* both in the presence and absence of metabolic activation. The peak response was seen after 24 hours of exposure at a high (20,000) ppm concentration. The control frequencies appear

to be unusually low and the induced response was within the control values reported by others (Martinez et al., 2000; Damment et al., 2005). Additionally, a weak positive response (statistically significant but well less than a twofold increase) for *E. coli* WP2*uvr*A was reported by Norpoth et al. (1980) at high levels (about 25,000 ppm) in another gas phase exposure study.

A stronger mutagenic response was seen when carbon tetrachloride was tested in the repair-proficient WP2/pKM101 strain of *E. coli*. A doubling in mutant frequency was observed at the 5000 ppm carbon tetrachloride concentration and reached a fivefold increase compared to pooled controls at the 20,000 ppm concentration. The increase was seen in experiments with and without metabolic activation as well as with S9 plus reduced glutathione. Because the WP2 strains of *E. coli* have an AT base pair at the critical mutation site within the *trpE* gene, they have been recommended for screening oxidizing mutagens (Gatehouse et al., 1994; Martinez et al., 2000). This increased sensitivity to oxidative damage may help explain both the Araki et al. (2004) and the DeFlores et al. (1984) isolated positive results, although some aspects of the studies are still unusual. The greater response in the repair-proficient strain seen in the Araki et al. (2004) study as compared to the repair-deficient strain was unexpected, and led the authors to postulate that a cross-linking metabolite might be responsible. If true, this could also be related to oxidative damage as lipid peroxidation-derived products have been shown to form DNA and DNA-protein cross-links (Kurtz and Lloyd, 2003; Niedernhofer et al., 2003). Again, the control frequencies reported by Araki (2004) are lower than those reported by others (Watanabe et al., 1998), but in this case, the induced mutant frequencies substantially exceed the control range of either group. Araki et al. (2004) reported a tenfold increase in mutants in the WP2/pKM101 experiments without S9. However, approximately half of the observed increase appeared to be due to an unusually low mutant frequency. Also, it should be noted that the results were not statistically analyzed as the experiments were not performed in triplicate.

Some caution should be exercised in the interpretation of these and other in vitro studies as a number of the factors listed in Table 4-12 could potentially influence the outcome of the assays and contribute to both positive and negative results. For example, the bioactivation of carbon tetrachloride to a mutagenic species can be affected in a variety of ways. The initial step in the bioactivation of carbon tetrachloride is a cytochrome P450 monooxygenase-mediated formation of the trichloromethyl radical (Halliwell and Gutteridge, 1999; Weber et al., 2003). This radical is highly reactive, and as a result, may not be able to cross the bacterial cell wall or membranes to access the bacterial DNA. The trichloromethyl radical or a derived species can also react with and inactivate the monooxygenase activation system (Weber et al., 2003), which could also affect the outcome of the in vitro assays. In addition, many of the commonly used vehicle solvents used for in vitro testing such as methanol, DMSO, and ethanol are also metabolized by the cytochrome P450 2E1 isoform CYP2E1 (Hyland et al., 1992), the isoform primarily involved in carbon tetrachloride metabolism, and may have interfered with the

bioactivation of carbon tetrachloride in these test systems. In addition, DMSO can act as a free radical scavenger (Halliwell and Gutteridge, 1999).

Similarly, when standard inducing procedures (Arochlor 1254 or the combination of phenobarbitone and *beta*-naphthoflavone) have been used, the levels of CYP2E1 in the rat liver are markedly suppressed (Burke et al., 1994). This would lead to a decrease in CYP2E1 in the S9 used for the test and could potentially contribute to the observed negative results. Furthermore, although carbon tetrachloride has been evaluated many times in the standard *Salmonella* test strains, it has not been tested in either TA102 or TA104 and only a few times in the *E. coli* WP2 strains, the strains that would be the most sensitive to the oxidative DNA damage likely to be generated during carbon tetrachloride toxicity. Because of the many possible confounding factors, the in vitro carbon tetrachloride results should be interpreted cautiously.

Table 4-12. Challenges in evaluating carbon tetrachloride genotoxicity

- Large number of genotoxicity studies
- Elevated error rates related to multiple statistical tests and comparisons
- Requirement to test to high levels of toxicity to ensure a true negative response
- Non-specific effects that can occur at very high chemical concentrations
- Potential volatility from culture media
- Requirement for metabolic activation
- Downregulation of CYP2E1 synthesis shortly after carbon tetrachloride administration
- Inhibition of cytochrome P450 monoxygenases by primary carbon tetrachloride metabolite(s)
- Competitive inhibition of CYP2E1 by common solvents used as vehicles (ethanol, methanol, DMSO)
- Free radical-scavenging properties of common vehicles such as DMSO
- Possible inability of reactive trichloromethyl radical generated extracellularly by rat postmitochondrial supernatant to cross the bacterial cell wall or eukaryotic cell membrane and damage the DNA of the cell being tested
- Commonly used enzyme inducers suppress CYP2E1 levels in the rat liver S9
- Possible influence of dosing vehicle (corn oil, olive oil) in vivo
- Concurrence of cytotoxicity and genotoxicity
- Occurrence of DNA breakage during apoptotic and necrotic cell death
- Occurrence of multiple reactive species and potential mechanisms of genotoxicity
- Difficulties in distinguishing direct and indirect genotoxic effects
- Generation of genotoxic products secondary to lipid peroxidation
- Genotoxic responses occurring secondary to inflammatory responses

4.4.2.2. *Genotoxicity Studies: Non-Mammalian Eukaryotic Organisms*

Carbon tetrachloride has also been tested in the yeast *Saccharomyces cerevisiae* and the mold *Aspergillus nidulans* (Table 4-9). In contrast to the bacterial results, the majority of the studies conducted in these species have yielded positive results. However, the results obtained from the two fungal species differ significantly, most likely due to the test strains selected and the endpoints chosen for examination. In initial studies by Callen et al. (1980), carbon tetrachloride induced strong (>20-fold) increases in gene conversion and mitotic conversion and a weak (2.5-fold) increase in reverse mutations when tested at high concentrations in the yeast D7 strain in a preincubation assay employing capped tubes. The increases were only seen at the highest test concentration of 34 mM, one that caused extensive toxicity (90%). These initial results were followed by a series of studies by Schiestl and co-workers using yeast strains that were designed to detect intrachromosomal recombination (DEL assay) that results from double stranded DNA breakage. Interchromosomal recombination can also be measured in these strains. In the initial study using the DEL assay (Schiestl et al., 1989), carbon tetrachloride at a concentration of 8000 μg/mL induced a strong (25-fold) increase in intrachromosomal recombinants with no increase in interchromosomal recombination. Toxicity was greater than 99% at the highest test concentration where the increase in recombinants was seen. Follow-up studies showed that the induced recombinants occurred during the G1 and G2, but not S phase of the cell cycle, and in some cases an increase in interchromosomal recombination was also seen. The dose-response curves tended to be steep and occurred concurrently with significant toxicity (Galli and Schiestl, 1996; Galli and Schiestl, 1995). Since carbon tetrachloride did not induce recombination during S phase even though it was toxic, the authors suggested that carbon tetrachloride acted by prematurely pushing G1 cells into S phase and G2 cells into cell division (Galli and Schiestl, 1998). The inability to completely repair damaged DNA prior to replication or cell division might result in DNA strand breakage and subsequent recombination. Brennan and Schiestl (1998) showed that yeast cells treated with carbon tetrachloride showed an increase in oxidative radical species as measured by the intracellular oxidation of 2,7-dichlorofluorescein diacetate. N-acetylcysteine did not exhibit a protective effect on carbon tetrachloride-induced DEL recombination, although the results are difficult to interpret as increased toxicity was seen in cells jointly treated with carbon tetrachloride and this sulfhydryl-containing agent.

In contrast to the recombinogenic effects seen with *Saccharomyces cerevisiae*, the assays using *Aspergillus nidulans* primarily detected an abnormal segregation of chromosomes. Following treatment with high concentrations (0.5%) of carbon tetrachloride, Gualandi (1984) observed a significant (>20-fold) increase in abnormal chromosome segregation but only a weak (~2.5-fold) increase in forward mutations. Toxicity at the test concentration was approximately 70%. Additional studies showed a strong correlation between toxicity and altered segregation leading to aneuploid cells. Cysteamine (a free-radical scavenger) was also co-administered with carbon tetrachloride and showed some protection against the induced alterations in chromosome

segregation. In a series of related studies, carbon tetrachloride was consistently shown to interfere with chromosome segregation leading to aneuploidy. Crebelli et al. (1988) demonstrated that carbon tetrachloride induced a ten-fold increase in chromosome segregation at the highest (0.08%) concentration tested. Toxicity at this concentration was 72%. More modest effects (~3-fold) were seen beginning at lower concentrations (0.04%) that were less toxic (18%). Notably, no increase in crossing over was seen in these experiments. Similar results both on chromosome segregation and crossing over were observed in a follow-up study using a narrower and somewhat lower dose range (0.01 to 0.03%; Benigni et al., 1993). In a related quantitative structure-activity-relationship study of carbon tetrachloride and 23 other chlorinated aliphatic hydrocarbons, the ease at which the compounds were able to accept electrons, as characterized by the energy of lowest unoccupied molecular orbital, was the best predictor of their aneuploidy-inducing properties (Crebelli et al., 1992).

As indicated in Table 4-9, the genotoxic effects were seen in both *Saccharomyces* and *Aspergillus* experiments without the use of exogenous metabolic activation. This is consistent with studies that have shown actively growing cells of both species contain cytochrome P450 monooxygenase enzymes capable of bioactivating promutagens to mutagens (Bignami et al., 1981; Callen et al., 1980). As indicated above, the studies in *Saccharomyces* detected primarily recombination whereas those in *Aspergillus* detected primarily alterations in chromosome segregation. This difference in outcome appears to be due primarily to the nature of the specific strains used and the endpoints selected for evaluation by the investigators. There was a close association seen between cytotoxicity and the recombinogenic and aneugenic effects measured in the two systems.

Additionally, carbon tetrachloride did not produce sex-linked recessive lethal mutations in *Drosophila melanogaster* (Foureman et al., 1994).

4.4.2.3. *Genotoxicity Studies: Mammalian Cells In Vitro*

Numerous studies have been performed to evaluate the ability of carbon tetrachloride to cause genotoxic effects or precursor lesions in mammalian cells in vitro (Table 4-10). These studies have been performed using both model cell systems frequently with exogenous metabolic activation and hepatocytes that retain their xenobiotic-metabolizing capabilities.

Studies in non-target mammalian cells. In studies using peripheral blood lymphocytes or lymphoblastoid cells, carbon tetrachloride yielded mixed results. As part of a study of fumigants, Garry et al. (1990) exposed G_o lymphocytes to carbon tetrachloride for 30 minutes, then cultured the lymphocytes and measured the frequencies of chromosome aberrations and sister chromatid exchanges (SCEs). No increases in structural aberrations or SCEs were seen. Tafazoli et al. (1998) used the micronucleus assay to measure chromosome loss or breakage in the peripheral lymphocytes obtained from two donors. Exposure to different concentrations of

carbon tetrachloride ranging from 1 to 40 mM did not induce a statistically significant increase in micronucleated cells at any concentrations except at 10 mM in one donor with S9 mix and at 5 mM in the second donor without S9 mix. Cell division was not affected at these mutagenic concentrations; however, the authors identified a cytotoxic concentration of 40 mM both with and without S9 mix in one donor. To measure the amount of DNA strand breaks, Tafazoli et al. used the in vitro Comet assay with isolated lymphocytes from the donors. No statistically significant response was found for either tail length or tail moment at concentrations tested (5 to 20 mM) either with or without S9 mix. Carbon tetrachloride was also reported to be negative when assayed for unscheduled DNA synthesis (UDS) in lymphocytes (Perocco and Prodi, 1981). Each of these studies either used high carbon tetrachloride concentrations (>1500 µg/mL) or tested to toxic concentrations.

In contrast, when tested at relatively low concentrations, Sivikova et al. (2001) reported that cultured ovine peripheral lymphocytes exposed to carbon tetrachloride exhibited modest twofold increases in micronuclei in both the absence and presence of S9, and an approximately 25% increase in SCEs in the absence of S9. Under similar conditions, no increase in structural chromosome aberrations was seen although a decrease in the mitotic index was detected. Interestingly for both the MN and SCE experiments, the addition of vitamin E and selenium to the cultures protected against the increases in MN and SCE, implicating a role for free radicals in the observed genotoxic effects. In spite of the protective effects of the antioxidants, these studies would still appear to be anomalous given the observations of effects at fairly low concentrations and the greater activity in the absence of S9.

Doherty et al. (1996) reported that carbon tetrachloride induced micronuclei in two human lymphoblastoid cell lines – one expressing CYP2E1 (h2E1) and the other expressing CYP1A2, 2A6, 3A4, 2E1 and microsomal epoxide hydrolase (MCL-5) – but not the CYP1A1-expressing AHH-1 cell line. Treatment of the cells with 10 mM carbon tetrachloride resulted in a five- and a ninefold increase in micronucleated cells in the h2E1 and the MCL-5 cell lines, respectively. The increases occurred mostly in kinetochore-positive micronuclei, indicating an origin from chromosome loss. Smaller increases (~two to fourfold) in micronuclei originating from chromosomal breakage (kinetochore-negative) were also seen. At the 10 mM concentration, the percentage of binucleated cells, an indicator of cell proliferation and an indirect indicator of cytotoxicity, was 6 – 7% of the control values indicating that the increase in micronuclei occurred primarily under conditions producing potent cytotoxic or cytostatic effects.

In other studies involving non-target cell culture systems, carbon tetrachloride was negative for inducing structural chromosome aberrations and SCEs in Chinese hamster ovary (CHO) cells (Loveday et al., 1990). However, in a number of other assays using CHO and V79 cells, carbon tetrachloride in the absence of exogenous activation was reported to produce modest increases in c-mitoses, generate multipolar spindles and lagging chromosomes during

anaphase, and interfere with chromosome segregation resulting in aneuploidy (Onfelt, 1987; Coutino, 1979).

Carbon tetrachloride was also tested for its ability to induce morphological transformation in Syrian hamster embryo cells (Amacher and Zelljadt, 1983). In the transformation assay, carbon tetrachloride was tested in both RPMI 1640 media with horse serum and DMEM with fetal bovine serum. It was negative in the RPMI medium with 0 transformants among 2665 colonies. In DMEM, one transformed colony was seen in 2003 colonies scored. Although this was considered a positive result by the authors, the increase is not statistically significant, does not meet current criteria for a positive result (Kerckaert et al., 1996), and falls within the normal control frequencies of 0 to 0.8% reported for this type of transformation assay (LeBoeuf et al., 1996).

In studies using mouse lymphoma (L5178Y) cells with exogenous activation, carbon tetrachloride was inactive in inducing mutations at the *tk* locus when tested up to toxic concentrations (Wangenheim and Bolcsfoldi, 1988). In a follow-up study employing similar cells and conditions, DNA strand breaks were induced as measured by the alkaline elution assay. The increases in strand breaks were accompanied by increases in cytotoxicity (Garberg et al., 1988).

Studies in liver cells. Carbon tetrachloride has also exhibited mixed results when tested in vitro using isolated hepatocytes or cell lines derived from the rat liver. In early studies by Dean and Hodgson-Walker, carbon tetrachloride was negative for inducing structural chromosome aberrations or SCEs when tested at a low concentration in a metabolically competent rat liver cell line (Dean and Hodson-Walker, 1979). Similarly, no increase in UDS was seen by Selden et al. (1994) in their studies using rat hepatocytes or by Butterworth et al. (1989) in their UDS studies employing primary hepatocyte cultures from four human donors. In contrast, using an alkaline elution assay on isolated rat hepatocytes, Sina and colleagues reported a 3.1- to 5.0-fold increase in strand breaks at the highest concentration tested (3 mM), a dose that also resulted in approximately 50-60% toxicity (Sina et al., 1983). A modest dose-related increase in DNA strand breaks was also seen in the single cell gel electrophoresis (Comet) assay by Beddowes et al. (2003). The increase in breaks reported by Beddowes was accompanied by similar increases in the formation of the oxidative DNA adducts, 8-oxodeoxyguanosine and a malondialdehyde deoxyguanosine adduct.

The ability of bioactivated carbon tetrachloride to react directly with DNA has been investigated by a number of investigators using isolated DNA and nuclear preparations obtained from hepatocytes. Initial studies by Rocchi and colleagues demonstrated that when radiolabeled carbon tetrachloride was incubated with microsomes from uninduced and 3-methylcholanthrene-induced mice and rats, modest increases in radiolabel were recovered following extensive washing and extraction of the DNA with several solvents (Rocchi et al., 1973). This binding was

greater in the incubations containing the 3-methylcholanthrene-induced microsomes. Similarly DiRenzo et al. (1982) reported that significant binding of carbon tetrachloride to DNA (0.39 nmol/mg DNA) occurred following the incubation of radiolabeled carbon tetrachloride with pronase-pretreated calf thymus DNA and microsomes from phenobarbital-induced rats. The incubation was performed under a N_2 atmosphere using conditions that in previous studies had resulted in maximal binding to proteins and lipids. Oruambo and Van Duuren (1987) investigated the binding of radiolabeled carbon tetrachloride to various regions of mouse chromatin. Following a two-hour incubation with mouse hepatic microsomes, hepatic chromatin, and radiolabeled carbon tetrachloride, the authors concluded that the carbon tetrachloride metabolite(s) bound equally to both DNase I-sensitive and -resistant regions. After 4 hours of incubation, more radiolabel was recovered associated with DNase I-resistant DNA than with DNase I-sensitive DNA. This preferential binding to transcriptionally inactive (DNase I-resistant) sites in chromatin was seen as unique among carcinogens, and could be attributable to changes in chromatin conformation or differential DNA repair. In addition, Castro et al. (1989) investigated the ability of radiolabeled carbon tetrachloride to bind to the DNA of purified nuclear preparations obtained from the livers of Sprague-Dawley rats, a strain resistant to carbon tetrachloride carcinogenicity, and C3H mice and Syrian golden hamsters, two strains that are sensitive to carbon tetrachloride hepatocarcinogenesis. Low levels of binding were observed, which were increased in the mouse and hamster incubations when NADPH was included in the microsomal incubation. The authors noted that there was no correlation between sensitivity to carbon tetrachloride carcinogenesis (hamster ≥ mouse >> rat) and the binding of carbon tetrachloride metabolites to DNA, either in vitro or in vivo (in vivo: hamster = mouse = rat; in vitro with NADPH: hamster = mouse = rat; in vitro without NADPH: rat > mouse = hamster).

Overall, these data indicate that under certain conditions carbon tetrachloride can induce genotoxic effects in mammalian cells exposed in vitro. Although numerous negative studies were seen, there are indications from multiple studies that at high doses, bioactivated carbon tetrachloride is able to cause DNA breaks leading in some cases to chromosome breakage. There are also multiple studies indicating that carbon tetrachloride is able to interfere with chromosome segregation resulting in modest levels of chromosome loss and aneuploidy. However, since exogenous bioactivation was required in some studies and not others, the observed effects may result from both specific and non-specific mechanisms, some of which may not be operable in vivo. The binding studies using radiolabeled carbon tetrachloride have significant weaknesses (for discussion, see the following sections), but provide limited evidence that bioactivated carbon tetrachloride can bind directly to DNA. The overall magnitude of the covalent binding appears to be low. As seen in non-mammalian assay systems, in most cases where genotoxic effects were observed, they occurred concurrently with significant cytotoxicity.

4.4.2.4. *Genotoxicity Studies: Mammalian Cells In Vivo*

Carbon tetrachloride has been extensively tested for genotoxicity in mammalian systems in vivo (Table 4-11). A number of these studies have been conducted using standard protocols and examined genotoxicity in highly proliferating non-target organs such as the bone marrow. In addition, a large number of studies have examined genotoxic effects or precursor lesions such as DNA adducts occurring in the rodent liver. A summary of the important studies by target organ and endpoint is presented below.

Chromosomal alterations and DNA breakage in non-target organs. In studies of chromosomal alterations occurring in the bone marrow, carbon tetrachloride has shown negative results for the induction of structural chromosome aberrations in the bone marrow of male Sprague-Dawley rats and 101/H mice (Rossi et al., 1988; Lil'p, 1982), as well as for the formation of micronuclei in the bone marrow and peripheral blood erythrocytes of male BDF1 mice (Suzuki et al., 1997; Morita et al., 1997). Negative results were also seen for the induction of micronucleated erythrocytes in the bone marrow and peripheral blood of both male and female CD-1 mice (Crebelli et al., 1999). In the Comet assay, no evidence of DNA breakage was seen in the nucleated cells of the stomach, kidney, bladder, lung, brain or bone marrow of male CD-1 mice administered 2000 mg/kg carbon tetrachloride with sampling at 0, 3 and 24 hours after dosing (Sasaki et al., 1998). In these same animals, significant increases in DNA breakage were seen in the liver, although this was considered by the authors to be a false positive result because it was accompanied by evidence of necrosis in the liver. In a biomarker study, carbon tetrachloride was also reported to induce an isolated significant increase in DNA breakage in the Comet assay in nucleated peripheral blood cells of male F344 rats (Kadiiska et al., 2005). The increase is of questionable relevance as it was only seen at one of the three time points tested and only at the lower of the two doses tested.

DNA breakage in rodent liver cells. Within the rodent liver, carbon tetrachloride has been evaluated for a range of genotoxic effects across a considerable dose range. Fourteen studies employed the alkaline elution or similar method to determine if carbon tetrachloride is able to induce DNA breaks in liver cells in vivo. Negative results were seen in eight of the studies, equivocal or weak responses were seen in two, and positive results were seen in four studies. When positive or equivocal responses were seen, they consistently occurred at doses where extensive toxicity or regenerative proliferation was manifest. For most of the studies that showed a positive response, the responses appear to be more related to a general cytotoxic effect rather than a specific genotoxic effect. A brief overview of each of the positive studies is provided below.

Nakamura and Hotchi (1992) observed a modest increase in DNA breakage in their studies of DNA breakage in non-parenchymal cells. The DNA breaks were identified using an in

situ nick translation approach at time points ranging from 12 hours to 18 weeks after dosing. Although breaks were seen, the authors argued that the breaks were most likely physiological in nature, reflecting changes in proliferation and gene expression rather than direct carbon tetrachloride-mediated DNA damage. In another series of experiments involving the adaptation of the liver to long-term continuous carbon tetrachloride administration to mice, Gans and Korson (1984) noted changes in the DNA synthesis of the liver nuclear DNA. As one aspect of the study, the authors used an alkaline elution approach to study DNA damage in the liver of CD-1 mice. A maximal increase in DNA damage was seen 18 hours after administration. The normal pattern of sedimentation was restored by 24 to 36 hours. The authors stated that "these changes were observed only following doses of carbon tetrachloride which resulted in liver necrosis. Doses of carbon tetrachloride which did not produce necrosis did not result in a shift in the sedimentation of DNA."

Similarly, Cabre and associates detected DNA breaks in rats treated with two high doses of carbon tetrachloride using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) technique (Cabre et al., 1999). The TUNEL assay is commonly used to measure DNA strand breaks occurring in apoptotic cells but also detects breaks occurring in necrotic cells (Higami et al., 2004). Similarly, Yasuda and colleagues used the TUNEL assay to study necrotic cell death induced by carbon tetrachloride and dimethylnitrosamine (Yasuda et al., 2000). In the Yasuda studies of carbon tetrachloride-treated livers, TUNEL staining was closely associated with the release of lysosomal enzymes into the cytoplasm, and an intranuclear localization of lysosomal enzymes occurred at an early stage of subcellular damage. This pattern was notably different from that seen with the alkylating agent, dimethylnitrosamine. Given the high doses administered and the known hepatotoxicity of carbon tetrachloride, the observed detection of DNA strand breaks in these and the other studies is not surprising. As mentioned earlier and for the same reason, Sasaki et al. (1998) considered the DNA strand breaks that they observed using the Comet assay to be false positives and not relevant to assessing genotoxic potential since evidence of necrosis was present.

Unscheduled DNA synthesis in the rodent liver. A number of studies have been performed to investigate the ability of carbon tetrachloride to induce UDS in the liver of rats and mice treated in vivo. In an initial study of de novo and repair replication of DNA in the livers of treated rats, Craddock and Henderson (1978) reported that oral administration of 4000 mg/kg carbon tetrachloride increased the synthesis of DNA in non-replicating hydroxyurea-treated hepatocytes 17 hours, but not 2 hours, after treatment. In the absence of the hydroxyurea treatment, extensive DNA synthesis was seen at the 17-hour time point. Diethylnitrosamine, ethyl ethanesulfonate, aflatoxin, and retrosine induced DNA repair replication at the earlier two-hour sampling. The delay seen with carbon tetrachloride was suggested by the authors as indicating that the repair was associated with damage caused by an indirect mechanism such as

deoxyribonuclease activity resulting from lysosomal damage; however, the extensive DNA synthesis occurring at the 17-hour time point is almost certainly due to proliferation following extensive cell death induced by carbon tetrachloride. Under these conditions, it is not clear how efficient the hydroxyurea inhibition of DNA synthesis would be. In a more recent study using the hydroxyurea approach, Ikegwuonu and Mehendale (1991) saw similar results, although they saw no increase in DNA breakage using an alkaline elution technique in a parallel study. The observations of DNA repair in the absence of detectable DNA breaks are inconsistent and the authors concluded that the hydroxyurea repair results were attributable to induced de novo synthesis (post replication repair) rather than true DNA repair. It should also be noted that the use of the hydroxyurea method to measure UDS is generally not recommended because of the complex effects of hydroxyurea in the cell and its ability to directly induce UDS (for additional details, see Madle et al., 1994).

Six other studies have been conducted using the currently recommended and more reliable autographic method of detecting UDS. No increase in UDS induced by carbon tetrachloride was seen even at doses exhibiting significant toxicity. With the autographic method, DNA uptake is measured in individual cells allowing UDS to be clearly distinguished from de novo synthesis.

To summarize the UDS results, eight in vivo studies have been performed investigating UDS in the rodent liver following carbon tetrachloride administration. Two major methods for measuring UDS were employed, the autographic method that allows UDS in individual cells to be measured and that is considered to be more reliable, and a less reliable method that measures DNA synthesis in the presence of hydroxyurea, an inhibitor of global de novo DNA synthesis. The six studies that used the autoradiographic method yielded negative results whereas the two that used the hydroxyurea method produced results most appropriately characterized as false positives.

Chromosome aberrations and micronuclei in rodent liver cells. In cytogenetic assays of hepatocytes isolated from treated rodents, carbon tetrachloride produced mixed, largely negative results. In an early study by Curtis and Tiley (1968), no increase in chromosomal fragments or bridges occurring in anaphase cells was seen in liver squash preparations of mice treated with a high (8000 mg/kg) dose of carbon tetrachloride. Similar negative results for structural chromosome aberrations, SCEs and micronuclei were reported at all time points in time course studies conducted by Sawada et al. (1991). Negative results were also reported for micronucleus formation and altered ploidy by Uryvaeva and Delone (1995).

In two studies conducted by Van Goethem and colleagues, however, an increase in micronuclei was reported. In their initial study investigating the early stages of hepatic carcinogenesis (Van Goethem et al., 1993), carbon tetrachloride was administered to male Wistar rats at 3200 mg/kg and the frequency of micronuclei was measured in hepatocytes harvested 72

hours later. Initial studies of the mitotic index and the percent binucleated cells indicated that 72 hours was the optimal time to harvest hepatocytes for the detection of micronuclei. High intra-animal variability was seen, but the results suggested that the hepatocytes of the carbon tetrachloride- (and CT+NaCl-) treated mice exhibited an increase in micronuclei (1.7-7.2%) as compared to those of control (and NaCl-treated) mice (0.2-1%). In a follow-up study, Van Goethem and associates repeated portions of their earlier experiment (Van Goethem et al., 1995). Three animals received carbon tetrachloride and three served as controls. The frequency of micronucleated hepatocytes increased from 1.5% in the controls to 7.6% in the carbon tetrachloride-treated rats, a significant fivefold difference. Using fluorescence in situ hybridization with a multi-centromeric rat probe, the authors attributed the increase in MN primarily to chromosomal breakage. Based on the frequencies given in the paper, chromosome breakage can be calculated to be 5.5-fold over the control whereas chromosome loss can be calculated as a 3.5-fold increase. It should be noted that the observed difference in the proportion of centromere-containing and -lacking micronuclei in the study is attributable to a low frequency of centromere-containing micronuclei in only one rat and is unlikely to be either statistically or biologically significant. Based on their work and that of others (Craddock and Henderson, 1978), the authors attributed the results to chemically-induced oxidative cellular damage, and suggested that free radicals produced from carbon tetrachloride may disrupt cytoplasmic organelles releasing DNase and tissue-destructive hydrolases within the cell leading to DNA strand breaks and tissue damage. Although the sample sizes of the studies are quite small, the two studies indicate that the micronucleus results are reproducible and that under regenerative conditions following toxicity, an increase in chromosome breakage and possibly chromosome loss can be detected in the regenerating cells of carbon tetrachloride-treated rats.

In should also be noted that Sarkar et al. (1999) reported that the administration of carbon tetrachloride to mice over a five-week period resulted in increases in structural chromosome aberrations in liver cells. However, there appear to be numerous and significant methodological issues with these experiments. For example, the methods section does not adequately explain how metaphases were obtained from either the treated or control mice that would allow structural chromosome aberrations to be scored. Given the low number of mitotic cells in the untreated mouse liver, it would be very difficult if not impossible without mitotic stimulation to obtain 50 well spread metaphases without the use of colchicine or other spindle-disrupting agent. In addition, the reported frequencies of structural aberrations including some classes of aberration, such as ring chromosomes, are unusually high (32-48% including gaps) when compared to other studies. Because of these concerns, this paper has not been included in Table 4-11.

Mutations in transgenic mice. The ability of carbon tetrachloride to induce mutations in hepatocytes in vivo has been investigated in three studies using transgenic mice. Negative results were seen in each of the three studies. As reported by Mirsalis and coworkers, transgenic

B6C3F1 lacI mice were treated with 5 daily doses of carbon tetrachloride at 35 mg/kg-day and the animals were sacrificed 7 days after the first dose (Mirsalis et al., 1994; Mirsalis, 1995). Mice were implanted with an osmotic pump that released [^{3}H]thymidine at the beginning of the study to measure the percent of hepatocytes in S phase (labeling index). Controls had a labeling index of 0.07% and a mutant frequency of $\leq 6 \times 10^{-5}$. Carbon tetrachloride produced a nearly 1000-fold increase in the labeling index with no increase in the mutant frequency. The authors concluded that short bursts of cell proliferation induced by carbon tetrachloride do not result in mutations in the liver.

As part of another study to investigate the impact of cell proliferation on liver mutagenesis, carbon tetrachloride at 80 mg/kg was administered by i.p. injection to *lacz* transgenic CD2F1 mice (Muta™Mice) and the animals were sacrificed 14 days later (Tombolan et al., 1999; Lambert et al., 2005). The mutant frequency in the carbon tetrachloride-treated animals (8.6×10^{-5}) was not significantly increased over that seen in the controls (5.4×10^{-5}). In non-transgenic CD2F1 mice receiving an intragastric dose of carbon tetrachloride, significant increases in absolute and relative liver weights were seen beginning two days after treatment. The percent of hepatocytes labeling with BrdU during the last two hours before sacrifice peaked at 59 times that of the controls at 3 days after treatment and returned to control levels by day 7.

In the third study reported by Hachiya and Motohashi (2000), the frequency of mutations the *lacZ* transgene in liver of male CD2F1 *lacZ* transgenic mice (Muta™Mice) was determined 14 days after administration of 700 mg/kg carbon tetrachloride (by oral gavage) or 7, 14, or 28 days after administration of 1400 mg/kg. A small increase in mutant frequency, considered biologically insignificant by the authors, was seen. The mutant frequencies for six of the nine carbon tetrachloride-treated animals were within the control range (53×10^{-6} to 100.4×10^{-6}). The mutant frequencies for the other three mice exceeded the upper end of the control range by 3 to 49%. The results as analyzed by Fishers exact test were statistically significant in part because of the large number of plaques evaluated and the fact that the Fisher's exact test does not account for animal-to-animal variability. The authors concluded that no biologically significant increase in the mutant frequency was seen in the carbon tetrachloride-treated mice. Other reviewers have concurred with this conclusion (Lambert et al., 2005).

As indicated in Heddle et al. (2000), a commonly used cut-off value for a positive response in this type of transgenic assay is at least a twofold increase over the historical negative control mutant frequency. Although a historical control range for the Hayashi and Motohashi lab was not presented, the range for the concurrent controls was 5.3×10^{-5} to 10×10^{-5} with a mean of 8.2×10^{-5}. For comparison, a general control range suggested by Heddle et al. (2000) used for sample size calculations is 4×10^{-5} to 7×10^{-5}. Using this as a historical control, no treatment group exceeded twofold that of the control and only one treated animal in the study was outside of this range. As a caveat, the numbers of animals used in the three studies were small, and the dosing and sampling protocols did not follow those currently recommended (Heddle et al., 2000;

Lambert et al., 2005). However, the results of these three in vivo studies are consistent and provide no evidence for the formation of carbon tetrachloride-induced mutations in the liver following acutely toxic doses.

DNA binding by carbon tetrachloride-derived metabolites. A number of studies have investigated the potential of carbon tetrachloride to bind covalently to DNA. Additional studies have investigated whether DNA adducts derived from reactive oxygen species or from lipid peroxidation-derived products are elevated following carbon tetrachloride administration. DNA adducts from both pathways have been reported in carbon tetrachloride-treated mice, rats and hamsters.

In initial studies, Rocchi et al. (1973) investigated the ability of ^{14}C-labeled carbon tetrachloride to bind to the DNA, RNA and proteins in the liver of male Wistar rats and male Swiss mice. Carbon tetrachloride was injected i.p. at 56 mg/kg and the animals were sacrificed 12 hours later and the livers from the treatment groups were pooled. Half of the animals had been previously treated with 3-methylcholanthrene to induce hepatic metabolism. Radiochemical binding to nuclear and cytoplasmic proteins but not DNA was seen in the 3-methylcholanthrene-pretreated and non-pretreated rats. Binding to rRNA was also seen in the 3-methylcholanthrene-pretreated rats. In the mouse studies, DNA binding was seen in the livers of mice pretreated with 3-methylcholanthrene but not in mice not previously pretreated. Protein binding was seen in both groups of mice. Since the livers of the treatment groups were pooled for analysis, no measure of variability or statistical significance could be established. In addition, although the article mentions that the counts per minute (cpm) of the samples was at least twice that of the background, there is no mention of controls nor information on how the samples were corrected for radioactivity in the control samples.

Diaz Gomez and Castro (1980a) also studied the ability of ^{14}C-labeled carbon tetrachloride to bind to DNA, nuclear proteins and nuclear lipids in the liver of male Sprague Dawley rats and male Strain A/J mice. Carbon tetrachloride was injected i.p. at 1.4 mg/kg, and the animals were sacrificed 16 hours later. Three samples, each comprised of one rat liver or the pooled livers from 10 mice, were measured per experimental group. A small but significant increase in radiocarbon binding was seen in both the mouse and rat samples in this experiment. Binding to nuclear proteins and lipids was also seen in parallel experiments. In another series of experiments, mice previously treated with phenobarbital or 3-methylcholanthrene to induce hepatic metabolism were administered carbon tetrachloride at 1.4 mg/kg. Another group was administered a higher (3200 mg/kg) toxic carbon tetrachloride dose. Radiochemical binding to mouse liver DNA was reported for the phenobarbital and 3-methylcholanthrene-pretreated mice as well as for the mice treated with the toxic carbon tetrachloride dose. DNA binding was slightly increased in the 3-methylcholanthrene-pretreated mice (0.84 pmol/mg) and the high-dose mice (2.803 pmol/mg) as compared to the low-dose carbon tetrachloride-treated mice (0.72

pmol/mg). The levels of low-dose carbon tetrachloride binding to DNA were considered to be quite low in both species with the binding in the mouse liver slightly higher than that in the rat liver. Negative control information was not presented. In place of a true negative control, the background radioactivity counted in the presence of DNA of 78 dpm (disintegrations per minute). This was approximately double the background of 38 detected in the absence of DNA and was deducted from each experimental determination.

In a follow-up study, Castro et al. (1989) investigated the relationship between the intensities of covalent binding to liver DNA and nuclear proteins in vivo in samples obtained from C3H mice, Syrian golden hamsters, and Sprague-Dawley rats – three species with different susceptibilities to carbon tetrachloride-induced liver cancer – administered 1200 mg/kg radiolabeled carbon tetrachloride ($[^{14}C]CCl_4$). The authors reported that there was no correlation between the intensity of the carcinogenic effects in these species and DNA binding, either in vitro or in vivo. However, a good correlation was found between carcinogenicity and covalent binding to total nuclear proteins both in vitro and in vivo. Covalent binding to liver DNA in all three species was similar [(2.2-2.3 pmol carbon tetrachloride/mg DNA or 1.4-1.5 mol nucleotides/mol carbon tetrachloride metabolites (x 10^6)]. Higher levels of covalent binding to nuclear proteins, particularly the acidic nuclear protein fractions, were seen when expressed on a pmol per mg basis. The authors discussed that the acidic nuclear proteins often have regulatory functions in gene expression and that this may be important in carbon tetrachloride-induced carcinogenesis. Again, the authors indicated that they subtracted for background radioactivity (35 dpm), but presented no data on control binding or how they corrected for control radioactivity – a serious limitation for the use of this and other studies in assessing genotoxic potential.

Levy and Brabec (1984) also investigated the ability of radiolabeled carbon tetrachloride to bind to different types of DNA. After the administration of a single dose of ^{14}C- carbon tetrachloride to male Sprague-Dawley rats, elevated levels of radioactivity were recovered bound to purified mitochondrial and nuclear DNA. At both a low non-necrotizing and a high dose, 20- to 50-fold more radioactivity was recovered bound to mitochondrial DNA than to nuclear DNA. Binding to mitochondrial DNA also occurred when radiolabeled carbon tetrachloride was incubated anaerobically with isolated mitochondria. Carbon tetrachloride is known to be bioactivated in the mitochondria (Weber et al., 2003), so this report of elevated binding close to the site of activation seems plausible. Again, there is no mention of a negative control or how the samples were corrected for control radioactivity or counts. There is also no indication of variability, the number of samples analyzed, or statistical significance of the results.

As described above, four studies have reported that following administration of radiolabeled carbon tetrachloride, detectable amounts of radioactivity were recovered bound to the extracted nuclear DNA. Significant methodological problems with each of the studies create difficulties in interpreting the results. For one or two of the studies, basic information on sample

size, variability and statistical significance is not provided. In addition, all studies failed to provide data for untreated controls or indicate that the treatment samples were corrected for control radioactivity (or dpm). For agents that bind weakly to DNA such as carbon tetrachloride, even small increases in dpm in the controls can substantially alter the amount of binding attributed to the chemical treatment.

Following the administration of a radiolabeled compound to an animal, the recovery of radioactivity strongly associated with the isolated and extracted DNA is assumed to represent covalent binding of the chemical or its metabolite to DNA. However, binding to proteins or lipids can occur and may be recovered as contaminants within the DNA preparation (Kitta et al., 1982). In addition, metabolic incorporation of the radiocarbon into DNA can also occur through entry into the carbon pool of the cell with subsequent incorporation into DNA (Phillips et al., 2000). This is a concern with carbon tetrachloride as metabolic studies have shown that complete dechlorination of carbon tetrachloride can occur during cellular metabolism (Weber et al., 2003; Halliwell and Gutteridge, 1999). It is therefore possible that part of the radiolabel recovered in the in vivo ^{14}C studies represents carbon tetrachloride-derived carbon that was incorporated into DNA. For both of these reasons, it is important to identify the carbon tetrachloride-derived DNA adducts to confirm that they occur in vivo. Unfortunately, this has not yet occurred. Studies in nonaqueous model systems have shown that the trichloromethyl radical can adduct nucleotides (Castro et al., 1994; Diaz Gomez and Castro, 1981), but it is not clear to what extent this would occur in aqueous systems or in vivo. Assuming that all of the radiocarbon recovered represents adducts and that the levels of radioactivity in the controls are equivalent to background, the magnitude of the DNA binding even at high toxic concentrations is relatively low (Castro et al., 1989; Lutz, 1986; Levy and Brabec, 1984; Diaz Gomez and Castro, 1980a; Lutz, 1979; Rocchi et al., 1973). Overall, there is limited evidence for the ability of carbon tetrachloride metabolites to bind covalently to DNA in vivo.

Oxidative- and lipid peroxidation-derived DNA adducts. Since reactive oxygen species as well as lipid peroxidation-derived degradation products are also known to bind covalently to DNA, numerous investigators have investigated whether oxidative adducts can be detected following the administration of carbon tetrachloride to animals. Adducts derived from both reactive oxygen and lipid peroxidation have been detected. Four studies employing a wide range of doses attempted to detect DNA adducts derived from the lipid peroxidation product malondialdehyde (MDA) or similar reactive species, in the hepatic DNA of rats or hamsters. Of the four studies, two were positive, one was equivocal, and one produced negative results. In addition, two studies detected DNA adducts formed in the liver (as well as other tissues) from *trans*-4-hydroxy-2-nonenal (4-HNE), another reactive species formed during lipid peroxidation. A brief description of the individual studies follows.

In the initial study, Hadley and Draper (1990) briefly mention that the excretion of a newly identified guanine-malondialdehyde adduct in the urine was increased 2.5-fold after the oral administration of carbon tetrachloride to rats. No data were provided. In a later study using a sensitive mass spectrometric method, Chaudhary et al. (1994) demonstrated that four days after the administration of a 0.1 mg/kg oral dose of carbon tetrachloride to Sprague Dawley rats, the liver levels of the major endogenous malondialdehyde deoxyguanosine adduct increased 1.8-fold from 2.1 per 10^7 bases in the controls to 3.8 per 10^7 bases. The level of isoprostane, another product of lipid peroxidation, was increased 16-fold in the treated animals.

In the report by Draper et al. (1995), the concentration of a deoxyguanosine-malondialdehyde adducts in the liver was determined 48 hours after oral administration of 160 mg/kg carbon tetrachloride to a group of five rats. A significant decrease in the level of this adduct was seen in the carbon tetrachloride-treated rats as compared to controls. The authors suggested that in some undetermined fashion the liver DNA was protected from the increasing amounts of malondialdehyde formed. They noted that under the same conditions, previous studies have shown that large concentrations of malondialdehyde adducts with lysine, but not deoxyguanosine-malondialdehyde, are excreted in the urine.

As part of another study to identify DNA adducts contributing to lipid hydroperoxide-mediated carcinogenesis, Wang and Liehr (1995) performed ^{32}P-postlabeling to measure and quantify the influence of carbon tetrachloride on the presence of endogenous adducts in Syrian golden hamsters four hours after treatment with 160 mg/kg and 1600 mg/kg carbon tetrachloride. Treatment of the hamsters with the 160 mg/kg dose resulted in a doubling of renal and liver lipid hydroperoxide levels. At the higher dose, renal lipid hydroperoxide levels were raised by 30% but those in the liver were lowered by 50%, presumably due to lipid hydroperoxide-mediated inactivation of metabolic enzymes required for the activation of carbon tetrachloride. The levels of lipid hydroperoxide-derived DNA adducts in the kidney and liver varied in a comparable manner; the measured endogenous adducts in the liver increased from ~9 in the controls to ~14 (expressed as relative adduct level x 10^8 adducts) at the low dose and decreased to ~8 at the high carbon tetrachloride dose. Adduct levels in the kidney increased from ~11 in the controls to ~25 at the low dose and ~16 at the high dose. A very good correlation between measured lipid hydroperoxide levels and endogenous adducts was seen. The authors noted that the decreased levels that were seen at the high dose were consistent with decreases in polar adducts observed by Nath et al. (1990) in the livers of mice treated with carbon tetrachloride at 1200 mg/kg. The observed decrease is also similar to the decrease in the deoxyguanosine-malondialdehyde adduct seen by Draper et al. (1995). It would appear that at times there can be an unusual relationship between carbon tetrachloride dose and lipid peroxide-derived DNA adducts.

Using ^{32}P postlabeling combined with high-performance liquid chromatography, the formation of trans-4-hydroxy-2-nonenal-derived cyclic adducts with deoxyguanosine was seen in untreated rat and human tissues indicating that they are endogenous in origin (Chung et al.,

2000). Significant increases in the formation of the HNE-dG adduct were seen in the livers of F344 rats treated with a single 3200 mg/kg dose of carbon tetrachloride. Twenty-four hours after treatment the levels of the HNE-dG adducts were increased 37-fold as compared to those of control animals (104 nmol/mol guanine vs. 2.8 nmol/mol guanine). The adducts appeared to be persistent as significant levels of the HNE-dG adducts (88 nmol/mod guanine) were present 72 hours after dosing.

The formation of 1,N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal (HNE-dGp-adducts) were measured in tissues of rats treated with carbon tetrachloride and compared to those in control rats (Wacker et al., 2001). Carbon tetrachloride at a dosage of 500 mg/kg was administered by a single i.p. injection with sacrifices at 4, 16 and 24 hours post injection, or by 4 injections at 24-hour intervals with the sacrifice occurring 8 hours after the final dose. In the single injection studies, increases in HNE-dGp adducts were seen in the lung and colon at various times and in the forestomach at all three time points. HNE-dGp adduct levels also showed a nonsignificant increase in the liver and no change in the kidney. The maximum increases seen were approximately 1.5 to 2-fold. In the multi-dose studies, significant increases were seen in the liver (2.2-fold) and the forestomach (1.7-fold). The levels of HNE-dGp adducts detected in the liver (2.8 per 10^7 normal nucleotides) in this study were of the same order of magnitude as the adduct levels formed from malondialdehyde (MDA) in the liver after treatment with carbon tetrachloride (3.8 per 10^7 normal nucleotides; Chaudhary et al., 1994) and HNE adducts found in the liver (22 per 10^7 normal nucleotides; Chung et al., 2000).

The formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of many adducts formed between reactive oxygen species and DNA. Because of its prevalence and ease of measurement, it is frequently used as a measure of oxidative DNA damage. Four studies have attempted to measure 8-OHdG following the administration of carbon tetrachloride to rats or mice. All four of the studies were positive although the response in one was relatively weak.

In the initial study by Takahashi et al. (1998), the suitability of an antibody to detect 8-OHdG for immunohistochemistry was determined by measuring adduct levels in hepatocyte nuclei in a time-course study following the treatment of rats with carbon tetrachloride. Rats were administered carbon tetrachloride at 3200 mg/kg by gavage and sacrificed at 6 hours, 12 hours, 1, 2, 3, and 7 days. Severe centrilobular necrosis was present by day 1. By days 2 and 3, anti-8-OHdG antibody staining was present in the mononuclear cells infiltrating the necrotic centrilobular regions as well as in the hepatocytes in the midzonal and periportal regions, and sinusoidal endothelial cells. At the day 2 time point, the formation of 8-OHdG in DNA and 8-oxo-dGTPase mRNA expression were also increased by 5.1- and 1.7-fold, respectively. MDA plus 4-NHE showed peaks at 6 hours and 3 days. The findings suggested that increased lipid peroxidation, rather than an excessive formation of 8-OHdG, was the main contributing factor in the massive hepatic necrosis observed. The observed increase in 8-OHdG was attributed to the infiltrating mononuclear cells.

In the studies reported by Iwai et al. (2002), carbon tetrachloride was administered by subcutaneous injection to rats twice a week at a dose of 200 mg/kg for the first 10 weeks, then at 400 mg/kg for the next 10 weeks. The rats were sacrificed at the end of week 22. At week 1, an approximately twofold increase in 8-OHdG was seen in liver DNA of the treated rats when compared with untreated controls. Consistent with this, the treated rats also exhibited higher levels of 8-oxo-guanine DNA glycosylase 1 (OGG1) mRNA when measured using reverse-transcriptase PCR.

Recently as part of an investigation into the susceptibility of young and old mice to oxidative stressors, Lopez-Diazguerrero et al. (2005) administered carbon tetrachloride at a dose of 43 mg/kg by i.p. injection on 3 consecutive days to young (2 month old) and older (14 month old) female CD-1 mice. Twenty-four hours post-treatment, liver DNA in carbon tetrachloride-treated young and old mice exhibited significant increases in 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG). The 8-oxodG levels increased from 0.5 residues/10^6 dG in the young controls to 7.4 residues/10^6 dG in the carbon tetrachloride-treated young animals. In the older animals, the 8-oxo-dG levels increased from 2.6 residues/10^6 dG in the controls to 10.1 residues/10^6 dG in the treated animals. The 8-oxodG levels between the treated young and old animals did not differ significantly.

Similarly as part of a larger study of oxidative biomarkers, Kadiiska et al. (2005) measured the levels of 8-OHdG in the urine of male Fischer 344 rats previously administered carbon tetrachloride at 120 mg/kg and 1200 mg/kg by i.p. injection (urine collected 2-7 hours and 7-16 hours after carbon tetrachloride injection). Significant increases in 8-OHdG compared to the control were seen for the low dose at 16 hours and the high dose at both sample times. The high dose resulted in a seven- and threefold increase in the excreted adducts at the two successive time points.

Available studies provide considerable evidence of DNA adducts derived from reactive oxygen species or lipid peroxidation following in vivo administration. In some cases, the relationship between dose and adduct levels appeared to be complex, without a monotonic relationship between dose and response. In comparing the results from the various binding studies, it should be remembered that the binding measured in radiocarbon binding studies reflects all DNA adducts that contain the ^{14}C label. In contrast, 8-OHdG and MDA and 4-HNE adducts represent only a few of the many types of oxidative adducts (De Bont and van Larebeke, 2004; Halliwell and Gutteridge, 1999). When increases in these marker adducts are seen, the total number of oxidative DNA adducts is undoubtedly much larger. The overall consistency and magnitude of the results from the oxidative adduct studies indicate that they likely represent the major class of DNA lesion occurring in the rodent liver following carbon tetrachloride administration.

Endogenous adducts. Using the ^{32}P-post-labeling assay, Nath et al. (1990) investigated the effects of carbon tetrachloride on presence of hepatic "I" spots (DNA adducts believed to be formed from endogenous compounds) in both acute and long-term studies using 10-12 month-old ICR mice. For the acute study, carbon tetrachloride was injected i.p. at a dose of 1200 mg/kg. Twenty-four hours after the injection, the intensity of non-polar I-spots in the liver DNA was increased as compared to those in corn oil-treated controls while the intensity of one polar I spot was reduced. In contrast, in a long-term study of carbon tetrachloride, mice given two consecutive injections of carbon tetrachloride (1200 mg/kg) and sacrificed at 1, 4, 8, 12, and 22 weeks after the final injection, the total liver I compound levels were reduced to 17-49% of the corresponding controls. Although there was a trend in recovery between weeks 8 and 22, the I-compound levels remained significantly lower at week 22. The authors suggested that the persistent reduction in I-compound levels may point towards a nongenotoxic effect of carbon tetrachloride contribution to hepatocarcinogenesis. As mentioned by the authors, previous studies have shown a significant reduction in I-compound levels following treatment with a number of nongenotoxic carcinogens and by other treatments/conditions associated with rat liver carcinogenesis. Of particular note, the authors reported that "neither the acute nor the chronic experiments with carbon tetrachloride produced extra spots indicative of DNA adducts" indicating that exogenous adducts were not seen in the carbon tetrachloride-treated mice.

Altered DNA methylation. Following carbon tetrachloride administration, a number of studies have reported alterations in liver DNA methylation. In early studies performed by Barrows and Shank (1982), increases in 7-methylguanine and O^6-methylguanine were seen in liver DNA 12 hours after rats were administered a single 1000 mg/kg dose of carbon tetrachloride. This increase was also seen in hydrazine- and ethanol-treated rats, and there was some evidence in the hydrazine-treated rats that S-adenosylmethionine (SAM) was the methyl donor. Based on the observed results, the authors suggested that aberrant DNA methylation may be a non-specific response to chemical injury to the liver.

More recently, Varela-Moreiras et al. (1995) investigated the effect of short-term administration of carbon tetrachloride on hepatic DNA methylation and on SAM and S-adenosylhomocysteine (SAH) in male Wistar rats administered 800 mg/kg carbon tetrachloride by i.p. injection 2 times/week, for 3 weeks. Rats treated with carbon tetrachloride exhibited hypomethylation of their hepatic DNA as measured by the extent to which the liver DNA from the treated animals could be methylated in vitro using [^{3}H-methyl]-SAM as a methyl donor. In addition, decreased levels of SAM, methionine and folate as well as increased levels of SAH and homocysteine were seen. No changes were observed in the levels of cystathionine, reduced glutathione, or in the activity of SAM-synthetase. The magnitude of the observed changes was substantially reduced in animals co-administered SAM with carbon tetrachloride. The authors proposed that "carbon tetrachloride disrupts the distribution of homocysteine between

remethylation and its degradation via the transsulphuration pathway, and that SAM, by resetting the methylation ratio, restores this equilibrium." In eukaryotic and mammalian cells, gene expression is influenced by the extent and patterns of DNA methylation, so the observed changes in hepatic DNA methylation could represent an epigenetic alteration that could contribute to carbon tetrachloride carcinogenesis.

4.4.2.5. *Genotoxicity Studies: Summary of the Evidence for Genotoxic and Mutagenic Effects*

EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) identify a number of criteria that should be considered in judging the adequacy of mechanistic data. These include mechanistic relevance, number of studies of each endpoint, consistency of results in different test systems and species, conduct of the tests according to generally accepted protocols, and degree of consensus and general acceptance among scientists regarding the interpretation of the results. In addition to these general considerations, evaluation of the genotoxicity data on carbon tetrachloride poses some unique challenges. First, the genotoxicity data for carbon tetrachloride are derived from a large number of experiments performed over a period spanning almost 40 years. Some assays were at early stages of development when performed, whereas others were conducted under well-established protocols. As a result, the quality of the data varies widely. In spite of this, most studies provide worthwhile information that can provide insights into the potential of carbon tetrachloride to cause genotoxic effects. In addition, because of the large numbers of tests performed, one would expect a number of studies to be positive due to random chance or elevated error rates resulting from multiple comparisons. Some of the unique challenges associated with evaluated carbon tetrachloride genotoxicity are outlined in Table 4-12.

In accordance with the EPA mutagenicity risk assessment guidelines (U.S. EPA, 1986b), when evaluating genotoxicity results, more weight has been given to tests performed in vivo in mammalian systems than to those performed in vitro using mammalian cells or in sub-mammalian systems such as yeast and bacteria. Preference has also been given to results seen in the rodent liver over those seen in other non-target tissues. This prioritization scheme is also consistent with the current EPA carcinogen risk assessment guidelines (U.S. EPA, 2005a), which state "Although important information can be gained from in vitro test systems, a higher level of confidence is generally given to data that are derived from in vivo systems, particularly those results that show a site concordance with the tumor data."

As indicated in Tables 4-8 to 4-11, well over 100 studies have been performed to assess the genotoxic and mutagenic effects of carbon tetrachloride. A few experiments have been conducted using human cells but none were located describing genotoxic effects in humans. A summary evaluation by major type of genetic alteration is presented below.

Gene mutations. Intragenic or point mutations have been found in many cancer-related

genes and have been shown to play a determining role in chemical carcinogenesis (Stanley, 1995; Anderson et al., 1992; Harris, 1991). The ability of a chemical to form mutations in model systems is an important consideration in establishing whether an agent acts through a mutagenic mode of action. There is little direct evidence that carbon tetrachloride induces intragenic or point mutations in mammalian systems. The mutation studies that have been performed using transgenic mice have yielded negative results, as have the vast majority of the mutagenesis studies that have been conducted in bacterial systems. Since oxidative DNA adducts can be converted into mutations, the inability to detect mutations in the transgenic mouse assays may be an indication of efficient repair of oxidative lesions, a preferential formation of large chromosomal mutations that are inefficiently detected in the transgenic models, or a reflection of the limitations and sensitivity of the specific assays that were performed with carbon tetrachloride. The two positive mutation/DNA damage studies conducted in *E. coli* were seen in strains that are particularly sensitive to oxidative damage. Moreover, the intrachromosomal recombination induced by carbon tetrachloride in *S. cerevisiae* is believed to result from double stranded DNA breaks leading to deletion mutations. These results are consistent with DNA breakage originating from oxidative or peroxidative stress that occurs concurrently with cytotoxicity.

DNA strand breakage. DNA strand breakage is not a measure of mutation per se, but can be a useful indicator of DNA damage and can contribute to an evaluation of an agent's mutagenic potential. However, DNA breaks can also be formed during apoptotic and necrotic cell death even by noncarcinogenic agents (Higami et al., 2004; Bergman et al., 1996; Grasl-Kraupp et al., 1995; Elia et al., 1994), so the potential contribution of cytotoxicity to the observed results needs to be carefully evaluated in studies reporting DNA damage. There is some evidence that carbon tetrachloride administration results in DNA breakage and fragmentation in the liver of treated mice and rats; however, extensive hepatotoxicity was seen in each of the studies where DNA damage has been reported. While some of the damage may be due to reactive species formed during carbon tetrachloride metabolism and lipid peroxidation, much of observed damage appears to be more related to a cytotoxic response associated with cell death than a genotoxic response leading to mutation. Indeed, the TUNEL assay used in two of the positive carbon tetrachloride studies is commonly used as an early indicator of apoptotic and necrotic cell death (Higami et al., 2004; Grasl-Kraupp et al., 1995).

Structural and numerical chromosome aberrations. Non-random structural and numerical chromosomal aberrations are commonly seen in cancer cells and are believed to play an important role in carcinogenesis (Pedersen-Bjergaard et al., 2002; Solomon et al., 1991; Hansen and Cavenee, 1987; Oshimura and Barrett, 1986; Yunis, 1983). Furthermore, elevated frequencies of chromosomal aberrations have been observed in humans exposed to

environmental chemicals, and recent investigations have indicated that individuals with elevated levels of these alterations have increased risks of developing cancer (Hagmar et al., 2004; Hagmar et al., 1998; Sorsa et al., 1992). Chromosomal alterations, measured in cell culture systems or in animals treated in vivo, are commonly induced by carcinogenic agents, and the evaluation of chromosomal aberrations or micronuclei is an important component of commonly accepted genotoxicity testing schemes (Muller et al., 1999). Although less prone to problems of cytotoxicity than the DNA breakage assays, under conditions of severe toxicity or stress, increases in structural chromosome aberrations and micronuclei have been shown to occur through indirect mechanisms (Galloway, 2000; Galloway et al., 1987). While aberrations formed by noncarcinogenic agents under extreme conditions are not believed to be relevant to mutagenic risks (Galloway, 2000), the significance of aberrations formed by carcinogens under such conditions is less clear. For screening new chemicals, protocols have been established, at least in vitro, to limit genotoxicity testing to concentrations that do not exhibit high toxicity (Muller and Sofuni, 2000).

In the genotoxicity studies conducted on carbon tetrachloride, there is no evidence for chromosomal damage when carbon tetrachloride has been tested in conventional assays for chromosomal damage in the rat or mouse bone marrow. There is some evidence that following high cytotoxic doses of carbon tetrachloride, increases in chromosome breakage and loss can occur in the rat liver. It has not been established, however, whether these represent independent genotoxic events due to the formation of reactive metabolites or the result of chromosomal damage occurring at an early stage of necrosis or apoptosis. Regardless of their origin, the increases that have been observed have occurred exclusively at hepatotoxic doses and have been limited in magnitude.

DNA adducts. The formation of DNA adducts within the liver following carbon tetrachloride exposure is indicative of DNA damage occurring in the target organ. Because adducts may be converted into mutations or DNA strand breaks, but can also be efficiently repaired or remain unchanged in less critical non-coding sequences of DNA, these DNA adducts represent precursor lesions rather than specific mutagenic or genotoxic effects. It is generally recognized that the types of DNA adducts formed after exposure can also provide valuable insights into the mechanisms underlying an agent's genotoxic and mutagenic effects. There is strong evidence of increases in DNA adducts formed from reactive oxygen species (i.e., 8-OHdG) and lipid peroxidation products such as MDA and 4-HNE in the liver of rodents following administration of carbon tetrachloride. Based on both in vivo and in vitro studies, there is some (limited) evidence for the formation of DNA adducts derived directly from carbon tetrachloride; however, this has not been adequately established and serious methodological problems limit the interpretation and usage of the results from the existing studies.

Unscheduled DNA synthesis. The unscheduled synthesis of DNA is a measure of DNA repair and is commonly used to assess DNA damage produced by mutagenic chemicals in the livers of treated animals. Based on the reliable studies conducted to date, there is no evidence of UDS in the livers of carbon tetrachloride-treated rats or mice even when tested under conditions producing significant hepatotoxicity.

4.4.3. Neurotoxicity Studies

High-dose, acute toxicity studies in humans and animals reported neurotoxic effects of carbon tetrachloride. Human case reports mention headache, drowsiness, comas, or seizures occurring after exposure by ingestion or inhalation (Stewart et al., 1965; New et al., 1962; Norwood et al., 1950). Lehmann and Schmidt-Kehl (1936) reported neurological symptoms occurring after exposures of 30 mg/L (≈4800 ppm) or higher. In an acute inhalation study in rats, signs of central nervous system depression occurred at ≥4600 ppm (Adams et al., 1952).

Frantik et al. (1994) quantified the air concentrations of carbon tetrachloride and other solvents that would produce an acute neurotoxic effect in rats and mice. Whole-body exposures at various concentrations were undertaken for groups of four male albino Wistar rats for 4 hours or female H mice for 2 hours; animals were then tested for the inhibition of propagation and maintenance of an electrically evoked seizure discharge. Testing was conducted by application of a short electrical impulse (0.2 seconds, 50 Hz, 180 volts in rats and 90 volts in mice) through ear electrodes. The most consistent sensitive measure was the duration of tonic extension through the hind limbs in rats and the velocity of toxic extension (reciprocal of latency) in mice. The authors reported the "isoeffective concentration" of carbon tetrachloride in air by interpolating to the level that would produce one-third of the maximum effect. The isoeffective concentrations were 611 ppm (one-tailed 90% CI: 98 ppm) for rats and 1370 ppm (one-tailed 90% CI: 465 ppm) for mice.

4.4.4. Immunotoxicity Studies

Immunological effects of carbon tetrachloride have been evaluated in mice and rats exposed by the parenteral (Kaminski et al., 1990, 1989), oral (Guo et al., 2000; Ladics et al., 1998; Ahn and Kim, 1993; Smialowicz et al., 1991; Kaminski et al., 1989), and inhalation (Ban et al., 2003) routes. Results of available studies indicate that carbon tetrachloride produces adverse effects on T-cell-dependent immunity at doses that are hepatotoxic. However, it is important to note that immunological effects appear to be, at least in part, secondary to hepatotoxicity and the process of hepatic repair. Information regarding the mechanism of immune system effects and the relationship of immunotoxicity to hepatotoxicity, inflammation, and repair, including activation of Kupffer and stellate cells, is reviewed in Section 4.5.6.

Effects of parenteral exposure of mice to carbon tetrachloride on immune function was studied by Kaminski et al. (1990, 1989). Carbon tetrachloride was injected intraperitoneally to

female B6C3F1 mice at doses of 0, 500, 1000, or 1500 mg/kg-day in corn oil for 7 consecutive days. Systemic toxicity endpoints included body weight, selected organ weights (liver, spleen, lung, kidney, and thymus), and serum chemistry. Humoral antibody responses (the number of antibody-forming cells) to T-cell-dependent antigen (sheep erythrocytes) and T-cell-independent antigen (DNP-ficoll) were evaluated in vivo and in vitro. Treatment with carbon tetrachloride had no significant effect on survival, clinical signs, body weight gain, or organ weights, except for a decrease in thymus weight at ≥500 mg/kg-day. There were significant increases in serum ALT and bilirubin at ≥500 mg/kg-day, albumin at ≥1000 mg/kg-day, and total protein at 1500 mg/kg-day. In vivo response to T-cell-dependent antigen was suppressed in a dose-related manner: by 36% at 500 mg/kg-day to 53% at 1500 mg/kg-day. The in vivo response to T-cell-independent antigen was suppressed by 16% at the highest dose. T-cell-dependent responses were more vulnerable to carbon tetrachloride than were T-cell-independent responses.

Kaminski et al. (1990) conducted a series of immunotoxicity experiments in female B6C3F1 mice given carbon tetrachloride by i.p. injection or gavage in corn oil. Oral or i.p. administration of 500–5000 mg/kg-day for 7 consecutive days significantly reduced in vivo T-dependent antibody response to sheep erythrocytes; the route of administration had no significant effect. Intraperitoneal injection of 25 mg/kg-day for 30 consecutive days also significantly reduced the in vivo T-dependent antibody response. Intraperitoneal injection at 500 or 1000 mg/kg-day on 8 consecutive days significantly increased serum ALT (by five- and sevenfold, respectively), but treatment at 250 mg/kg-day had no effect; no effects on body or organ weights (spleen, liver, or thymus) were observed. Intraperitoneal injection with 5–1000 mg/kg-day on 7 consecutive days significantly reduced the total microsomal protein content per gram of liver. Whereas treatment at 25–100 mg/kg-day for 3 days had no effect on the T-cell-dependent antibody response, pretreatment with 4 g/kg ethanol caused significant immunosuppression at 50 or 100 mg/kg-day. The authors concluded that immunosuppression following treatment with carbon tetrachloride is related to its bioactivation by microsomal enzymes.

The effects of oral exposure to carbon tetrachloride have been studied in mice (Guo et al., 2000; Ahn and Kim, 1993) and rats (Ladics et al., 1998; Smialowicz et al., 1991). Guo et al. (2000) administered carbon tetrachloride at doses of 0, 50, 100, 500, or 1000 mg/kg-day by gavage in corn oil to B6C3F1 mice on 14 consecutive days. Mice were examined for gross pathology at which time organ weights were recorded for thymus, lungs, liver, spleen, and kidneys with adrenals. Blood was collected for hematology and serum chemistry analyses. Immunological endpoints included quantification of T- and B-cells in the spleen and spleen immunoglobulin (IgM) antibody-forming cell response and antibody titers to a T-dependent antigen, sheep red blood cells; in addition, cellular-mediated immunity was evaluated in host responses to infection by two bacterial strains. Treatment had no effect on mortality, the incidence of clinical signs, body weight gain, or the weights of brain, spleen, lung, thymus, and kidneys and no biologically significant effect on hematology parameters. Absolute liver weight

was significantly increased by 23% at 500 mg/kg-day compared with that in vehicle controls. Significant, dose-related increases in relative liver weights were observed at ∃50 mg/kg-day. Treated groups showed histopathology in the liver (cloudy swelling of hepatocytes and centrilobular necrosis) but not in other organs. Significant dose-related changes in serum parameters included increases in ALT (19-fold at 50 mg/kg-day), total protein (9% at 100 mg/kg-day), BUN (34% at 500 mg/kg-day), and globulin (20% at 1000 mg/kg-day) and a decrease in glucose (by 20% at 1000 mg/kg-day). Exposure to carbon tetrachloride had no effect on the mixed leukocyte response, cytotoxic T-lymphocyte activity, or natural killer (NK) cell activity. Exposure to carbon tetrachloride reduced the humoral immune response; the IgM antibody-forming cell response to sheep erythrocytes was suppressed at ≥50 mg/kg-day, maximally by 43% at 1000 mg/kg-day. IgM serum titers to sheep erythrocytes were significantly reduced at ≥100 mg/kg-day. Absolute numbers of $CD4^{+}CD8^{+}$ T-cells were reduced by 40% in all dosed groups compared with vehicle controls; absolute numbers and percentages of $CD4^{+}CD8^{-}$ T-cells were reduced in the 500 mg/kg-day group. Treatment with carbon tetrachloride reduced host resistance to both *Streptococcus pneumoniae* and *Listeria monocytogenes* at 500 and ≥50 mg/kg-day, respectively. In mice, the low dose of 50 mg/kg-day was a LOAEL for immunotoxic effects of carbon tetrachloride by oral exposure, affecting primarily T-cell-dependent responses.

The immunotoxicity of carbon tetrachloride was investigated in male ICR mice administered 1 mL/kg (1590 mg/kg) carbon tetrachloride in olive oil twice weekly by gavage (Ahn and Kim, 1993) for 4 weeks. Systemic endpoints included relative weights of liver, spleen, and thymus. Immune response to sheep erythrocytes was assessed using hemagglutinin (HA) titers, assays of plaque-forming cells (PFCs) and delayed-type hypersensitivity reaction, and measurement of NK cell and phagocytic activity. Compared with control (olive oil) mice, relative liver weights were significantly increased by 12% in mice treated with carbon tetrachloride. Relative weight of thymus and spleen were significantly decreased by 6 and 25%, respectively, compared with that in controls. The HA titer against sheep erythrocytes and the PFC response, both measures of T-cell-dependent antibody response, were significantly inhibited by 56 and 40%, respectively, in mice treated with carbon tetrachloride. The delayed-type hypersensitivity response, a measure of in vivo cell-mediated immunity, was significantly increased by carbon tetrachloride treatment, indicating that carbon tetrachloride alters T-helper cell function. In carbon tetrachloride-treated mice, the number of rosette-forming cells (1.90%) was significantly decreased compared with controls (4.18%). Natural killer cell activity, activity of phagocytic cells, and the number of circulating leukocytes were significantly decreased by 61, 40, and 34%, respectively, in carbon tetrachloride-treated mice compared with controls. These results demonstrate that treatment with carbon tetrachloride alters humoral and cell-mediated immune functions.

The effect of carbon tetrachloride on humoral immunity was assessed by the IgM response to intravenously injected sheep erythrocytes in male CD rats administered 0, 12.5, or 25 mg/kg carbon tetrachloride (eight rats per group) in corn oil by gavage 5 days/week for 30 or 90 days (Ladics et al., 1998). Carbon tetrachloride-induced hepatotoxicity was assessed by examination of the liver by light microscopy and measurement of serum SDH activity in rats injected with sheep erythrocytes or control vehicle. In rats treated for 30 days, administration of 12.5 and 25 mg/kg carbon tetrachloride decreased sheep erythrocyte-specific serum IgM levels by 42 and 45%, respectively. In contrast, sheep erythrocyte-specific serum IgM levels were unchanged compared with controls in the 12.5 mg/kg group and increased by 50% in the 25 mg/kg group in rats treated for 90 days. The authors proposed that time-dependent decreases in metabolism of carbon tetrachloride contributed to the increased IgM response observed after 90 days of treatment with 25 mg/kg. Exposure to carbon tetrachloride did not alter the population of splenic lymphocyte subsets (numbers of T-helper cells, T-cyt/sup cells, total T-cells, total B-cells) or weights or morphology of lymphoid organs (spleen and thymus). Exposure to 25 mg/kg carbon tetrachloride for 30 or 90 days and to 12.5 mg/kg for 90 days produced hepatotoxicity, as indicated by increased relative liver weight, histopathological alterations (centrilobular fatty changes), and increases in serum SDH activity. Results of hepatotoxicity assessments in rats treated with sheep erythrocytes were similar to controls, indicating that exposure to sheep erythrocytes did not interfere with the histopathological examination or measurement of serum SDH activity.

Smialowicz et al. (1991) evaluated immunotoxicity in male F344 rats given carbon tetrachloride by gavage at doses of 0, 5, 10, 20, or 40 mg/kg-day on 10 consecutive days. Endpoints included body weight gain, organ weights (liver, kidney, spleen, and thymus), hepatic microsomal protein levels, serum chemistry, and the histopathology of liver and kidney. Immunological endpoints included NK cell activity of splenocytes, cytotoxic T-lymphocyte responses, and proliferative responses of splenic lymphocytes to T-cell mitogens (phytohemagglutinin and concanavalin A), a B-cell mitogen (*S. typhimurium*), and a T- and B-cell mitogen (pokeweed mitogen). Primary antibody responses to a T-cell-dependent antigen (sheep erythrocytes) were also tested following treatment with carbon tetrachloride at 0, 40, 80, or 160 mg/kg-day for 10 days. Treatment at ≥80 mg/kg-day significantly reduced body weight gain; separate analysis by two-way analysis of variance of 40 mg/kg-day groups and their respective controls in three experiments indicated a significant decrease in body weight gain. Treatment had no significant effect on the absolute or relative weights of the spleen, thymus, or kidney or on absolute liver weight; relative liver weight was significantly increased at 40 mg/kg-day. There were dose-related increases in AST and ALT: 47% and twofold, respectively, at 20 mg/kg-day. Whereas no hepatic histopathology was detected in control rats, there were dose-related increases in the incidence and severity of vacuolar degeneration (minimal at 5 mg/kg-day to mild/moderate at 40 mg/kg-day) and hepatic necrosis (none-to-minimal at 10 mg/kg-day to

minimal/mild at 40 mg/kg-day). Treatment had no significant effect on kidney histopathology or renal serum parameters. Treatment had no effect on immunological parameters in rats at doses that caused hepatic toxicity.

The effects of inhaled carbon tetrachloride on systemic and local immune response were investigated in female BALB/c mice exposed to 0, 100, 200, or 300 ppm (630, 1260, or 1890 mg/m^3) of carbon tetrachloride vapor (Ban et al., 2003). Exposure duration was not reported; however, the maximum exposure period was most likely less than 24 hours. Immune function was assessed for systemic (spleen) and local (lung-associated lymph nodes) effects using the IgM response to sheep erythrocytes and interferon-γ (IFN-γ) production by spleen and lung-associated lymph node cells isolated from exposed mice. Assessments of other systemic effects of carbon tetrachloride (e.g., hepatotoxicity) were not conducted. The IgM response of spleen cells to sheep erythrocytes, as measured by the number of PFCs, was unaffected by carbon tetrachloride treatment. In lung-associated lymph nodes, the PFC number was significantly increased (1.7-fold increase) in mice exposed to 300 ppm carbon tetrachloride compared with controls, but no differences were observed in the 100 or 200 ppm carbon tetrachloride groups. In spleen cells, carbon tetrachloride exposure had no effect on IFN-γ release, whereas IFN-γ release from lung-associated lymph node cells was significantly increased by 150 to >600% of controls in all carbon tetrachloride groups. Results of this study indicate that inhaled carbon tetrachloride exerts immunotoxicity at the point of entry.

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

There is considerable in vivo and in vitro evidence that may contribute to an understanding of the mode of action by which carbon tetrachloride produces toxic effects in animals (Weber et al., 2003; Jaeschke et al., 2002; Plaa, 2000; Omura et al., 1999; Mehendale, 1990; Recknagel et al., 1989; DiRenzo et al., 1982; Slater, 1982; Gillette, 1973; Recknagel and Glende, 1973; Castro et al., 1973, 1972; Castro and Diaz Gomez, 1972). Representative studies that provide information on the roles of metabolism, lipid peroxidation, and disruption of calcium homeostasis are summarized below.

4.5.1. Metabolism Is Required for Toxicity

Numerous studies show that metabolism of carbon tetrachloride is required for toxicity. As discussed in Section 3.3, the initial step of carbon tetrachloride metabolism is reductive dehalogenation by CYP450, primarily CYP2E1. Studies using CYP450 inhibitors (e.g., SKF-525A, colchicine, silymarin, and allylisopropylacetamide) have shown that these compounds, which inhibit activity of CYP450 enzymes and consequently prevent metabolism of carbon tetrachloride, prevent carbon tetrachloride-induced liver damage (Martinez et al., 1995; Letteron et al., 1990; Mourelle et al., 1988; Bechtold et al., 1982; Weddle et al., 1976).

Carbon tetrachloride itself has been shown to temporarily protect against carbon tetrachloride toxicity by inhibiting activity of CYP450 and reducing its own metabolism. Glende (1972) found that rats pretreated with a small, nonlethal dose of carbon tetrachloride were protected against toxicity from a subsequent large and ordinarily lethal challenge dose of carbon tetrachloride. Protection was not yet evident when the challenge occurred only 6 hours after the initial dose but was complete for challenge doses administered 1–3 days after pretreatment and was gradually less effective for subsequent challenge doses. CYP450 activity measured in this study showed a sharp decline after the initial dose that reached a minimum at 1 day after treatment. Gradual increases in CYP450 activity were observed at 4 days and later. The close parallel between time course of effects on CYP450 activity and toxicity in this study is further evidence that metabolism of carbon tetrachloride by CYP450 is required for toxicity.

Wong et al. (1998) demonstrated the specific significance of CYP2E1 to carbon tetrachloride-induced hepatotoxicity in mice using CYP2E1 knockout mice (*cyp2e1*$^{-/-}$). Twenty-four hours after i.p. injection of 1 mL/kg (1.59 g/kg) of carbon tetrachloride to wild type mice (*cyp2e1*$^{+/+}$), there were no significant effects on survival or liver/body weight ratios, but there was a 422-fold increase in serum ALT, a 125-fold increase in serum AST, and significant necrosis in the centrilobular hepatocytes. In *cyp2e1*$^{+/+}$ mice, serum ALT was found to be significantly increased at 12 hours and peaked 24 hours after carbon tetrachloride dosing (Avasarala et al., 2006). Administration of the same dose to knockout mice (*cyp2e1*$^{-/-}$) resulted in no increase in AST, only a slight elevation in serum ALT (within normal range), and absence of liver histopathology. Additionally, Badger et al. (1997) demonstrated that treatment of Sprague-Dawley rats with gadolinium chloride ($GdCl_3$) decreased CYP450 levels in liver preparations from these animals, which may explain the protective role of $GdCl_3$ in carbon tetrachloride-treated animals (See Section 4.5.6).

Conversely, it has been demonstrated that chemical inducers of CYP450 that increase the activity of CYP450, and particularly those that induce the activity of CYP2E1 specifically, potentiate carbon tetrachloride hepatotoxicity. See Section 4.8.6 for a list of chemical CYP450 inducers, and associated references, shown to potentiate carbon tetrachloride hepatotoxicity. In vitro, it has been shown that hepatocyte cell lines that over-express CYP450 have increased levels of carbon tetrachloride-induced cytotoxicity (Jaeschke et al., 2002; Takahashi et al., 2002; Dai and Cederbaum, 1995).

4.5.2. Role of Free Radicals

The products of carbon tetrachloride metabolism by CYP2E1 include trichloromethyl and trichloromethyl peroxy radicals (see Section 3.3). Studies with radical scavengers, such as N-acetylcysteine, and spin-trapping agents, such as *N-tert*-butyl-α-(4-nitrophenyl)nitrone, have shown that these agents confer a protective effect against carbon tetrachloride-induced liver toxicity (Brennan and Schiestl, 1998; Stoyanovsky and Cederbaum, 1996; Slater, 1982),

indicating that free radicals released via metabolism of carbon tetrachloride may contribute to carbon tetrachloride toxicity.

The trichloromethyl and trichloromethyl peroxy radicals are highly reactive species that may produce cellular damage by covalently binding to cellular macromolecules to form nucleic acid, protein, and lipid adducts (Recknagel and Glende, 1973). Studies using radiolabeled carbon tetrachloride have shown irreversible binding to cellular DNA, proteins, nuclear proteins, and lipids, following bioactivation in various in vitro and in vivo systems (Boll et al., 2001b; Azri et al., 1991; Castro et al., 1989; DiRenzo et al., 1982; Diaz Gomez and Castro, 1980a; Castro and Diaz Gomez, 1972; Gordis, 1969). Pulse radiolysis experiments showed that the trichloromethyl peroxy radical is far more reactive towards cellular macromolecules than the trichloromethyl radical (Slater, 1981; Packer et al., 1978). The trichloromethyl radical binds to macromolecules strongly but more slowly than the more reactive trichloromethyl peroxy radical. However, Slater (1981) concluded that most covalent binding involved the trichloromethyl radical, because binding with the trichloromethyl peroxy radical, although faster, produces a less stable product. This process involving the binding of the trichloromethyl radical to macromolecules is known as haloalkylation (Dianzani, 1984).

4.5.3. Lipid Peroxidation

Under oxygen rich conditions, the trichloromethyl radical is converted to the more reactive trichloromethyl peroxy radical. The trichloromethyl peroxy radical can attack polyenoic (polyunsaturated) fatty acids in the cellular membrane, forming fatty acid free radicals that initiate subsequent autocatalytic lipid peroxidation through a chain reaction (see Figure 4-3).

Although the trichloromethyl radical can also initiate lipid peroxidation, it does so at a very slow rate compared to the more reactive trichloromethyl peroxy radical (Slater, 1981). In this process, the trichloromethyl peroxy radical abstracts a hydrogen from the methylene carbon between two double bonds in the polyunsaturated fatty acid, generating a lipid free radical. Rearrangement of the double bonds into a conjugated pattern shifts the location of the free radical electron to an adjacent tetrahedral carbon, and reaction of the free radical carbon with molecular oxygen produces a peroxylipid free radical. The peroxylipid radical can abstract a hydrogen from a donor molecule, forming a lipid hydroperoxide, a first step in the oxidation of the fatty acid. If the hydrogen donor is another polyunsaturated fatty acid, the process begins again, perpetuating the lipid peroxidation (Klaassen, 1996). If the donor is a small hydrocarbon free radical, an alkane can form.

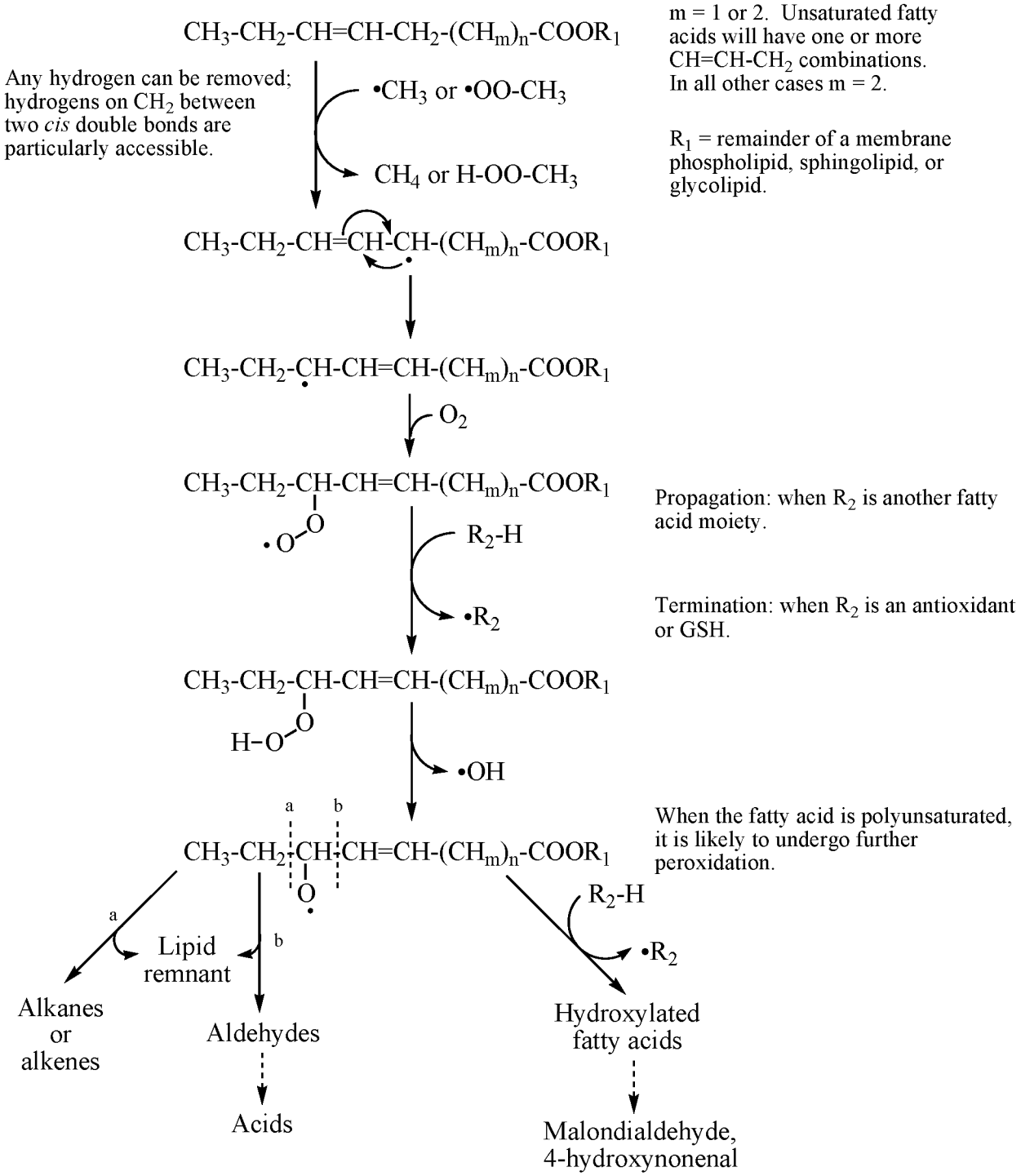


Figure 4-3. Lipid peroxidation.

Numerous studies have demonstrated the occurrence of lipid peroxidation following carbon tetrachloride exposure, either by detection of conjugated dienes (a characteristic marker of lipid peroxidation) in liver lipids (Tribble et al., 1987; Lee et al., 1982; Recknagel and Glende, 1973; Rao and Recknagel, 1969), increased exhalation of ethane or pentane (end degradation products of peroxidized T-3 and T-6 polyunsaturated fatty acids, respectively) in treated rats (Younes and Siegers, 1985; Gee et al., 1981), or occurrence of reactive aldehydes, such as malonaldehyde and 4-hydroxyalkenals, frequently measured as thiobarbituric acid-reactive substances (TBARS) (de Zwart et al., 1997; Gasso et al., 1996; Ichinose et al., 1994; Fraga et al., 1987; Comporti, 1985; Comporti et al., 1984). TBARS form when the oxidation of the fatty acid progresses from the hydroperoxide, facilitated by the oxidation of Fe^{2+} to Fe^{3+} in a Fenton reaction, leading to breaks in the fatty acid chain and the formation of aldehydes from the fatty acid fragments (Klaassen, 1996). Among the many different aldehydes formed from lipid peroxidation are 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA).

In vitro studies have shown 4-HNE at high concentrations (> 10 μM) is a cytotoxic product of liver microsomal lipid peroxidation because of degradation of T-6 unsaturated fatty acids (Esterbauer et al., 1991; Van Kuijk et al., 1990). The formation of HNE-dGp-adducts may be relevant to the formation of cancer when these promutagenic lesions are insufficiently repaired (Wacker et al., 2001). Wacker et al. (2001) developed a sensitive detection method for 1,N2-propanodeoxyguanosine adducts of HNE (promutagenic adducts), a specific marker for genotoxic interaction of reactive oxygen species and lipid peroxidation products. Background levels of adducts in various tissues in F344 rats were found in the range of 18–158 adducts/10^9 nucleotides. Levels of endogenous DNA adducts were higher in the liver, and lower levels were found in kidney, lung, and colon. After induction of lipid peroxidation by a single i.p. application of 50 μL carbon tetrachloride at a dosage of 500 mg/kg body weight, levels of HNE-dG-adducts in the liver were elevated 1.5- to twofold compared with those in controls. The authors concluded that these promutagenic adducts are evidence of radical-initiated lipid peroxidation, which if not repaired effectively can lead to cancer. Other studies have also indicated that lipid peroxidation by-products could inhibit certain DNA repair systems and thus indirectly increase the rate of spontaneous mutations (Curren et al., 1988; Krokan et al., 1985).

Chung et al. (2000) identified lipid peroxidation as the cause of the 37-fold increase of HNE-dG adducts in liver tissue DNA of F344 rats after treatment with 3.2 g/kg carbon tetrachloride via i.p. administration. Wang and Liehr (1995) found that MDA induced DNA adducts in hamsters treated with an oral administration of 0.1 mL/kg carbon tetrachloride, and the levels of adducts formed were directly correlated with lipid hydroperoxide concentrations. These reactive aldehydes can form DNA adducts causing frameshift or base mispairing (G to T and G to A mutations).

Similar to 4-HNE, MDA is a result of oxidative degradation of polyunsaturated fatty

acids with more than two methylene-interrupted double bonds. In mammalian tissues, precursors for MDA are arachidonic acid and docosahexenoic acid.

Ichinose et al. (1994) compared the in vitro production of MDA per mg microsomal protein from hepatic microsomes in several species. The rat generated the highest amount of MDA over 2 hours, followed by monkey, mouse, pig, cow, rabbit, sheep, horse, and dog. Using tissue slices from male Sprague-Dawley rats incubated in 1 mM carbon tetrachloride for 2 hours, Fraga et al. (1987) found significant increases over control values in TBARS (nmol/g tissue) released from treated liver (~fourfold), kidney (~threefold), spleen (~twofold), and testis (~fivefold). Abraham et al. (1999) reported significantly elevated lipid peroxide levels in the lung (65%), testis (200%), kidney (85%), and liver (200%) of Wistar rats exposed to carbon tetrachloride vapor over a 12-week period. The results of Fraga et al. (1987) and Abraham et al. (1999) show that lipid peroxidation can occur in other tissues besides the liver, specifically in the kidney, testis, spleen, and lung.

Lipid peroxidation has been proposed to disrupt cellular membranes, resulting in loss of membrane integrity (Recknagel and Glende, 1989) and the production of reactive aldehydes that can attack tissues and form protein and DNA adducts (Comporti, 1985; Comporti et al., 1984). These aldehydes may diffuse from the membranes and traverse intracellularly or extracellularly away from the point of origin to attack distant targets, acting as secondary toxicants. Immunohistochemical procedures using antibodies directed against MDA- and 4-HNE protein adducts have been used to detect adducts in rat liver sections treated with carbon tetrachloride (Bedossa et al., 1994). Abraham et al. (1999) reported significantly elevated protein carbonyl content, a measure of protein adduct formation, in the liver (238%), lungs (51%), and testis (21%) of carbon tetrachloride vapor-treated rats compared with controls.

Hartley et al. (1999) studied the temporal relationship between carbon tetrachloride-initiated lipid peroxidation, hepatocellular damage, and formation of 4-HNE and MDA-hepatic protein adducts, using immunohistochemical detection of aldehyde-adducted proteins in liver sections and immunoprecipitation and immunoblotting procedures to detect and characterize 4-HNE and MDA-adducted proteins in liver homogenates from male highly alcohol-sensitive rats treated with 1 mL/kg (1.59 g/kg) of carbon tetrachloride in mineral oil by gavage. Mineral oil alone elicited subtle centrilobular steatosis, a slight increase in necrosis at 12 hours, and a slight elevation of serum ALT at 24 hours. The livers of rats treated with carbon tetrachloride in mineral oil exhibited a significant number of ballooned hepatocytes and inflammatory cells at 12 hours and progressive, massive centrilobular steatosis, inflammation, and necrosis at 18–48 hours. There was a fivefold increase in serum ALT at 6 hours after treatment, peaking at 36 hours with a 32-fold increase in ALT over control. Between 18 and 36 hours posttreatment, TBARS values in liver homogenates of treated rats were maximal at a 2.5-fold increase over controls. MDA-amine and 4-hydroxynonenal-sulfhydryl protein adducts were detectable at 6 hours in the midzonal region and in the centrilobular region at 12–36 hours. The

correspondence in time course and location for lipid peroxidation, production of protein adducts, and liver damage suggests that protein adducts resulting from lipid peroxidation contribute to hepatocellular injury in carbon tetrachloride-treated rats.

Evidence of the relationship between hepatotoxicity and lipid peroxidation was also reported by Younes and Siegers (1985). These researchers found that administration of an iron-chelating agent, deferoxamine, suppressed both lipid peroxidation (ethane exhalation) and hepatotoxicity (serum ALT and SDH levels) in GSH-depleted mice treated with carbon tetrachloride. This result suggests that the observed hepatotoxic effect was secondary to lipid peroxidation. Administration of the antioxidant vitamin E (α-tocopherol) was shown to reduce lipid peroxidation (pentane exhalation) and metabolism (chloroform generation) in another rat study (Gee et al., 1981).

Lipid peroxidation by-products can also form promutagenic DNA adducts and modify double-stranded DNA by formation of amino-imino propene crosslinks between the NH_2 group of the guanosine base and complementary cytosine base. In rat hepatocytes cultured with 0.25, 1 or 4 mM carbon tetrachloride, Beddowes et al. (2003) showed that carbon tetrachloride caused a dose-dependent increase in the formation of DNA strand breaks, 8-oxodG and MDA-DNA adducts. The increased formation of DNA strand breaks and MDA-DNA adducts was statistically significant at 1 and 4 mM. The level of 8-oxodG was statistically elevated only at 4 mM, a concentration that caused a decrease in cellular viability. Carbon tetrachloride induced lipid peroxidation carbonyl product formation (>2-fold) at 4 mM; lower concentrations were not studied. The formation of MDA-DNA adducts appeared to correlate with the ability of carbon tetrachloride to induce lipid peroxidation, although failure to measure lipid peroxidation at the two lower concentrations (0.25 and 1 mM) somewhat limits the ability to establish this correlation.

4.5.4. Depletion of Glutathione

Reduced glutathione is capable of donating a hydrogen to quench a free-radical chain reaction and can play a key role in limiting the damage to cellular membranes caused by lipid peroxidation. The efficacy of reduced glutathione in quenching a free radical reaction is dependent on the activity of GSH peroxidase, the enzyme that facilitates the transfer of hydrogen to hydrogen peroxide with the formation of glutathione disulfide (GSSG) and water. Cellular levels of reduced glutathione are restored through the activity of GSH reductase using NADPH + H^+ as the hydrogen donor (Klaassen, 1996a).

Cabre et al. (2000) assessed the temporal relationships between hepatic lipid peroxidation, GSH metabolism, and development of cirrhosis in groups of 10 male Wistar rats exposed to carbon tetrachloride. Rats were injected intraperitoneally with 0.5 mL of carbon tetrachloride in olive oil twice weekly for 9 weeks to induce hepatic cirrhosis. By the second week, 10/10 livers were fibrotic. Cirrhosis appeared in all 10 animals by week 9. Hepatic GSH

levels were significantly reduced, beginning at week 5, and GSH peroxidase activity was significantly decreased at week 7 in carbon tetrachloride-treated rats; the activity of GSH peroxidase is dependent on a sufficient level of GSH. Cytosolic GSH S-transferase activity was also significantly inhibited in rats receiving carbon tetrachloride at week 1. TBARS (lipid peroxides) began to be elevated by week 7. The findings of this study show that induction of cirrhosis in rats by carbon tetrachloride produces a decrease in several components of the hepatic GSH antioxidant system. Impairment of this hepatoprotective system was related to an increased generation of lipid peroxides.

Gorla et al. (1983) confirmed that oral pretreatment of male Sprague-Dawley rats with 2 g/kg of GSH 30 minutes before an i.p. injection of carbon tetrachloride (1.59 mg/kg) partially prevented the hepatic necrosis that normally occurs 24 hours after carbon tetrachloride dosing. Treatment with cysteine, which is a precursor of GSH and, like GSH, is able to conjugate phosgene (from chloroform) produced from carbon tetrachloride, also protected against carbon tetrachloride hepatotoxicity when given orally 30 minutes before or 1 hour after i.p. injection of carbon tetrachloride (de Ferreyra et al., 1974).

Gasso et al. (1996) investigated the effects of S-adenosylmethionine (SAM) availability on lipid peroxidation and liver fibrogenesis in male Wistar rats with carbon tetrachloride-induced cirrhosis. SAM is essential for the production of the GSH precursor homocysteine, which provides the sulfur for the endogenous synthesis of cysteine (the source of the reactive -SH functional group in glutathione). A SAM deficiency can also limit transmethylation reactions that function in DNA and RNA methylation and the production of thymine for DNA repair. Gasso et al. (1996) found that depletion of GSH triggers a feedback mechanism, leading to inactivation of SAM synthetase, which in turn causes a further decrease in GSH. SAM synthetase is responsible for the endogenous production of SAM from the essential amino acid methionine. The deficit of SAM could be corrected by exogenous administration of SAM but not methionine. Accordingly, the deficit appeared to be the result of enzyme inhibition rather than methionine availability.

Carbon tetrachloride-treated rats receiving SAM for 6 weeks had significantly higher SAM synthetase activity (156 ± 5.6 pmol/minute/mg protein) than rats treated with carbon tetrachloride alone (89.4 ± 3.4 pmol/minute/mg protein) (Gasso et al., 1996). The hepatic GSH was significantly decreased in carbon tetrachloride-treated rats (2.7 ± 13 nmol/g tissue) and returned to normal in rats receiving SAM for 3 or 6 weeks (3.7 ± 0.13 and 3.9 ± 0.11 nmol/g tissue). Carbon tetrachloride-treated rats receiving SAM for 6 weeks had significantly lower liver toxicity (collagen and propyl hydroxylase activity, reduced lipid peroxidation, and less advanced liver fibrosis). The hepatic TBARS, markers of lipid peroxidation, were also significantly lower in rats treated with carbon tetrachloride and SAM for 6 weeks (98 ± 5 nmol/g tissue) than rats treated with only carbon tetrachloride (134 ± 12 nmol/g tissue). In rats treated with carbon tetrachloride and SAM for 6 weeks, serum AST (76 ± 6 U/L) and ALT (57 ± 4 U/L)

were lower than rats treated with only carbon tetrachloride (321 ± 33 U/L and 185 ± 21 U/L, respectively). These data provide evidence that hepatic lipid peroxidation is increased during hepatic fibrogenesis and that exogenous SAM may lead to an increase of GSH levels, which could prevent SAM synthetase inactivation, inhibit lipid peroxidation, and, consequently, attenuate the development of liver fibrosis and cirrhosis.

Will et al. (1999) demonstrated in 11 untreated mammalian cell lines that the intrinsic levels of GSH expression were inversely correlated with the background level of oxidative DNA modifications, such as 8-hydroxyguanine. Depletion of GSH with buthionine sulphoximine, an inhibitor of γ -glutamyl-cysteine, the precursor to GSH (Edgren and Revesz, 1987), increased the basal levels of oxidative DNA base modifications. Schisandrin B, a compound that enhances the GSH antioxidant status in hepatic mitochondria, was hepatoprotective against carbon tetrachloride exposure in Balb/c mice (Chiu et al., 2003).

4.5.5. Disruption of Calcium Homeostasis

Calcium plays an essential role in cellular physiology. Levels of calcium in the cell are maintained far below extracellular levels by resistance of the plasma membrane to passive diffusion of calcium across the membrane and by active transport of calcium across the cell membrane and into the extracellular space (Klaassen, 1996). Calcium within the cell is actively transported across the microsomal membrane into the endoplasmic reticulum and across the mitochondrial membrane into the mitochondria. Maintenance of calcium homeostasis is vital to cellular function, and interference with calcium homeostasis is suspected to cause cell death (Farber, 1981).

Calcium ATPase helps maintain calcium-level homeostasis within the cell. When cytosolic calcium levels are highly elevated, the calcium ATPase, located in the plasma membrane, is activated. Activation of calcium ATPase triggers the transport of two calcium ions from the cytosol to the endoplasmic reticulum hydrolyzing one ATP in this process. This process also requires Mg^{2+} to be tightly complexed to ATP. A rise in cytosolic calcium also induces the binding of calcium ions to regulatory calcium-binding proteins, like calmodulin (a 148-residue protein found in many cells and an essential subunit of the plasma membrane calcium ATPase). Binding of cytosolic calcium to calmodulin triggers an allosteric activation of calcium ATPase that accelerates the uptake of calcium ions from the cytosol by the endoplasmic reticulum to maintain a low cytosolic concentration of less than 1 μM calcium. While calmodulin complements calcium ATPase, it also modulates the activities of a large number of calcium-dependent proteins (Garrett and Grisham, 1999).

Studies conducted with carbon tetrachloride have reported 100-fold or more increases in the cytosolic concentration of calcium following exposure (Agarwal and Mehendale, 1986, 1984; Long and Moore, 1986; Kroner, 1982). In a study in which hepatocytes were incubated in a medium containing EGTA, a calcium-specific chelator, but no added calcium, treatment with

carbon tetrachloride elicited an increased calcium-dependent conversion of glycogen phosphorylase "b" to phosphorylase "a" by phosphorylase kinase, which is stimulated by increased intracellular calcium levels (Long and Moore, 1986). The lack of extracellular calcium in this experimental system indicates that the carbon tetrachloride exposure released sequestered calcium, probably from microsomes. The authors suggested that calcium could contribute to cell death by the overstimulation of calcium-responsive cellular enzymes that initiate a cascade of events, resulting in irreversible cell injury.

Hepatocytes treated with carbon tetrachloride had an impaired ability to maintain proper calcium levels that was associated with inactivation of the calcium ATPase of the endoplasmic reticulum (Lowrey et al., 1981; Moore, 1980). Administration of carbon tetrachloride caused an 85% reduction of ATP-dependent calcium uptake and calcium-sequestering capacity of the hepatocyte endoplasmic reticulum (Moore et al., 1976). Hemmings et al. (2002) showed that carbon tetrachloride decreased active calcium transport across the plasma and mitochondrial membranes, as well as the endoplasmic reticulum, in rat liver. In vitro experiments confirmed that inhibition of the plasma membrane calcium transport system by carbon tetrachloride was rapid (within a minute) and strong (>90%) (Hemmings et al., 2002).

Carbon tetrachloride can also increase cytoplasmic calcium levels by opening certain calcium transport channels in membranes. Liver endoplasmic reticulum contains ryanodine-sensitive calcium-binding sites (Feng et al., 1992). Ryanodine is an alkaloid, usually found in the skeletal and cardiac sarcoplasmic reticulum, that induces calcium release from liver microsomes by binding to certain calcium release channels. Stoyanovsky and Cederbaum (1996) showed that hepatic ryanodine-sensitive calcium channels may be involved in the elevation of cytosolic calcium levels in the liver following carbon tetrachloride dosing. These researchers observed elevated cytosolic calcium levels after treatment of hepatic microsomes with 50 μM of carbon tetrachloride. Ruthenium red, a specific inhibitor of the ryanodine receptor calcium release channel, has been shown to block the carbon tetrachloride-induced release of calcium.

Activation of calcium-dependent cysteine proteases and phospholipases

The increase in cytosolic calcium and inhibition of the calcium pump can activate a number of calcium-dependent cysteine proteases (e.g., calpains, known for their involvement in proteolysis of proteins during mitosis, apoptosis, and necrosis) and phospholipases (particularly phospholipase A_2) that preferentially hydrolyze membrane lipids. Activation of these enzymes can contribute to toxicity of carbon tetrachloride in the liver.

When calcium homeostasis has been disrupted because of the loss of microsomal membrane integrity, increased levels of calcium leakage activate a number of cytosolic and lysosomal degradative enzymes that are also leaked out into the extracellular space from dying cells; these degradative enzymes can subsequently attack neighboring cells. Limaye et al. (2003) demonstrated the involvement of calpain, a calcium-dependent cytosolic neutral cysteine

protease that leaks out from injured hepatocytes, in degrading cytoskeletal and membrane proteins (e.g., α-fodrin, talin, filamin) and other macromolecules crucial to maintaining cellular integrity, culminating in cell lysis and hepatocyte cell death. Calpain causes cell death by attacking the plasma membrane, and, once the integrity of the membrane is lost, cells are rendered highly vulnerable to destruction. Limaye et al. (2003) showed how calpain inhibition with calpain-specific inhibitor N-benzyloxycarbonyl-valine-phenylalanine methyl ester (CBZ) after carbon tetrachloride treatment substantially reduced the progression of injury and improved animal survival. After 48 hours, the elevation in calpain activity was substantially in the carbon tetrachloride + CBZ-treated rats than the carbon tetrachloride + DMSO-treated rats. [DMSO was the vehicle used for CBZ administration.] More significantly, in rats challenged with a normally lethal dose of carbon tetrachloride (3 mL/kg, i.p.), 75% of the male Sprague-Dawley rats that received CBZ (60 mg/kg) 1 hour after carbon tetrachloride administration survived, while rats treated with carbon tetrachloride alone or carbon tetrachloride and DMSO experienced 75% mortality. All control rats survived.

This study also evaluated the degradative effect of calpain on α-fodrin, a membrane protein (Limaye et al., 2003). Calpain is known to degrade the 240-kDa fodrin to produce a 150-kDa fragment. In rats receiving CBZ after carbon tetrachloride, the breakdown of α-fodrin was similar to that in controls, indicating that inhibition of calpain released from dying hepatocytes resulted in lower cellular damage. To confirm that cell death was caused by calpain, fresh hepatocytes were incubated with calpain and 2.5 mM calcium. By the end of 240 minutes, cell viability was decreased to 75%. Dying cells were found to develop plasma membrane blebs, indicating cytotoxicity, which is typical of cytoskeletal damage induced by calpain. In the presence of CBZ, hepatocytes were completely protected from calpain-mediated cell death. Additional experiments with E64, a cell-impermeable inhibitor of calpain, also significantly reduced plasma ALT levels, suggesting that the presence of calpain in the extracellular space is responsible for the damage to some hepatocytes.

While these results suggest that calpain is a major contributor in the progression of liver injury, other degradative enzymes are also released into the extracellular space, such as nucleases, acid phosphatases, and phospholipases. Loss of calcium sequestration capacity caused by in vitro metabolism of carbon tetrachloride by isolated rat liver microsomes (e.g., Lowrey et al., 1981) correlates with carbon tetrachloride-dependent activation of phospholipase A_2, measured by lysophosphatide formation or release of arachidonic acid from the hydrolysis of esterified arachidonic acid from the sn-2 position of hepatocyte phospholipids (Glende and Pushpendran, 1986). Studies with rat hepatic microsomes demonstrated a progressive loss of phospholipid after incubation in 5 mM $CaCl_2$, with time-dependent losses of microsomal protein activity (G6Pase and CYP450) that reached 80% by 3 hours (Chien et al., 1980). Quinacrine, a phospholipase A_2 inhibitor at 150 mg/kg i.p., has been shown to prevent carbon tetrachloride-induced liver necrosis at 24 hours when administered 30 minutes before or 6 or 10 hours after

carbon tetrachloride exposure (2.5 mL/kg orally) (Gonzalez Padron et al., 1993). The authors of this study concluded that phospholipase A_2 plays a major role in carbon tetrachloride-induced liver necrosis.

Glende and Pushpendran (1986) prelabeled hepatocytes with ^{3}H-arachidonic acid or [^{14}C]-ethanolamine and subsequently incubated the cells with carbon tetrachloride. Calcium-activated phospholipase A_2 activity was determined by measuring the release of ^{3}H-arachidonic acid from cellular phospholipids labeled with arachidonate or the formation of [^{14}C]-lysophospholipids from cellular phospholipids labeled with ethanolamine. Treatment with 0.23–1.3 mM of carbon tetrachloride increased the endogenous phospholipase A_2 activity 1.4- to 5.3-fold beginning within 30–60 minutes. A similar study in isolated hepatocytes revealed that carbon tetrachloride stimulated phospholipase A_2 activity (monitored by production of lysophosphatidylethanolamine) within 15 minutes, succeeded within 15 minutes by hepatotoxicity, as measured by the release of LDH from the cells into the medium (Glende and Recknagel, 1992). This same study demonstrated that related compounds (chloroform, bromotrichloromethane, and 1,1-dichloroethylene) similarly activate phospholipase A_2 activity in hepatocytes. The authors suggested that phospholipase A_2 could contribute to hepatocyte pathology by two different means: by increasing the hydrolysis of membrane lipids at rates exceeding the rate of repair and/or by the phospholipase A_2-dependent generation of toxic prostanoids via initiation of the arachidonic acid cascade.

4.5.6. Immunological and Inflammatory Effects

Immunological effects of carbon tetrachloride appear to be, at least in part, secondary to hepatotoxicity and the process of hepatic repair. Carbon tetrachloride induces a regenerative response in the liver similar to that observed following administration of other hepatotoxic chemicals (e.g., acetaminophen) or partial hepatectomy (Jeon et al., 1997; Delaney et al., 1994). The regenerative process involves complex interactions among several cell types and cell mediators, including the hepatic synthesis and release of serum-borne growth factors (hepatotrophic factors) that act directly on liver cells to induce mitosis (Luster et al., 2000). Hepatotrophic factors also appear to act on peripheral organs, most notably the spleen (Delaney and Kaminski, 1994; Delaney et al., 1994). Results of studies on the effects of hepatotrophic factors indicate that immune effects of carbon tetrachloride, and other hepatotoxic chemicals, may be mediated by tumor growth factor (TGF)- β1 released from the liver during the regenerative process (Jeon et al., 1997; Delaney et al., 1994; Delaney and Kaminski, 1993).

A series of experiments conducted by Delaney and coworkers suggest that carbon tetrachloride-induced suppression of T-cell function is mediated through serum-borne factors (Delaney et al., 1994; Delaney and Kaminski, 1993). Serum from B6C3F1 mice treated with 250 or 500 mg/kg carbon tetrachloride in corn oil by gavage for 7 days, a dose regimen that produced hepatotoxicity, suppressed the sheep erythrocyte-induced antibody response of carbon

tetrachloride-naive spleen cells in vitro (Delaney and Kaminski, 1993). In a subsequent study, Delaney et al. (1994) demonstrated that carbon tetrachloride-induced suppression of the T-cell-dependent humoral response is at least partially mediated by TGF- β1. Suppression of the sheep erythrocyte antibody response of naive spleen cells in vitro by serum of mice exposed to carbon tetrachloride (single oral dose of 500 or 1000 mg/kg carbon tetrachloride in corn oil) was abolished upon addition of TGF- β1-specific antibodies to the assay. Jeon et al. (1997) reported elevations of TGF- β1 mRNA in the liver of B6C3F1 mice treated with a single hepatotoxic dose (500 mg/kg) of carbon tetrachloride within 24 hours of exposure. Although direct effects of carbon tetrachloride on the immune system by carbon tetrachloride have not been ruled out, results of in vitro and in vivo studies suggest that immunotoxicity is, in part, mediated by TGF-β1 secreted by the liver during tissue repair.

Inflammation contributes to the development of chemical-induced hepatotoxicity and possibly to immunotoxic effects. Kupffer cells are hepatic macrophages that respond to signals from injured hepatocytes by releasing biologically active mediators, such as prostaglandins, reactive oxygen species, and cytokines (Luckey and Petersen, 2001). Factors released by Kupffer cells after activation by carbon tetrachloride include nitric oxide, tumor necrosis factor-α, TGF- β, and interleukins-6, -8, and -10. The mediators produced by Kupffer cells are involved in the regulation of the inflammatory response and fibrotic response following hepatic injury. As discussed earlier, TGF- β1 released from the liver plays an important role in the immunotoxic effects of carbon tetrachloride, providing a possible link between hepatic inflammation and Kupffer cell activation by immunotoxic events.

Stellate cells are hepatic fat-storing cells that respond to liver injury by proliferating, migrating towards damaged areas, releasing nitric oxide and extracellular signal-regulated kinases that perform various functions in different tissues, and increasing production of extracellular matrix, thereby promoting fibrosis (Weber et al., 2003; Marra et al., 1999). Stellate cells are activated by TGF-α. Acute treatment with carbon tetrachloride increases the activity of extracellular signal-regulated kinases from stellate cells (Marra et al., 1999).

Carbon tetrachloride has been shown to stimulate increases in the numbers of immunodetectable Kupffer cells in the livers of treated rats, as well as increases in releases of various cytokines and reactive oxidative species, corresponding to different stages of liver histopathology (Luckey and Petersen, 2001; Alric et al., 2000). Towner et al. (1994) reported that i.p. administration of 1275 mg/kg of carbon tetrachloride to male Wistar rats was characterized by hepatic edema from the accumulation of vacuoles and lipid droplets in parenchymal cells and accumulation of phagosomes (large secondary lysosomes) and extrusion of pseudopods in enlarged Kupffer cells. With a 1-hour intravenous pretreatment with 10 mg/kg gadolinium trichloride ($GdCl_3$), an inhibitor of Kupffer cell activation, the parenchymal cells were normal and Kupffer cells contained only a few secondary lysosomes. The protective effect of $GdCl_3$ was not associated with a change in detectability of carbon tetrachloride-generated

trichloromethyl radical by electron spin resonance spectroscopy.

The effects of $GdCl_3$ on carbon tetrachloride-induced hepatic toxicity were evaluated in other studies. Muriel et al. (2001) treated male Wistar rats with 4000 mg/kg of carbon tetrachloride by gavage in corn oil, with or without i.p. injection of 2000 mg/kg $GdCl_3$. Twenty-four hours later, rats treated with carbon tetrachloride showed typical hepatotoxicity (increased serum enzymes and bilirubin, 2.5-fold increase in hepatic lipid peroxidation, and liver histopathology: ballooning necrotic hepatocytes). Treatment with $GdCl_3$ eliminated the increases in serum biomarkers of membrane damage and hepatic lipid peroxidation and significantly reduced the severity of hepatic necrosis. In a follow-up study of similar design, male Wistar rats were treated with carbon tetrachloride (400 mg/kg by i.p. injection in mineral oil 3 times/week), $GdCl_3$ (20 mg/kg i.p. in saline daily), or both for 8 weeks (Muriel and Escobar, 2003). Cotreatment with $GdCl_3$ resulted in partial or complete protection against the effects of carbon tetrachloride on serum ALT, GGT, ALP, and bilirubin; liver MDA content (index of lipid peroxidation); liver hydroxyproline content (index of collagen content and fibrosis); and histopathology (both necrosis and fibrosis). Depletion of liver glycogen by carbon tetrachloride was not affected by $GdCl_3$, and $GdCl_3$ itself produced a significant depletion of glycogen.

Although multiple studies have indicated that $GdCl_3$ treatment reduces or inhibits carbon tetrachloride-induced hepatotoxicity through inactivation of Kupffer cells, $GdCl_3$ may also reduce carbon tetrachloride toxicity through other cellular mechanisms. Rose et al. (2001) demonstrated both in vivo and in vitro that $GdCl_3$ stimulated hepatocyte proliferation through a mitogenic mechanism involving TNF-α, and promoted recovery from liver damage. $GdCl_3$ has also been shown to inhibit free radical-induced hepatocyte damage by nonselective blockage of Na+ channels that induce necrosis in an in vitro model (Barros et al., 2001). Critical to carbon tetrachloride-induced toxicity is the generation of reactive metabolites by CYP2E1 for which $GdCl_3$ downregulates the gene expression in vivo (Okamoto, 2000; Badger et al., 1997). Overall, multiple cellular mechanisms have been demonstrated by which $GdCl_3$ reduces carbon tetrachloride-induced toxicity and indicates that toxicity is not mediated exclusively through inactivation of Kupffer cells.

4.5.7. Changes in Gene Expression

Changes in gene expression in response to exposure to carbon tetrachloride have been investigated in the liver of rats and mice and in the human hepatoma cell line (Jessen et al., 2003; Fountoulakis et al., 2002; Bartosiewicz et al., 2001; Holden et al., 2000; Columbano et al., 1997; Menegazzi et al., 1997). Many of the known upregulated genes are related to stress, DNA damage and repair, and signal transduction, but for the most part their specific contributions to hepatotoxicity are not known. Fountoulakis et al. (2002) reported a fivefold increase in expression of some genes related to stress and DNA damage repair in the livers of male Wistar rats 6 hours after they received 400 mg/kg carbon tetrachloride. Rats receiving 3190 mg/kg showed 10-fold increases in expression in some genes. Some of the stress- and DNA-damage-related genes upregulated by both doses at 24 hours included GADD45, GADD153, heat-shock proteins, heme oxygenase, p53, *c-myc*, and *c-jun*. There were some qualitative differences in altered gene expression at 6 and 24 hours between the two doses administered in this study, which possibly provides a basis for the different hepatocellular responses to carbon tetrachloride-induced injury. The hepatic expression of the Cdk inhibitor p21 in mice treated with carbon tetrachloride occurs just prior to necrosis at 6 hours, and mice deficient in that gene do not exhibit necrosis in response to carbon tetrachloride (Kwon et al., 2003); p21 also contributes to the cessation of cellular proliferation that occurs later.

Intraperitoneal injection of Sprague-Dawley rats with 160 mg/kg of carbon tetrachloride in corn oil activated c-*fos* and c-*jun* gene expression in the liver within 30 minutes (Gruebele et al., 1996). Pretreatment of rats with diallyl sulfide, an inhibitor of CYP2E1, 3 hours before dosing with carbon tetrachloride reduced c-*jun* mRNA levels by 76%. Treatment with carbon tetrachloride also increased hepatic nuclear levels of the NF-6B transcription factor, which regulates genes involved in responses to inflammation, apoptosis, hepatocyte proliferation, and liver regeneration.

Columbano et al. (1997) investigated the relationship between immediate early genes and hepatocyte proliferation through comparison of the hepatic levels of c-fos, c-jun and LRF-1 transcripts during mouse liver cell proliferation under two conditions: (1) direct hyperplasia induced by the primary mitogen (and hepatocarcinogen) 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), and (2) compensatory regeneration caused by a necrogenic dose of carbon tetrachloride (single intragastric dose of 2 mg/kg in oil) or by performing a 2/3 partial hepatectomy. A striking difference in the activation of early genes was observed. In spite of a rapid stimulation of S phase by the mitogen TCPOBOP, there were no changes in the expression of c-fos, c-jun and LRF-1 or in steady state mRNA hepatic levels of IGFBP-1 (a gene highly expressed in rat liver following partial hepatectomy), and only a slight increase in c-myc and PRL-1. In contrast, a rapid, massive and transient increase in the hepatic mRNA levels of all these genes was observed during carbon tetrachloride-induced regeneration

that was comparable to those seen following 2/3 partial hepatectomy. In similar research from the same laboratory, the pattern of immediate early gene and growth factor gene expression in the rat liver induced by primary mitogens (including lead nitrate (LN), cyproterone acetate, or nafenopin) was shown to differ from that observed following compensatory liver regeneration occurring after cell loss/death and direct hyperplasia resulting from a partial 2/3 hepatectomy or a necrogenic dose (2 mL/kg) of carbon tetrachloride (Menegazzi et al., 1997). In this study, the following indicators of gene expression were examined: modifications in the activation of two transcription factors, NF-kappaB and AP-1; steady-state levels of tumor necrosis factor alpha (TNF-alpha) messenger RNA (mRNA); and induction of the inducible nitric oxide synthase (iNOS). Liver regeneration after treatment with carbon tetrachloride was associated with an increase in steady-state levels of TNF-alpha mRNA, activation of NF-kappaB and AP-1, and induction of iNOS. LN induced NF-kappaB, TNF-alpha and iNOS mRNA but not AP-1, whereas direct hyperplasia induced by the other two primary mitogens occurred in the complete absence of modifications in the hepatic levels of TNF-alpha mRNA, activation of NF-kappaB and AP-1, or induction of iNOS, although the number of hepatocytes entering S phase 18 to 24 hours after NAF was similar to that seen after PH. The findings from these two studies indicate that regenerative proliferation alone does not explain the tumorigenic response associated with carbon tetrachloride in chronic bioassays, but these data do not preclude regenerative proliferation as a biologically based marker of such causal events.

4.5.8. Mechanisms of Kidney Toxicity

Limited data suggest that some of the same mechanisms by which carbon tetrachloride produces damage to the liver can also operate in the kidney. Dogukan et al. (2003) observed moderate renal histopathology (tubular necrosis, dilatation, atrophy, glomerular hypercellularity, capillary obliteration, and interstitial fibrosis) in male Wistar rats subcutaneously injected three times/week with 240 mg/kg of carbon tetrachloride in olive oil for 7 weeks. The tissue damage was associated with a significant increase in renal MDA (+34%), indicating lipid peroxidation, and the researchers attributed the effects to oxidative stress. The tissue damage was also accompanied by a significant decrease in renal GSH peroxidase, indicating a depletion of renal GSH as contributing to the observed tissue damage. Studies by Fraga et al. (1987) using rat tissue slices in vitro and Abraham et al. (1999) in rats in vivo also showed lipid peroxidation in the kidney resulting from carbon tetrachloride exposure.

Ozturk et al. (2003) evaluated the levels of antioxidants in the kidney of Sprague-Dawley rats subcutaneously injected with 1594 mg/kg-day of carbon tetrachloride on 4 consecutive days. Compared with control kidneys, treated kidneys had significantly elevated activity levels for superoxide dismutase (+30%) and catalase (+46%) but reduced activity for GSH peroxidase (~44%) 24 hours after the last injection. The authors attributed the reduced activity of GSH peroxidase to decreased availability of renal GSH in its reduced form. Treated kidneys showed

severe and extensive cortical histopathology: focal glomerular necrosis, tubular dilation, epithelial vacuolization or necrosis (with detachment from the basement membrane), and protein casts. A parallel group treated with carbon tetrachloride and betaine (a methyl group donor) showed no differences from the control group for superoxide dismutase or GSH peroxidase, whereas catalase was significantly elevated (+34%). Kidneys of rats treated with carbon tetrachloride plus betaine had normal glomerular histology and only sparse tubular dilatation, epithelial vacuolization, and few cell detachments. The authors suggested that the beneficial effect of betaine on renal histology and GSH peroxidase activity was related to its promotion of SAM levels, as has been demonstrated in the liver by other investigators. This study suggests that similar toxicological mechanisms may occur in the liver and kidney of rats treated with carbon tetrachloride.

Cytosolic phospholipase A_2 levels were significantly elevated in the renal cortex and medulla of rats with carbon tetrachloride-induced cirrhosis and ascites (Niederberger et al., 1998). The authors attributed the increase in phospholipase A_2 to the increased renal production of prostaglandins in cirrhosis.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

Hepatic and renal effects are the most sensitive noncancer effects of oral or inhalation exposure to carbon tetrachloride in humans and animals.

4.6.1. Oral Exposure

No long-term toxicity data are available for humans with quantified oral exposures to carbon tetrachloride, but case reports identify the liver and kidney as the primary target organs following acute exposures. Evidence of acute oral hepatotoxicity in humans comes from observations of liver enlargement, elevated serum enzyme (AST and/or ALT), bilirubin levels, or histopathology (hepatocyte degeneration) (Ruprah et al., 1985; Stewart et al., 1963; Docherty and Nicholls, 1923; Docherty and Burgess, 1922). Other acute oral effects in humans include renal toxicity, usually delayed relative to hepatic toxicity (New et al., 1962) and lung effects secondary to renal failure (Umiker and Pearce, 1953). The prominence of hepatic injury in acutely exposed humans suggests that hepatic toxicity observed in subchronic animal studies is an important and relevant consideration for human health risk assessment of carbon tetrachloride.

Studies in laboratory animals indicate that hepatic toxicity is the predominant noncancer effect of subchronic or chronic oral exposure to carbon tetrachloride (Table 4-13). In these studies, evidence of hepatic damage included liver histopathology (fatty degeneration, necrosis, fibrosis, cirrhosis, inflammation, and regenerative activity), along with increases in liver weight and serum markers for hepatotoxicity (ALT, AST, OCT, SDH, and bilirubin) (Koporec et al., 1995; Allis et al., 1990; Bruckner et al., 1986; Condie et al., 1986; Hayes et al., 1986; NCI, 1977,

1976a, b; Weisburger, 1977; Litchfield and Gartland, 1974; Della Porta et al., 1961; Eschenbrenner and Miller, 1946; Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941). Liver damage was produced at doses as low as 7–9 mg/kg-day in rats and mice in 90-day corn oil gavage studies (Table 4-13). The corresponding NOAEL values were 0.7–0.9 mg/kg-day (Bruckner et al., 1986; Condie et al., 1986). The lowest dose to produce hepatotoxicity in 90-day aqueous gavage studies was 18 mg/kg-day (Koporec et al., 1995).

Table 4-13. Oral toxicity studies for carbon tetrachloride

Species	Dose/duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects at the LOAEL	Reference
Subchronic studies					
Dog (6/sex)	28 days in gelatin capsule: 797 mg/kg-day	Not determined	797	Increased ALT, OCT; fatty vacuolization with single cell necrosis in liver	Litchfield and Gartland, 1974
Dog (3 F)	8 weeks in gelatin capsule: 32 mg/kg-day	32	Not determined	No increases in serum enzymes; no liver histopathology	Litchfield and Gartland, 1974
Rat (15–16 M/ group)	5 days/week for 12 weeks by corn oil gavage: 0, 1, 10, or 33 mg/kg-day	1 [0.71][a]	10 [7.1][a]	Two- to threefold increase in SDH; mild centrilobular vacuolization in liver	Bruckner et al., 1986
Rat (6 M/ group and sacrifice time)	5 days/week for 12 weeks by corn oil gavage: 0, 20, or 40 mg/kg-day; sacrificed at intervals from 1-15 days post-exposure	Not determined	20 [14.3][a]	Increased liver weight, ALT, AST, LDH; reduced liver CYP450; cirrhosis, necrosis, and degeneration in liver	Allis et al., 1990
Rat (11 M/ group)	5 days/week for 13 week by corn oil gavage: 0, 25, or 100 mg/kg-day	Not determined	25 [17.8[[a] (FEL)	10% Mortality; increased ALT, SDH; slight hepato-cellular vacuolization and minimal fibrosis in liver	Koporec et al., 1995
Rat (11 M/ group)	5 days/week for 13 weeks by gavage in 1% Emulphor: 0, 25, or 100 mg/kg-day	Not determined	25 [17.8][a] (FEL)	25% Mortality; increased ALT, SDH; slight hepato-cellular vacuolization and minimal fibrosis in liver	Koporec et al., 1995
Mouse (12/sex/ group)	5 days/week for 12 weeks by corn oil gavage: 0, 1.2, 12, or 120 mg/kg-day	1.2 [0.86][a]	12 [8.6][a]	Increased ALT; mild to moderate hepatic lesions (hepatocytomegaly, necrosis, inflammation)	Condie et al., 1986
Mouse (12/sex/ group)	5 days/week for 12 weeks by gavage in 1% Tween-60: 0, 1.2, 12, or 120 mg/kg-day	12 [8.6][a]	120 [86][a]	Increased liver weight, ALT, AST, LDH; hepato-cytomegaly, vacuolation, inflammation, necrosis, and fibrosis in liver	Condie et al., 1986

Table 4-13. Oral toxicity studies for carbon tetrachloride

Species	Dose/duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects at the LOAEL	Reference
Mouse (20/sex/ group)	7 days/week for 13 weeks by corn oil gavage: 0, 12, 120, 540, or 1200 mg/kg-day	Not determined	12	Increased liver weight, ALT, AST, ALP, LDH, 5'-nucleotidase; fatty change, hepatocytomegaly, necrosis, and hepatitis	Hayes et al., 1986
Mouse (5/sex/ group)	30 times in 120 days by olive oil gavage: 0, 40, 80, or 160 mg/kg-day	40	80	Necrosis in liver	Eschenbrenner and Miller, 1946
Chronic studies					
Rat (50/sex/ group)	5 days/week for 78 weeks by corn oil gavage: 0, 47, or 80 mg/kg-day for males; 0, 94, or 159 mg/kg-day for females	Not determined	47	Increased mortality; cirrhosis in liver	NCI, 1977, 1976a, b
Mouse (50/sex/ group)	5 days/week for 78 weeks by corn oil gavage: 0, 1250, or 2500 mg/kg-day	Not determined	1250	Markedly increased mortality; cirrhosis and other toxic lesions in liver; adrenal pheochromocytoma	NCI, 1977, 1976a, b
Gestational exposure studies					
Rat (29 gravid F)	2 days on GDs 7–11 by corn oil gavage: 478 mg/kg-day	Not determined	478	21% Maternal mortality; 59% of dams had no offspring, 38% because of full-litter resorption	Wilson, 1954
Rat (9–14 gravid F/ group)	GDs 6–19 by corn oil gavage: 0, 112.5, or 150 mg/kg-day	Not determined	112.5	Reduced maternal weight gain; markedly increased full-litter resorption	Narotsky and Kavlock, 1995
Rat (12–14 gravid F/ group)	GDs 6–15 by corn oil gavage: 0, 25, 50, or 75 mg/kg-day	25	50	Piloerection; markedly increased full-litter resorption	Narotsky et al., 1997b
Rat (12–14 gravid F/ group)	GDs 6–15 by gavage in 10% Emulphor: 0, 25, 50, or 75 mg/kg-day	25	50	Piloerection; slightly increased full-litter resorption	Narotsky et al., 1997b
Mouse (≥8 gravid F/ group)	GDs 1–5 by gavage in corn oil: 0, 83, or 826 mg/kg-day	826	Not determined	No effect on dams or pups	Hamlin et al., 1993

[a]Duration adjusted dose provided in brackets (e.g., 1 mg/kg-day x (5 days per week/7 days per week) = 0.71 mg/kg-day.

Subchronic oral studies that also examined nonhepatic endpoints (Bruckner et al., 1986; Hayes et al., 1986) did not observe effects in the kidneys or other organs. There was some evidence for impairment of T-cell-dependent immunity in mice treated with 40 mg/kg-day for 14 days but not in rats at hepatotoxic doses (160 mg/kg-day for 10 days) (Guo et al., 2000; Smialowicz et al., 1991; Kaminski et al., 1990).

There is no direct evidence for effects on reproduction or development in humans exposed orally to carbon tetrachloride. One epidemiological study (Bove et al., 1995, 1992a, b) suggested associations between maternal exposure to carbon tetrachloride in drinking water and adverse birth outcomes (the strongest relationship was for low term birth weight), but subjects were exposed to multiple chemicals and the study included only a limited characterization of exposure. Studies in animals have found that relatively high oral doses of carbon tetrachloride (50 mg/kg-day and above) given on days 6–15 of gestation produce significant prenatal loss by increasing the incidence of full-litter resorptions (Narotsky et al.,1997a, b, 1995; Narotsky and Kavlock, 1995; Wilson, 1954); some evidence exists that reproductive effects are a consequence of a maternally mediated response to alterations in hormonal levels (Narotsky et al., 1995, 1997a). The doses producing litter resorption also produced overt toxic effects in dams (piloerection, kyphosis (or rounded upper back), and marked weight loss) and are well above the LOAELs for liver toxicity with longer-term exposure. Although the NOAELs and LOAELs were the same, both the clinical signs and litter resorptions were more pronounced when carbon tetrachloride was administered in corn oil versus aqueous emulsion. Mice treated with carbon tetrachloride early in gestation apparently did not show these effects (Hamlin et al., 1993).

Adrenal adenoma and pheochromocytomas were observed in mice exposed to carbon tetrachloride by gavage in an NCI bioassay in which carbon tetrachloride was used as a positive control for liver tumors (Weisburger, 1977); none of these tumors were specifically identified as malignant. These tumors may indicate a potential noncancer health risk, as well as a cancer risk. Benign pheochromocytomas are tumors that originate in chromaffin cells of the adrenal gland medulla and secrete excessive amounts of catecholamines, usually epinephrine and norepinephrine. Because pheochromocytomas are not innervated, catecholamine secretion is unregulated, producing sustained sympathetic nervous system hyperactivity leading to hypertension, tachycardia, and cardiac arrhythmias (Hansen, 1998). Health effects related to pheochromocytoma formation in mice were not assessed in the NCI (1977) cancer bioassay. Therefore, the potential for secondary effects of pheochromocytoma on the cardiovascular system can only be inferred. The lowest exposure level associated with benign pheochromocytomas in mice (LOAEL of 1250 mg/kg-day, 5 days/week [approximately 900 mg/kg-day]) is approximately 2 orders of magnitude higher than levels at which liver effects become apparent in experimental animals. Therefore, the available data do not identify the

adrenal gland as a sensitive target organ for carbon tetrachloride by oral administration.

Effect of Dosing Vehicle on Carbon Tetrachloride Toxicity

A number of investigators have demonstrated that the vehicle used in gavage studies to administer carbon tetrachloride and other chlorinated solvents may affect the test chemical's toxicity. Several investigators reported that carbon tetrachloride toxicity was enhanced if administered in corn oil compared to an aqueous solution (Narotsky et al., 1997; Condie et al., 1986), whereas Kaporec et al. (1995) found that corn oil as a vehicle (compared to an aqueous vehicle) did not significantly alter carbon tetrachloride hepatotoxicity following subchronic exposure, and Kim et al. (1990b) observed that administration in an aqueous solution enhanced carbon tetrachloride toxicity as compared to corn oil. Raymond and Plaa (1997) and Narotsky et al. (1997) found that the influence of vehicle could be dose-dependent. In their study of developmental toxicity, Narotsky et al. (1997) reported that maternal toxicity was slightly more pronounced when carbon tetrachloride was administered in aqueous vehicle, but at higher doses was more pronounced when administered in corn oil vehicle. Sanzgiri and Bruckner (1997) found that Emulphor, a polyethoxylated vegetable oil used as an emulsifier for VOCs and other lipophilic compounds, had no significant effect on carbon tetrachloride acute hepatotoxicity in Sprague-Dawley rats (as measured by elevation of serum enzyme activities of SDH and ALT) when carbon tetrachloride was administered as a single oral doses at two dose levels (10 and 180 mg/kg) and at four concentrations of Emulphor (1, 2.5, 5 and 10%). Blood carbon tetrachloride concentrations in these rats (measured at intervals up to 12 hours postdosing) revealed no significant differences as a function of Emulphor concentration, suggesting that Emulphor did not significantly affect carbon tetrachloride absorption or distribution.

A number of explanations of the influence of vehicle on the oral toxicity of carbon tetrachloride have been offered. Kim et al. (1990b) reported that corn oil delays carbon tetrachloride absorption from the digestive track and thereby decreases its arterial blood concentration. Such alterations in carbon tetrachloride pharmacokinetics could influence the resulting toxicity. It is possible that the preservation state of corn oil might influence toxicity; cell membranes could be altered by older oil stored under improper conditions and contaminated with peroxides or by heated and oxygenated corn oil that could lead to the formation of reactive oxygen radicals (Raymond and Plaa, 1997). It has been proposed that corn oil might induce CYP450 metabolizing enzymes that could enhance metabolism of carbon tetrachloride to reactive, cytotoxic forms (Raymond and Plaa, 1997; Kaporec et al., 1995). High lipid intake could possibly increase lipid levels in the liver, thereby enhancing target organ deposition of lipophilic carbon tetrachloride. Corn oil could also directly affect the lipid composition of cell membranes; the effects of carbon tetrachloride-derived trichloromethyl free radicals on hepatic microsomal proteins and lipids might then be enhanced (Kim et al., 1995).

Kaporec et al. (1995) proposed that a possible explanation for the observation of less pronounced hepatotoxicity in mice dosed with halocarbons in aqueous media involves method preparation. Even using methods to minimize carbon tetrachloride loss, Kaporec et al. (1995) found that there was typically about a 20% loss of carbon tetrachloride from an aqueous emulsion (Emulphor), but none from corn oil dosing solutions. Thus, findings of less severe toxicity with an aqueous vehicle than corn oil vehicle may have been the result of animals receiving a lower daily dose.

Thus, it is possible that the vehicle used in oral gavage studies to administer carbon tetrachloride could be a potential confounding factor in toxicity assays; however, the magnitude of the confounding and the nature of the interaction of corn oil remain uncertain.

4.6.2. Inhalation Exposure

Case reports of acute high-level exposure to carbon tetrachloride vapor or long-term occupational exposure provide evidence of hepatotoxic and nephrotoxic effects of carbon tetrachloride in humans. Observations indicative of an effect on the liver in these cases include jaundice, increased serum enzyme levels, and, in fatal cases, necrosis of the liver (Stewart et al., 1965; New et al., 1962; Kazantzis and Bomford, 1960; Norwood et al., 1950). Delayed effects on the kidney have also been reported in acute overexposure cases. Other effects associated with carbon tetrachloride exposure in humans are gastrointestinal symptoms (nausea and vomiting, diarrhea, and abdominal pain) and neurological effects indicative of central nervous system depression (headache, dizziness, and weakness). Tomenson et al. (1995) conducted a cross-sectional epidemiology study of hepatic function in workers exposed to carbon tetrachloride. They found suggestive evidence of an effect of occupational carbon tetrachloride exposure on serum enzymes indicative of hepatic effects at workplace concentrations in the range of 1 to 4 ppm.

The liver and kidney are the most prominent targets of carbon tetrachloride in subchronic and chronic inhalation studies of laboratory animals. Hepatic toxicity in these studies was demonstrated by histopathology (centrilobular fatty degeneration, necrosis, fibrosis, cirrhosis, hepatitis, and regenerative activity) as well as increases in liver weight and serum markers for liver damage (Nagano et al., 2007a,b; Benson and Springer, 1999; JBRC, 1998; Prendergast et al., 1967; Adams et al., 1952; Smyth et al., 1936). Hepatic effects were observed in animals exposed to carbon tetrachloride concentrations as low as 2 ppm (adjusted to continuous exposure, see Table 4-14). Renal damage was reported less frequently in these animal studies and generally at higher concentrations than liver damage. The JBRC chronic bioassay (Nagano et al., 2007b; JBRC, 1998) found renal damage, as evidenced by histopathology (increased severity of chronic nephropathy in the rat and protein casts in the mouse) and changes in serum chemistry and urinalysis variables at a concentration of 4 ppm (adjusted to continuous exposure, see Table 4-14). There is evidence that liver effects produced by carbon tetrachloride are

proportional to the product of concentration and time (C × T) (Plummer et al., 1990) and, therefore, that the duration adjusted exposure concentration is the most appropriate dose metric to use as the basis for comparison among studies. This is assumed to be the case for other systemic targets as well.

Table 4-14. Inhalation toxicity studies for carbon tetrachloride

Species	Duration/ concentration	NOAEL (ppm)	LOAEL (ppm)	Effects at the LOAEL	Reference
Subchronic studies					
Rat (24 mixed sex/group)	8 hours/day, 5 days/week for 10.5 months: 0, 50, 100, 200, or 400 ppm	Not determined	50 [12][a]	Fatty change in liver; effects in other organs were reported, but the LOAEL for these effects was unclear	Smyth et al., 1936
Guinea pig (24 mixed sex/group)	8 hours/day, 5 days/week for 10.5 months: 0, 25, 50, 100, 200, or 400 ppm	Not determined	25 [6][a]	Increased mortality; reduced body weight gain; fatty change in liver; effects in other organs were reported, but LOAEL for these effects was unclear	Smyth et al., 1936
Monkey (4/group)	8 hours/day, 5 days/week for 10.5 months: 0, 50, or 200 ppm	Not determined	50 [12][a]	Mild fatty change and degeneration in liver; effects in other organs were reported, but LOAEL for these effects was unclear	Smyth et al., 1936
Rat (15–25/sex/ group)	7 hours/day, 5 days/week for 6 months: 0, 5, 10, 25, 50, 100, 200, or 400 ppm	5 [1][a]	10 [2][a]	Increased liver weight; fatty degeneration in liver	Adams et al., 1952
Guinea pig (5–9/sex/ group)	7 hours/day, 5 days/week for 6 months: 0, 5, 10, 25, 50, 100, 200, or 400 ppm	5 [1][a]	10 [2][a]	Increased liver weight; fatty degeneration in liver	Adams et al., 1952
Rabbit (1–2/sex/ group)	7 hours/day, 5 days/week for 6 months: 0, 5, 10, 25, 50, or 100 ppm	10 [2][a]	25 [5][a]	Increased liver weight; fatty degeneration and slight cirrhosis in liver	Adams et al., 1952
Monkey (1–2/group)	7 hours/day, 5 days/week for 6 months: 0, 5, 10, 25, 50, or 100 ppm	50 [10][a]	100 [21][a]	Slight fatty degeneration and increased lipid content in liver	Adams et al., 1952
Rat (15/group)	24 hours/day, 7 days/week for 13 weeks: 0, 1 (in n-octane), or 10 ppm	1	10	Reduced body weight gain; enlarged liver with fatty change	Prendergast et al., 1967

Table 4-14. Inhalation toxicity studies for carbon tetrachloride

Species	Duration/ concentration	NOAEL (ppm)	LOAEL (ppm)	Effects at the LOAEL	Reference
Guinea pig (15/group)	24 hours/day, 7 days/week for 13 weeks: 0, 1 (in n-octane), or 10 ppm	1	10	Three died; reduced body weight gain; enlarged liver with fatty change	Prendergast et al., 1967
Rabbit (3/group)	24 hours/day, 7 days/week for 13 weeks: 0, 1 (in n-octane), or 10 ppm	1	10	Reduced body weight gain; enlarged liver with fatty change	Prendergast et al., 1967
Dog (2/group)	24 hours/day, 7 days/week for 13 weeks: 0, 1 (in n-octane), or 10 ppm	1	10	Reduced body weight gain; fatty change in liver	Prendergast et al., 1967
Monkey (3/group)	24 hours/day, 7 days/week for 13 weeks: 0, 1 (in n-octane), or 10 ppm	1	10	Visibly emaciated; enlarged liver with fatty change	Prendergast et al., 1967
Rat (10/ sex/group)	6 hours/day, 5 days/week for 13 weeks: 0, 10, 30, 90, 270, or 810 ppm	Not determined	10 [2][a]	Increased liver weight; fatty change in liver	Nagano et al., 2007a; JBRC, 1998
Mouse (10/ sex/group)	6 hours/day, 5 days/week for 13 weeks: 0, 10, 30, 90, 270, or 810 ppm	Not determined	10 [2][a]	Slight cytological alterations in the liver	Nagano et al., 2007a; JBRC, 1998
Rat (10 M/ group)	6 hours/day, 5 days/week for 12 weeks: 0, 5, 20, or 100 ppm	20 [4][a]	100 [18][a]	Increased ALT, SDH; necrosis in liver	Benson and Springer, 1999
Mouse (10 M/ group)	6 hours/day, 5 days/week for 12 weeks: 0, 5, 20, or 100 ppm	5 [0.9][a]	20 [4][a]	Increased ALT, SDH; necrosis and cell proliferation in liver	Benson and Springer, 1999
Hamster (10 M/ group)	6 hours/day, 5 days/week for 12 weeks: 0, 5, 20, or 100 ppm	20 [4][a]	100 [18][a]	Increased ALT, SDH; necrosis and cell proliferation in liver	Benson and Springer, 1999
Chronic studies					
Rat (50/sex/ group)	6 hours/day, 5 days/week for 104 weeks: 0, 5, 25, or 125 ppm	5 [0.9][a]	25 [4][a]	Reduced body weight gain; increased AST, ALT, LDH, GPT, BUN, CPK; lesions in the liver (fatty changes, fibrosis, cirrhosis) and kidney (progressive glomerulonephrosis)	Nagano et al, 2007b; JBRC, 1998

Table 4-14. Inhalation toxicity studies for carbon tetrachloride

Species	Duration/ concentration	NOAEL (ppm)	LOAEL (ppm)	Effects at the LOAEL	Reference
Mouse (50/sex/ Group)	6 hours/day, 5 days/week for 104 weeks: 0, 5, 25, or 125 ppm	5 [0.9][a]	25 [4][a]	Reduced survival late in study (because of liver tumors); reduced body weight gain; increased ALT, AST, LDH, ALP, protein, total bilirubin, and BUN; decreased urinary pH; increased liver weight; lesions in the liver (degeneration), spleen (extra medullary hematopoiesis), and kidney (protein casts); benign pheochromocytoma (males)	Nagano et al., 2007b; JBRC, 1998
Gestational exposure study					
Rat (22–23 gravid F/group)	7 hours/day on GDs 6–15: 0, 334, or 1004 ppm	Not determined	334 [97][a]	Dam: reduced body weight; increased liver weight and ALT; altered gross appearance of liver Fetus: reduced body weight and crown-rump length	Schwetz et al., 1974

[a]Duration adjusted concentration is provided in brackets (e.g., 10 ppm x (6 hours per day/24 hours per day x 5 days per week/7 days per week) = 2 ppm).

In the subchronic studies, effects on the kidneys were generally observed at concentrations above the LOAEL for liver effects and thus are not listed in Table 4-14. With chronic exposure, the sensitivity of the kidney and liver as target organs appears to be comparable in the rodent. The JBRC chronic rat study (Nagano et al., 2007b, JBRC, 1998) reported liver toxicity (serum enzyme changes, fatty liver, fibrosis, cirrhosis) and kidney toxicity (increases in BUN, creatinine, inorganic phosphorus, and severity of chronic progressive nephropathy [CPN]) at exposure concentrations of 25 ppm (≥4 ppm, duration adjusted) (Table 4-14). An increase in the severity of proteinuria was reported in male and female rats at the lowest tested concentration of 5 ppm (0.9 ppm, duration adjusted). While the increased severity of proteinuria could be related to the nephropathy observed at ≥25 ppm, the biological significance of the finding of proteinuria at 5 ppm is unclear. Proteinuria (or protein in the urine) was found in essentially 100% of the rats – both control and carbon tetrachloride-exposed, and

90% or more of all rats – again control and carbon tetrachloride-exposed – had protein content in the urine graded as either 3+ or 4+ (see Table 4-2). In the carbon tetrachloride-exposed animals, however, rats showed an increase in the severity of proteinuria relative to controls (i.e., relatively more carbon tetrachloride-exposed animals had protein content in urine graded 4+ than 3+). After two years of exposure to carbon tetrachloride, proteinuria in 5-ppm rats did not appear to progress, i.e., rats at this concentration did not show treatment-related increases in incidence or severity of renal changes recognized as clearly adverse (e.g., progressive glomerulonephrosis [or CPN] or measures of impaired glomerular function, including increased levels of BUN, creatinine, and inorganic phosphorus) that were observed at higher exposure concentrations.

Complicating interpretation of kidney effects in this study is the fact that the F344 rat is known for its high incidence of spontaneous, age-related CPN (Hard and Seely, 2005; Chandra and Frith, 1993/94). Chandra and Firth (1993/94) reported a background incidence of CPN of 88.8% in male and 74.5% in female F344 rats based on an examination of 491 controls from several 2-year carcinogenicity/chronic toxicity bioassays. CPN can be seen as early as 3 months and severity of the lesion increases with age. The presence of CPN can confound kidney lesion diagnosis (Hard and Seely, 2005). Kidney lesions in the JBRC 13-week study of carbon tetrachloride (Nagano et al., 2007a; JBRC, 1998) were examined with the thought that the confounding encountered in older (2-year old) rats would be minimized and treatment-related lesions could be more easily distinguished from spontaneous old-age renal lesions. In the 13-week study, the severity of proteinuria was statistically significantly increased at a concentration of ≥90 ppm in females and ≥270 ppm in males; histopathological changes in the kidney occurred in both sexes at ≥810 ppm. These effect levels are approximately 20- to 50-fold higher than the 5-ppm concentration in the chronic study at which an increase in severity of proteinuria was observed. It is unexpected that the effect level for kidney effects would decrease by such a large margin between subchronic and chronic exposure durations. Thus, the findings from the subchronic study by JBRC (Nagano et al., 2007a; JBRC, 1998) are not clearly consistent with a LOAEL for renal toxicity following chronic exposure of 5 ppm. Finally, the body of literature for carbon tetrachloride suggests that the rat liver is a more sensitive target organ that the kidney following exposures of subchronic duration (e.g., Nagano et al., 2007a; JBRC, 1998; Bruckner et al., 1986; Adams et al., 1952); there are no adequate chronic studies of carbon tetrachloride (beyond JBRC, 1998) to confirm whether the kidney may be a more sensitive target organ than the liver following chronic exposure. The above uncertainties raise questions as to the relevance of the finding of proteinuria in 5-ppm rats to human health assessment.

In addition to adverse effects on the liver and kidney, the observation of benign pheochromocytomas in mice exposed to carbon tetrachloride by inhalation in the JBRC chronic study (Nagano et al., 2007b; JBRC, 1998) may indicate a potential noncancer health risk. As noted in Section 4.6.1, benign pheochromocytomas are tumors that originate in chromaffin cells of the adrenal gland medulla and secrete excessive amounts of catecholamines, usually

epinephrine and norepinephrine. Because pheochromocytomas are not innervated, catecholamine secretion is unregulated, producing sustained sympathetic nervous system hyperactivity leading to hypertension, tachycardia, and cardiac arrhythmias (Hansen, 1998). Health effects related to pheochromocytoma formation in mice were not assessed in the JBRC chronic inhalation exposure study. Therefore, the potential for secondary effects of pheochromocytoma on the cardiovascular system can only be inferred. Exposure levels associated with benign pheochromocytomas in mice (LOAELs of 4 and 22 ppm, duration adjusted, in male and female mice, respectively) were equal to or greater than levels associated with hepatic and renal toxicity; thus, the adrenal gland does not appear to be the most sensitive target organ for carbon tetrachloride following inhalation exposure.

There is no evidence for reproductive or developmental toxicity in humans exposed by inhalation to carbon tetrachloride. One epidemiological study found no association between maternal occupational exposure to carbon tetrachloride and infants born small for gestational age (Seidler et al., 1999). Carbon tetrachloride has been found to produce effects in mouse testis (Bergman, 1983), testicular atrophy, and reduced fertility in rats exposed intermittently to high concentrations (≥200 ppm) for 6 or more months (Adams et al., 1952; Smyth et al., 1936). Testicular degeneration has also been reported in rats following repeated i.p. doses of 1.5 mL/kg (Kalla and Bansal, 1975; Chatterjee, 1966). A definitive reproductive toxicity study has not been performed, however. In a developmental toxicity study, Schwetz et al. (1974) found significant reductions in fetal body weight and crown-rump length in rats exposed to carbon tetrachloride vapor in the air during gestation but at a high concentration (334 ppm, 7 hours/day) that also produced hepatotoxicity and reduced growth in the dams.

4.6.3. Mode of Action Information

The mode of action of carbon tetrachloride-induced hepatotoxicity has been the subject of extensive research. Mechanistic studies (described in Section 4.5) provide evidence that metabolism of carbon tetrachloride via CYP2E1 to highly reactive free radical metabolites plays a role in its mode of action (Wong et al., 1998; Martinez et al., 1995; Letteron et al., 1990; Mourelle et al., 1988; Bechtold et al., 1982; Weddle et al., 1976). The primary metabolites, trichloromethyl and trichloromethyl peroxy free radicals, are highly reactive and are capable of covalently binding to cellular macromolecules (Boll et al., 2001b; Azri et al., 1991; DiRenzo et al., 1982; Diaz Gomez and Castro, 1980a; Castro and Diaz Gomez, 1972; Gordis, 1969).

Because the toxicity of carbon tetrachloride is secondary to its metabolism, the liver is expected to be an important target organ on the basis of its high CYP2E1 content. Subchronic gavage studies report that the liver is the sole target organ (see Section 4.6.1), probably related to a first-pass effect. The literature for carbon tetrachloride also suggests that the rat liver is a more sensitive target organ compared to the kidney following exposures of subchronic duration (e.g., Nagano et al., 2007a; JBRC, 1998; Bruckner et al., 1986; Adams et al., 1952). Additionally,

there are no adequate chronic studies of carbon tetrachloride (other than Nagano et al., 2007b; JBRC, 1998) to confirm whether the kidney may be a more sensitive target organ than the liver following chronic exposure (see Section 4.6.2).

The trichloromethyl peroxy and trichloromethyl radical may induce multiple cellular effects including lipid peroxidation (de Zwart et al., 1997; Gasso et al., 1996; Ichinose et al., 1994; Tribble et al., 1987; Lee et al., 1982; Recknagel and Glende, 1973; Rao and Recknagel, 1969) decreases in antioxidant levels (Cabre et al., 2000; Gasso et al., 1996 Gorla et al., 1983), alterations in calcium homeostasis, and activation of calcium dependent phospholipases as discussed in Section 4.5 (Limaye et al., 2003; Hemmings et al., 2002; Gonzalez Padron et al., 1993; Agarwal and Mehendale, 1986, 1984; Long and Moore, 1986; Kroner, 1982; Moore et al., 1976). Additionally, products of lipid peroxidation include reactive aldehydes that can form protein adducts that *may* contribute to hepatotoxicity (Beddowes et al., 2003; Abraham et al., 1999; Hartley et al., 1999; Bedossa et al., 1994; Comporti, 1985; Comporti et al., 1984). At this time it is uncertain the exact sequence or contribution of cellular mechanisms leading from the key event of metabolism to carbon tetrachloride-induced hepatotoxicity (cell death). A description of how the noncancer mode of action coincides with the carcinogenic mode of action can be found in Figure 4-4.

Although most mechanistic studies for carbon tetrachloride have concentrated on hepatic effects, some studies provide evidence for a similar mode of action for noncancer effects in the kidney. The distribution study of Bergman (1983) provided evidence that nonvolatile metabolites of carbon tetrachloride accumulate in the kidney as well as the liver of mice immediately following a 10-minute inhalation exposure (see Section 3.2). Like the liver, the kidney contains both CYP2E1 and CYP3A, which are able to metabolize carbon tetrachloride to the trichloromethyl radical (Warrington et al., 2004; Koch et al., 2002; Haehner et al., 1996). Histopathological examination in multiple studies revealed clear evidence of treatment-related glomerular damage (increased in severity of glomerulonephrosis, BUN, proteinuria, tubular degeneration, organ weight, and protein casts) in male and female rats exposed to carbon tetrachloride (Nagano et al., 2007a,b; Benson and Springer, 1999; JBRC, 1998; Prendergast et al., 1967; Adams et al., 1952; Smyth et al., 1936). Mechanistic similarities also exist between the liver and kidney regarding increases in lipid peroxidation products (Natarajan et al., 2006; Dogukan et al., 2003; Abraham et al., 1999; Fraga et al., 1987), reductions in GSH peroxidase activity, attributable to depleted stores of GSH (Natarajan et al., 2006; Dogukan et al., 2003; Ozturk et al., 2003) and increased levels of cytosolic phospholipase A_2 (Niederberger et al., 1998). Based on the available data, the kidney and liver effects associated with carbon tetrachloride appear to operate via a similar mode-of-action pathway.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight-of-Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), carbon tetrachloride can be classified as *likely to be carcinogenic to humans* by all routes of exposure. This cancer weight of evidence determination is based on (1) inadequate evidence of carcinogenicity in humans and (2) sufficient evidence in animals (i.e., concordant expression of hepatic tumors in several species and by several routes of exposure in response to carbon tetrachloride and evidence of pheochromocytomas in mice by two routes of exposure).

Carbon tetrachloride has been shown to be a liver carcinogen in rats, mice, and hamsters in eight bioassays of various experimental design by oral and inhalation exposure. A general correspondence has been observed between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors. At lower exposure levels, this correspondence is less consistent. In particular, in the JBRC 2-year inhalation cancer bioassay in the mouse (Nagano et al., 2007b, JBRC, 1998), the lowest exposure concentration tested (5 ppm or 0.9 ppm adjusted; see Tables 4-5 and 4-6) was not hepatotoxic, whereas the incidence of liver adenomas in female mice at this exposure concentration displayed a statistically significant increase compared to concurrent and historical controls.

A hypothesized carcinogenic mode of action for carbon tetrachloride has been proposed and includes the following key events: (1) metabolism to the trichloromethyl radical by CYP2E1 and subsequent formation of the trichloromethyl peroxy radical, (2) radical-induced mechanisms leading to hepatocellular cytotoxicity, and (3) sustained regenerative and proliferative changes in the liver in response to hepatotoxicity. A substantial amount of data exists that supports these key events in the cancer mode of action for carbon tetrachloride. A weight of evidence analysis of the genotoxicity literature suggests that carbon tetrachloride is more likely an indirect than direct mutagenic agent. Results of extensive testing for genotoxic and mutagenic potential are largely negative. There is little direct evidence that carbon tetrachloride induces intragenic or point mutations in mammalian systems. The mutagenicity studies that have been performed using transgenic mice have yielded negative results, as have the vast majority of the mutagenesis studies that have been conducted in bacterial systems. Under highly cytotoxic conditions, bioactivated carbon tetrachloride can exert genotoxic effects. These tend to be modest in magnitude and are manifested primarily as DNA breakage and related sequelae. Chromosome loss leading to aneuploidy may also occur to a limited extent. The fact that carbon tetrachloride overall has not been found to be a potent mutagen and that positive genotoxic results are found only at high exposure levels and generally in concert with cytotoxic effects (see Tables 4-8 to 4-11) indicates that carbon tetrachloride does not likely induce genotoxic effects through direct binding or damage to DNA. The majority of genotoxicity studies, however, have been conducted at relatively high exposure levels, which does not provide information regarding low doses of carbon tetrachloride (e.g., as potentially elicited through biological mechanisms such as

the formation of DNA adducts through carbon tetrachloride-induced oxidative stress, lipid peroxidation, and direct modification of DNA). Because carbon tetrachloride is metabolized to reactive species (trichloromethyl and trichloromethyl peroxy radical), the potential exists for these biologically active metabolites of carbon tetrachloride to react with macromolecules at low exposures (i.e., exposure levels below doses that are cytotoxic). Data to characterize this low-exposure activity are limited. Therefore, the mode of action of carbon tetrachloride at low exposure levels can be hypothesized, but is unknown at this time.

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

Studies in humans are inadequate to show an association between exposure to carbon tetrachloride and carcinogenicity. There is some evidence for certain types of cancer in occupational populations thought to have had some exposure to carbon tetrachloride, including NHL (Blair et al., 1998; Spirtas et al., 1991), lymphosarcoma and lymphatic leukemia (Checkoway et al., 1984; Wilcosky et al., 1984), esophageal and cervical cancer (Blair et al., 1990, 1979), breast cancer (Cantor et al., 1995), astrocytic brain cancer (Heineman et al., 1994), and rectal cancer (Dumas et al., 2000). In these cases exposure to carbon tetrachloride was poorly characterized and confounded by simultaneous exposures to other chemicals. Additionally, these studies were designed to evaluate tetrachloroethylene and trichloroethylene and had only limited ability to examine other chemical exposures such as carbon tetrachloride. None of the human epidemiology studies reported associations to cancer of the liver, which is the main site of carcinogenicity in animal studies, but this may be because of a lack of power to detect a relatively rare human tumor.

Carbon tetrachloride has been shown to induce hepatocellular carcinomas in rodents by oral, inhalation, and parenteral exposure. Researchers at the NCI conducted a series of gavage studies in mice of various strains and found large increases in the incidence of liver tumors in treated mice (Andervont, 1958; Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941). A similar result was obtained in hamsters (Della Porta et al., 1961). These animal studies were generally conducted using a single high dose of carbon tetrachloride, but one early study was conducted with multiple dose levels in order to investigate dose-response relationships for induction of liver tumors (Eschenbrenner and Miller, 1946). This study was conducted using small groups of five mice of each sex per group and two dosing regimens (gavage administration in olive oil daily or every 4 days for 4 months) that gave the same total exposure. Liver tumors (hepatomas) were found in all strain A male and female mice that received average daily doses as low as 20 mg/kg-day. No gross or microscopic tumors were found in mice receiving only 10 mg/kg-day. Interestingly, the incidence of hepatomas was somewhat higher in mice dosed daily, whereas liver necrosis appeared to be somewhat more prevalent in mice treated intermittently (every 4 days). Liver necrosis and hepatomas were not clearly concordant as would be expected. The fact that mice were sacrificed one month after dosing ended complicates interpretation of the

necrosis findings.

Oral bioassays of carbon tetrachloride using groups of 50 animals/sex were conducted in mice and rats by NCI (1977, 1976a, b) as a positive control for bioassays of chloroform, trichloroethylene, and 1,1,1-trichloroethane. The bioassay in mice employed very high doses (1250 or 2500 mg/kg, 5 day/week for 78 weeks) that produced close to 100% incidence of hepatocellular carcinoma. The incidence of adrenal adenoma and pheochromocytoma was also significantly increased in both dose groups in male and female mice. The bioassay in rats (47 or 94 mg/kg for males and 80 or 159 mg/kg for females, 5 days/week for 78 weeks) produced only a low incidence of liver tumors, but high early mortality, particularly in the high-dose group, may have affected the power of this study to detect a carcinogenic effect. Even so, the increase in carcinomas was statistically significant in low-dose females (4/49) in relation to pooled controls (1/99).

Carbon tetrachloride produced clear evidence of carcinogenicity in inhalation bioassays in rats and mice (Nagano et al., 2007b; JBRC, 1998). In rats, intermittent exposure (6 hours/day, 5 days/week) to 125 ppm for 2 years produced marked significant increases in the incidence of hepatocellular carcinomas and adenomas in both males and females. The incidence of tumors was not increased in rats exposed to 5 or 25 ppm by the same protocol although the incidence of liver carcinoma (3/50) in 25-ppm females exceeded the range of historical control incidence from JBRC bioassays. In mice, marked significant increases in hepatocellular carcinomas and (to a lesser extent) adenomas occurred at both 25 and 125 ppm in both sexes. Also, a statistically significant increase in the incidence of liver adenomas in female mice at 5 ppm (0.9 ppm adjusted) was observed compared to the concurrent control and exceeded the historical control range for hepatocellular adenomas from JBRC 2-year bioassays. The assays in mice also found significant increases in the incidence of benign adrenal pheochromocytomas in males at 25 or 125 ppm and females at 125 ppm, exposure levels at or above those associated with liver hepatocellular carcinoma and adenoma. Specifically, pheochromocytomas were identified in 32/50 high-dose male mice, only one of which was classified as malignant (the remaining 31 pheochromocytomas were benign) (JBRC, 1998). Benign pheochromocytomas were identified in 22/49 high-dose female mice; none were malignant. In addition to the potential cancer risk suggested by these tumors, benign pheochromocytomas may represent a noncancer health risk because of the excessive secretion of catecholamines, leading to sustained and unregulated sympathetic nervous system hyperactivity (see Section 4.6.2).

Some data from parenteral studies are also available. Subcutaneous injections of carbon tetrachloride at an average dose of 0.29 mg/kg-day for 33–47 weeks induced hepatocellular carcinomas in Osborne-Mendel, Japanese, and Wistar rats but not in Sprague-Dawley or black rats (Reuber and Glover, 1970, 1967a, b). Intraperitoneal injections at an average of 86 mg/kg-day induced hepatomas in C3H mice (Kiplinger and Kensler, 1963).

Overall, carbon tetrachloride has been extensively studied for its genotoxic and

mutagenic effects with largely negative results. There is little direct evidence that carbon tetrachloride induces intragenic or point mutations in mammalian systems (Section 4.4.2). The mutagenicity studies that have been performed using transgenic mice have yielded negative results, as have the vast majority of the mutagenesis studies that have been conducted in bacterial systems. However, since oxidative DNA adducts can be converted into mutations, the inability to detect mutations in the transgenic mouse assays may be an indication of efficient repair of oxidative lesions, a preferential formation of large chromosomal mutations that are inefficiently detected in the transgenic models, or a reflection of the limitations and sensitivity of the specific assays that were performed with carbon tetrachloride (see Table 4-12). The two positive mutation / DNA damage studies conducted in *E. coli* were seen in strains that are particularly sensitive to oxidative damage. Moreover, the intrachromosomal recombination induced by carbon tetrachloride in *S. cerevisiae* is believed to result from double stranded DNA breaks leading to deletion mutations. These results are consistent with DNA breakage originating from oxidative stress or lipid peroxidation products that occur concurrently with cytotoxicity.

An evaluation based on the weight of evidence suggests that carbon tetrachloride is more likely an indirect than a direct mutagenic agent. In general, genotoxic effects have been observed in a consistent and close relationship with cytotoxicity, lipid peroxidation, and/or oxidative DNA damage. Mutagenic effects, if they occur, are likely to be generated through indirect mechanisms resulting from oxidative stress or lipid peroxidation products. Under highly cytotoxic conditions, bioactivated carbon tetrachloride can exert genotoxic effects. These tend to be modest in magnitude and are manifested primarily as DNA breakage and related sequelae. Chromosome loss leading to aneuploidy may also occur to a limited extent.

Challenges in evaluating the carbon tetrachloride genotoxicity database must be acknowledged (e.g., see Table 4-12). Although the cellular effects of carbon tetrachloride are described adequately at doses at or above those that induce cytotoxicity, there is a paucity of data describing DNA damaging events at doses below those that are cytotoxic. Additionally, there exists some level of uncertainty as to whether assays used to assess the genotoxicity of carbon tetrachloride were of sufficient quality to assess genotoxicity at doses that do not induce cytotoxicity.

The database for carbon tetrachloride provides evidence that hepatic regeneration is related to hepatic carcinogenicity. Acute toxicity studies on rodents treated orally with carbon tetrachloride report hepatic necrosis within 6–24 hours of dosing and evidence of compensatory hepatocellular proliferation (mitosis, BrdU-positive labeling, or increases in DNA-synthesizing enzymes and increases in cells in S-phase) at the same time or within 48 hours (Lee et al., 1998; Wang et al., 1997; Steup et al., 1993; Doolittle et al., 1987; Nakata et al., 1975; Eschenbrenner and Miller, 1946). Table 4-15 shows the necrotic and regenerative lesions observed in subchronic and chronic oral and inhalation studies of carbon tetrachloride (only studies explicitly reporting necrotic or regenerative lesions are included). In these studies, hepatic necrosis or

degeneration was usually found in conjunction with some type of proliferative lesion, either regenerative hepatocellular changes (Nagano et al., 2007a,b; Benson and Springer, 1999; JBRC, 1998; Prendergast et al., 1967) or proliferation or hyperplasia of the bile duct (Nagano et al., 2007a; JBRC, 1998; Koporec et al., 1995; Bruckner et al., 1986; Hayes et al., 1986; NCI, 1977, 1976a, b; Prendergast et al., 1967). The only detailed study of both chronic toxicity and carcinogenicity of carbon tetrachloride was the JBRC inhalation study in rats and mice (Nagano et al., 2007b; JBRC, 1998). The occurrence of liver adenomas in female mice exposed to carbon tetrachloride at 5 ppm (0.9 ppm adjusted) makes the relationship of the hypothesized key events cytotoxicity and regenerative proliferation with tumor formation less clear since there are no available data that supports these key events at this dose level (see Table 4-15). Eschenbrenner and Miller (1946) reported development of tumors in mice at doses that did not evidently produce necrosis, but the design of this study may have influenced this result, as animals were sacrificed and examined one month after the end of the main treatment period (animals were, however, given one last dose 24 hours prior to sacrifice). Currently, there are no data to characterize the liver changes that may have occurred and what effect this would have on eliciting or abating cellular cytotoxicity 24 hours prior to terminal sacrifice. The investigators noted that all doses that induced hepatomas were likely to have caused initial necrosis based on separate studies using one or two doses. Regenerative changes were not investigated in this part of the study.

Table 4-15. Exposure levels for necrosis/degeneration and hyperplasia/ regeneration in liver following subchronic or chronic exposure to carbon tetrachloride by gavage or inhalation

Strain, species	Exposure	Hepatic necrosis/ degeneration	Hyperplasia/ regeneration	Reference
Sprague-Dawley rat (male)	Oral, 12 weeks 24 mg/kg-day (adjusted)	Necrosis	Bile duct hyperplasia	Bruckner et al., 1986
F344 rat (male)	Oral, 12 weeks 14 mg/kg-day (adjusted)	6/6 Necrosis		Allis et al., 1990
Sprague-Dawley rat (male)	Oral, 13 weeks 71 mg/kg-day (adjusted)	Necrosis	Nodular hepatic, bile duct, and oval cell hyperplasia	Koporec et al., 1995
CD-1 mouse	Oral, 13 weeks 12 mg/kg-day	Necrosis	Bile duct hyperplasia	Hayes et al., 1986
CD-1 mouse	Oral, 12 weeks 8.6 mg/kg-day (adjusted)	Necrosis		Condie et al., 1986
Strain A mouse	Oral, 120 days (30 doses) 80 mg/kg-day[b]	Necrosis		Eschenbrenner and Miller, 1946
B6C3F1 mouse	Oral, 78 weeks, 892 mg/kg-day[b] (adjusted)		Bile duct proliferation	NCI, 1977, 1976a, b

Table 4-15. Exposure levels for necrosis/degeneration and hyperplasia/ regeneration in liver following subchronic or chronic exposure to carbon tetrachloride by gavage or inhalation

Strain, species	Exposure	Hepatic necrosis/ degeneration	Hyperplasia/ regeneration	Reference
F344 rat (male)	Inhalation, 12 weeks 18 ppm (adjusted)[a]	Necrosis	BrdU-negative hepatocytes	Benson and Springer, 1999
B6C3F1 mouse (male)	Inhalation, 12 weeks 4 ppm (adjusted)[a]	Necrosis	BrdU-positive hepatocytes	Benson and Springer, 1999
Syrian hamster (male)	Inhalation, 12 weeks 18 ppm (adjusted)[a]	Necrosis	BrdU-positive hepatocytes	Benson and Springer, 1999
Wistar rat	Inhalation, 6 months 42 ppm	Necrosis		Adams et al., 1952
Hartley guinea pig	Inhalation, 13 weeks 10 ppm (continuous)	Hepatocellular degeneration	Hepatocellular regeneration	Prendergast et al., 1967
Hartley guinea pig; Sprague-Dawley or Long-Evans rat	Inhalation, 6 weeks 20 ppm (adjusted)[a]	Necrosis, hepatocellular degeneration	Hepatocellular regeneration, bile duct proliferation	Prendergast et al., 1967
F344 rat	Inhalation, 13 weeks 2 ppm (adjusted)[a]		Mitosis, bile duct proliferation, foci	Nagano et al., 2007a; JBRC, 1998
BDF1 mouse	Inhalation, 13 weeks 5-48 ppm (adjusted)[a]		Bile duct proliferation: 5 ppm, F; 16 ppm, M; mitosis: 16 ppm, M; 48 ppm, F; foci: 48 ppm both sexes	Nagano et al., 2007a; JBRC, 1998
F344 rat	Inhalation, 104 weeks 5-22 ppm (adjusted)[a]		Foci: 5 ppm, F; 22 ppm, M[a]	Nagano et al., 2007b; JBRC, 1998
BDF1 mouse	Inhalation, 104 weeks 5 ppm[b] (adjusted)[a]	Degeneration in males; necrosis in females		Nagano et al., 2007b; JBRC, 1998

[a] This concentration was adjusted to continuous exposure, e.g., a factor of 6/24 x 5/7 applied used for an inhalation exposure administered 6 hours/day, 5 days/week.
[b] Hepatic tumors detected at this level.

In summary, repeated oral or inhalation exposure to carbon tetrachloride causes degeneration or necrosis of the liver, and there is evidence for hepatocellular regeneration in repeated inhalation exposure studies.

4.7.3. Mode of Action Information for Liver Tumors

4.7.3.1. *Hypothesized Mode of Action and Identification of Key Events*

The hypothesized mode of action for carbon tetrachloride-induced liver tumors is described graphically in Figure 4-4. Key events in the carcinogenicity of carbon tetrachloride include: (1) metabolism to the trichloromethyl radical by CYP2E1 and subsequent formation of the trichloromethyl peroxy radical, (2) radical-induced mechanisms leading to hepatocellular toxicity, and (3) sustained regenerative and proliferative changes in the liver in response to hepatotoxicity. In rodent models (Nagano et al., 2007b; JBRC, 1998; NCI, 1977, 1976a, b; Della Porta et al., 1961; Andervont, 1958; Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941), tumorigenicity occurs consistently in the liver. Metabolism of carbon tetrachloride is identified as a key event based on the following: (1) reactive metabolites are present in the liver (Stoyanovsky et al., 1999; Conner et al., 1986), (2) CYP450 inhibitors prevent carbon tetrachloride-induced liver damage (Martinez et al., 1995; Letteron et al., 1990; Mourelle et al., 1988; Bechtold et al., 1982; Weddle et al., 1976), (3) treatment of knockout mice specific for CYP2El (cyp2el $^{-/-}$) with carbon tetrachloride does not result in hepatocellular cytotoxicity as compared to wild type (cyp2el $^{+/+}$) mice, and (4) treatment with compounds that induce CYP450's result in potentiating effects to carbon tetrachloride-induced toxicity (Section 4.8.6). The resulting hepatocellular cytotoxicity has been demonstrated in numerous studies (Table 4-15) as measured by increases in liver enzymes (i.e., ALT, AST, SDH, and LDH) in plasma or by histopathological examination. As a result of cytotoxicity in the liver of carbon tetrachloride-treated animals, significant regenerative cellular proliferation occurs to compensate for the necrotic or damaged tissue. As discussed in Section 4.7.2, there is a general correlation (particularly at higher doses) between occurrence of hepatotoxicity and/or regenerative/proliferative lesions and development of tumors. Findings from the study by JBRC (Nagano et al., 2007b; JBRC, 1998), the only detailed study of both chronic toxicity and carcinogenicity of carbon tetrachloride available, are generally consistent with the hypothesis that liver tumors occur at exposure levels that produced hepatotoxicity in both rats and mice. Liver adenomas did occur, however, in female mice at exposures below which hepatotoxicity was observed. Tumorigenesis through this hypothesized mode of action resulting from carbon tetrachloride-induced toxicity is believed to require persistent hepatocellular cytotoxicity and regenerative cellular proliferation for tumor formation. Other biological mechanisms (e.g., mutagenicity) may contribute to the tumorigenic response and may represent additional key events or other operable modes of action; however, the contribution of these key events or modes of action has not been fully established.

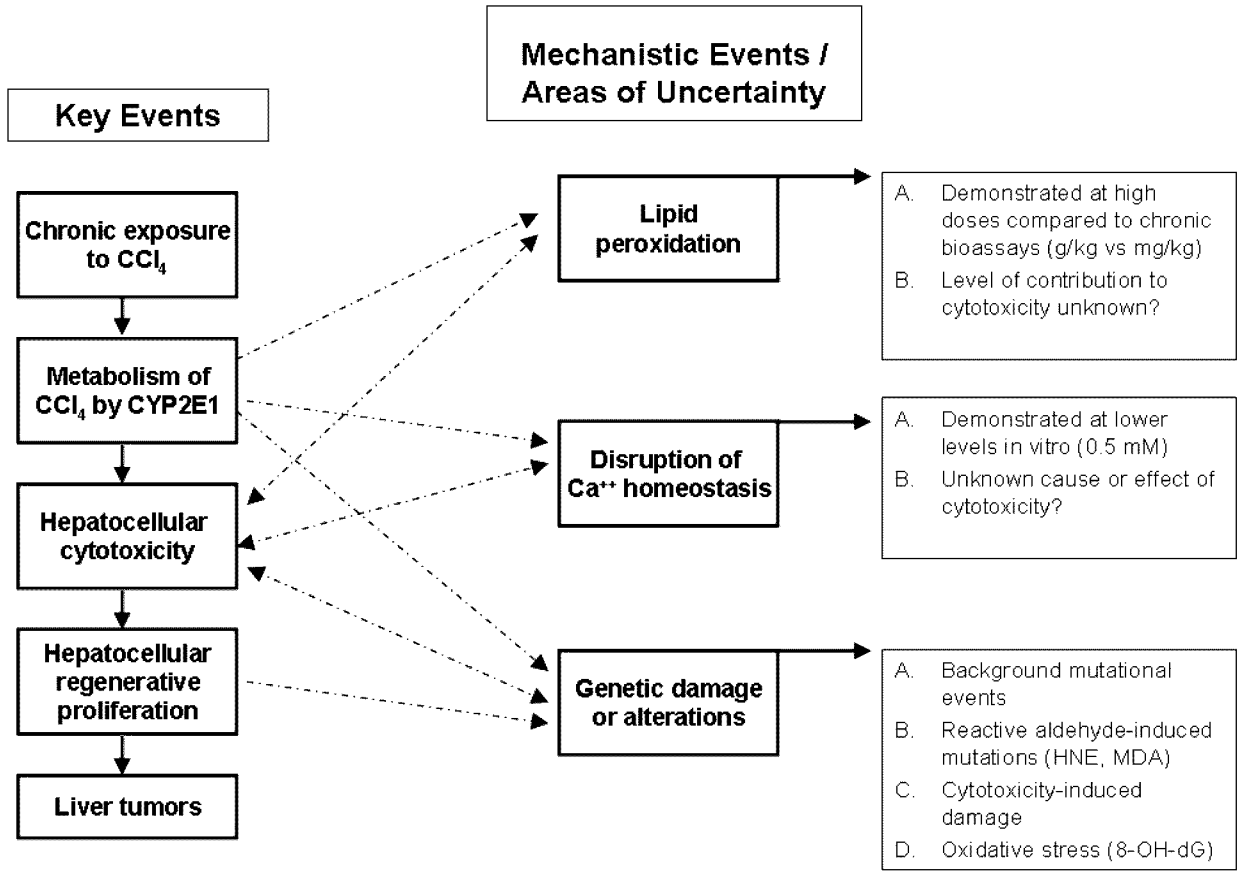


Figure 4-4. Hypothesized carcinogenic mode of action

As mentioned above there exist several important mechanistic events, including lipid peroxidation, disturbances in calcium homeostasis, and genetic damage, that are possibly involved in this biological process; however, the available data at doses below those that induce cytotoxicity are not adequate to evaluate their relative significance. Therefore, whether these mechanistic events represent key events remain areas of uncertainty in carbon tetrachloride's carcinogenic mode of action.

In general, mechanistic studies that have evaluated the induction of lipid peroxidation have been conducted at doses that induce significant levels of cytotoxicity (see Table 4-15). Representative studies evaluating the occurrence of lipid peroxidation are provided in Table 4-16, Section 4.7.3.2.2. These studies are not definitive regarding the cellular responses that occur below those that have been found to induce tumors in chronic bioassays. Additionally, it is not clear at what dose lipid peroxidation or generation of reactive aldehydes would begin to contribute to the toxicity of carbon tetrachloride. Although carbon tetrachloride is not considered likely to be directly genotoxic, it is possible that lipid peroxidation products

generate compounds (reactive aldehydes) that may covalently bind to DNA. The low molecular weight aldehydes generated by lipid peroxidation have sufficiently long biological half-lives to diffuse from their site of formation to other parts of the cell (Slater, 1982, 1981). Nuclear DNA adducts to these aldehydes in hepatocytes have been demonstrated in a number of studies (Beddowes et al., 2003; Wacker et al., 2001; Chung et al., 2000; Wang and Liehr, 1995; Chaudhary et al., 1994). One of these compounds, malonaldehyde, has been shown to be tumorigenic in Swiss mice when applied repeatedly to the skin (Shamberger et al., 1974). In cultured rat hepatocytes, however, the lowest concentration producing a statistically significant increase in DNA breaks and DNA adducts generated by lipid peroxidation approached the concentration that induced cytotoxicity (LDH leakage) (Beddowes et al., 2003). The possibility exists that reactive aldehydes generated at low levels of carbon tetrachloride could result in increased levels of endogenous MDA and 4-HNE DNA adducts that may contribute to the genotoxicity of carbon tetrachloride. Additionally, based on current data sets that characterize the generation of lipid peroxidation induced by carbon tetrachloride (Table 4-16, Section 4.7.3.2.2), the doses at which this effect have been demonstrated do not allow a clear determination as to whether lipid peroxidation induces cytotoxicity or whether cytotoxicity induces lipid peroxidation.

Disruption of calcium homeostasis as a process by which carbon tetrachloride may induce toxicity is an area of extensive research (Hemmings et al., 2002; Long and Moore, 1986; Kroner, 1982; Moore et al., 1976). Similar to research conducted on carbon tetrachloride-induced lipid peroxidation, it is not established if disruption of calcium homeostasis is a cause or an effect of cellular cytotoxicity. Some studies present evidence that disturbances in calcium homeostasis may not be a necessary event for cell death to result from carbon tetrachloride (Albano et al., 1989; Clawson, 1989). Likewise, evaluation of the carbon tetrachloride dose required to induce disturbances in calcium homeostasis does not confirm this as a key event (Hemmings et al., 2002; Long and Moore, 1986; Kroner, 1982; Moore et al., 1976).

Genetic damage or alteration to the DNA represents an additional area of uncertainty for the mode(s) of action for carbon tetrachloride that is at the present time not adequately characterized. Several cellular processes have been proposed that may account for how genetic damage may occur, ultimately leading to genotoxic events. The trichloromethyl and trichloromethyl peroxy free radicals are capable of covalently binding to nucleic acids. However, the reactivity of these radicals is such that they are not expected to diffuse very far from their site of formation (Slater, 1982, 1981). As a result, the amount reaching the cell nucleus from microsomes would be negligible. Studies have indicated small increases in covalent binding of trichloromethyl radical to nuclear DNA, as well as nuclear proteins and lipids, apparently as a result of bioactivation of carbon tetrachloride by CYP450 in the nuclear membrane (Fanelli and Castro, 1995; Castro et al., 1989; Levy and Brabec, 1984; Diaz Gomez and Castro, 1980a, b; Rocchi et al., 1973). However, there are significant methodological

problems with each of these studies that create difficulties in interpreting the results (see Section 4.4.2.4). Additionally, the fact that carbon tetrachloride overall has not been found to be a potent mutagen and that the few positive genotoxic results are found only at high exposure levels and generally in concert with cytotoxic effects (Tables 4-8 to 4-11) indicates that carbon tetrachloride does not likely induce genotoxic effects through direct binding or damage to DNA. As for lipid peroxidation-induced DNA damage, development of mutations by this mechanism could occur and would likely result from the production of radicals exceeding the cell's capacity to quench and/or repair these alterations.

Genetic damage could also result from background or spontaneous mutations. In vivo studies have estimated that background mutation frequencies may increase many fold over the lifetime of an organism (Morley and Turner, 1999). It is generally accepted that sustained cell proliferation in response to cell death from toxicity or other causes is a significant risk factor for cancer (Holsapple et al., 2005). Hepatic regeneration following injury from carbon tetrachloride has the potential to result in carcinogenesis as a result of replication errors becoming fixed mutations before DNA repair can be completed.

Many studies have characterized the formation of endogenously produced DNA adducts (Beddowes et al., 2003; Wacker et al., 2001; Chung et al., 2000; Wang and Liehr, 1995; Chaudhary et al., 1994), DNA strand breaks (Kadiiska et al., 2005; Yasuda et al., 2000; Gans and Korson, 1984), chromosomal aberrations (Sawada et al., 1991), and micronucleus formation (Uryvaeva and Delone, 1995; Van Goethem et al., 1995). However, to date no measure of genetic damage to DNA has been well characterized at or below doses at which tumors are observed (Nagano et al., 2007b; JBRC, 1998; NCI, 1977, 1976a, b; Eschenbrenner and Miller, 1946). Adequate dose-response studies for assays that measure genetic damaging events at or below dose levels for which carbon tetrachloride induces tumors in chronic bioassays would help clarify whether or not carbon tetrachloride is carcinogenic at dose levels that do not cause cytotoxicity and cell regeneration. In the absence of adequate characterization of genetic damaging events that may lead to or contribute to background mutational levels, EPA is choosing to characterize the full range of carcinogenic potential for human exposure to carbon tetrachloride (see Section 5.3).

4.7.3.2. *Experimental Support for the Hypothesized Mode of Action*

4.7.3.2.1. ***Strength, consistency, specificity of association.*** Carcinogenicity studies of carbon tetrachloride have consistently reported carcinogenicity in the liver, independent of species, gender, or route of administration. Hepatic toxicity (cytotoxicity), necrosis, and regenerative proliferation have generally been reported in animals exposed to carbon tetrachloride orally or by inhalation and are correlated with the CYP450 content. However, it remains to be established if genotoxic events could occur at dose levels below those that induce tumors or are induced by cytotoxicity and clonal expansion due to regenerative proliferative events and could represent a primary mode of action for carbon tetrachloride. In the 2-year inhalation studies in rats and mice, which are the best documented of chronic studies, livers with adenomas or carcinomas also expressed nonneoplastic lesions (Nagano et al., 2007b; JBRC, 1998) with the exception of liver adenomas at the lowest dose (5-ppm or 0.9-ppm adjusted) in female mice. Additionally, multiple reports confirm the occurrence of adenomas and carcinomas in carbon tetrachloride-treated animals (NCI 1977, 1976a, b; Della Porta et al., 1961; Andervont, 1958; Eschenbrenner and Miller, 1946; Edwards and Dalton, 1942; Edwards et al., 1942; Edwards, 1941) (see also Table 4-15).

4.7.3.2.2. ***Dose-response concordance.*** Carbon tetrachloride-induced liver tumors were seen in rats, mice, and hamsters after oral bolus dosing in oil and in rats and mice exposed by inhalation. Several oral studies were conducted using only a single-dose level (i.e., studies in the mouse by Edwards, 1941; Edwards and Dalton, 1942; Edwards et al., 1942; and a study in the hamster by Della Porta et al., 1961) and, therefore, did not provide information on the relationship between dose and tumor induction. The NCI (1977, 1976a, b) bioassay included two dose levels, but high early mortality in the rat study, particularly at the high dose, limited interpretation of the results. In the mouse study, liver carcinomas were produced at .100% incidence in male and female mice of both dose groups (179/182 mice). Eschenbrenner and Miller (1946) observed liver tumors in all mice treated daily with 20 mg/kg-day or more (n = 29), but none in the 10 mice treated with 10 mg/kg-day. In the inhalation studies (Nagano et al., 2007b; JBRC, 1998), liver tumors were markedly increased at 125 ppm in male and female rats and 25 and 125 ppm in male and female mice. In both species at the next lower exposure concentration (i.e., 25 ppm in rats or 5 ppm in mice) the liver tumor incidence was elevated only in female animals. In the 25-ppm female rat, the incidence of hepatocellular carcinomas did not differ statistically from the concurrent controls, but exceeded the historical control range from the research center that conducted the bioassay. In the 5-ppm female mouse, the incidence of hepatocellular adenomas was statistically elevated over the concurrent control and historical controls for that research center. In male mice, the incidence of carcinomas was very high in the 25 ppm group (88%), and there was only a modest increase as the concentration was increased to 125 ppm (94%). In the female mice, the

incidence of carcinomas was lower in the 25-ppm group (66%), and there was a larger increase as the concentration was increased to 125-ppm (98%).

The dose-response relationship between hepatic cytotoxicity and tumor formation is best demonstrated by the JBRC cancer bioassay in rats and mice, which examined histopathological changes to the liver after 13 and 104 weeks and tumor formation after 104 weeks of exposure to carbon tetrachloride by inhalation (Nagano et al., 2007a,b; JBRC, 1998). Carbon tetrachloride concentrations evaluated were 0, 10, 30, 90, 270, and 810 ppm in the 13-week study and 0, 5, 25, and 125 ppm in the 104-week study. In rats exposed for 13 weeks, histopathological changes indicative of cellular damage ("fatty change") and inflammation (granulation) were observed in all carbon tetrachloride treatment groups. At concentrations ≥30 ppm, proliferative (pleomorphism and increased mitosis) and regenerative (fibrosis, proliferative ducts, cirrhosis) responses occurred. At concentrations ≥270 ppm, eosinophilic and basophilic foci, which are associated with hyperplastic or preneoplastic changes, were observed. Similar nonneoplastic hepatic lesions (fatty changes, granulation, cirrhosis) were observed in livers of rats exposed to ≥25 ppm for 104 weeks; the incidence of nonneoplastic lesions in rats exposed to 5 ppm for 104 weeks appeared similar to that in controls. The incidence of liver tumors in rats was significantly increased only in the 125-ppm group compared with that in concurrent controls, although an increase in hepatocellular carcinomas in 25-ppm female rats exceeded the historical control range. Thus, liver tumors were observed at an exposure level associated with hepatotoxicity following subchronic and chronic exposure, but tumors were not observed at an exposure level below the level that induced cytotoxicity (<10 ppm for 13-week exposure and 5 ppm for 104-week exposure). A similar dose-response relationship for cytotoxicity and tumor formation was observed for mice (Nagano et al., 2007b; JBRC, 1998), although the relationship was less consistent at the lowest exposure concentration. In mice exposed for 13 weeks, dose-dependent histopathological findings indicative of cytotoxicity, damage, proliferation, and preneoplastic changes (e.g., fatty liver, pleomorphism, increased mitosis, proliferative ducts, and eosinophilic and basophilic foci) were observed; "cytological alterations" occurred at the lowest concentration tested (10 ppm). Similar histopathological findings were observed in mice exposed to ≥25 ppm for 104 weeks. The incidence of liver adenomas and carcinomas in mice was increased compared to concurrent controls at ≥25 ppm; thus, an increase in hepatic tumors in mice was observed at an exposure level that produced cytotoxicity. Additionally, at 5 ppm (0.9-ppm adjusted), where hepatocellular damage was not observed in the 104-week study, the incidence of hepatocellular adenomas was statistically elevated in female mice compared to concurrent controls.

Thus, results of this dose-response analysis suggest that liver tumors generally occur at the same or higher exposure levels as cytotoxicity. Although cytotoxicity was not observed at the 5-ppm (0.9-ppm adjusted) exposure level, evidence for cytotoxicity does exist at the 25-ppm level (5-ppm adjusted) (Nagano et al., 2007b) and regenerative proliferation at the 30-ppm level

(7-ppm adjusted) (Nagano et al., 2007a). The dose-response relationships between cytotoxicity and liver tumors demonstrated by the JBRC bioassay in rats and mice generally support cytotoxicity, regeneration, and proliferation as the predominant mode of action for carbon tetrachloride-induced carcinogenesis at higher exposure levels but may not adequately characterize critical events that would support other plausible modes of action, particularly at lower exposure levels.

As summarized in Table 4-15, several subchronic inhalation and oral studies have demonstrated that carbon tetrachloride produces hepatic toxicity and regeneration. In rodents exposed to carbon tetrachloride vapor for 12 weeks to 6 months, LOAELs for tissue damage were reported at concentrations ranging from 4 to 42 ppm (adjusted) and for hyperplasia/ regeneration at concentrations ranging from 4 to 20 ppm (adjusted). Thus, results of subchronic exposure studies are consistent with results of the JBRC study in rats, showing cytotoxicity at ≥10 ppm (≥2 ppm adjusted) and hyperplasia/proliferation at ≥30 ppm (≥5.4 ppm adjusted) after 13 weeks of exposure (Nagano et al., 2007a; JBRC, 1998) and cytotoxicity and hyperplasia/regeneration at ≥25 ppm (≥4.5 ppm adjusted) after 104 weeks of exposure (Nagano et al., 2007b; JBRC, 1998). In rats and mice exposed orally to carbon tetrachloride for 12–17 weeks, LOAELs for tissue necrosis ranged from 8.6 to 80 mg/kg-day and for hyperplasia/regeneration ranged from 12 to 71 mg/kg-day. Durations of the subchronic studies were too short to evaluate tumor formation; thus, data from subchronic studies do not allow for further definition of the dose-response relationship and time course for cytotoxicity and tumor formation.

Significant research has been conducted on the mechanistic events that precede carbon tetrachloride-induced hepatocellular cytotoxicity (see Section 4.5). Much of this research has focused on lipid peroxidation (de Zwart et al., 1997; Gasso et al., 1996; Ichinose et al., 1994; Tribble et al., 1987; Lee et al., 1982; Recknagel and Glende, 1973; Rao and Recknagel, 1969), decreases in antioxidant levels (Cabre et al., 2000; Gasso et al., 1996 Gorla et al., 1983), alterations in calcium homeostasis, and activation of calcium-dependent phospholipases (Limaye et al., 2003; Hemmings et al., 2002; Gonzalez Padron et al., 1993; Agarwal and Mehendale, 1986, 1984; Long and Moore, 1986; Kroner, 1982; Moore et al., 1976). Compared to doses that result in tumor formation in chronic bioassays (5-125 ppm: Nagano et al., 2007b; JBRC, 1998; 20 mg/kg-day: Eschenbrenner and Miller, 1946), these mechanistic studies were conducted at relatively high exposure levels (see Table 4-16). In most, if not all, mechanistic studies, exposure levels greatly exceeded those used in chronic bioassays (e.g., on the order of grams per kilogram (in vivo) or millimolar concentrations (> 1 mM) of carbon tetrachloride). The relevance of the mechanistic findings at these high exposure levels to toxicologically relevant exposures is uncertain (Weber et al., 2003; Clawson, 1989; Recknagel and Glende, 1989; Dolak et al., 1988). The degree to which lipid peroxidation, depletion of cellular antioxidants, alterations in calcium homeostasis, and activation of calcium-dependent phospholipases

contribute to the process of cytotoxicity, regenerative proliferation, and tumorigenesis, and the possible reversibility of these effects, constitutes an area of uncertainty (Weber et al., 2003; Rikans et al., 1994; Kefalas and Stacey, 1989; Dolak et al., 1988; Sandy et al., 1988; Stacey and Klaassen, 1981).

Table 4-16. Dose considerations of mechanistic studies of carbon tetrachloride

End point	Dose of Carbon Tetrachloride	Test System	Result	Reference
Lipid peroxidation	1 ml/kg (1590 mg/kg)[a]	Sprague-Dawley rats; three strains of mice (A/J, BALB/cJ, and C57B1/6J	Increased conjugated dienes in carbon tetrachloride (CCl_4) treated animals compared to controls	Lee et al., 1982
Lipid peroxidation	0.5 ml/kg (800 mg/kg)[a]	Rats and mice	Ethane production increased in CCl_4-treated animals; iron binding eliminated lipid peroxidation (ethane) in CCl_4-treated animals	Younes and Siegers, 1985
Lipid peroxidation	0.5 ml (2.38 ml/kg) injected i.p. (800 mg/kg)[a]	Male Wistar rats	TBARS significantly lower in animals receiving SAM	Gasso et al., 1996
Lipid peroxidation	1 mM (154 mg/L)	In vitro, liver microsomes (multiple species)	Increased MDA DNA adducts	Ichinose et al., 1994
Lipid peroxidation	1 mM (154 mg/L)	Liver slices from Sprague-Dawley Rats	Significant increase in TBARS	Fraga et al., 1987
Lipid peroxidation	500 mg/kg	Female F344 rats	2-fold induction of HNE-dG adducts	Wacker et al. 2001
Lipid peroxidation	3200 mg/kg	Female F344 rats	37-fold induction of HNE-dG adducts	Chung et al. 2000
Lipid peroxidation	0.1 ml/kg (160 mg/kg)[a]	Hamsters	MDA DNA adducts	Wang and Liehr, 1995
Lipid peroxidation	1590 mg/kg	Rat	Significant increase in 4-HNE and MDA adducts in liver	Hartley et al., 1999
Lipid peroxidation	2.5 ml/kg p.o. or 1 ml/kg (5 ml/kg as a 20% solution.) injected i.p. (3980 or 1590 mg/kg)[a]	Male Sprague-Dawley rats	Conjugated dienes or incorporation of C^{14} labeled CCl_4 was not significantly prevented by several antioxidants	de Ferreyra et al., 1975
Lipid peroxidation	1590 mg/kg	Rat	2.5-fold increase TBARS over	Hartley et al., 1999

Table 4-16. Dose considerations of mechanistic studies of carbon tetrachloride

End point	Dose of Carbon Tetrachloride	Test System	Result	Reference
			controls	
Lipid peroxidation	1 and 4 mM (154 and 615 mg/L)	In vitro rat hepatocytes	Significant increase in MDA adducts	Beddowes et al., 2003
Protein carbonyl (protein adducts)	1 and 4 mM (154 and 615 mg/L)	In vitro rat hepatocytes	2.5-fold increase at 4 mM	Beddowes et al., 2003
GSH modulation	0.5 ml (2.38 ml/kg) injected i.p. (800 mg/kg)[a]	Wistar rats	GSH decreased at 5 weeks	Cabre et al., 2000
GSH modulation	Pretreated with 2 g/kg GSH 30 minutes prior to 1590 mg/kg i.p. carbon tetrachloride	Male Sprague-Dawley rats	GSH pretreatment partially prevented hepatic necrosis	Gorla et al., 1983
GSH modulation	1600 mg/kg, twice weekly for 6 weeks, i.p.	Rat	Significant decrease in GSH; SAM partially prevented liver toxicity	Gasso et al., 1996
GSH modulation	0.1 ml/kg (160 mg/kg)[a]	Female Balb/c mice	Schisandrin B-partially prevented hepatotoxicity and GSH depletion	Chiu et al., 2003
Altered Ca++ homeostasis	0.3 – 10 mM (46-1540 mg/L)	In vitro hepatocytes	Increased activity of phosphorylase a and decreased activity of endoplasmic reticulum Ca++ pump; effects only observed at concentrations > 1mM	Long and Moore, 1986
Altered Ca++ homeostasis	1ml/kg injected i.p. (1590 mg/kg)[a]	Female Wistar rats	Significant decrease in microsomal Ca++ concentration; significant increase in mitochondrial Ca++ concentration	Kroner, 1982
Altered Ca++ homeostasis	2.5 ml/kg oral dose by feeding tube (3980 mg/kg)[a]	Male Sprague-Dawley rats	85% reduction in ATP-dependent Ca++ uptake and endoplasmic reticulum capacity	Moore et al., 1976

Table 4-16. Dose considerations of mechanistic studies of carbon tetrachloride

End point	Dose of Carbon Tetrachloride	Test System	Result	Reference
Altered Ca++ homeostasis	0.03 ml/100 g to 0.125 ml /100 g body weight (1.25 ml /kg by feeding tube) (0.48 - 1990 mg/kg)[a]	Male F344 rats	Decreased Ca++ transport across plasma membrane and mitochondria	Hemmings et al., 2002
Altered Ca++ homeostasis	50 µM (7.7 mg/L)	In vitro hepatocytes	Elevated cytosolic Ca++ levels	Stoyanovsky and Cederbaum, 1996
Phospholipase activity	3 ml/kg i.p. (4770 mg/kg)[a]	Male Sprague-Dawley rats	Co-treated with CBZ (calpain inhibitor), decreased mortality 50% from lethal dose of CCl4	Limaye et al., 2003
Phospholipase activity	1 ml/kg injected i.p. (1590 mg/kg)[a]	Male Sprague-Dawley rats	Pretreated with quinacrine (phospholipase A_2 inhibitor)	Gonzalez Padron et al., 1993
Phospholipase activity	0.23 – 1.3 mM (35-200 mg/L)	In vitro hepatocytes	Increased phospholipase A2 activity 1.4- to 5.3-fold	Glende and Pushpendaran, 1986
Phospholipase activity	1.2 mM (185 mg/L)	In vitro hepatocytes	Increased phospholipase A2 activity and hepatocyte degeneration (LDH release)	Glende and Recknagel, 1992

[a] Dose in mg/kg estimated using a density for carbon tetrachloride of 1.5940 g/ml at 20 °C.

An additional area of significant uncertainty for dose-response concordance is the possibility of genetically damaging events occurring at or below doses that induce tumors in laboratory rodents. Because genotoxicity and mechanistic data in this portion of the dose-response curve are limited, the possible contribution of genetic damage to the formation of liver tumors due to carbon tetrachloride exposure cannot be established.

4.7.3.2.3. ***Temporal relationship.*** Carbon tetrachloride is metabolized to trichloromethyl and peroxy free radicals, which may result in radical-induced mechanisms including lipid peroxidation and disruption of calcium homeostasis leading to hepatocellular cytotoxicity. Initial metabolism of carbon tetrachloride to reactive radicals and subsequent events leading to cytotoxicity are ongoing processes that occur throughout exposure.

The temporal progression of nonneoplastic liver lesions following acute and subchronic exposure is consistent with the hypothesized cytotoxic-proliferative mode of action. Initial histopathological changes in the liver following acute exposure to carbon tetrachloride include fatty degeneration, inflammatory cell infiltration, and necrosis (Lee et al., 1998; Wang et al., 1997; Steup et al., 1993). Hepatocyte regeneration has been observed within 24 hours of exposure (Lee et al., 1998). As reviewed in Sections 4.2.1.1 and 4.2.2, numerous subchronic exposure studies report histopathological findings consistent with an ongoing cycle of hepatic damage, repair, and proliferation (e.g., fatty vacuolization and degeneration, necrosis, nuclear pleomorphism, hyperplasia, fibrosis, and cirrhosis) (Nagano et al., 2007a; JBRC, 1998; Allis et al., 1990; Bruckner et al., 1986; Condie et al., 1986; Litchfield and Gartland, 1974). Smyth et al. (1936), Adams et al. (1952), and Benson and Springer (1999) clearly show a progression of liver toxicity from fatty degeneration of the liver to liver cirrhosis and hepatocellular proliferation only at doses that produce necrotic damage.

A temporal and dose-related progression of key events (hepatotoxicity, repair, proliferation, and tumor development) is supported by the results of the JBRC inhalation cancer bioassay in rats (Nagano et al., 2007b; JBRC, 1998), in which the development of hyperplastic or preneoplastic lesions (eosinophilic and basophilic foci) following subchronic exposure to cytotoxic levels, with subsequent development of liver tumors, is demonstrated (see Table 4-17). Thus, in the rat, the temporal relationship of the key events is consistent with the mode of action for carbon tetrachloride carcinogenesis. This relationship, however, is not as clearly defined for the increased incidence of liver adenomas in female mice (Nagano et al., 2007a,b).

Table 4-17. Temporal sequence and dose-response relationship for key events and liver tumors in male and female F344 rats exposed to carbon tetrachloride vapor for 13 and 104 weeks (6 hours/day, 5 days/week)

Key event (time ➔)						
Exposure level [a] (ppm)	Metabolism & formation of •O-O-CCl_3 (immediate and ongoing)	13 weeks		104 weeks		Liver tumors (104 weeks)
		Hepato-toxicity [c]	Regeneration and proliferation [d]	Hepato-toxicity [c]	Regeneration and proliferation [d]	
5 (0.9)	+ [b]			—	—	—
10 (1.8)	+ [b]	+/— [e]	—			
25 (4.5)	+ [b]			+	—	+/— [f]
30 (5.4)	+ [b]	+	+			
90 (16.1)	+ [b]	+	+			
125 (22.3)	+ [b]			+	+	+
270 (48.2)	+ [b]	+	+			
810 (145)	+ [b]	+	+			

[a] The exposure concentration in parentheses is the concentration adjusted to continuous exposure (i.e., multiplied by 5/7 x 6/24)
+ [b] = Studies demonstrating key event were not conducted as part of the JBRC 13- and 104-week bioassays. Based on data from acute exposure and in vitro studies (Avasarala et al., 2006; Zangar et al., 2000; Raucy et al., 1993), metabolism of carbon tetrachloride to reactive metabolites has been demonstrated and is assumed to occur immediately and continue throughout the duration of exposure to carbon tetrachloride at all exposure levels. Although metabolism to reactive metabolites has been specifically demonstrated at relatively high doses, it can reasonably be assumed that such metabolism would occur at lower exposures.
[c] As indicated based on histopathological findings, including fatty change, fibrosis, cirrhosis, and/or necrosis.
[d] As indicated based on histopathological findings, including proliferation and hyperplasia (and in the 13-week study, mitosis).
[e] An increased incidence of fatty change was observed that was not statistically significant.
[f] The incidence of hepatocellular carcinomas in female 25-ppm rats was not statistically elevated compared to concurrent controls, but did exceed the historical control range for female rats from JBRC (0-2%), and increase that was statistically significant compared to the historical control.
Note: Different exposure concentrations were used in the 13-week and 104-week JBRC bioassays. Blank cells indicate exposure concentrations not tested in either the 13-week or 104-week study.
+ = Evidence demonstrating key event.
— = No evidence demonstrating key event.
+/— = equivocal

Source: Nagano et al., 2007a,b; JBRC, 1998.

4.7.3.2.4. ***Biological plausibility and coherence.*** The theory that sustained cell proliferation to replace cells killed by toxicity or viral or other insults, such as physical abrasion of tissues, can be a significant risk factor for cancer is plausible and generally accepted (Correa, 1996). It is logical to deduce that sustained cytotoxicity and regenerative cell proliferation may result in a

greater likelihood of mutations (whether spontaneous, or directly or indirectly induced by the chemical) being perpetuated, with the possibility of one or more of these resulting in loss of cell cycle control and tumor development. It may also be that continuous stimulus of proliferation by growth factors involved in inflammatory responses (e.g., TGF-α in the hepatic response to carbon tetrachloride) increases the probability that damaged cells may slip through cell cycle checkpoints carrying DNA alterations that would otherwise be repaired. Current views of cancer processes support both possibilities. A high proliferation rate alone is not assumed to cause cancer; tissues with naturally high rates of turnover do not necessarily have high rates of cancer, and tissue toxicity in animal studies does not invariably lead to cancer. Nevertheless, regenerative proliferation associated with persistent cytotoxicity appears to be a risk factor of consequence.

4.7.3.3. *Other Possible Modes of Action*

Section 4.4.2 provides a critical review of the genotoxicity literature for carbon tetrachloride. Various confounding factors and other challenges in evaluating genotoxicity studies are highlighted in Table 4-12; these general features of the carbon tetrachloride literature as well as various methodological and reporting issues in individual studies were taken into account in the current review of the genotoxicity literature. Many of the positive genotoxicity findings, including the following, are consistent with compounds that induce oxidative events leading to genetic damage: (1) two positive mutation/DNA damage studies in *E. coli* strains particularly sensitive to oxidative damage; (2) intrachromosomal recombination induced by carbon tetrachloride in *S. cerevisiae* consistent with DNA breakage originating from oxidative stress that occurs concurrent with cytotoxicity; (3) evidence from in vitro and in vivo assays of DNA breakage and fragmentation in association with extensive hepatotoxicity; and (4) DNA adducts formed from reactive oxygen species and lipid peroxidation products (e.g., MDA and 4-HNE) in the liver of rodents following carbon tetrachloride administration. A limited number of positive genotoxicity findings in the absence of cytotoxicity have been reported (see Section 4.4.2 and Table 4-8 to 4-11); methodological or reporting issues with many of these studies have been identified.

As a whole, the literature suggests that carbon tetrachloride is more likely an indirect than direct mutagenic agent and that mutagenic effects, if they occur, are likely to be generated through indirect mechanisms resulting from oxidative damage stress or lipid peroxidation by-products, which have been observed with cytotoxicity at high doses of carbon tetrachloride (Table 4-16). Nevertheless, uncertainties in this complex database must be acknowledged. To that end, if carbon tetrachloride-associated DNA damage occurs above background levels and contributes to low-dose mutagenic activity, some nonzero risk of carcinogenicity at doses below those associated with cytotoxicity would be predicted. Under this scenario, a quantitative approach that accounts for possible carbon tetrachloride-associated DNA damage in a mode of

action would apply and would dictate an approach consistent with a nonthreshold response (see Section 5.3.2).

Thus, the possible contribution of a low-dose mutagenic effect in the mode of action or alternative modes of action cannot be excluded.

4.7.3.4. *Conclusions About the Hypothesized Mode of Action*

The weight of evidence supports reductive dehalogenation of carbon tetrachloride by CYP2E1, sustained cytotoxicity, and regenerative cell proliferation as key events in the mode of action for carbon tetrachloride-induced tumors of the liver. A wide range of evidence across different species, sexes, and routes of exposure implicates reductive dehalogenation by CYP2E1 as the initial step leading to hepatic toxicity. Hepatocellular cytotoxicity leading to regenerative proliferation and subsequent tumorigenesis as key events have experimental support at high exposure levels in terms of strength, consistency and specificity of association; dose-response concordance; temporal relationship; and biological plausibility and coherence. Considerable empirical evidence provides support for a hypothesis that liver carcinogenicity is presumed to occur at exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response, and that exposures that do not cause hepatotoxicity are not expected to result in liver cancer. This hypothesized mode of action for carbon tetrachloride liver carcinogenicity is consistent with a nonlinear approach to low-dose extrapolation (see Section 5.3.1).

However, the temporal relationship for cytotoxicity, regenerative hyperplasia, and liver tumors in the female mouse (Nagano et al., 2007b) is not consistent with the above mode of action (see Section 4.7.3.2.3 for additional discussion). Furthermore, relatively little mode of action information is available at lower exposure levels (i.e., exposures that are not cytotoxic). Whether carbon tetrachloride-induced biological events occur at low exposures that could lead to increased cancer risk is uncertain. Possible mechanistic events that could be operating at low doses include mutagenicity as a key event or a mode of action in the formation of tumors. These events could be operating at all exposure levels. Currently, information on such events is unavailable at low exposure levels. At higher exposures there are some studies indicating equivocal, or possibly positive, genetic toxicity. Additionally, at high exposures both the cytotoxicity-based mode of action and the mutagenicity-based mode of action may be operative, but it is not possible to delineate the contribution of these possible mode(s) of action to carbon tetrachloride tumor response. It is important to establish the presence of mutational events following carbon tetrachloride treatment, and the mechanistic role such events may play in tumor response, especially at low exposure levels. Thus, alternative nonthreshold approaches (i.e., low-dose linear extrapolation procedures) to carbon tetrachloride carcinogenicity have also been considered (see Section 5.3.2).

In summary, biological support exists for a hypothetical mode of action involving

metabolism of carbon tetrachloride by CYP2E1, sustained cytotoxicity, and regenerative cell proliferation as a major mode of action driving the steep nonlinear increase in liver tumor dose-response at relatively high carbon tetrachloride exposures. Inconsistencies and uncertainties at the low end of the experimental exposure range suggest that other (or another) mode(s) of action that are independent of cytotoxicity and regenerative cell proliferation may be operative in this range.

4.7.3.5. *Relevance of the Hypothesized Mode of Action to Humans*

Although there is no evidence for hepatic cancer resulting from exposure to carbon tetrachloride in humans, the potential modes of action are considered relevant to humans. Humans express ethanol-inducible CYP2E1 and phenobarbital-inducible CYP3A in the liver, both of which are associated with the generation of trichloromethyl radical in animals exposed to carbon tetrachloride. The antioxidant systems in animals and humans are similar. Therefore, both the mode of action and the endogenous protective mechanisms likely have related processes in animals and humans. Furthermore, humans exhibit the same signs of liver toxicity that have been observed in animal studies (cirrhosis, fibrosis, steatosis, necrosis, and liver enzyme changes). Finally, the types of tumors, hepatocellular adenoma and carcinoma, expressed consistently in several animal species exposed to carbon tetrachloride are also found in humans.

4.7.4. Mode of Action Information for Pheochromocytomas

An increased incidence of pheochromocytomas (a neuroendocrine tumor of adrenal chromaffin cells) associated with carbon tetrachloride administration has been observed in male and female mice by oral (NCI, 1977, 1976a,b; Weisburger, 1977; NTP, 2007) and inhalation exposure (Nagano et al., 2007b; JBRC, 1998), but not in rats by either route of exposure. The mode of action by which carbon tetrachloride induces pheochromocytomas in mice is not known. Unlike liver tumors, it is not known whether the parent compound or an active metabolite is responsible for tumor induction, and none of the key events in the development of carbon tetrachloride-induced pheochromocytomas has been elucidated. In general, few chemicals have been reported to cause pheochromocytomas in mice. Of 514 technical reports published by the National Toxicology Program (NTP), only seven chemicals have been associated with pheochromocytomas in mice with no apparent common denominator (Tischler et al., 2004; Hill et al., 2003).

In the absence of any information on mode of action for carbon tetrachloride induction of pheochromocytomas, the framework for evaluating a hypothesized mode(s) of action as described in U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) has not been applied.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

Age (e.g., childhood, senescence), gender, nutritional status, disease status, and exposure to other chemicals are all factors that might influence susceptibility to carbon tetrachloride. Each of these factors is described below in more detail.

4.8.1. Possible Childhood Susceptibility

Key events leading to carbon tetrachloride-induced liver toxicity and carcinogenicity (e.g., metabolism to trichloromethyl radical by CYP2E1 and subsequent formation of trichloromethyl peroxy radical, cytotoxicity, and sustained regenerative and proliferative changes in the liver in response to hepatotoxicity; or possibly, DNA damage and fixation leading to mutagenic activity) involve metabolic and cellular processes common to cells at all life stages, and are therefore considered plausible in the developing organism. For these key events, limited data are available to evaluate the relative susceptibility of children to carbon tetrachloride.

As observed in adult animals, the initiating event for liver toxicity and carcinogenicity is metabolism of carbon tetrachloride by CYP2E1 to reactive metabolites. Assuming that this is the initiating key event in the mode of action for all age groups, susceptibility to carbon tetrachloride at all life stages is related to the presence of functional microsomal enzymes (particularly CYP2E1 but also CYP3A). Hepatic concentrations of CYP2E1 do not achieve adult levels until sometime between 1 and 10 years, although large increases in hepatic CYP2E1 protein occur postnatally between 1 and 3 months in humans (Vieira et al., 1996). Thus, age-related differences in CYP450, as described below, could potentially affect susceptibility. To the extent that hepatic CYP2E1 levels are lower, infants and children would be less susceptible to free radical-induced liver injury from carbon tetrachloride than adults. There is some evidence from the therapeutic drug literature that CYP3A levels also change with age, but in a pattern different from CYP2E1. Based on half-life results for several therapeutic drugs metabolized by the CYP3A family (Ginsberg et al., 2002), enzyme levels appeared to be lower than the adult up to 2 months of age, but from 6 months to 2 years of age were significantly higher than the adult. To the extent that CYP3A levels are relatively higher than the adult and CYP3A plays a significant role in carbon tetrachloride metabolism, infants and young children could be relatively more susceptible to liver injury from carbon tetrachloride. Work conducted by Zangar et al. (2000), however, suggests that CYP2E1 is the major human enzyme in the adult responsible for carbon tetrachloride bioactivation at lower, environmentally relevant levels (i.e., levels that are not hepatotoxic). Only at higher carbon tetrachloride levels, CYP3A and possibly other CYP450 forms may contribute to carbon tetrachloride metabolism. Therefore, assuming CYP2E1 is the more effective metabolizing enzyme in children as it is in adults at environmentally-relevant exposure levels, infants and children would likely be less susceptible to liver injury from carbon tetrachloride than adults to the extent that hepatic CYP2E1 levels are lower. Carbon tetrachloride-specific enzyme data for younger populations are not available, however, to confirm these assumptions.

There is no information to suggest that key events subsequent to metabolism of carbon tetrachloride would exhibit age-dependence.

Low levels of CYP2E1 mRNA begin to be elevated in human fetal brains after week 7 and increase thereafter to week 16 (the oldest stage examined) (Brzezinski et al., 1999). CYP2E1 function was analyzed in prenatal human brain and liver tissues (7 to 17 weeks of gestation) using three assays (Boutelet-Bochan et al., 1997). Low levels of CYP2E1 expression were detected in fetal brain tissue, with some evidence for increasing expression at later stages of gestation; weaker levels were identified in fetal liver. In fetal brain, CYP2E1 was not detected with the less sensitive assay (Northern blot), and expression measured with the two more sensitive assays (RT-PCR and RNase protection assays) were considerably weaker than those measured in adult human or rat liver samples. The results suggested that, during gestation weeks 8–17, the fetal brain might be more vulnerable than the liver to toxic effects from exposure to carbon tetrachloride. Carpenter et al. (1996) detected functional CYP2E1 in human fetal livers at 19 weeks of gestation. However, when related to weight unit of microsomal protein, the CYP2E1 content of fetal livers was considerably lower than in adults. In an in vitro experiment, exposure to ethanol or clofibrate induced expression of CYP2E1 in hepatocytes from a 20-week fetus, which suggests that maternal alcohol intake might enhance CYP2E1 in the human fetus. Given that the maternal liver mass and hepatocellular CYP2E1 content are so much higher than the fetal values, it would seem that fetuses would have only a slight vulnerability from maternal exposure to carbon tetrachloride at low levels. Because for inhalation exposures the arterial blood flow does not perfuse the liver before reaching the fetus, this observation may apply more to oral exposures than to inhalation exposures.

An unknown factor in fetal vulnerability is the expression of CYP450 in the placenta. Two different laboratories have detected CYP2E1 in human placentas. Hakkola et al. (1996) detected several different enzymes in human placentas, including CYP2D6, CYP4B1, and several forms of CYP3A and CYP2E1; there was considerable variation in expression among the different individuals. Rasheed et al. (1997) compared the levels of CYP2E1 protein in western immunoblots of microsomes taken at delivery from placentas of 12 African-American women. None of the women who abstained from ethanol had detectable levels of placental CYP2E1, whereas the protein was detectable in blots for 6/8 drinkers. The median head circumference at birth was significantly smaller (33.2 cm) in children with detectable CYP2E1 compared with those without detectable enzyme (37 cm, $p = 0.04$). The study provides suggestive evidence that placental CYP2E1 is inducible by alcohol consumption, although there are individual variations. Theoretically, fetuses of mothers who drink ethanol would be potentially more susceptible to injury from carbon tetrachloride exposure.

Carpenter et al. (1996) measured the amount and activity of CYP2E1 in fetal (GD 20) and maternal rat liver and brain, following maternal exposure to a 5% ethanol diet. Rates of metabolism for chlorzoxazone and N-nitrosodimethylamine were used to evaluate functional

activity of CYP2E1. In untreated or pair-fed rats, the amount of CYP2E1 in maternal or fetal brain was several hundred-fold lower than in the respective livers. Ethanol exposure increased the level of CYP2E1 protein by 1.4-fold in the maternal liver and 2.4-fold in the fetal liver compared with the untreated or pair-fed groups but had no effect on CYP2E1 levels in maternal or fetal brain. Hepatic CYP2E1 function, as exemplified by chlorzoxazone 6-hydroxylation, was elevated 2.1-fold in ethanol-exposed maternal liver but not significantly in fetal liver. Demethylation of N-nitrosodimethylamine was elevated about 1.5-fold in maternal and fetal livers after ethanol exposure. Cambon-Gros et al. (1986) demonstrated the formation of trichloromethyl radicals in maternal and fetal rat liver exposed to carbon tetrachloride on GD 20. The results of these studies suggest that maternal ethanol ingestion might increase the susceptibility of fetuses to hepatotoxicity from exposure to carbon tetrachloride.

Developmental studies in rats demonstrated that total litter loss was the primary effect of maternal exposure between GDs 6 and 15 (Narotsky et al., 1997b; Narotsky and Kavlock, 1995). The mode of action for developmental effects has not been explored, so it is unknown whether placental expression of CYP2E1 may contribute to the litter loss, as CYP2E1 contributes to liver cytotoxicity.

While some information is available on the activity of enzymes involved in the metabolism of carbon tetrachloride in children, little lifestage-specific information on the levels of antioxidants (e.g., GSH) was identified.

In summary, there is no direct evidence for increased or decreased susceptibility to carbon tetrachloride in children. The relatively lower activity of CYP2E1 (the major human enzyme responsible for carbon tetrachloride bioactivation at environmentally-relevant exposure levels) in infants and children compared to adults suggests the possibility of lower susceptibility to carbon tetrachloride-induced liver injury for younger life stages. Too little is known, however, about changes in activity of other enzyme levels with age to support a conclusion that children are at decreased risk. CYP3A levels are higher in children 6 months to 2 years than in adults (although CYP3A is less likely to contribute to carbon tetrachloride metabolism at environmentally-relevant exposure levels than CYP2E1). Further, little lifestage-specific information on the levels of antioxidants (e.g., GSH), another factor likely to contribute to susceptibility to carbon tetrachloride toxicity, is available. No information is available to support an evaluation of differences in childhood susceptibility to possible effects of carbon tetrachloride on the adrenal medulla (as suggested by the increased incidence of pheochromocytomas in mice).

4.8.2. Possible Effects of Aging

The overall vulnerability to carbon tetrachloride is affected directly by the rate of generation of reactive intermediates, a function of microsomal CYP activity, and inversely by the antioxidant content. Compared with young/mature adults, older organisms exhibit changes,

usually decreases, in these parameters that vary independently in different tissues.

Studies evaluating the capacity for drug metabolism in the human liver during different life stages reported a reduction in activity for CYP3A3/4 and CYP2E1 in the elderly (i.e., individuals older than 65 years) (reviewed in Tanaka, 1998). Total immunoreactive CYP3A protein (the sum of CYP3A4 and CYP3A5) per mg hepatic microsomal protein was significantly reduced by 90% in samples from men aged 61–72 (n = 5) compared with those from men aged 21–40 years (n = 5) (Patki et al., 2004). McLean and Le Couteur (2004) suggested that the reduction in phase I enzyme activity may be related not to deficits intrinsic to the liver microsomal monooxygenase systems, but rather to structural changes in the liver with age (e.g., thickening and defenestration of the sinusoidal endothelium of the liver) that may reduce oxygen availability for phase I enzymes that are directly dependent on oxygen supply as a substrate.

Studies in experimental animals also provide evidence of age-related changes in CYP activity. Although no significant age-related variations in hepatic CYP2E1 mRNA content were noted in adult (18-month-old) male Wistar rats compared with 8-month-old rats, CYP2E1 microsomal protein levels were 20% reduced (not statistically significant), and CYP2E1 activity (assayed as chlorzoxazone oxidation) was significantly reduced by 46% in the older group (Wauthier et al., 2004); this study found no age-related changes for hepatic CYP3A1, 3A2, 3A9, or 3A23 mRNA or protein levels in rats. Wauthier et al. (2004) attributed age-related reductions in hepatic CYP2E1 activity to posttranslational modifications, possibly from the reactive oxygen species commonly generated by this CYP. A photoperiodicity study reported that increases in hepatic CYP3A-dependent erythromycin N-demethylase activity, which is elevated after Wistar rats are exposed to a dark cycle, were twofold lower in the livers of 22-month-old rats compared with 10-week-old rats (Martin et al., 2003). Total immunoreactive CYP3A content was reduced in the hepatic microsomes of 2-year-old compared with 1-year-old male CD-1 mice and was associated with a reduced clearance of the substrate midazolam (Warrington et al., 2000). These results suggest that the metabolism of carbon tetrachloride would be slower in the liver of old compared with younger organisms.

Warrington et al. (2004) compared age-related changes in microsomal CYP3A and NADPH-reductase in the liver and kidney in male F344 rats at 2–4 months (young), 13–14 months (intermediate), and 25–26 months (old). Expression of CYP3A protein in the kidney was only 1% of that in the liver. The net CYP3A content of the liver was significantly reduced in old rats compared with young or intermediate rats and involved both immunodetectable bands in western blots. Conversely, a 50% increase in one isoform of CYP3A was detected in the kidneys of old rats compared with the intermediate group; an 11% net increase in renal CYP3A was not statistically significant. Age-related decreases (by 23–36%) in the expression of NADPH-reductase occurred in the liver and kidney of male F344 rats, but compared with that in young rats the decline was statistically significant only in the liver of old rats (Warrington et al., 2004). The results of this study suggest that the capacity to initiate the metabolism of carbon

tetrachloride is reduced in the liver but possibly increased in the kidney of older organisms compared with younger animals.

Antioxidant content is also reduced in aging animals compared with younger life stages. Hepatic GSH content was 35% lower in 24–28-month-old male F344 rats compared with 2–5-month-old rats (Suh et al., 2004); the decline was related to significant decreases in the level and activity of γ -glutamylcysteine ligase (GCL), the rate-controlling enzyme in the synthesis of GSH. The ultimate reduction in enzyme activity in old rats was related to an age-related decrease in a transcription factor, nuclear factor erythroid-related factor 2, that governs the expression of GCL (Suh et al., 2004). In the liver of 18-month-old male Wistar rats, the GSH content was significantly reduced by 34% compared with 8-month-old rats, and the level of TBARS was increased by 287% compared with that in 3-month-old rats (Wauthier et al., 2004). One study reported a significant age-related reduction in GSH peroxidase activity in the kidney, but not the liver, of 24-month-old male F344 rats compared with 6-month-old rats (Tian et al., 1998). Significant decreases in GSH (~20% and ~15%), GSH peroxidase activity (~59% and ~37%), and increases in TBARS (+54% and +23%) were noted, respectively, in the liver and kidney of 22-month-old Wistar rats compared with those of 10-week-old animals (Martin et al., 2003). These studies suggest that older animals are at greater risk than younger animals of oxidative damage following exposure to carbon tetrachloride. Studies vary as to whether the age-related changes are more significant in the kidney or liver, possibly because of strain differences.

In general, aging is associated with constriction of the kidney arterioles and reduced renal blood flow as well as with reductions in kidney mass and the number of functioning nephrons (U.S. EPA, 2001b). The result of these changes is a decrease in glomerular filtration rate. Because of their reduced glomerular function, aged adults are likely to be more sensitive than younger adults to a chemical, such as carbon tetrachloride, that targets the glomerulus. The manifestations of renal disease in 2-year-old rats that had been exposed to high concentrations of carbon tetrachloride in air for most of their lifetimes were increased severity of glomerular lesions associated with aging (progressive glomerulonephrosis) and impaired glomerular function (decreased glomerular filtration rate, as indicated by increases in serum levels of BUN, creatinine, and inorganic phosphorous) in comparison with untreated concurrent controls.

Whether older populations would likely be more susceptible to carbon tetrachloride toxicity is difficult to determine. Evidence for a reduction in CYP3A and CYP2E1 activity in the liver with age would suggest an age-related reduction in the generation of reactive metabolites from carbon tetrachloride and possibly a corresponding reduction in susceptibility; however, evidence for reduction in antioxidant content in aging animals would result in a relative increased risk of oxidative damage in older animals. Functional changes in the kidney with age and increases in kidney CYP3A activity (as suggested by experimental animal studies) indicate that older populations may be at greater risk of carbon tetrachloride-associated kidney damage.

4.8.3. Possible Gender Differences

The extent to which men and women differ in susceptibility to carbon tetrachloride toxicity is not known. No human data are available to suggest there are gender differences in the toxicity or carcinogenicity of carbon tetrachloride.

Animal subchronic and chronic toxicity studies by the oral or inhalation route did not report any significant gender differences in susceptibility to cancer or noncancer effects from carbon tetrachloride. One study in rats exposed by i.p. injection measured a 2.5-fold increase in the serum level of hepatic enzymes, a longer period of hepatic injury, and more evidence of hepatic regeneration in females compared with males (Moghaddam et al., 1998); male livers had 20% more CYP2E1 activity than female livers. The significance of this observation is uncertain, given the modest difference and the absence of other corroborating data. There appears to be no basis for assuming gender differences in susceptibility.

4.8.4. Nutritional Status

Fasting or food deprivation has been shown to increase the toxicity of carbon tetrachloride, as demonstrated by histopathology of the liver, increased serum enzyme levels, or increased generation of chloroform (Qin et al., 2007; Seki et al., 2000; Shertzer et al., 1988; Sato and Nakajima, 1985; Pentz and Strubelt, 1983; Yoshimine and Takagi, 1982). Decreasing levels of GSH have been detected in food-restricted animals (Gonzalez-Reimers et al., 2003; Harris and Anders, 1980; Nakajima and Sato, 1979). The basis for the increased toxicity caused by fasting is the increase in lipolysis, which generates acetone, an inducer of CYP2E1 (Bruckner et al., 2002). Bruckner et al. (2002) established that a circadian rhythmicity of vulnerability to carbon tetrachloride in rats was based on the increased levels of acetone that occur during overnight fasting. Peak levels of serum SDH, ALT, and isocitrate dehydrogenase were significantly higher in fasted rats than in fed rats (for example, peak SDH levels were seven times higher with fasting). Fasted rats also showed significantly more covalent binding of radiolabeled carbon tetrachloride to microsomal protein and significantly higher CYP2E1 activities.

Carbon tetrachloride toxicity is also affected by the level of antioxidants in the diet. Rats fed a diet low in vitamin E, methionine, and selenium (a cofactor for GSH reductase) showed an increase in lipid peroxidation and liver damage that was reversed by supplementing the diet with one or more of the antioxidants (Parola et al., 1992; Sagai and Tappel, 1978; Hafeman and Hoekstra, 1977; Taylor and Tappel, 1976). Addition of vitamin A (retinoic acid or retinol) to basal diet reduced the hepatic effects of carbon tetrachloride in mice (Rosengren et al., 1995; Kohno et al., 1992), although it had the opposite effect in rats (Badger et al., 1996; El Sisi et al., 1993a, b).

Dietary mineral content can also be important. Rats fed a diet deficient in zinc showed an increase in hepatotoxicity from carbon tetrachloride (DiSilvestro and Carlson, 1994). Cabre

et al. (2000) assessed the time course of hepatic lipid peroxidation and GSH metabolism in Wistar rats injected with 0.5 mL of carbon tetrachloride to induce hepatic cirrhosis. Inclusion of zinc in the diet delayed the appearance of cirrhosis and prevented the rise in lipid peroxides. The protective effect of zinc was independent of GSH levels, which were reduced by carbon tetrachloride.

4.8.5. Disease Status

Based on experimental findings from rodent studies, there is some reason to suspect that people with diabetes may have altered susceptibility to hepatotoxic effects from carbon tetrachloride. Studies in rats have found that rats made diabetic by pretreatment with the diabetogenic agents alloxan or streptozotocin display markedly enhanced hepatotoxicity in comparison with nondiabetic rats (Sawant et al., 2007, 2004; Watkins et al., 1988; Hanasono et al., 1975). The relevance of this finding to humans is uncertain, although it has been reported that diabetics have nearly twofold higher risk of acute liver failure due to drug-induced toxicities and chronic liver disease (Sawant et al., 2007). Streptozotocin-induced diabetes not only failed to enhance the hepatotoxicity of carbon tetrachloride but actually protected against lethality of the compound in mice (Shankar et al., 2003; Gaynes and Watkins, 1989).

There has been some investigation of the mechanism by which diabetes potentiates carbon tetrachloride hepatotoxicity in rats. Diabetic rats do not gain weight as normal rats do, raising the possibility that the enhanced toxicity in diabetic rats is a result of associated starvation (see Section 4.8.4). However, data for a pair-fed control group in the Hanasono et al. (1975) study showed that the restriction in food intake could account for only a small portion of the observed hepatotoxicity in diabetic Sprague-Dawley rats. [Diabetes was induced by treatment with alloxam monohydrate or streptozotocin.] Treatment of diabetic rats with insulin controlled the diabetic state and prevented any enhancement of carbon tetrachloride hepatotoxicity in these rats (Watkins et al., 1988; Hanasono et al., 1975), suggesting the diabetic state and not the presence of inducer chemicals potentiates carbon tetrachloride hepatotoxicity. Serum glucose levels in the diabetic rats were not sensitive predictors of the extent of hepatotoxicity in the Hanasono et al. (1975) study (e.g., 40 mg alloxan and 65 mg streptozotocin produced similar plasma glucose levels, but the increase in serum ALT associated with carbon tetrachloride treatment was twofold higher in the latter experiment), suggesting that other metabolic effects of diabetes are more important to the effect on carbon tetrachloride toxicity.

Because ketones and compounds metabolized to ketones have been found to potentiate the toxicity of carbon tetrachloride and other haloalkanes (see Section 4.8.6), presumably by enhancing expression of CYP2E1 leading to increased activation of the hepatotoxicant, it has been suggested that ketosis associated with diabetes might be responsible for the observed effect (Hewitt et al., 1980). However, there are several lines of evidence suggesting that ketonemia and increased bioactivation may not be the critical features of diabetes leading to enhanced toxicity

of carbon tetrachloride. In the study by Hanasono et al. (1975), alloxan and streptozotocin both potentiated carbon tetrachloride-induced hepatotoxicity, even though alloxan-induced diabetes in rats is characterized by a marked persistent increase in ketone bodies and streptozotocin-induced diabetes is not. Both alloxan and streptozotocin have been reported to decrease CYP450 activity (Watkins et al., 1988; Hanasono et al., 1975). Sawant et al. (2004) found no effect on hepatic microsomal CYP2E1 levels or activity, lipid peroxidation, GSH, or covalent binding of carbon tetrachloride in the liver in rats with streptozotocin-induced diabetes. Time course studies performed by Sawant et al. (2004) found that the initial liver injury produced by carbon tetrachloride in diabetic rats was similar to that in nondiabetic rats but that the effect progressed only in the diabetic rats. Sawant et al. (2007) reported that liver injury initiated by non-lethal doses of carbon tetrachloride progressed to hepatic failure and death of diabetic Sprague-Dawley rats because liver cells failed to advance from G_0/G_1 to S-phase, thereby unabling S-phase DNA synthesis (a critical step in cell division) and inhibiting tissue repair. A more detailed understanding of the mechanism would be needed to predict how diabetes might affect carbon tetrachloride toxicity in humans.

4.8.6. Exposure to Other Chemicals

Factors that increase the expression of CYP2E1 or CYP3A are likely to increase susceptibility to carbon tetrachloride exposure (all other things being the same) because the relatively higher rate of metabolism on a per cell basis would significantly increase the rate of generation of trichloromethyl radicals in the liver and kidney. Heavy consumers of ethanol, which induces CYP2E1, are therefore more vulnerable to carbon tetrachloride (Manno et al., 1996). Manno et al. (1996) described case reports of two workers who consumed 120 or 250 grams of ethanol per day and were the only individuals to develop severe hepatonephrotoxicity following a 2-hour exposure to carbon tetrachloride vapors used in a fire extinguisher (Manno et al., 1996); their nonsymptomatic colleagues, who also were exposed, consumed less than 50 grams of ethanol per day. Cases of acute carbon tetrachloride poisoning often involved individuals who were alcohol consumers (New et al., 1962). Enhanced toxicity from concomitant or preceding ethanol consumption and exposure to carbon tetrachloride has been verified in animal studies (Wang et al., 1997; Plummer et al., 1994; Hall et al., 1991; Ikatsu et al., 1991; Kniepert et al., 1990; Reinke et al., 1988; Sato and Nakajima, 1985; Strubelt, 1984; Teschke et al., 1984; Harris and Anders, 1980; Sato et al., 1980).

Potentiation of carbon tetrachloride hepatotoxicity has also been observed following exposure to other chemical inducers of CYP450, including isopropanol which converts to acetone (Rao et al., 1996; Folland et al., 1976; Traiger and Plaa, 1971), methanol (Allis et al., 1996; Harris and Anders, 1980), 2-butanol (Traiger and Bruckner, 1976), tert-butanol (Ray and Mehendale, 1990; Harris and Anders, 1980), and other aliphatic alcohols (Ray and Mehendale, 1990); acetone, methyl ethyl ketone, methyl isobutyl ketone, 2-butanone, and other ketones

(Raymond and Plaa, 1995; Charbonneau et al., 1986; Pilon et al., 1986; Plaa and Traiger, 1972); phenobarbital (Abraham et al., 1999; Sundari et al., 1997; Hocher et al., 1996; Cornish et al., 1973; Garner and McLean, 1969); DDT (McLean and McLean, 1966); polychlorinated and polybrominated biphenyls (Kluwe et al., 1979); and mirex and chlordecone (Soni and Mehendale, 1993; Kodavanti et al., 1992; Mehendale, 1992, 1991, 1990; Bell and Mehendale, 1987, 1985; Curtis et al., 1979). Coexposure to nicotine in drinking water also increased hepatic effects of carbon tetrachloride, although this was thought to be because of a synergistic effect on lipid peroxidation produced by both chemicals rather than induction of CYP450 (Yuen et al., 1995).

There is also limited evidence for a reduction in carbon tetrachloride hepatotoxicity associated with reduced bioactivation of the chemical. Coexposure to carbon tetrachloride and carbon disulfide both in rats and human workers resulted in hepatic and neurological effects associated with carbon disulfide but no effects characteristic of carbon tetrachloride (Peters et al., 1987; Seawright et al., 1980). The researchers attributed this result to destruction of CYP450 by carbon disulfide and reduced bioactivation of carbon tetrachloride. Pretreatment with lead nitrate reduced the hepatotoxicity of carbon tetrachloride, apparently because of the ability of lead to inhibit CYP450 (Calabrese et al., 1995).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

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

Epidemiological studies of long-term exposure to carbon tetrachloride are inadequate to establish whether an association exists between oral exposure and adverse birth outcomes (the only health outcome evaluated in these studies). Case reports of human poisoning identify the liver and kidney as primary target organs of acute carbon tetrachloride exposure, but do not provide data useful for dose-response analysis.

Several subchronic oral toxicity studies, including Bruckner et al. (1986), Condie et al. (1986), Hayes et al. (1986) and Allis et al. (1990), provide liver toxicity data that was considered for dose-response analysis. Hayes et al. (1986) and Allis et al. (1990) reported liver toxicity at the lowest dose tested (i.e., a NOAEL was not identified) and thus are less suitable for defining a point of departure (POD) for the RfD. Further, in the Hayes et al. (1986) study, which included both a vehicle (corn oil) and untreated control group, the vehicle controls themselves had significantly elevated serum enzyme levels, altered organ weights, and increased incidence of liver necrosis. This type of corn oil vehicle response was not seen in other studies. The Allis et al. (1990) protocol also provided data less amenable to dose-response analysis. Male rats were sacrificed in groups of six at various time points after exposure was terminated (1, 3, 8, and 15 days), and results at these various time points could not be combined.

Subchronic gavage studies by Bruckner et al. (1986) in male rats and Condie et al. (1986) in male and female mice provided the best available characterizations of the dose response for ingested carbon tetrachloride at low doses. Bruckner et al. (1986) identified a NOAEL of 1 mg/kg and a LOAEL of 10 mg/kg in rats treated by gavage in corn oil, while Condie et al. (1986) identified a NOAEL of 1.2 mg/kg and a LOAEL of 12 mg/kg in similarly treated mice. In both studies, the LOAEL of 10–12 mg/kg (average daily dose of 7–9 mg/kg-day) produced hepatotoxicity, indicated by increased serum activity of enzyme markers of liver damage and direct histopathological determination of liver lesions. More marked effects on the liver were found at higher doses in both studies. Liver effects were also observed in numerous other studies in animals. The LOAELs from Bruckner et al. (1986) and Condie et al. (1986) are consistent with the LOAELs from Hayes et al. (1986) [12 mg/kg-day] and Allis et al. (1990) [14.3 mg/kg-day].

5.1.2. Methods of Analysis—Including Models

The most sensitive endpoints identified for effects of carbon tetrachloride by oral exposure relate to liver toxicity in the subchronic corn oil gavage studies of Bruckner et al. (1986) in male rats and Condie et al. (1986) in male and female mice. Sensitive endpoints in

both studies were evaluated for suitability for benchmark dose (BMD) modeling. For suitable data sets, BMD modeling methodology (U.S. EPA, 2000c, 1995) was used to analyze the data.

The Bruckner et al. (1986) study identified serum enzyme changes and liver histopathology as the most sensitive endpoints for carbon tetrachloride. Serum chemistry data from Bruckner et al. (1986) are presented in Table 5-1. Of the enzymes monitored, only SDH shows a clear statistically and biologically significant increase in the 10 mg/kg dose group. The data for the 10- and 12-week blood draws are similar. The 10-week data were used for BMD modeling because the precise group sizes were not known for the 12-week data (a range of 7–9 rats per group was reported).

Table 5-1. Serum enzyme data in male rats after 10- or 12-week exposure to carbon tetrachloride

Daily dose (mg/kg-day)	SDH (IU/mL)[a]		OCT (nmol CO_2/mL)[a]		ALT (IU/mL)[a]	
	10 weeks	12 weeks	10 weeks	12 weeks	10 weeks	12 weeks
0	3.5 ± 0.4	3.2 ± 0.4	28 ± 8	45 ± 4	18 ± 1	20 ± 0.3
1	2.3 ± 0.6	1.9 ± 0.1	23 ± 3	61 ± 12	20 ± 1	19 ± 1
10	7.6 ± 2.5[b]	8.7 ± 2.0[b]	55 ± 10	69 ± 16	23 ± 1	27 ± 2[b]
33	134.8 ± 15.0[b]	145.7 ± 57.9[b]	148 ± 48[b]	247 ± 31[b]	617 ± 334	502 ± 135[b]

[a]Values presented are mean standard error for groups of five rats at 10 weeks and seven to nine rats at 12 weeks.
[b]$p<0.05$.

Source: Bruckner et al., 1986.

All of the models for continuous data in U.S. EPA's BMD software (BMDS) (version 1.4.1) (U.S. EPA, 2007) were fit to the 10-week SDH data. An increase in SDH activity two times the control mean, representing an increase in serum enzyme level considered to be biologically significant, was used as the benchmark response (BMR). Several expert organizations, particularly those concerned with early signs of drug-induced hepatotoxicity, have identified an increase in liver enzymes compared with concurrent controls of two to fivefold as an indicator of concern for hepatic injury (EMEA, 2006; Boone et al., 2005; FDA Working Group, 2000). Dr. James Bruckner, University of Georgia and principal investigator of the study used to derive the RfD, considered a twofold increase in SDH to be an indication of a toxicologically significant response (personal communication, November 7, 2006, with Susan Rieth, U.S. EPA). Because ALT is the liver enzyme that is generally measured clinically, most expert organizations similarly focus on ALT as an indicator of liver injury in preclinical (animal) studies. Because SDH, like ALT, is one of the more specific indicators of hepatocellular damage in most animal species and generally parallels changes in ALT in toxicity studies where liver

injury occurs, a similar twofold increase in SDH is considered indicative of liver injury in experimental animals.

BMD modeling results are summarized in Appendix B. The 3rd degree polynomial and power models provided adequate fits of the 10-week SDH data (based on a goodness-of-fit p-value of ≥0.1). The power model provided the better fit of the data (based on the lower AIC value) and therefore was selected as the basis for a candidate POD; this model estimated a BMD_{2X} of 7.32 mg/kg-day and $BMDL_{2X}$ of 5.46 mg/kg-day.

BMD modeling was also performed using the 10-week OCT and ALT data from Bruckner et al. (1986). OCT data could not adequately be fit by the models available in BMDS. The power model provided an adequate fit of the 10-week ALT data; however, as shown in Table 5-1, the standard error of the mean ALT for the high-dose (33 mg/kg-day) male rats was extremely large (617 ± 334). Bruckner et al. (1986) noted: "There was a pronounced rise in GPT [ALT] at 10 and 12 weeks. Scrutiny of values of individual animals revealed that dramatic increases in two rats at each time point were largely responsible for the late increase in GPT [ALT] activity." In light of the large variation in response at 33 mg/kg-day, using the ALT data set for quantitative analysis was not considered appropriate.

Condie et al. (1986) also reported liver enzyme changes in carbon tetrachloride-exposed mice; however, the median of 8 to 12 determinations was reported without a standard error (SE) or standard deviation (SD) (only the minimum and maximum of the range were reported). Without a mean and SE or SD, BMD analysis cannot be performed.

Liver lesion incidence data from the Bruckner et al. (1986) study in male rats and the Condie et al. (1986) study in male and female mice support a nonlinear induction of hepatic lesions due to carbon tetrachloride somewhere below 10–12 mg/kg. Table 5-2 presents liver pathology data from the Bruckner et al. (1986) study. Data were displayed as mean severity scores. Incidence data were not presented directly, although it can be inferred that incidence was 0% where severity is 0. In addition, a statement in the text implied that incidence was 100% for lipid vacuolation in the 10 mg/kg dose group.

Table 5-2. Severity of liver lesions in male rats after 12-week exposure to carbon tetrachloride

Daily dose (mg/kg-day)	Lipid vacuolation[a]	Nuclear and cellular pleomorphism[a]	Bile duct hyperplasia[a]	Periportal fibrosis[a]
0	0[b]	0	0	0
1	0	0	0	0
10	3.7[c]	0	0	0
33	4	5.7	4	3.7

[a]Severity graded from 0 (absent) to 8 (severe); values presented are means for groups of 6–7 rats.
[b]Severity score of 0 implies incidence of 0%.
[c]Text reports that "each animal" in this group showed the lesion, implying incidence of 100%.

Source: Bruckner et al., 1986.

It can be seen that lipid vacuolation was the only lesion to occur in the 10 mg/kg group, making this the most sensitive pathology endpoint in the study, and that the incidence (not reported but assumed from the text of the paper) of this lesion increased from 0% at 1 mg/kg to 100% at 10 mg/kg.

In the Condie et al. (1986) study, exposure to carbon tetrachloride by gavage in corn oil or Tween-60 aqueous emulsion produced a variety of liver lesions (hepatocellular vacuolization, inflammation, hepatocytomegaly, necrosis, portal bridging fibrosis) in male and female mice at the high dose of 120 mg/kg. However, only necrosis (minimal to mild) in males and hepatocytomegaly (severity unranked) in males and females treated using corn oil vehicle occurred with statistically elevated incidence in the 12 mg/kg dose group. Incidence data for these lesions, which represent the most sensitive effects of carbon tetrachloride in mice, are shown in Table 5-3.

Table 5-3. Incidence of selected liver lesions in mice treated with carbon tetrachloride for 90 days

Sex	Vehicle	Lesion	Incidence at daily dose			
			0 mg/kg-day	1.2 mg/kg-day	12 mg/kg-day	120 mg/kg-day
M	Corn oil	Necrosis	0/10	0/9	9/10[a]	9/10[a]
M	Corn oil	Hepatocytomegaly	0/10	0/9	8/10[a]	10/10[a]
F	Corn oil	Hepatocytomegaly	0/10	0/9	6/10[a]	9/9[a]

[a]$p<0.05$ by Fisher's Exact test conducted for EPA.

Source: Condie et al., 1986.

For all three of these data sets, incidence increased from 0% in the 1.2 mg/kg group to 60–90% in the 12 mg/kg group.

The histopathology data from Bruckner et al. (1986) and Condie et al. (1986) are, therefore, consistent with a POD between 1 and 10 mg/kg in male rats and 1.2 and 12 mg/kg in mice but do not provide sufficient information on response in the vicinity of the BMR (typically 10% for quantal data) (U.S. EPA, 2000c) to objectively inform the shape of the dose-response curve in the region of interest. At the LOAEL in these studies, the response rate was 60 to 100%, whereas the response at the dose below the LOAEL was 0%. The incident data do, however, support the BMD_{2X} of 7.32 mg/kg and $BMDL_{2X}$ of 5.46 mg/kg estimated from the increase in 10-week serum SDH observed in the Bruckner et al. (1986) study.

Serum activity of SDH is widely used in toxicity studies as an indicator of hepatocellular injury. It is a specific and sensitive biomarker of liver damage. SDH is located in the cytosol and mitochondria of liver cells. It is found at low levels in normal serum and erythrocytes. Presence of increased activity in serum indicates leakage from hepatocytes secondary to cell damage. In acute studies with carbon tetrachloride, serum SDH activity was a particularly sensitive indicator of liver toxicity, with increases found at doses similar to, or even lower than, those producing cellular damage visible by light microscopy (Paustenbach et al., 1986b; Korsrud et al., 1972). In the Bruckner et al. (1986) study, the lowest administered dose at which serum SDH activity was increased was also the lowest dose at which liver lesions were observed.

Use of elevated serum SDH activity as the critical effect for derivation of the RfD is supported by results of a study examining the use of serum liver enzymes as predictors of hepatotoxicity (Travlos et al., 1996). The relationship between the activity of serum liver enzymes (ALT, SDH, ALP, and TBA [total bile acids]) and liver histopathology was examined for 50 chemicals and three chemical mixtures using 1-, 2-, 3-, and 13-week clinical chemistry measurements and 13-week histopathology assessments in male and female F344 rats, although carbon tetrachloride was not tested. Treatment-related changes in serum liver enzymes were determined using the Jonksheere-Terpstra trend test at the 0.05 level or Dunn's test at the 0.01 level; serum liver enzyme activities were not reported. An association was observed between treatment-related increases in SDH and ALT activities and the development of histopathological changes to the liver. SDH appeared to be a more sensitive predictor of histopathological changes than ALT, with SDH activity predicting 13-week histopathological changes in rats of both sexes with 76–92% accuracy, compared with 56–83% accuracy for ALT. If both SDH and ALT were elevated, positive terminal histopathological changes were predicted with 100% accuracy from the 2-, 3-, and 13-week clinical chemistry measurements. TBA and ALP were predictive of histopathology results with 20–85% accuracy and 29–82% accuracy, respectively. Based on these findings, statistically significant elevations in serum SDH and ALT activity appear to be sensitive markers for liver toxicity, with SDH predicting histopathological changes to the liver

with higher accuracy than ALT. As shown in Table 5-1, serum liver enzyme activity for SDH in the Bruckner et al. (1986) study was significantly elevated after 10 and 12 weeks of exposure in the mid- and high-dose groups and ALT was significantly elevated in the mid- and high-dose groups after 12-weeks of exposure. In addition, treatment-related histopathologic findings were observed in the mid-dose group (lipid vacuolization), with more extensive findings in the high-dose group (lipid vacuolization, nuclear and cellular pleomorphism, bile duct hyperplasia, and periportal fibrosis) after 12-weeks of exposure. Thus, carbon tetrachloride-induced elevations in SDH and ALT appear to be valid markers of histopathological changes to the liver in the Bruckner et al. (1986) study.

The $BMDL_{2X}$ of 5.46 mg/kg-day estimated from the increase in serum SDH activity in male rats in the Bruckner et al. (1986) study was used as the POD for derivation of the RfD. Use of the modeled BMDL provides an inherent advantage over use of a NOAEL or LOAEL by making greater use of all of the data. The BMDS was able to achieve adequate fit to the SDH data, providing a better estimate of the dose-response relationship for this endpoint than for other endpoints monitored, which were less sensitive and/or had data less suited to dose-response analysis. The BMD results based on SDH are supported by the histopathology data both in rats and mice. Serum SDH activity is a specific and sensitive biomarker of hepatic injury by carbon tetrachloride, comparable to histopathology in terms of sensitivity.

Consideration of PBPK Models for Interspecies Extrapolation

Three PBPK models of oral exposures have been reported; two rat models (Semino et al., 1997; Gallo et al., 1993) and a mouse model (Fisher et al., 2004). These models implement different approaches to simulate the complex kinetics of absorption of carbon tetrachloride that follows an oral gavage dose of carbon tetrachloride in corn oil or emulsifiers (e.g., Emulphor). Oral absorption of carbon tetrachloride in corn oil (and Emulphor) exhibits a pulsatile behavior, evident from multiple peaks of carbon tetrachloride concentrations in blood that occur during the first 12-20 hours following an oral gavage dose (Fisher et al., 2004; Semino et al., 1997; Gallo et al., 1993). Semino et al. (1997) successfully modeled this pulsatile behavior in the rat with a multi-compartment model in which first-order absorption from 6-9 compartments was scheduled at different times following the dose (i.e., absorption was zero until the scheduled activation of each compartment). The scheduling was accomplished using the SCHEDULE commend in ACSL, which cannot be implemented repeatedly; therefore, the implementation is not directly amenable to continuous simulation of multiple exposures. The approach also required calibration of the model against blood concentration kinetics for a specific dose of carbon tetrachloride (e.g., 25 mg/kg). The dose-dependence of the resulting parameter values was not evaluated and, therefore, extrapolation to other dose levels would be highly uncertain. Gallo et al. (1993) successfully simulated the oral absorption of carbon tetrachloride in corn oil with multiple zero-order absorption rates (e.g., μg/hr) that were estimated by fitting to observed blood

carbon tetrachloride kinetics. Although this approach successfully reproduced the blood carbon tetrachloride absorption kinetics following a 25 mg/kg dose to the rat, implementation of this approach would require calibration of the zero-order absorption rates to each data set (i.e., blood kinetics following the dose levels of interest). Fisher et al. (2004) simulated oral absorption of carbon tetrachloride in an aqueous emulsion vehicle (similar to Emulphor) in the mouse with a 2-compartment, 3-parameter model (see Figure 3-2). Rate coefficients were estimated by visually fitting these parameters to blood kinetics following single oral gavage doses of carbon tetrachloride. One of the parameters in the absorption model was varied with dose in order to simulate dose-dependent absorption kinetics; as a result, similar to the Gallo et al. (1993) approach, implementation of the 2-compartment, 3-parameter model would require calibration to blood kinetics for the dose levels of interest.

The above approaches to simulating oral absorption kinetics of carbon tetrachloride were not implemented in the dosimetry analysis of oral bioassay data for two major reasons:
(1) predictions of oral absorption kinetics of carbon tetrachloride would be highly uncertain for doses other than those to which the above models had been specifically calibrated; and
(2) extrapolation of these absorption models to humans also would be highly uncertain. An alternative approach that simulates a time-averaged daily absorption rate and bioavailability might suffice for simulating long-term average blood (arterial) concentrations of carbon tetrachloride that would result from repeated oral exposures to carbon tetrachloride. Estimates of liver metabolism rates would be less certain, however, since carbon tetrachloride is simulated in the PBPK models as a non-linear function of carbon tetrachloride delivery to the liver (i.e., from absorption and from arterial blood). (See additional discussion of PBPK modeling in Section 5.4.2.3.4). As a result, large fluctuations in absorption rate could result in similarly large fluctuations in metabolism rates that may not be accurately represented by simulations of time-averaged rates of absorption.

The $BMDL_{2X}$ of 5.46 mg/kg-day was derived from a study (Bruckner et al., 1986) with an intermittent dosing schedule. In the absence of a suitable PBPK model, the BMDL is adjusted to an average daily dose according to the following equation:

$$\begin{aligned} BMDL_{2X\text{-}ADJ} &= BMDL_{2X} \times 5 \text{ days}/7 \text{ days} \\ &= 5.46 \text{ mg/kg-day} \times 5 \text{ days}/7 \text{ days} \\ &= 3.9 \text{ mg/kg-day} \end{aligned} \tag{5-1}$$

5.1.3. RfD Derivation—Including Application of Uncertainty Factors

An RfD of 0.0039 mg/kg-day for carbon tetrachloride is derived by applying a composite uncertainty factor (UF) of 1000 to the $BMDL_{2X\text{-}ADJ}$ of 3.9 mg/kg-day, as follows:

$$
\begin{aligned}
RfD &= BMDL_{2X\text{-}ADJ}/UF \\
&= 3.9 \text{ mg/kg-day}/1000 \\
&= 0.004 \text{ mg/kg-day} \qquad (5\text{-}2)
\end{aligned}
$$

The composite UF of 1000 includes a factor of 3 ($10^{0.5}$) to extrapolate from a subchronic to chronic duration of exposure, a factor of 10 to protect susceptible individuals, a factor of 10 to extrapolate from rats to humans, and a factor of 3 to account for an incomplete database, lacking an adequate multigeneration study of reproductive function.

- A default 10-fold UF for intraspecies differences was selected to account for variability in susceptibility among members of the human population in the absence of quantitative information on the variability of human response to carbon tetrachloride. Factors that could contribute to a range of human response to carbon tetrachloride were discussed in Section 4.8. Variations in CYP450 levels because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes) could alter susceptibility to carbon tetrachloride toxicity. Individual variability in nutritional status, alcohol consumption, or the presence of underlying disease could also alter metabolism of carbon tetrachloride or antioxidant protection systems. To account for these uncertainties, a factor of 10 was included for individual variability.

- A default 10-fold UF for interspecies extrapolation was selected to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans. Metabolism of carbon tetrachloride to reactive species is the initial key event in the development of carbon tetrachloride toxicity. Also critical to carbon tetrachloride toxicity are cellular antioxidant systems that function to quench the lipid peroxidation reaction, thereby preventing damage to cellular membranes. PBPK models available for carbon tetrachloride were found unsuitable for repeat-dose oral scenarios, and could not be used for interspecies extrapolation. In the absence of data to quantify specific interspecies differences for key events of the mode of action and a suitable PBPK model, a UF of 10 is included.

- A UF of 3 ($10^{0.5}$) for subchronic to chronic extrapolation was selected based on the following: (1) Qualitative information demonstrating that the target of toxicity following chronic oral exposure is the liver. The NCI oral cancer bioassay in rats and mice (NTP, 2007; NCI, 1977, 1976a,b; Weisburger, 1977) did not include an adequate evaluation of low-dose exposures; in rats, there was marked hepatotoxicity at the lowest dose tested, and in mice survival was low in dosed animals because of the high incidence of liver tumors. For these reasons, the bioassay was not suitable for dose-response analysis.

Nevertheless, complete nonneoplastic incidence data available through an NTP (2007) database of neoplastic and nonneoplastic data did not identify carbon tetrachloride-related histopathological changes in any organ systems or tissues other than the liver. Therefore, the NCI bioassay clearly identified the liver as a target organ following chronic exposures, consistent with the findings from subchronic oral studies and subchronic and chronic inhalation studies.

(2) Knowledge of the relationship between effect levels in subchronic and chronic inhalation studies. The JBRC inhalation bioassay, which included 13-week and 2-year inhalation studies in rats and mice (Nagano et al., 2007a,b; JBRC, 1998), provides information on the relationship between NOAELs and LOAELs from subchronic and chronic exposure durations. In the 13-week study, liver toxicity (increased liver weight and fatty liver) was observed in rats and mice at the lowest exposure concentration tested (LOAEL = 2 ppm, duration adjusted). Following chronic exposure, the LOAEL based on liver and kidney effects was 4 ppm (duration adjusted) and the NOAEL was 0.9 ppm (duration adjusted); the LOAEL concentration in the chronic study was, in fact, twofold higher than the LOAEL from the subchronic study. Other subchronic inhalation studies in rats and mice support a NOAEL in the range of 0.9 to 4 ppm (see Table 4-14), which is similar to or within fourfold of the NOAEL from the JBRC chronic inhalation bioassay. Thus, the inhalation data do not support a full default UF of 10.

(3) Early onset of liver toxicity. Cytotoxicity occurs early in the sequence of events. For example, Bruckner et al. (1986) observed increases in liver enzymes and liver cell vacuolization after four days of exposure in an 11-day oral toxicity study, and increases in liver enzymes at week two in a 12-week oral toxicity study. Thus, early appearance of liver toxicity in carbon tetrachloride-exposed animals similarly does not support a full 10-fold UF for subchronic to chronic extrapolation.

- An UF to account for extrapolation from a LOAEL to a NOAEL was not used because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR represented by an increase in SDH activity two times the control mean was selected under an assumption that it represents a minimal biologically significant change.

- A database UF of 3 ($10^{0.5}$) was selected. The oral database for this chemical includes extensive testing for subchronic toxicity in animals, a number of tests of immunotoxic potential, limited chronic oral bioassays in both rats and mice, and limited human data.

Developmental toxicity testing by the oral route has been conducted. Testing for developmental toxicity by two groups of investigators (Narotsky and Kavlock, 1995; Wilson, 1954) found full-litter resorption at doses accompanied by some degree of maternal toxicity, ranging from piloerection to mortality. Because both studies used relatively high doses, neither study identified a NOAEL. The low dose of carbon tetrachloride (25 mg/kg-day) used in Narotsky et al. (1997b) caused neither maternal nor developmental effects when administered in either aqueous or corn oil vehicles, albeit the group sizes (12-14 dams/dose level) were smaller than the group size used in the typical developmental toxicity study. Nevertheless, the NOAEL in this developmental study (25 mg/kg-day) exceeds the POD for the RfD based on liver effects by over 6-fold and the LOAEL (50 mg/kg-day) by 13-fold, and is consistent with developmental toxicity endpoints as less sensitive than measures of hepatotoxicity. Also, as noted in Section 4.8.1 (Possible Childhood Susceptibility), the available life stage information on microsomal enzyme activity, and in particular CYP2E1, suggests that the developing organism would be no more susceptible to free radical-induced liver injury from carbon tetrachloride than adults. The carbon tetrachloride database lacks an adequate multigeneration study of reproductive function by any route of exposure. A database UF of 3 was applied to account for the lack of a multigeneration reproductive toxicity study.

5.1.4. RfD Comparison Information

PODs and oral RfDs based on selected studies included in Table 4-13 are arrayed in Figures 5-1 to 5-3, and provide perspective on the RfD supported by Bruckner et al. (1986). These figures should be interpreted with caution because the PODs across studies are not necessarily comparable, nor is the confidence in the data sets from which the PODs were derived the same. PODs in these figures may be based on a NOAEL, LOAEL, or BMDL (in the case of the principal study), and the nature, severity, and incidence of effects occurring at a LOAEL are likely to vary. To some extent, the confidence associated with the resulting RfD is reflected in the magnitude of the total UF applied to the POD (i.e., the size of the bar); however, the text of Sections 5.1.1 and 5.1.2 should be consulted for a more complete understanding of the issues associated with each data set and the rationale for the selection of the critical effect and principal study used to derive the RfD.

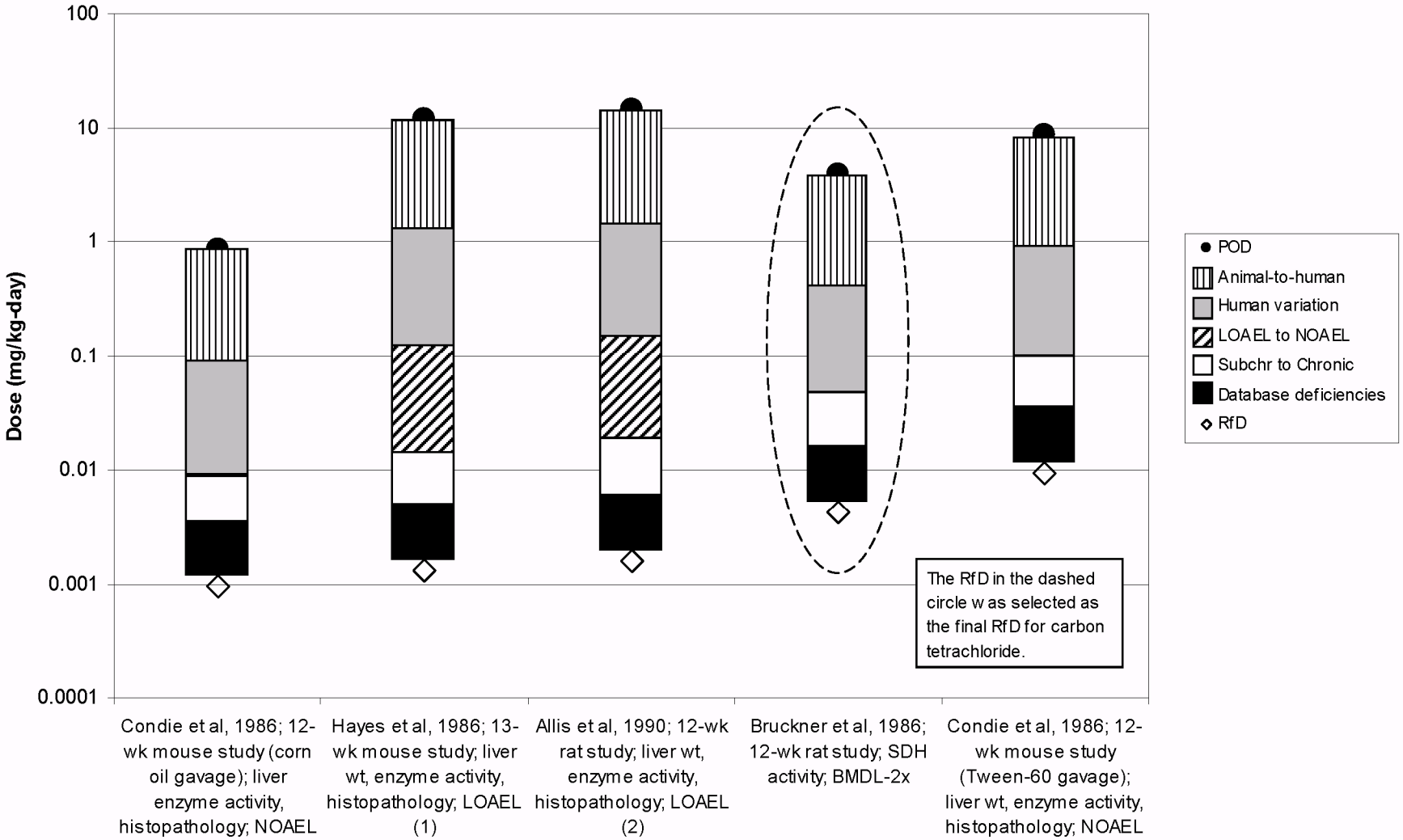
The predominant noncancer effect of subchronic and chronic oral exposure to carbon tetrachloride is hepatic toxicity. Figure 5-1 provides a graphical display of dose-response information from five studies that reported liver toxicity in experimental animals following subchronic oral exposure to carbon tetrachloride, including the PODs that could be considered in deriving the oral RfD. As discussed in Sections 5.1.1 and 5.1.2, among those studies that demonstrated liver toxicity, the study by Bruckner et al. (1986) provided the data set most appropriate for deriving the RfD. Possible RfDs that might be derived from each of these studies

are also presented. Although the RfD based on Bruckner et al. (1986) is not the lowest among candidate studies, it is considered the most scientifically rigorous. The POD is based on BMD methods, which has an inherent advantage over use of a NOAEL or LOAEL by making greater use of all the data from the study. Because the studies by Hayes et al. (1986) and Allis et al. (1990) identified only a LOAEL for liver effects, the RfD associated with these studies is driven lower by use of a larger composite UF.

Studies in experimental animals have also found that relatively high doses of carbon tetrachloride during gestation can produce prenatal loss; these doses also produced overt toxic effects in the dams. A graphical display of dose-response information from three developmental studies is provided in Figure 5-2.

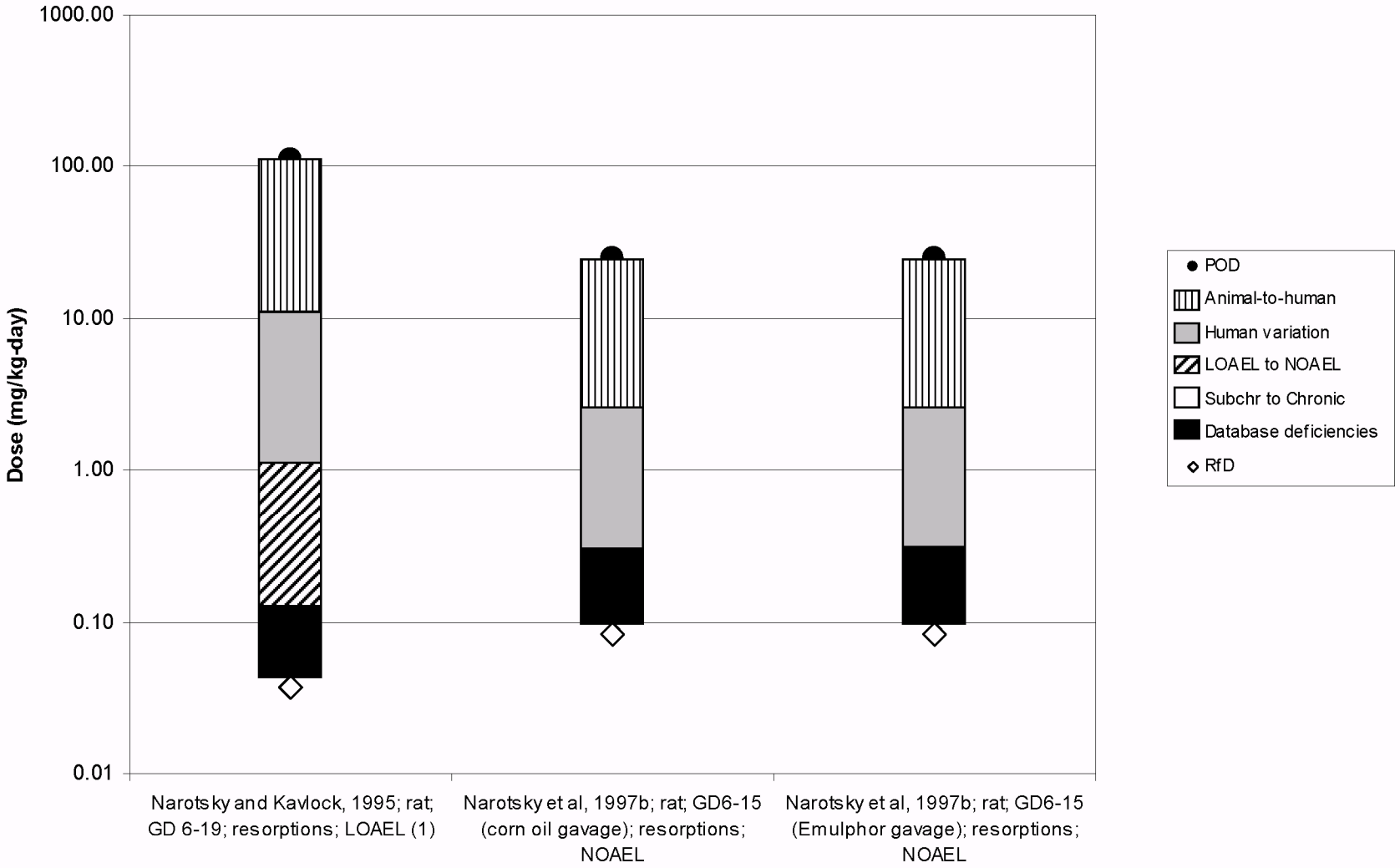
Figure 5-3 displays PODs for the major targets of toxicity associated with oral exposure to carbon tetrachloride. For the reasons discussed in Section 5.1.2, liver effects in the rat observed in the study by Bruckner et al. (1986) are considered the most appropriate basis for the carbon tetrachloride RfD. The POD is lower than that for developmental toxicity, and the resulting RfD should adequately protect against developmental effects of carbon tetrachloride.

Figure 5-1. Liver toxicity: oral



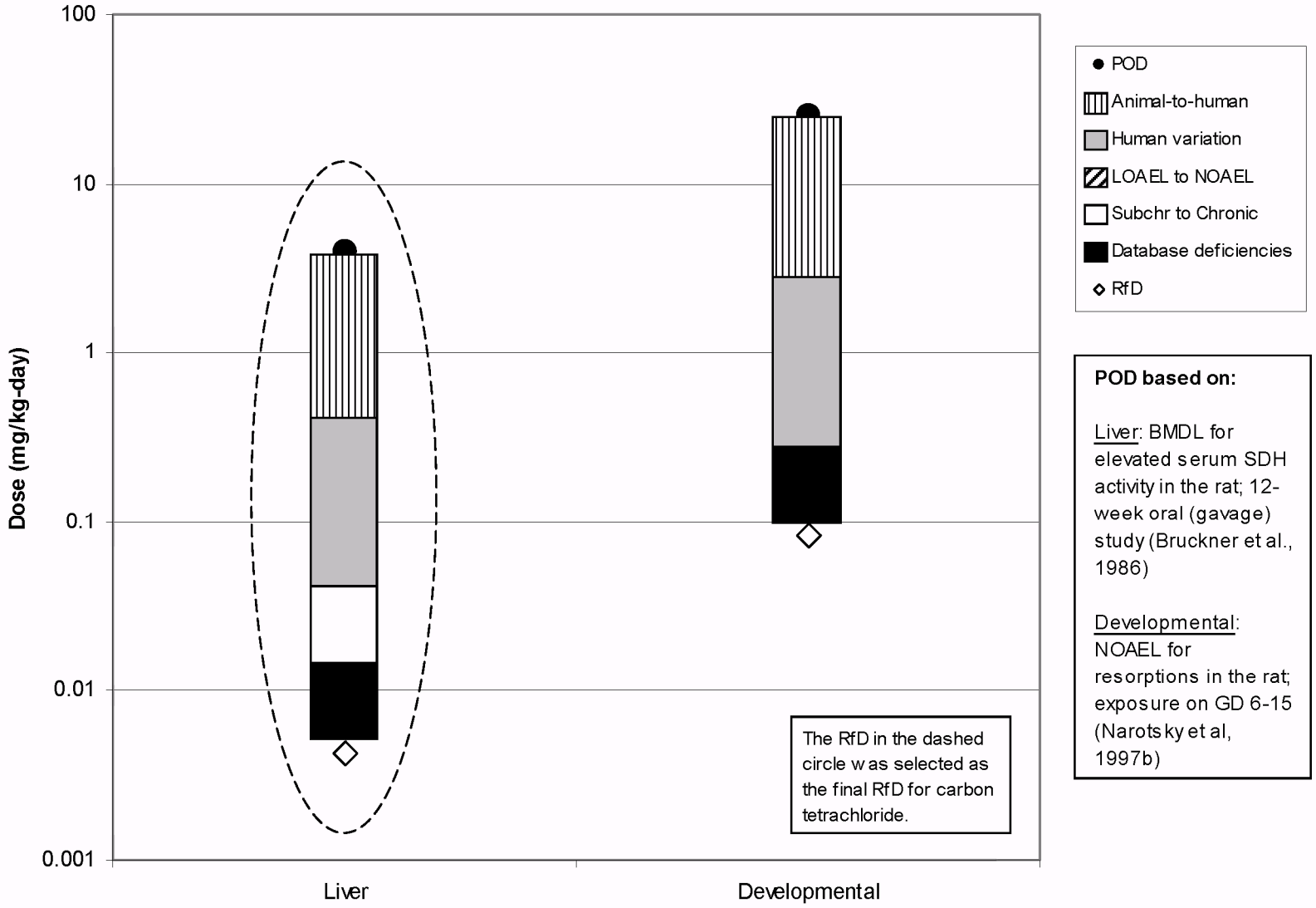
(1) Magnitude of effect at the LOAEL: liver weight (↑ 15-19%); enzyme activity (↑ ≤6-fold); 100% necrosis.
(2) Magnitude of effect at the LOAEL: liver weight (↑ 30%); enzyme activity (↑ 3-5X); 100% necrosis.

Figure 5-2. Developmental toxicity: oral



(1) Magnitude of effect at the LOAEL: 44% resorptions

Figure 5-3. Organ-specific oral RfDs



5.1.5. Previous RfD Assessment

The previous oral RfD for carbon tetrachloride (verified on 05/20/85 and posted on the IRIS database in 1987) was 0.0007 mg/kg-day, based on the NOAEL of 1 mg/kg (daily dose of 0.7 mg/kg-day) and the LOAEL of 10 mg/kg (daily dose of 7 mg/kg-day) for liver lesions (evidenced by mild centrilobular vacuolation and significantly increased serum SDH activity) in rats treated for 12 weeks (5 days/week) with carbon tetrachloride by gavage in corn oil by Bruckner et al. (1986). [A 1983 draft of the Bruckner et al. (1986) study was used as the basis for the RfD by the RfD Work Group. The published version of the study did not necessitate a change to the RfD.] The RfD of 0.0007 mg/kg-day was calculated by applying a UF of 1000 (three factors of 10 to account for interspecies and interhuman variability and extrapolation from subchronic to chronic exposure) to the NOAEL of 0.7 mg/kg-day.

The current RfD relies on the same principal study as the previous RfD, but applies benchmark dose analysis to derive the POD (3.9 mg/kg-day), whereas the previous RfD used the NOAEL (0.7 mg/kg-day) as the POD. Both RfDs were derived using a total UF of 1000, although some of the individual UFs differed. The previous RfD incorporated a UF of 10 to account for extrapolation from subchronic to chronic exposure, whereas the current RfD includes a subchronic to chronic UF of 3 based from a more thorough analysis of the available oral and inhalation literature. The current RfD also includes a database UF of 3; the previous RfD (posted in 1987) predated the institution of the database UF.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

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

As noted in Section 4.6.2., the predominant targets of toxicity of carbon tetrachloride in humans (based on case reports of acute, high-level exposure or long-term occupational exposure) and experimental animals following inhalation exposure are the liver and kidney. Only one cross-sectional epidemiological study of hepatic function in workers (Tomenson et al., 1995) provides data that can be considered for use in dose-response analysis.

Tomenson et al. (1995) conducted a cross-sectional study of hepatic function in 135 carbon tetrachloride-exposed workers in three chemical plants in northwest England and in a control group of 276 unexposed workers. The exposure assessment was based on historical personal monitoring data for various jobs at the three plants. Subjects were placed into one of three exposure categories—low (≤1 ppm), medium (1.1–3.9 ppm), or high (≥4 ppm)—according to their current jobs. Multivariate analysis, based on simultaneous consideration of ALT, AST, ALP, and GGT as dependent variables, revealed a statistically significant ($p<0.05$) difference between exposed and unexposed workers. Univariate analyses (in which each dependent variable was assessed separately) showed evidence of increased levels of ALP and GGT in the medium and high exposure groups, with the differences between the medium exposure group and controls being statistically significant ($p < 0.05$). In an alternative analysis, the proportion of

exposed workers exceeding the normal range (i.e., the 2.5 and 97.5% quantiles of the control group) was significantly elevated for ALT (8%) and GGT (11%) but not for the other serum chemistry variables. There was little difference between the low carbon tetrachloride exposure group (≤1 ppm estimated exposure levels) and the control group on any of the liver enzymes. Overall, this study provides suggestive evidence of an effect of occupational carbon tetrachloride exposure on the liver at exposures in the range of >1 to 3.9 ppm (6.3 to 24.5 mg/m^3); this exposure range is considered to be a LOAEL. The low exposure category in this study (≤1 ppm or ≤6.3 mg/m^3) appears to be a NOAEL. Because of study uncertainties described in Section 4.1.2.2, these values of the NOAEL and LOAEL must be considered similarly uncertain.

A number of experimental animal studies that identified liver and kidney as targets of carbon tetrachloride toxicity were considered as the basis for RfC derivation. The most robust study was the 2-year inhalation bioassay by JBRC (Nagano et al., 2007b; JBRC, 1998), which used 50 animals/sex/group and examined an extensive set of endpoints of toxicity. The exposure concentration of 25 ppm, 6 hours/day, 5 days/week in this study (corresponding to a continuous exposure level of 4.5 ppm)[f] produced evidence of liver and renal toxicity in both male and female F344/DuCrj rats. The lowest exposure concentration in this study, 5 ppm (0.9 ppm, adjusted to continuous exposure), was considered to be a NOAEL. As described in Section 4.2.2.2., carbon tetrachloride-induced liver toxicity at ≥25 ppm was evidenced by serum chemistry changes (including significant increases in ALT, AST, LDH, LAP and GGT) and histopathologic changes (fatty change, fibrosis, and cirrhosis) (see Table 4-3). In the kidney, there was a dose-related increase in the severity of chronic nephropathy (progressive glomerulonephrosis or CPN) (see Table 4-3) and a significant increase in BUN in rats exposed to ≥25 ppm. Because of the high spontaneous rate of chronic nephropathy in F344 rats, the incidence of chronic nephropathy was close to 100% in all dose groups, including the control, and a dose-related increase in incidence could not be demonstrated. As discussed in Section 4.6.2, the severity (but not incidence) of proteinuria was increased in all carbon tetrachloride-exposed rats. Because this observation was difficult to interpret and its biological significance was uncertain, it was not used to define the NOAEL and LOAEL for kidney effects. For these reasons, hepatic effects in this study were considered the more appropriate and sensitive measure of carbon tetrachloride-related toxicity.

Hepatic effects observed in the chronic rat inhalation study are consistent with the overall carbon tetrachloride database. Subchronic studies in a number of experimental species (Adams et al., 1952; Prendergast et al., 1967; Benson and Springer, 1999) identified a NOAEL for liver effects in the range of 0.9 to 4 ppm (adjusted to continuous exposure). These subchronic studies used exposure durations of 12 to 26 weeks (versus 104 weeks in the JBRC bioassay) and

[f] The exposure of 25 ppm for 6 hours/day, 5 days/week was adjusted to continuous exposure as follows: 25 ppm x 6 hours/24 hours x 5 days/7 days = 4.5 ppm

experimental protocols that were less rigorous than the JBRC bioassay. Therefore, these studies were considered less appropriate as the basis for the RfC. In the chronic mouse study by JBRC (Nagano et al., 2007b; JBRC, 1998), the NOAEL for liver toxicity was 0.9 ppm (adjusted to continuous exposure). This NOAEL is the same as that for rats in the JBRC bioassay; however, the incidences of specific liver lesions in the mouse were lower than those in the rat. Hepatic toxicity as the critical effect is also consistent with the epidemiological literature, in particular a cross-sectional study of hepatic function in carbon tetrachloride-exposed workers (Tomenson et al., 1995). Tomenson et al. (1995) reported suggestive evidence of carbon tetrachloride-associated effects on hepatic serum enzymes.

Renal effects were observed in the JBRC chronic mouse study (Nagano et al., 2007b; JBRC, 1998) and in subchronic animal studies, but generally at concentrations higher than those that produced liver effects or occurred at a lower incidence than liver effects.

At the lowest tested concentration of 5 ppm in the JBRC study (corresponding to a continuous exposure level of 0.9 ppm), an increase in severity of proteinuria in male and female rats was reported. As discussed in Section 4.6.2., the adversity of the proteinuria findings at this exposure concentration is uncertain, and the evidence as a whole does not support the finding of a LOAEL at 5 ppm based on proteinuria data.

In addition to proteinuria, the only other effect reported at 5 ppm in the chronic rat study was an increase in severity of eosinophilic change in the nasal cavity of the female rats (Nagano et al., 2007b; JBRC, 1998). A similar effect in males was seen only at 25 ppm and above. This change, by itself, is not considered to represent an adverse effect. Even in the high-exposure group that experienced severe renal and hepatic effects, the nasal lesion was graded at only moderate severity and was not accompanied by any other, more clearly adverse effects in the nasal cavity. Nonvolatile and partly nonextractable radioactivity was detected in the nasal mucosa after inhalation of radiolabeled carbon tetrachloride in mice (Bergman, 1983), suggesting that some inhaled carbon tetrachloride is metabolized in the nasal cavity. However, there are no other reports of lesions or irritant effects produced by carbon tetrachloride vapor in either humans or animals.

By inhalation, benign pheochromocytomas were reported in mice in the JBRC inhalation bioassay (Nagano et al., 2007b; JBRC, 1998). This benign tumor was observed only in mice (i.e., no increase in pheochromocytomas was observed in rats in either NCI (1977) or Nagano et al. (2007b)] and thus may represent a strain-specific finding. No data are available, however, to establish whether this response is species specific. Developmental toxicity (reduced fetal body weight and crown-rump length) was reported in a single inhalation study (Schwetz et al., 1974) at a concentration that also produced toxicity in the dam. Because neither benign pheochromocytomas nor developmental toxicity occurred at a concentration below those associated with liver toxicity and because level of response was less robust than for endpoints of liver toxicity, these endpoints were not considered most appropriate as the basis for the RfC.

The hepatic effects observed in the JBRC chronic inhalation bioassay (Nagano et al., 2007b; JBRC, 1998) were considered the most appropriate basis for RfC derivation. Fatty change in the liver of rats was selected as the specific endpoint for dose-response analysis because this histopathologic lesion is indicative of cellular damage and appears to be a more sensitive endpoint than other histopathologic changes that were also present in 25-ppm rats in the JBRC study. Liver serum enzyme activities were also increased in male and female rats and mice exposed to 25 ppm; however, serum enzyme levels were considered a less consistent and reliable indicator of liver damage in this study than histopathologic changes. In the mouse, the overall increase in liver enzyme levels was not monotonic (i.e., levels at 5 ppm were lower than control levels). In the rat, liver enzyme level increases at 25 ppm were considered modest (i.e., increases over control of only 40 to 90%). Further, reliable liver enzyme data were not available for 125-ppm rats or mice because of the high mortality at this exposure concentration (1 to 3 surviving animals/group at study termination) and because blood biochemistry was not performed on animals that died before study termination. Therefore, liver enzyme data were considered a less appropriate endpoint for dose-response analysis.

The occupational study by Tomensen et al. (1995) was also considered as the basis for RfC derivation, using the estimated LOAEL of 5.5 ppm (35 mg/m^3) as the POD. As discussed more fully in Section 4.1.2.2, exposures for almost two-thirds of the workers were estimated, so that there is some uncertainty in the value of the LOAEL. Although the data from the Tomensen et al. (1995) study was not used to derive the RfC, the study was considered in an examination of RfC values that would be obtained using alternative PODs (see Section 5.2.4).

5.2.2. Methods of Analysis—Including Models

Candidate RfCs for carbon tetrachloride were derived from data on fatty changes to the liver in male and female rats; incidence data are summarized in Table 5-4

Table 5-4. Nonneoplastic lesions (fatty change) in F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

Species	Sex	Lesion type	Lesion severity[a]	Number of rats with lesions			
				Dose			
				0 ppm	5 ppm	25 ppm	125 ppm
Rat	M[b]	fatty change	1+ and 2+	4	7	39	49
Rat	F[c]	fatty change	1+, 2+, and 3+	6	7	49	46

[a]Severity rating: +, slight; 2+, moderate; 3+ marked.
[b]Number of male rats examined: 50/group; number of male rats surviving to study termination: 0 ppm, 22/50; 5 ppm, 29/50; 25 ppm, 19/50; 125 ppm, 3/50.
[c]Number of female rats examined: 50/group; number of female rats surviving to study termination: 0 ppm, 39/50; 5 ppm, 43/50; 25 ppm, 39/50; 125 ppm, 1/50.

Source: Nagano et al., 2007b; JBRC, 1998.

The general procedure for analysis of the animal bioassay data is depicted in Figure 5-4. Exposure levels studied in the 2-year rat bioassay (Nagano et al., 2007b; JBRC, 1998) were converted to estimates of internal doses by application of a PBPK model. BMD modeling methodology (U.S. EPA, 2000c, 1995) was used to analyze the relationship between the estimated internal doses and response (i.e., fatty change of the liver). The resulting BMDL values were converted to estimates of equivalent human exposure concentrations (HECs) by applying a human PBPK model.

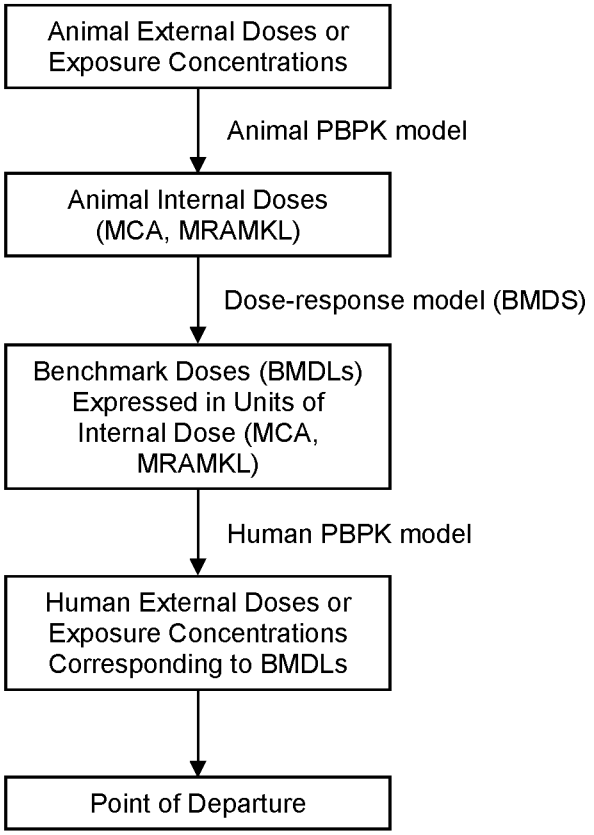


Figure 5-4. Process for analyzing animal bioassay data for deriving noncancer toxicity values and cancer unit risks and slope factors using PBPK modeling.

BMDL, lower confidence limit on benchmark dose; BMDS, Benchmark Dose Software; MCA, time-averaged arterial blood concentration of carbon tetrachloride (µmol/L); MRAMKL, time-average rate of metabolism of carbon tetrachloride (µmol/hr/kg liver); PBPK, physiologically-based pharmacokinetics model

5.2.2.1. *PBPK Modeling for Internal Dose Metrics*

Estimation of internal doses corresponding to the exposure concentrations studied in the 2-year rat bioassay (Nagano et al., 2007b; JBRC, 1998) was accomplished using a PBPK model for the rat (Thrall et al., 2000; Benson and Springer, 1999; Paustenbach et al., 1988) (see Sections 3.5 for description of the model). The review, selection and application of the chosen PBPK models was informed by an EPA report (U.S. EPA, 2006), which addresses the application and evaluation of PBPK models. The PBPK model was used to simulate internal dose metrics corresponding to exposure concentrations studied in the 2-year bioassay: 5, 25, and 125 ppm, 6 hours/day, 5 days/week (Nagano et al., 2007b; JBRC, 1998). Internal dose metrics were selected that were considered to be most relevant to the toxicity endpoints of interest (e.g., liver toxicity), based on consideration of evidence for mode of action of carbon tetrachloride.

Two dose metrics were selected based on available information on the mechanisms of carbon tetrachloride liver toxicity: (1) time-averaged arterial blood concentration of carbon tetrachloride (MCA, µmol/L); and (2) time-averaged rate of metabolism of carbon tetrachloride (MRAMKL, µmol/hr/kg liver). Liver metabolism rate was selected as the primary dose metric for liver effects, based on evidence that metabolism of carbon tetrachloride via CYP2E1 to highly reactive free radical metabolites plays a crucial role in its mode of action in producing liver toxicity (described in Section 4.5). Uncertainty regarding the accuracy of available PBPK models to simulate carbon tetrachloride is recognized. These uncertainties include the following: (1) estimates of the K_m and V_{max} for the CYP2E1 pathway in the rat and human and potential dose-dependence of these parameters (e.g., suicide inhibition); (2) relative contributions of extra-hepatic tissues to carbon tetrachloride metabolism (all of which is assigned to the liver in PBPK models used in this analysis); and (3) magnitude of direct contribution of carbon tetrachloride (i.e., parent compound) to liver toxicity. Given the above uncertainties, arterial blood concentration of carbon tetrachloride was also included in the analysis as a more proximal dose metric to liver metabolism.

The two dose metrics, MCA and MRAMKL, were simulated in the rat PBPK model as time-averaged values, with the averaging time being the chronic exposure period (e.g., 2 years). The time-averaged dose metrics were calculated as follows (Equations 5-1 and 5-2):

$$MCA = \frac{AUC_{CA}}{t} \qquad \text{Eq. (5-1)}$$

$$MRAMKL = \frac{AUC_{RAMKL}}{t} = \frac{AMKL}{t} \qquad \text{Eq. (5-2)}$$

where:
MCA = time-averaged arterial blood concentration of carbon tetrachloride (µmol/L)
AUC_{CA} = area under the arterial concentration (CA) – time profile (µmol·hr/L)
MRAMKL = time-averaged rate of metabolism of carbon tetrachloride (µmol/hr/kg liver weight)
AUC_{RAMKL} = area under the rate of metabolism (RAMKL) – time profile (µmol/kg liver weight)
AMKL = cumulative amount of carbon tetrachloride metabolized (µmol/kg liver)
t = time (hours)

Internal dose metrics corresponding to the exposure concentrations studied in the 2-year rat inhalation bioassay (Nagano et al., 2007b; JBRC, 1998) are presented in Table 5-5. Two values for V_{maxC} (maximum rate of hepatic metabolism of carbon tetrachloride) have been reported for the rat; both estimates are represented in the data presented in Table 5-5. Gargas et al. (1986) derived a value for V_{maxC} of 0.4 mg/hr/kg $BW^{0.70}$, based on the results of gas uptake studies in rats. Paustenbach et al. (1988) derived a value of 0.65 mg/hr/kg $BW^{0.70}$, based on a

reanalysis of data for a subset of the rats used in the Gargas et al. (1986) study. Increasing V_{maxC} from 0.4 to 0.65 mg/hr/kg $BW^{0.70}$ resulted in lower values for the MCA dose metric and higher values for the MRAMKL dose metric (Table 5-5). Comparisons of internal doses predicted for various exposure concentrations are shown in Figure 5-5. The effect of varying V_{maxC} on MRAMKL becomes more pronounced as exposure concentration increases. This pattern reflects the increasing influence of V_{max} on rate of metabolism at higher exposures concentrations that result in liver carbon tetrachloride concentrations that exceed the K_m.

Table 5-5. Comparisons of internal dose metrics predicted from PBPK rat models (Paustenbach et al., 1988; Thrall et al., 2000)

Exposure (ppm)	MCA (µmol/L)		MRAMKL (µmol/hr/kg liver)	
	V_{maxC}=0.40	V_{max}=0.65	V_{maxC}=0.40	V_{max}=0.65
5	0.128	0.116	3.813	4.991
25	0.708	0.653	12.092	17.626
125	3.892	3.775	24.320	36.266

Values are for 0.452 kg rat.
MCA, time-averaged arterial concentration of carbon tetrachloride; MRAMKL, time-averaged rate of metabolism of carbon tetrachloride per kg liver, V_{maxC}, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$)

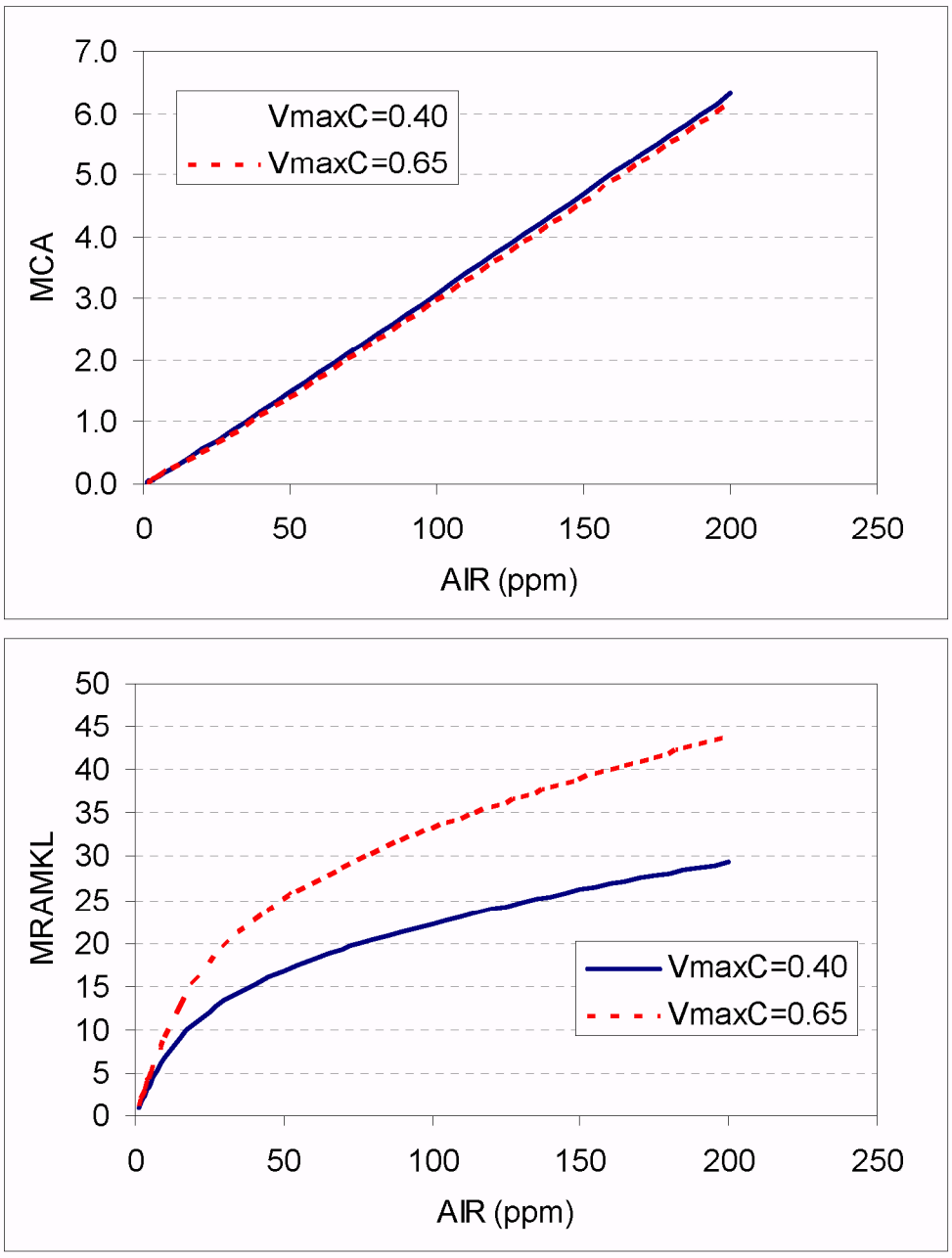


Figure 5-5. Internal dose metrics predicted by the PBPK rat model (Paustenbach et al., 1988; Thrall et al., 2000).

Dose metrics shown are time-averaged arterial concentration of carbon tetrachloride (MCA, µmol/L, upper panel), and time-averaged rate of metabolism of carbon tetrachloride (MRAMKL, µmol/hr/kg liver, lower panel). The dose metrics are plotted against exposure concentration (6 hours/day, 5 days/week, 2 years) for a 0.452 kg rat.

5.2.2.2. *Benchmark Dose Modeling*

BMD modeling methodology (U.S. EPA, 2000c, 1995) was used to analyze data on estimated internal doses (i.e., MCA, MRAMKL) and incidence data (e.g., fatty changes of the liver) from the 2-year rat bioassay (Nagano et al., 2007b; JBRC, 1998). All of the models for dichotomous data in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to the incidence data for rats.

Internal doses associated with a benchmark response (BMR) of 10% extra risk were calculated. A BMR of 10% extra risk of fatty changes in the liver was selected because the POD associated with this BMR fell near the low end of the range of experimental data points (see plots in Appendix D). As noted U.S. EPA (2000), "[t]he major aim of benchmark dose modeling is to model the dose-response data for an adverse effect in the observable range (i.e., across the range of doses for which toxicity studies have reasonable power to detect effects) and then select a 'benchmark dose' at the low end of the observable range to use as a 'point of departure'."

In the male rat, the best fit of the data was provided by the log-logistic model using MCA as the dose metric and the logistic model using MRAMKL as the dose metric (based on χ^2 $p \geq 0.1$ and lowest AIC value). For female rats, no models provided an adequate fit to the data when all dose groups were included, as assessed by the χ^2 goodness-of-fit test (i.e., application of the models in BMDS yielded χ^2 p values in all cases <0.1). After dropping the highest dose, the multistage model provided the best fit of the female incidence data (based on χ^2 $p \geq 0.1$ and lowest AIC value) using either dose metric. Summaries of the resulting BMD_{10} and $BMDL_{10}$ values for male and female rats are shown in Tables 5-6 and 5-7 (columns 3 and 4). Details of the BMD modeling are provided in Appendix D.

Table 5-6. HEC values corresponding to BMDL values for incidence data for fatty changes of the liver in male F344 rats

BMR	Metric	BMD modeling[a]		$VMAXC_H$	HEC	
		$VMAXC_R$=0.4	$VMAXC_R$=0.65		$VMAXC_R$=0.4	$VMAXC_R$=0.65
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)
0.1	MCA	BMD_{10}: 0.14 $BMDL_{10}$: 0.079	BMD_{10}: 0.12 $BMDL_{10}$: 0.071	0.40	5.396	4.830
0.1				0.65	5.712	5.113
0.1				1.49	6.338	5.671
0.1				1.70	6.436	5.760
0.1	MRAMKL	BMD_{10}: 3.26 $BMDL_{10}$: 2.59	BMD_{10}: 4.60 $BMDL_{10}$: 3.65	0.40	23.793	35.243
0.1				0.65	17.160	24.773
0.1				1.49	11.826	16.794
0.1				1.70	11.343	16.093

Rats were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Doses modeled correspond to exposure concentrations: 0, 5, 25, 125 ppm.
BMR, benchmark response; HEC, human equivalent concentration, mg/m^3; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; VMAXC, maximum rate of metabolism in humans (H) or rat (R), mg/hr/kg $BW^{0.70}$
[a] MCA, log-logistic model provided the best fit; MRAMKL, logistic model provided the best fit.
[b] Number in parentheses indicates the column number.

Table 5-7. HEC values corresponding to BMDL values for incidence data for fatty changes of the liver in female F344 rats (high dose dropped)

BMR	Metric	BMD modeling[a]		$VMAXC_H$	HEC	
		$VMAXC_R$=0.4	$VMAXC_R$=0.65		$VMAXC_R$=0.4	$VMAXC_R$=0.65
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)
0.1	MCA	BMD_{10}: 0.12 $BMDL_{10}$: 0.085	BMD_{10}: 0.11 $BMDL_{10}$: 0.078	0.40	5.815	5.298
0.1				0.65	6.156	5.608
0.1				1.49	6.831	6.222
0.1				1.70	6.937	6.319
0.1	MRAMKL	BMD_{10}: 3.77 $BMDL_{10}$: 2.82	BMD_{10}: 5.42 $BMDL_{10}$: 3.75	0.40	26.259	36.337
0.1				0.65	18.838	25.478
0.1				1.49	12.935	17.246
0.1				1.70	12.405	16.524

Rats were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Doses modeled correspond to exposure concentrations: 0, 5, 25 ppm (125 ppm dose dropped).
BMR, benchmark response; HEC, human equivalent concentration, mg/m^3; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; VMAXC, maximum rate of metabolism in humans (H) or rat (R), mg/hr/kg $BW^{0.70}$
[a] MCA, multistage (2); MRAMKL, multistage (3)
[b] Number in parentheses indicates the column number.

5.2.2.3. *PBPK Modeling of Human Equivalent Exposure Concentrations*

Interspecies extrapolation (i.e., rat-to-human) of carbon tetrachloride inhalation dosimetry was accomplished using a human PBPK model described in Paustenbach et al. (1988), Thrall et al. (2000), and Benson and Springer (1999). The human PBPK model was used to estimate continuous human equivalent concentrations (HECs, in mg/m^3) that would result in values for the internal dose metrics, MCA or MRAMKL, equal to the $BMDL_{10}$ values for fatty changes of the liver.

The approach used to derive the HECs for each dose metric was as follows:
(1) The human PBPK model was used to calculate internal doses corresponding to a series of exposure concentrations (EC, continuous exposure, mg/m^3). For the dose metric MCA, the human PBPK model was run at intervals over the range from 0.1 to 100 ppm (0.63 to 629 mg/m^3); for MRAMKL, the human PBPK model was run at intervals from 1 to 300 ppm (6.3 to 1887 mg/m^3).
(2) For each internal dose, conversion factors were calculated as the following corresponding ratios:

- EC/MCA (to relate a continuous chronic human inhalation exposure in mg/m^3 [EC] to an internal dose using MCA as the dose metric);
- EC/MRAMKL (to relate a continuous chronic human inhalation exposure in mg/m^3 [EC] to an internal dose using MRAMKL as the dose metric); and

(3) Conversion factors were calculated for each of four assumed values of V_{maxC} in the human PBPK model: 0.40, 0.65, 1.49, or 1.70 mg/hr/kg $BW^{0.70}$. These conversion factors are provided in Appendix C. Trend equations were also developed to permit the calculation of EC for any value of MCA or MRAMKL (see Appendix C).

Estimates of the dose metrics, MCA and MRAMKL, were sensitive to the value assigned to the V_{maxC} parameter (see Figure 5-5). Several values for V_{maxC} in animals and humans have been reported (Thrall et al., 2000, Benson and Springer, 1999; Paustenbach et al., 1988; Gargas et al., 1986); therefore, evaluation of uncertainty in this parameter was introduced into the analysis by assuming various reported values for V_{maxC} in the estimation of HECs. Thrall et al. (2000) and Benson and Springer (1999) derived a value of 1.49 mg/hr/kg $BW^{0.70}$ for humans, based on an analysis of data on in vivo (gas uptake) studies in rodents and in vitro studies of metabolism of carbon tetrachloride in rodent and human liver samples. Thrall et al. (2000) also derived a value of 1.7 mg/hr/kg $BW^{0.70}$ for hamsters, based on the results of closed chamber gas uptake studies. The value of 1.49 mg/hr/kg $BW^{0.70}$ for humans (Thrall et al., 2000; Benson and Springer, 1999), the value of 1.70 mg/hr/kg $BW^{0.70}$ for the hamster (Thrall et al., 2000), and the two values estimated for the rat (0.4, 0.65 mg/hr/kg $BW^{0.70}$; Paustenbach et al., 1988; Gargas et

al., 1986) were used in the estimation of HECs. Estimated values for HECs corresponding to $BMDL_{10}$ values for fatty changes of the liver as reported in the 2-year rat inhalation bioassay (Nagano et al., 2007b; JBRC, 1998) for alternative values of V_{maxC} in the rat and human are presented in Tables 5-6 and 5-7 (columns 6 and 7).

A human V_{maxC} estimated from in vitro human data can reasonably be presumed to be more relevant than a human V_{maxC} based entirely on rodent data. In addition, because the mode of action for carbon tetrachloride-induced hepatotoxicity involves metabolism to reactive metabolites in the liver, HECs based on the MRAMKL dose metric is the most proximate to the critical effect. Therefore, the human V_{maxC} estimated from in vitro human data (1.49 mg/hr/kg $BW^{0.70}$) and the dose metric MRAMKL are considered to yield the most appropriate estimate of the HEC. No information is available to establish a rat V_{maxC} of either 0.4 or 0.65 mg/hr/kg $BW^{0.70}$ as the more scientifically defensible value for this parameter. Therefore, HECs derived using these two rat V_{maxC} values were averaged to derive the POD for the carbon tetrachloride RfC. Accordingly, the POD based on male rat data was calculated as (11.826 + 16.794) ÷ 2 = 14.3 mg/m^3. In the female rat, the HEC was similarly calculated as (12.935 + 17.246) ÷ 2 = 15.1 mg/m^3. The HEC based on data for the male rat (14.3 mg/m^3) is the lower of the two values, and is selected as the POD for RfC derivation.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors

An RfC of 0.1 mg/m^3 for carbon tetrachloride is derived by applying a composite UF of 100 to the HEC of 14.3 mg/m^3, as follows:

$$\begin{aligned} RfC &= HEC/UF \qquad (5\text{-}3) \\ &= 14.3\ mg/m^3/100 \\ &= 0.143\ mg/m^3 \text{ or } 0.1\ mg/m^3 \end{aligned}$$

The composite UF of 100 includes a factor of 10 to protect susceptible individuals, a factor of 3 ($10^{0.5}$) to adjust for pharmacodynamic differences in the extrapolation from rats to humans, and a factor of 3 ($10^{0.5}$) to account for an incomplete database lacking an adequate multigeneration study of reproductive function.

- A default 10-fold UF for intraspecies differences was selected to account for variability in susceptibility among members of the human population in the absence of quantitative information on the variability of human response to carbon tetrachloride. Factors that could contribute to a range of human response to carbon tetrachloride were discussed in Section 4.8. Variations in CYP450 levels because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes)

could alter susceptibility to carbon tetrachloride toxicity. Individual variability in nutritional status, alcohol consumption, or the presence of underlying disease could also alter metabolism of carbon tetrachloride or antioxidant protection systems. To account for these uncertainties, a factor of 10 was included for individual variability.

- A UF of 3 ($10^{0.5}$) was selected for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. As pharmacokinetic and pharmacodynamic components are assumed to contribute equally to the uncertainty in interspecies extrapolation and the product of the two components is assumed by default to be 10, a numeric value of $10^{0.5}$ (3.2, expressed as the numeral 3 after rounding) is assigned to each component. Cellular antioxidant systems function to quench the lipid peroxidation reaction and prevent damage to cellular membranes. In the absence of data to quantify specific interspecies differences for cellular protective mechanisms, a UF of 3 is included to account for species differences in pharmacodynamics. A pharmacokinetic model was used to adjust for pharmacokinetic differences across species; therefore, an additional UF was not included for pharmacokinetic differences between species.

- An UF to account for extrapolation from a LOAEL to a NOAEL was not used because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in fatty changes of the liver was selected under an assumption that it represents a minimal biologically significant change.

- An UF to extrapolate from a subchronic to a chronic exposure duration was not necessary because the RfC was derived from a study using a chronic exposure protocol.

- A database UF of 3 ($10^{0.5}$) was selected. The inhalation database for this chemical includes extensive testing for subchronic toxicity in animals, 2-year chronic inhalation bioassays in both rats and mice, one study of immunotoxic potential, and human epidemiology data. Testing for developmental toxicity was limited to one inhalation study in the rat that found effects only at high, maternally toxic exposure concentrations. This study did not use an exposure concentration low enough to identify a NOAEL for either maternal or fetal toxicity. Nevertheless, the developmental effects at the LOAEL were modest, and were limited to decreased fetal body weight (7%) and decreased crown-rump length (3.5%). The LOAEL for developmental effects (in the presence of maternal toxicity) in this study (334 ppm) was 66-fold higher than the NOAEL from the principal study (5 ppm). Developmental toxicity has been tested more extensively by the oral

route, although all adequate studies were conducted in the same species (rat); the oral NOAEL for developmental toxicity exceeded both the oral NOAEL and LOAEL for liver toxicity. As noted in Section 4.8.1. (Possible Childhood Susceptibility), microsomal enzymes that are responsible for metabolizing carbon tetrachloride, particularly CYP2E1, are lower in the developing organism than the adult, and in humans do not achieve adult levels until sometime between one and 10 years. Thus, life stage information on microsomal enzyme activity suggests that the developing organism would be no more susceptible to free radical-induced liver injury from carbon tetrachloride than adults. On balance, the available information suggests that further developmental toxicity testing would not likely result in a POD smaller than that based on liver toxicity. The database lacks an adequate multigeneration study of reproductive function by any route of exposure.

5.2.4. RfC Comparison Information

PODs and inhalation RfCs based on selected studies included in Table 4-14 are arrayed in Figures 5-6 to 5-8, and provide perspective on the RfC supported by Nagano et al. (2007b; JBRC, 1998). These figures should be interpreted with caution because the PODs across studies are not necessarily comparable, nor is the confidence in the data sets from which the PODs were derived the same. PODs in these figures may be based on a NOAEL, LOAEL, or BMDL (in the case of the principal study), and the nature, severity, and incidence of effects occurring at a LOAEL are likely to vary. In addition, PBPK modeling for animal to human extrapolation was applied to data from the principal study, whereas the default approach (i.e., application of an UF of 10) was used for other animal data sets. To some extent, the confidence associated with the resulting RfC is reflected in the magnitude of the total UF applied to the POD (i.e., the size of the bar); however, the text of Sections 5.2.1 and 5.2.2 should be consulted for a more complete understanding of the issues associated with each data set and the rationale for the selection of the critical effect and principal study used to derive the RfC.

As discussed in Section 4.6.2, the liver and kidney are the predominant targets of carbon tetrachloride toxicity in subchronic and chronic inhalation studies in laboratory animals (Nagano et al., 2007a,b; Benson and Springer, 1999; JBRC, 1998; Prendergast et al., 1967; Adams et al., 1952; Smyth et al., 1936) and in humans based on case reports and studies in exposed workers. Benign pheochromocytomas from the adrenal gland medulla, that could represent a potential noncancer health hazard, were observed by inhalation only in mice in the JBRC chronic bioassay (Nagano et al., 2007b; JBRC, 1998). A single study of developmental toxicity (Schwetz et al., 1974) found significant reductions in fetal body weight and crown-rump length in rats at a carbon tetrachloride concentration that also produced hepatotoxicity and reduced growth in the dams. This set of literature was evaluated in selecting the most appropriate study and endpoint to use as the basis for the RfC, with particular consideration given to the overall strength of the

evidence for a given measure of toxicity, consistency of the finding across studies, relevance to humans, sensitivity of the endpoint, and rigor of a given study.

Figure 5-6 provides a graphical display of dose-response information from one occupational cross sectional study and five experimental animal data sets that reported liver toxicity; all animal studies identified a NOAEL for liver toxicity of approximately 6 mg/m^3 or 0.9 ppm (adjusted to continuous exposure) and the study of exposed workers (Tomensen et al., 1995) identified a LOAEL of approximately to 12.5 mg/m^3 or 2 ppm (also adjusted to continuous exposure).[g] As discussed in Section 5.2.1, the JBRC study in the rat (Nagano et al., 2007b; JBRC, 1998), which identified a NOAEL for liver toxicity of 5.7 mg/m^3 or 0.9 ppm (adjusted to continuous exposure), was determined to be a sensitive and well-conducted study of carbon tetrachloride toxicity, and was selected as the basis for the RfC. Dose-response analysis of the data from this study, which included BMD and PBPK modeling, yielded a POD of 14.3 mg/m^3. Possible RfCs that might be derived from other studies demonstrating liver toxicity are also presented in Figure 5-6. Although the RfC based on the JBRC rat data is not the lowest among candidate studies, it is considered to be the most scientifically rigorous and associated with a lower degree of uncertainty than other experimental animal studies. The POD is based on a study of chronic toxicity data (rather than the subchronic exposures used in Benson and Springer, 1999, and Adams et al., 1952), the application of BMD methods, which has an inherent advantage over the use of a NOAEL or LOAEL by making greater use of all the data from the study, and the use of PBPK modeling for interspecies extrapolation. As shown in Figure 5-6, the use of PBPK modeling also resulted in the application of a smaller composite uncertainty factor to the POD, i.e., smaller degree of uncertainty than with other data sets to which the default uncertainty factor of 10 for interspecies extrapolation was applied. The RfC derived using data from the JBRC rat study is consistent with the RfC derived from Tomensen et al. (1995). Tomensen et al. reported a statistically significant increase in two of four serum enzymes indicative of liver function in workers exposed to approximately 35 mg/m^3 (5.5 ppm) carbon tetrachloride (adjusted to continuous exposure: 12.5 mg/m^3). Using 12.5 mg/m^3 as the POD and applying a composite UF of 300 (10 for variation in sensitivity in the human population, 10 for extrapolation from a LOAEL to a NOAEL, and 3 for database deficiencies), the RfC is estimate to be 0.04 mg/m^3. Because the Tomensen et al. (1995) noted that "there was no evidence of effects of clear clinical significance on the liver function of workers exposed to carbon tetrachloride at the levels indicated," it could be argued that a UF for LOAEL to NOAEL extrapolation of 3 (rather than a full UF of 10) might be appropriate. In this case, the RfC estimated from Tomensen et al. (1995) serum enzyme data would be 0.1 mg/m^3. Thus, the RfC estimated from Tomensen et al. (1995) of 0.04 to 0.1 mg/m^3 is consistent with the RfC of

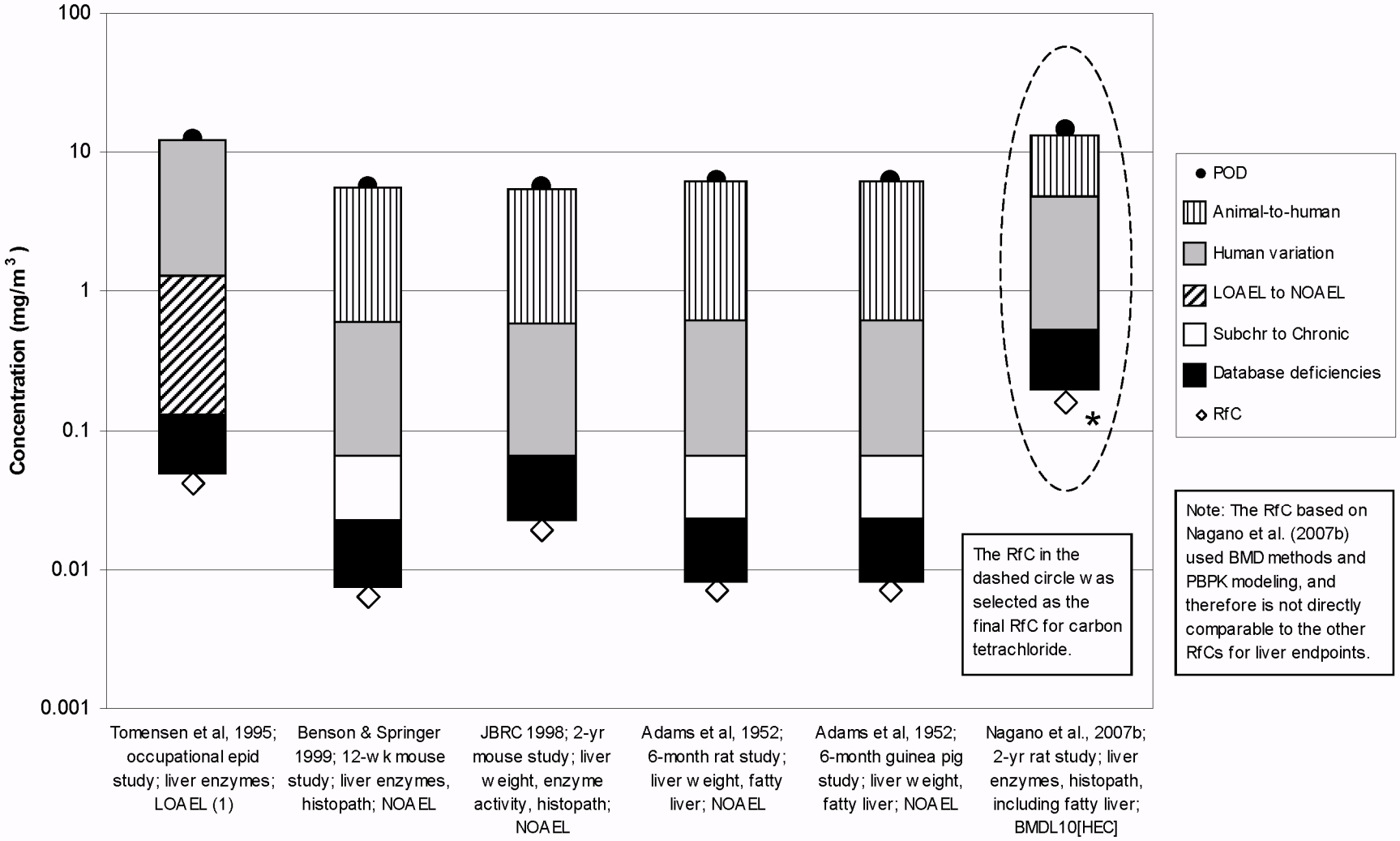
[g] The workplace exposure concentration of 35 mg/m^3 was adjusted to continous exposure by multiplying by (10 m^3/day ÷ 20 m^3/day) x (5 days/week ÷ 7 days/week), where 10 m^3/day is an estimate of an 8-hour time-weighted average occupational respiratory rate and 20 m^3/day an estimate of an average daily respiratory rate.

0.1 mg/m^3 derived from the JBRC rat bioassay (Nagano et al., 2007b; JBRC, 1998), and supports the RfC for liver effects derived from animal data.

The most sensitive study of kidney toxicity was the JBRC bioassay in the rat and mouse (Figure 5-7) (Nagano et al., 2007b; JBRC, 1998). As discussed in Section 5.2.1., kidney effects occurred at a concentration similar to liver effects, but at lower incidence.

Figure 5-8 displays PODs for all major targets of carbon tetrachloride toxicity by the inhalation route, including liver, kidney, adrenal gland, and developmental toxicity. For the reasons discussed in Section 5.2.1., liver effects in the rat observed in the JBRC study are considered the most appropriate basis for the carbon tetrachloride RfC. The POD based on liver effects is similar to the PODs associated with kidney effects and effects on the adrenal gland (benign pheochromocytomas); however, a smaller composite UF was applied to the POD for liver effects because PBPK modeling was used for interspecies extrapolation. The greatest degree of uncertainty is associated with the RfC for developmental toxicity. While this relatively large UF drives down the value of the RfC for developmental toxicity, the RfC based on liver effects should be adequately protective.

Figure 5-6. Liver toxicity: inhalation



(1) Magnitude of effect at the LOAEL: liver enzyme levels (↑ ≤23%)

Figure 5-7. Kidney toxicity: inhalation

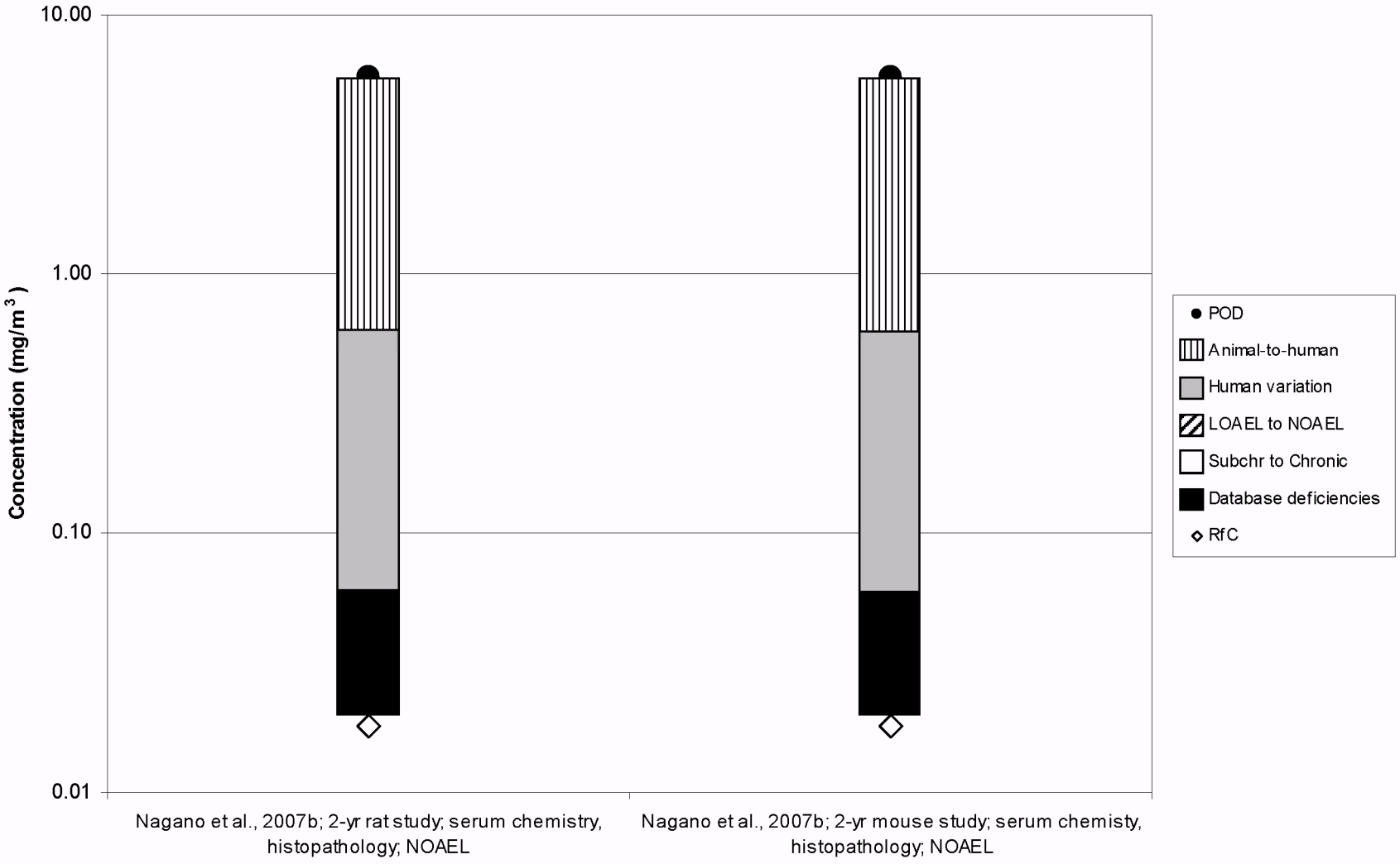
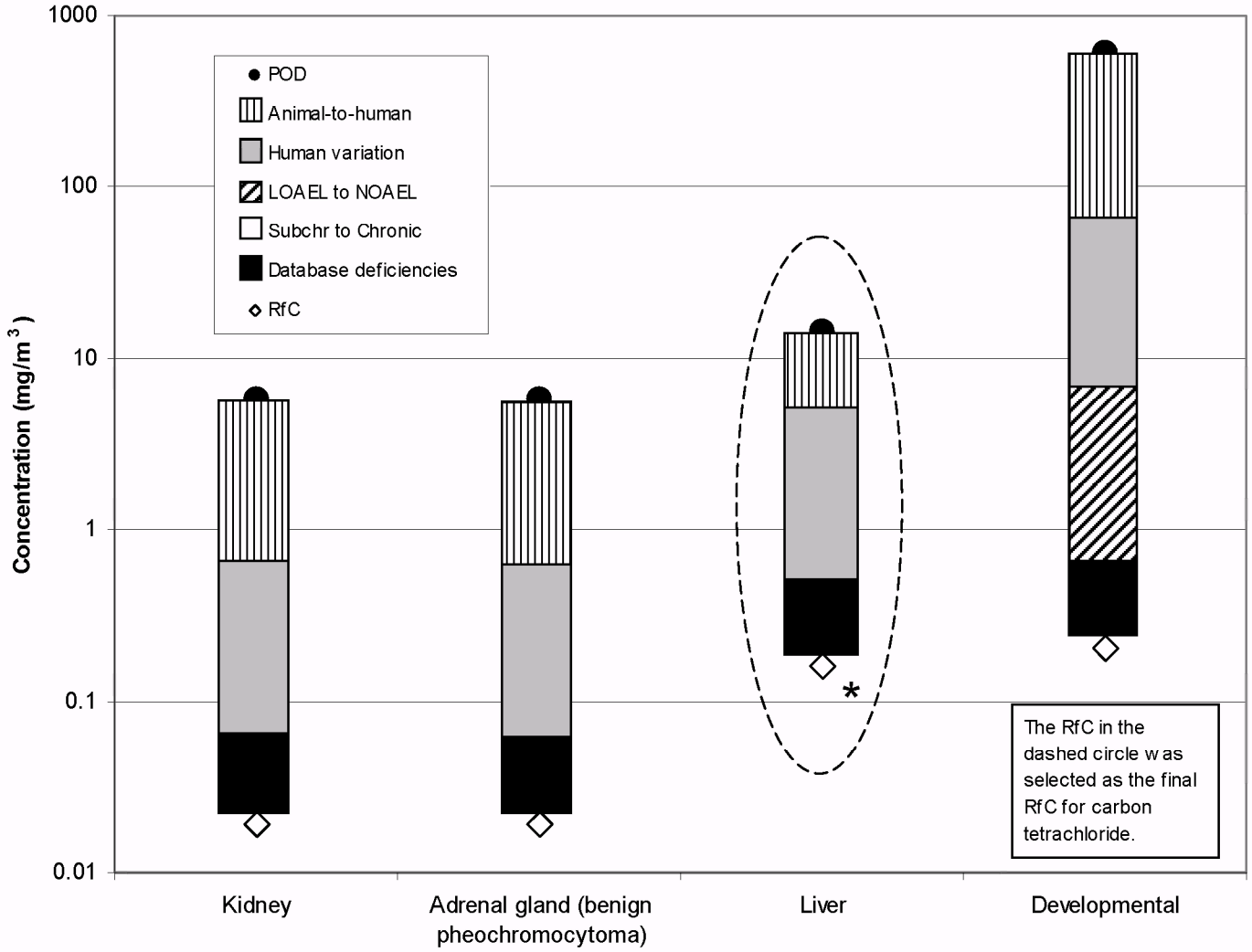


Figure 5-8. Organ-specific inhalation RfCs



POD based on:

Kidney: NOAEL for serum chemistry and histopathology changes in the rat and mouse; 2-year inhalation study (Nagano et al., 2007b)

Benign pheochromocytoma: NOAEL in the mouse; 2-year inhalation study (JBRC, 1998)

Liver: $BMDL_{[HEC]}$ for fatty liver in the rat; 2-year inhalation study (JBRC, 1998)

Developmental: LOAEL for decreased fetal body weight and crown-rump length; exposure on GD 6-15 (Schwetz et al., 1974)

Note: The RfC based on liver toxicity used BMD methods and PBPK modeling, and therefore is not directly comparable to the other organ-specific RfCs.

5.2.5. Previous RfC Assessment

An inhalation assessment for carbon tetrachloride was not previously available on IRIS.

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

Risk assessments need to describe associated uncertainty. The following discussion identifies uncertainties associated with the RfD and RfC for carbon tetrachloride. As presented earlier in this section (5.1.2 and 5.1.3 for the RfD; 5.2.2 and 5.2.3 for the RfC), the uncertainty factor approach (U.S. EPA, 2002, 1994b) was used to derive the RfD and RfC for carbon tetrachloride. Using this approach, the POD was divided by a set of factors to account for uncertainties associated with a number of steps in the analysis, including extrapolation from responses observed in animal bioassays to humans and from data from subchronic exposure to chronic exposure, a diverse population of varying susceptibilities, and to account for database deficiencies. Because information specific to carbon tetrachloride was unavailable to fully inform many of these extrapolations, default factors were generally applied.

A broad range of animal toxicity data and more limited range of human study data are available to assess carbon tetrachloride hazard (see Section 4). Human studies include case reports of acute human exposure (both oral and inhalation) and occupational epidemiology studies. The animal toxicology literature includes subchronic and chronic animal studies by the oral and inhalation routes, developmental toxicity studies by the oral and inhalation routes, studies of immunotoxic potential, extensive literature on genotoxicity, and numerous mechanistic toxicity studies. In addition, carbon tetrachloride has been used in hundreds of studies as a classic inducer of liver toxicity. Nevertheless, gaps in the carbon tetrachloride database have been identified; uncertainties associated with these data deficiencies are discussed more fully below.

Selection of the critical effect for reference value determination. Liver toxicity was selected as the critical effect for both the RfD and RfC (specifically, elevated liver enzymes [Bruckner et al., 1986] in the case of the RfD and fatty change of the liver [Nagano et al., 2007b; JBRC, 1998] in the case of the RfC. The liver has been established as a sensitive target of toxicity across animal species and routes of exposure. Case reports of human poisonings identify the liver as a target organ of acute carbon tetrachloride exposure, and an occupational epidemiology study of workers exposed to carbon tetrachloride (Tomenson et al., 1995) provides evidence of impaired liver function in humans following prolonged exposure. Thus, there is little uncertainty related to the relevance of the critical effect to human health assessment.

Kidney toxicity associated with carbon tetrachloride inhalation exposure has been seen less consistently in experimental animal studies. Nagano et al. (2007b; also reported as JBRC, 1998) reported an increase in the severity of proteinuria in rats at the lowest concentration tested

in a two-year bioassay. This kidney finding occurred at an exposure level lower than the concentration associated with fatty changes of the liver; however, given the uncertainties in this endpoint discussed in Section 4.6.2, proteinuria was not used as the critical effect for the RfC. Use of proteinuria data as the basis for the RfC would have yielded a lower POD than liver data.

Dose-response modeling. BMD modeling was used to estimate the POD for both the RfD and RfC. BMD modeling has advantages over a POD based on a NOAEL or LOAEL because, in part, the latter are a reflection of the particular exposure concentration or dose at which a study was conducted. A NOAEL or LOAEL lacks characterization of the dose-response curve and for this reason is less informative than a POD obtained from BMD modeling. The selected models—the power model in the case of the RfD and the logistic model in the case of the RfC—provided the best mathematical fits to the experimental data sets (as determined by the lowest AIC), but do not necessarily have greater biological support over the various models included in BMDS. Other models in BMDS yield estimates of the POD both higher and lower than the PODs used to derive the RfD and RfC in the current assessment.

Animal to human extrapolation. Extrapolating dose-response data from animals to humans is another source of uncertainty. The effect and the magnitude of the effect at the POD in rodents are extrapolated to human response. Uncertainty in interspecies extrapolation can be separated into two general areas—toxicokinetic and toxicodynamic. A UF of 3 was used to account for toxicodynamic difference between animals and humans. A PBPK model was available for the inhalation pathway and was used in deriving the RfC to address the toxicokinetic portion of interspecies extrapolation. Availability of an inhalation PBPK model generally reduces the toxicokinetic component of uncertainty associated with animal to human extrapolation by moving away from default assumptions about kinetic differences between animals and humans. Any PBPK model, however, has its own associated uncertainties. In the carbon tetrachloride RfC analysis, uncertainty was examined by using two dose metrics and alternative values of V_{maxC}. MRAMKL was considered the more scientifically appropriate dose metric for liver toxicity; MCA was included given uncertainties in modeling carbon tetrachloride metabolism and uncertainties regarding the magnitude of direct contribution of carbon tetrachloride (as parent compound) to liver toxicity. MRAMKL provided HEC (and thus RfC) values that were 2- to 7-fold higher than those derived using MCA (depending on the value of V_{maxC} used).

Estimates of the dose metrics, MCA and MRAMKL, were sensitive to the value assigned to the V_{maxC} parameter (see Figure 5-5 and Tables 5-6 and 5-7). Several values for V_{maxC} in animals and humans have been reported (Thrall et al., 2000, Benson and Springer, 1999; Paustenbach et al., 1988; Gargas et al., 1986); therefore, evaluation of uncertainty in this parameter was introduced into the analysis by assuming various reported values for V_{maxC} in the

estimation of HECs. Thrall et al. (2000) and Benson and Springer (1999) derived a value of 1.49 mg/hr/kg $BW^{0.70}$ for humans, based on an analysis of data on in vivo (gas uptake) studies in rodents and in vitro studies of metabolism of carbon tetrachloride in rodent and human liver samples. Thrall et al. (2000) also derived a value of 1.7 mg/hr/kg $BW^{0.70}$ for hamsters, based on the results of closed chamber gas uptake studies. The value of 1.49 mg/hr/kg $BW^{0.70}$ for humans (Thrall et al., 2000; Benson and Springer, 1999), the value of 1.70 mg/hr/kg $BW^{0.70}$ for the hamster (Thrall et al., 2000), and the two values estimated for the rat (0.4, 0.65 mg/hr/kg $BW^{0.70}$; Paustenbach et al., 1988; Gargas et al., 1986) were used in the estimation of HECs. In general, increasing V_{maxC} from 0.4 to 1.7 mg/hr/kg $BW^{0.70}$ resulted in higher values for HECs based on the MCA dose metric and lower values for HECs based on the MRAMKL dose metric. This pattern reflects the effect of higher rates of metabolism and blood clearance at any given exposure concentration that result from higher values for V_{max}. Higher rates of metabolism decrease the corresponding exposure concentration required to achieve a given value of MRAMKL and increase the corresponding exposure concentration required to achieve a given value of MCA. The effect of increasing V_{maxC} was more pronounced on HECs based on the MRAMKL dose metric. This pattern reflects the increasing influence of V_{max} on metabolism rate at higher exposure concentrations that result in liver carbon tetrachloride concentrations that exceed the K_m. The V_{maxC} upon which the RfC was based, i.e., a V_{maxC} based on in vitro human data, was considered most scientifically defensible; other values of V_{maxC} yielded HEC (and thus RfC) values that ranged from 4% smaller to 2-fold higher.

A sensitivity analysis was also performed for the human PBPK model (see Section C.4 in Appendix C). Other sensitive chemical-specific parameters included the blood:air partition coefficient and Michaelis-Menten coefficient for metabolism (K_{mX}) using MCA as the internal dose metric, and liver:blood, slowly-perfused:blood, and readily-perfused:blood partition coefficients for MRAMKL as the dose metric.

An adequate PBPK model for the oral pathway was not available and thus PBPK modeling could not be used for interspecies extrapolation in developing the RfD. In the absence of information to quantitatively assess oral toxicokinetic or toxicodynamic differences between animals and humans, a 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfD associated with this 10-fold UF.

The magnitude of possible over- or underestimation of interspecies differences introduced by the use of default factors cannot be determined.

Intrahuman variability. Heterogeneity among humans is another source of uncertainty. Carbon tetrachloride-specific data on human variation is not available. In addition, there is an absence of quantitative information on variation in hepatic levels of CYP2E1 or other metabolizing enzymes that can influence carbon tetrachloride toxicity, as well as an absence of quantitative information on levels of metabolizing enzymes in other tissues (e.g., brain) during

various stages of development. Accordingly, a default UF of 10 was used to account for uncertainty associated with human variation in the derivation of the RfD and RfC. Human variation may be larger or smaller; however, carbon tetrachloride-specific data to examine the potential magnitude of over- or underestimation is unavailable.

Subchronic to chronic exposure extrapolation. Because the available chronic oral toxicity studies for carbon tetrachloride were not considered adequate for derivation of the oral RfD, subchronic toxicity studies were used, and a UF of 3 was applied to extrapolate those data obtained from a study of subchronic exposure to chronic exposure. This UF is based on the assumption that an effect seen at a shorter duration will also be seen after a lifetime of exposure, but at a lower exposure level or with greater severity. In the absence of information to inform this extrapolation, a subchronic to chronic UF of 10 is typically applied. Inhalation data for carbon tetrachloride and other chemical-specific information (see Section 5.1.3) indicate that a full default UF of 10 would overestimate the difference in response following subchronic and chronic oral exposures. The availability of carbon tetrachloride-specific information reduces the uncertainty in extrapolating from subchronic to chronic exposure data.

Data gaps. To the extent that the database for carbon tetrachloride is incomplete, it is possible that certain endpoints of toxicity or certain sensitive lifestages have not been evaluated that could result in PODs lower than those for which study data are available. The carbon tetrachloride database lacks an adequate multigeneration study of reproductive toxicity by any route of exposure. The absence of these types of studies introduces uncertainty in the RfD and RfC. The magnitude of this uncertainty cannot be quantified.

Vehicle effects. The vehicle used in oral gavage studies to administer carbon tetrachloride could be a potential confounding factor in toxicity assays. Investigators have variably reported that (compared to an aqueous vehicle) corn oil either enhanced carbon tetrachloride toxicity (Narotsky et al., 1997; Condie et al., 1986), did not significantly affect toxicity (Kaporec et al., 1995), or reduced toxicity (Kim et al., 1990b), or that influences of vehicle could be dose-dependent (Raymond and Plaa, 1997; Narotsky et al., 1997). The polyethoxylated vegetable oil Emulphor has been shown not to influence carbon tetrachloride acute hepatotoxicity, absorption, or distribution (Sanzgiri and Bruckner, 1997). Thus, it is possible that the vehicle used in oral gavage studies to administer carbon tetrachloride could influence the observed toxicity; however, given the variable effects of corn oil (versus an aqueous vehicle), the magnitude of the confounding and the nature of the interaction of corn oil remain uncertain.

5.4. CANCER ASSESSMENT

Several epidemiological studies (including several case-control studies and one retrospective cohort study) have investigated potential associations between cancers of various types and exposure to carbon tetrachloride. In all the available studies, subjects experienced multiple chemical exposures and exposures were estimated qualitatively based on historical information. These studies, therefore, can provide only suggestive evidence for an association between carbon tetrachloride exposure and cancer, and are not useful for dose-response analysis.

Studies in experimental animals suggest that the primary cancer risk associated with exposure to carbon tetrachloride is development of liver cancer. Carbon tetrachloride produced hepatocellular carcinomas in rats, mice, and hamsters in oral studies and in rats and mice by inhalation exposure. In addition to liver tumors, adrenal pheochromocytomas were observed in male and female mice by oral and inhalation exposure (Nagano et al., 2007b; JBRC, 1998; Weisburger, 1977). No increase in pheochromocytomas was observed in rats.

Examination of rodent liver tumors reveals a general correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors, although at lower exposure levels this correspondence is somewhat less consistent. A weight of evidence analysis of the genotoxicity literature suggests that carbon tetrachloride is more likely an indirect than direct mutagenic agent; however, the nature of the genotoxicity database poses distinct challenges to the evaluation of carbon tetrachloride genotoxicity. Positive genotoxicity findings have generally been observed at exposures that induce cytotoxicity and regenerative cell proliferation. Due to the difficulties in detecting genotoxic effects following treatment with carbon tetrachloride, many studies were conducted at relatively high doses that lack information regarding dose-response. This has resulted in a database that does not characterize the role of genotoxicity at low doses of carbon tetrachloride.

The extensive mechanistic literature related to carbon tetrachloride-induced liver tumors informs the mode of action for liver tumors. As noted in Section 4.7.3 and in the following section, the empirical evidence for carbon tetrachloride, particularly data from relatively high-exposure studies, provides support for the hypothesis that liver carcinogenicity is presumed to occur at exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response, and that exposures that do not cause hepatotoxicity are not expected to result in liver cancer. This mode of action for carbon tetrachloride liver carcinogenicity is consistent with a nonlinear approach to low-dose extrapolation. A nonlinear low-dose extrapolation approach is presented in Section 5.4.1.

Although much of the empirical data is consistent with hepatocellular toxicity and a sustained regenerative and proliferative response as key events in the mode of action for rodent liver tumors, liver findings from the JBRC bioassay (Nagano et al., 2007b; JBRC, 1998) suggest that mouse hepatocarcinogenicity cannot simply be explained in terms of this mode of action. An increased incidence of hepatocellular adenomas occurred in the low-dose (5-ppm or 0.9-ppm

adjusted) female mouse in the absence of nonneoplastic liver toxicity, raising the possibility of another mode of action operating in addition to or in conjunction with the cytotoxic-proliferative mode of action. As discussed in Section 4.7, considerable evidence points to the involvement of highly reactive metabolites in the induction of liver toxicity and carcinogenicity by carbon tetrachloride. In addition, subsequent chemical reactions of carbon tetrachloride metabolites with cellular constituents lead to formation of reactive oxygen species that also can damage DNA and other macromolecules. Although the extensive genotoxicity database for carbon tetrachloride suggests that carbon tetrachloride is not likely a direct acting mutagen, the database is complex and raises various issues (see Table 4-12) that make it difficult to reach a firm judgement about the potential genotoxicity of carbon tetrachloride at exposures below which there is overt toxicity. In light of the mouse liver findings from the JBRC bioassay, the fundamental reactivity of both direct and indirect products of carbon tetrachloride metabolism, and limited information about carbon tetrachloride's biological activity at low exposures, an argument can be made for application of a nonthreshold approach to carbon tetrachloride carcinogenicity. Section 5.4.2 presents low-dose linear extrapolation approaches to carbon tetrachloride carcinogenicity.

5.4.1. Nonlinear Extrapolation Approach

As noted above, much of the empirical evidence for carbon tetrachloride, particularly from studies using relatively high exposure levels, supports a mode of action for liver tumors that includes the following key events: (1) metabolism to the trichloromethyl radical by CYP2E1 and subsequent formation of the trichloromethyl peroxy radical, (2) radical-induced mechanisms leading to hepatocellular toxicity, and (3) sustained regenerative and proliferative changes in the liver in response to hepatotoxicity. These key events are consistent with a hypothesis that liver carcinogenicity occurs at exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response, and that exposures that do not cause hepatotoxicity are not expected to result in liver cancer. For this hypothesized mode of action for carbon tetrachloride liver carcinogenicity, a nonlinear approach to low-dose extrapolation is considered appropriate.

The RfD of 0.004 mg/kg-day and RfC of 0.1 mg/m^3 derived in Sections 5.1 and 5.2 represent the outcome of nonlinear assessments based on hepatotoxicity associated with oral exposures (RfD) and inhalation exposures (RfC) to carbon tetrachloride. Consistent with the hypothesized mode of action for liver tumors consisting of metabolism, cytotoxicity, and sustained regeneration and proliferation, doses (or concentrations) of carbon tetrachloride below the RfD (or RfC) would not be expected to produce liver tissue damage and therefore would not be expected to produce an increase in liver cancer risk. This harmonized approach between noncancer and cancer endpoints transparently utilizes a key event (cytotoxicity or hepatotoxicity) in the hypothesized nonlinear mode of action to derive the RfD and RfC. Based on the mode of

action consistent with nonlinearity, the RfD of 0.004 mg/kg-day and RfC of 0.1 mg/m^3 can be used to assess the potential risk of liver cancer from carbon tetrachloride exposure.

The application of a nonlinear approach for liver tumors is based on mode of action information specific to that tumor type and does not apply to the occurrence of pheochromocytomas. As noted above, the pheochromocytomas in mice reported in the JBRC 104-week bioassay (Nagano et al., 2007b; JBRC, 1998) were, with one exception, characterized as benign rather than malignant. Unlike liver tumors associated with carbon tetrachloride exposure, which have been observed in numerous bioassays in multiple species and by multiple routes of exposure, pheochromocytomas have been observed only in the mouse. Thus, the finding of pheochromocytomas in the mouse may be a species-specific finding and, as such, may present a less certain human cancer risk than does the finding of liver tumors in experimental animals. Nevertheless, the RfD and RfC based on liver toxicity cannot be assumed to be protective for the potential cancer risk associated with carbon tetrachloride-induced pheochromocytomas in the mouse.

5.4.2. Linear Extrapolation Approach

This section develops estimates of carbon tetrachloride cancer risk using approaches incorporating low-dose linearity. Available data are not sufficient to support a biologically-based dose-response model for the relationship of carbon tetrachloride and cancer. Judgements regarding the potential dose-response relationships for carbon tetrachloride cancer risks at low dose are informed by bioassay and mechanistic data for carbon tetrachloride as well as broader scientific considerations.

The application of linear extrapolation is consistent with several pieces of evidence suggesting that carbon tetrachloride carcinogenicity may not be attributable to a nonlinear mode of action only. As noted above, the JBRC bioassay (Nagano et al., 2007b; JBRC, 1998) revealed an increased incidence of hepatocellular adenomas in the low-dose (5-ppm or 0.9-ppm adjusted) female mouse in the absence of cytotoxicity, suggesting that mouse hepatocarcinogenicity cannot be explained in terms of a cytotoxic-proliferative mode of action. In addition, carbon tetrachloride induced pheochromocytomas in male and female mice by oral (NTP, 2007; Weisburger, 1977) and inhalation (Nagano et al., 2007b; JBRC, 1998) exposure. Because the mode of action for pheochromocytomas in the mouse is unknown, linear low-dose extrapolation as a default approach is applied to data for this tumor type.

As discussed in Section 4.7, considerable evidence points to the involvement of highly reactive metabolites (with the capacity to chemically interact with DNA and other cellular macromolecules) in the processes of toxicity and carcinogenicity of carbon tetrachloride. In addition, subsequent chemical reactions of carbon tetrachloride metabolites with cellular constituents lead to formation of reactive oxygen species that also can damage DNA and other macromolecules. As evaluated in this assessment, the data available at this time do not

demonstrate that carbon tetrachloride or its metabolites are direct acting mutagens. However, the genotoxicity database, while large, is complex and there are various interpretive issues (see Table 4-12) regarding the potential for genotoxicity of carbon tetrachloride at doses below those associated with overt toxicity. In this situation, the fundamental reactivity of both direct and indirect products of carbon tetrachloride metabolism provides a cogent argument in favour of some degree of nonthreshold response to carbon tetrachloride carcinogenicity. Further research may inform both the dosimetry for DNA (or other macromolecules) exposure to direct and indirect reactive products resulting from carbon tetrachloride exposure and the dose-response relationships for subsequent events resulting from damage of DNA or other macromolecules.

Thus, in the case of liver tumors, bioassay evidence inconsistent with a nonlinear mode of action in the range of experimental observations and uncertainties in carbon tetrachloride's biological activity at low exposures suggest that other (or another) modes of action may be operative. Given a lack of understanding for the mode(s) of action for liver tumors, a default linear low-dose extrapolation approach to carbon tetrachloride cancer data (see Section 5.4.2) is presented in addition to the nonlinear approach (see Section 5.4.1) for carbon tetrachloride-induced liver tumors. Given a lack of understanding of the mode of action for pheochromocytomas, a default linear low-dose extrapolation approach to carbon tetrachloride cancer data is applied consistent with the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) (see Section 5.4.2).

Broader science considerations based on scientific literature not specific to carbon tetrachloride also support inferences about potential risks of carbon tetrachloride at lower doses. EPA guidance and reports from expert advisory bodies have provided broad and long-standing scientific arguments in favor of low-dose linear approaches to cancer risk assessment. This perspective is based on the following principles:

- A chemical's carcinogenic effects may act additively to ongoing biological processes, given that diverse human populations already have substantial background incidence of various tumors (e.g., Crump et al, 1976);

- A broadening of the dose-response curve in the human population (i.e., less rapid fall-off with dose) and, accordingly, a greater potential for risks from low-dose exposures (see Zeise et al., 1987; Lutz et al., 2005) would result for two reasons. First, even if there is a threshold concentration at the cellular level, that threshold is likely to be different among different individuals. Secondly, greater variability in response to exposures in the heterogeneous human population would be anticipated than in controlled laboratory species and conditions (due to, e.g., genetic variability, disease states, nutrition, age).

- The general use of linear extrapolation provides plausible upper-bound risk estimates and also provides consistency across assessments.

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

5.4.2.1.1. ***Inhalation data.*** As noted previously, epidemiological studies of populations exposed to carbon tetrachloride provide only suggestive evidence for an association between carbon tetrachloride exposure and human cancer and are not adequate for dose-response analysis.

The only chronic bioassay of carbon tetrachloride by the inhalation route is the 104-week inhalation bioassay in rats and mice conducted by JBRC (Nagano et al., 2007b; JBRC, 1998), a bioassay that provides data adequate for dose-response modeling. In this bioassay, F344 rats and BDF1 mice were exposed to 0, 5, 25, or 125 ppm carbon tetrachloride, 6 hours/day, 5 days/week, for 2 years. Carbon tetrachloride produced a statistically significant increase in hepatocellular adenomas and carcinomas in rats and mice of both sexes, and adrenal pheochromocytomas in mice of both sexes.

5.4.2.1.2. ***Oral data***. Studies of carbon tetrachloride carcinogenicity by the oral exposure route are not sufficient to derive a quantitative estimate of cancer risk using low-dose linear approaches. No epidemiological investigations of the possible carcinogenicity of carbon tetrachloride associated with oral exposure have been performed. The cancer studies by Edwards et al. (1942) in the mouse and Della Porta et al. (1961) in the hamster included a control and only one dose group, and animals were dosed for less than a lifetime (2 months and 30 weeks, respectively). Neither study provided body weight information, so that doses could not be estimated with certainty. Despite the relatively short dosing periods and the fact that animals were kept on study for less than a lifetime (approximately 6.5 months in the case of Edwards et al., 1942, and approximately 1 year in the case of Della Porta et al., 1961), liver tumor incidence was very high (71% in the case of Edwards et al., 1942, and 100% of the hamsters that died or were sacrificed between weeks 43 and 55 in the case of Della Porta et al., 1961). In the NCI bioassays (1977, 1976a,b), liver tumor incidence in the mouse was virtually 100% in both dose groups. In the rat, liver tumor incidence was low and failed to show a dose-response relationship (in the female rat, tumor incidence was higher in the low-dose group [4/46] than in the high-dose group [1/30], presumably because early mortality in the high-dose group precluded tumor formation). Thus, none of the available oral studies of carbon tetrachloride carcinogenicity provided data sets amenable to dose-response modeling.

5.4.2.2. ***Dose-Response Data***

5.4.2.2.1. ***Inhalation data***. Dose-response modeling was performed for five tumor responses from the JBRC bioassay: adenoma and carcinoma of the liver in female rats, adenoma and

carcinoma of the liver in male and female mice, and pheochromocytomas in male and female mice. Incidence data for liver tumors and pheochromocytomas are summarized in Tables 5-8 and 5-9 below.

Table 5-8. Incidence of liver tumors in F344 rats and BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

Tumor	Male				Female			
	0 ppm	5 ppm	25 ppm	125 ppm	0 ppm	5 ppm	25 ppm	125 ppm
RAT								
Hepatocellular adenoma or carcinoma	1/50[a]	1/50	1/50	40/50[b]	0/50[a]	0/50	3/50	44/50[b]
MOUSE								
Hepatocellular adenoma or carcinoma	24/50[a]	20/50	49/50[b]	49/50[b]	4/50[a]	9/49	44/50[b]	48/49[b]

[a] Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[b]Tumor incidence significantly elevated compared with that in controls by Fisher Exact test ($p \leq 0.01$).

Source: Nagano et al, 2007b; JBRC, 1998

Table 5-9. Incidence of adrenal tumors (pheochromocytomas) in BDF1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

Tumor	Male				Female			
	0 ppm	5 ppm	25 ppm	125 ppm	0 ppm	5 ppm	25 ppm	125 ppm
Adrenal pheochromocytoma[c]	0/50[a]	0/50	16/50[b]	32/50[b]	0/50[a]	0/49	0/50	22/49[b]

[a] Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[b]Tumor incidence significantly elevated compared with controls by Fisher Exact test ($p \leq 0.01$).
[c] All pheochromocytomas in the mouse were benign with the exception of one malignant pheochromocytoma in the 125-ppm male mouse group.

Source: Nagano et al, 2007b; JBRC, 1998

The male rat data for liver adenomas and carcinomas were not modeled because this data set lacked the resolution desired for dose-response modeling. Tumor frequency jumped from control levels to close to maximal response without any intervening dose levels having submaximal responses. In the female rat, lower but biologically significant levels of response were seen at intermediate dose levels. Further, the incidence of liver tumors was higher in the

female rat compared with the male rat, such that the female rat data would provide the higher estimate of risk of the two data sets.

For the female mouse, the bioassay data set contained two exposure concentrations (mid- and high-exposure concentrations) at which close to maximal responses were seen. Preliminary fitting of a multistage model revealed that: (1) a fit with an adequate chi square based p-value was not obtained, and (2) the fit and parameter estimates were highly sensitive to the precise finding of 48/49 tumors at the highest concentration. (A hypothetical shift of the data to 49/49 tumors led to a good model fit with different powers of the multistage model involved in the fit.) As these distinctions were not judged biologically based, multistage model fits below were conducted without use of the highest exposure concentration data, an approach commonly used in BMD modeling when very high dose data are not compatible with model fits.)

Dose-response modeling was also conducted for pheochromocytomas observed in the JBRC mouse bioassay. These tumors, with one exception, were characterized as benign rather than malignant. Unlike liver tumors associated with carbon tetrachloride exposure, which have been observed in numerous bioassays in multiple species, pheochromocytomas have been observed in only one species (mouse). Thus, the finding of pheochromocytomas in the mouse may present a less certain human health hazard than does the finding of liver tumors in experimental animals. The decision to develop dose-response models for pheochromocytomas was based on guidance provided in the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), which states that "benign tumors that are not observed to progress to malignancy are assessed on a case-by-case basis." The benign tumor type seen in the mouse has a human equivalent that is damaging to human health and can lead to fatal sequelae. In humans, pheochromocytomas are rare and usually benign neuroendocrine tumors, but may also present as or develop into a malignancy (Eisenhofer et al., 2004). Salmenkivi et al. (2004) noted that approximately 10% of pheochromocytomas in humans metastasize. The presence of one observed malignant tumor in the mouse study also suggests potential for these benign tumors to progress to malignancy. The oral NCI bioassay characterized adrenal gland tumors simply as "pheochromocytoma" (incidence data are provided in Weisburger, 1977, and NTP, 2007). This characterization suggests that the tumors were not malignant, although the status as benign or malignant was not clearly established. Finally, Salmenkivi et al. (2004) observed that while most pheochromocytomas are benign, differentiating a benign from a malignant tumor only by histological criteria is very difficult. Thus, it was considered appropriate to conduct dose-response modeling for pheochromocytomas and to address the potential cancer risk using linear extrapolation as a default approach.

In the analyses of the mouse and rat carbon tetrachloride inhalation data that follow, incidence reflects that of benign or malignant tumors combined. Data are not available to indicate whether malignant tumors developed specifically from progression of the benign tumors; however, etiologically similar tumor types, i.e., benign and malignant tumors of the

same cell type, were combined for these analyses because of the possibility that the benign tumors could progress to the malignant form (U.S. EPA, 2005a).

5.4.2.2.2. ***Oral data***. As noted above, oral cancer bioassay data for carbon tetrachloride are not adequate for dose-response analysis. Therefore, PBPK modeling was applied to extrapolate inhalation tumor data to the oral route. Because liver tumors and pheochromocytomas have been observed in experimental animals following both inhalation and oral exposures, the data sets evaluated as the basis for the inhalation unit risk were considered appropriate for estimation of an oral slope factor. The route-to-route extrapolation method is described further below.

5.4.2.3. ***Dose Adjustments and Extrapolation Methods***

5.4.2.3.1. ***General approach to modeling and extrapolation of animal data to humans***. Cancer risk estimates were obtained by straight line extrapolation from the POD to zero as described in the EPA's *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005a). As stated in the guidelines, "The linear approach is to draw a straight line between a point of departure from observed data, generally as a default, an LED [lower bound of effective dose] chosen to be representative of the lower end of the observed range, and the origin (zero incremental dose, zero incremental response)." Linear extrapolation is used as the approach in the absence of data supporting a biologically based model for extrapolation outside of the observed range (U.S. EPA, 2005a).

The general procedure for deriving the POD from animal bioassay data is the same as that used to derive the POD for RfC derivation and is depicted in Figure 5-4. Exposure levels studied in the 2-year rat and mouse bioassays (Nagano et al., 2007b; JBRC, 1998) were converted to estimates of internal doses by application of the rat and mouse PBPK models. BMD modeling methodology (U.S. EPA, 2000c, 1995) was used to analyze the relationship between the estimated internal doses and response (i.e., liver tumors and pheochromocytomas). The resulting BMDL values were converted to estimates of equivalent human exposure concentrations and doses (HECs and HEDs) by applying the human PBPK model.

5.4.2.3.2. ***PBPK modeling for internal dose metrics***. Estimation of internal doses corresponding to the exposure concentrations studied in the 2-year rat and mouse bioassays (Nagano et al., 2007b; JBRC, 1998) was accomplished using PBPK models of the rat (Thrall et al., 2000; Benson and Springer, 1999; Paustenbach et al., 1988) and mouse (Fisher et al., 2004; Thrall et al., 2000), respectively (see Sections 3.5 and Appendix C for description of the models). The review, selection and application of the chosen PBPK models was informed by an EPA report (U.S. EPA, 2006), which addresses the application and evaluation of PBPK models. The PBPK models were used to simulate internal dose metrics corresponding to exposure concentrations studied in the 2-year bioassays: 5, 25, and 125 ppm, 6 hours/day, 5 days/week

(Nagano et al., 2007b; JBRC, 1998). Internal dose metrics were selected that were considered to be most relevant to the toxicity endpoints of interest (i.e., liver tumors and pheochromocytomas), based on consideration of evidence for mode of action of carbon tetrachloride. Two dose metrics were selected based on available information on the mechanisms of carbon tetrachloride liver toxicity: (1) time-averaged arterial blood concentration of carbon tetrachloride (MCA, μmol/L); and (2) time-averaged rate of metabolism of carbon tetrachloride (MRAMKL, μmol/hr/kg liver). Liver metabolism rate was selected as the primary dose metric for liver effects based on evidence that metabolism of carbon tetrachloride via CYP2E1 to highly reactive free radical metabolites plays a crucial role in its mode of action in producing liver toxicity (described in Section 4.5). Because of acknowledged uncertainties regarding the accuracy of available PBPK models to simulate carbon tetrachloride (see Section 5.2.2.1), arterial blood concentration of carbon tetrachloride was also included in the analysis of liver tumor data as a more proximal dose metric to liver metabolism.

Data on incidence of adrenal pheochromocytomas in mice were also analyzed. The MRAMKL dose metric was excluded from consideration in the analysis of pheochromocytomas on the basis that reactive metabolites of carbon tetrachloride formed in the liver are unlikely to be sufficiently stable to contribute to toxicity or transformations of cells in the adrenal gland. Although it is possible that local generation of reactive metabolites may contribute to the production of pheochromocytomas, PBPK models available for this analysis do not simulate uptake and metabolism of carbon tetrachloride in the adrenal gland. [The model of Yoon et al. (2007) is the only one available that includes extra-hepatic metabolism, specifically in lung and kidney. Metabolism in each of these tissues was estimated to be less than 1% of that in the liver, and they had a negligible effect on MCA and MRAMKL.] It would be expected, however, that rates of metabolism in all tissues, including the adrenal gland, would be dependent on delivery of carbon tetrachloride to these tissues and, thereby, would be correlated with blood concentrations of carbon tetrachloride. Therefore, the MCA dose metric was used to represent the internal dose in BMD modeling of pheochromocytoma incidence in mice.

The two dose metrics, MCA and MRAMKL, were simulated in the rat and mouse PBPK models as time-averaged values, with the averaging time being the chronic exposure period (e.g., 2 years). See Equations 5-1 and 5-2 (Section 5.2.2.1) for the calculation of the time-averaged dose metrics.

Internal dose metrics corresponding to the exposure concentrations studied in the 2-year rat inhalation bioassay (Nagano et al., 2007b; JBRC, 1998) for two values of V_{maxC} were provided previously in Table 5-5 (see Section 5.2.2.1). Internal dose metrics corresponding to the exposure concentrations studied in the 2-year mouse inhalation bioassay (Nagano et al., 2007b; JBRC, 1998) as derived from the Fisher et al. (2004) and Thrall et al. (2000) PBPK models are presented in Table 5-10. The Fisher et al. (2004) model predicts lower values for MCA than the Thrall et al. (2000) model. This is at least partly explained by the higher values

for tissue:blood partition coefficients in the Fisher et al. (2004) model, which results in a larger fraction of the body burden outside of the vascular compartment. The Fisher et al. (2004) model predicts higher values for MRAMKL at exposure concentrations above approximately 40 ppm. At least two factors contribute to this pattern: (1) the higher liver:blood partition coefficient in the Fisher et al. (2004) model results in higher concentrations of carbon tetrachloride in the liver; and (2) the higher V_{maxC} in the Fisher et al. (2004) model results in increases in liver metabolism rate at any given liver concentration of carbon tetrachloride, with the more pronounced enhancement of metabolism at liver concentrations above the K_m. The exposure concentration-dependence of the dose metrics estimated from both models is shown in Figure 5-9.

Table 5-10. Internal dose metrics predicted from Fisher et al. (2004) and Thrall et al. (2000) PBPK mouse models

Exposure (ppm)	MCA (µmol/L)		MRAMKL (µmol/hr/kg liver)	
	Fisher	Thrall	Fisher	Thrall
5	0.111	0.213	12.666	15.456
25	0.603	1.226	41.675	43.599
125	3.315	6.856	71.589	63.596

Values are for 0.036 kg mouse.
MCA, time-averaged arterial concentration of carbon tetrachloride; MRAMKL, time-averaged rate of metabolism of carbon tetrachloride per kg liver.

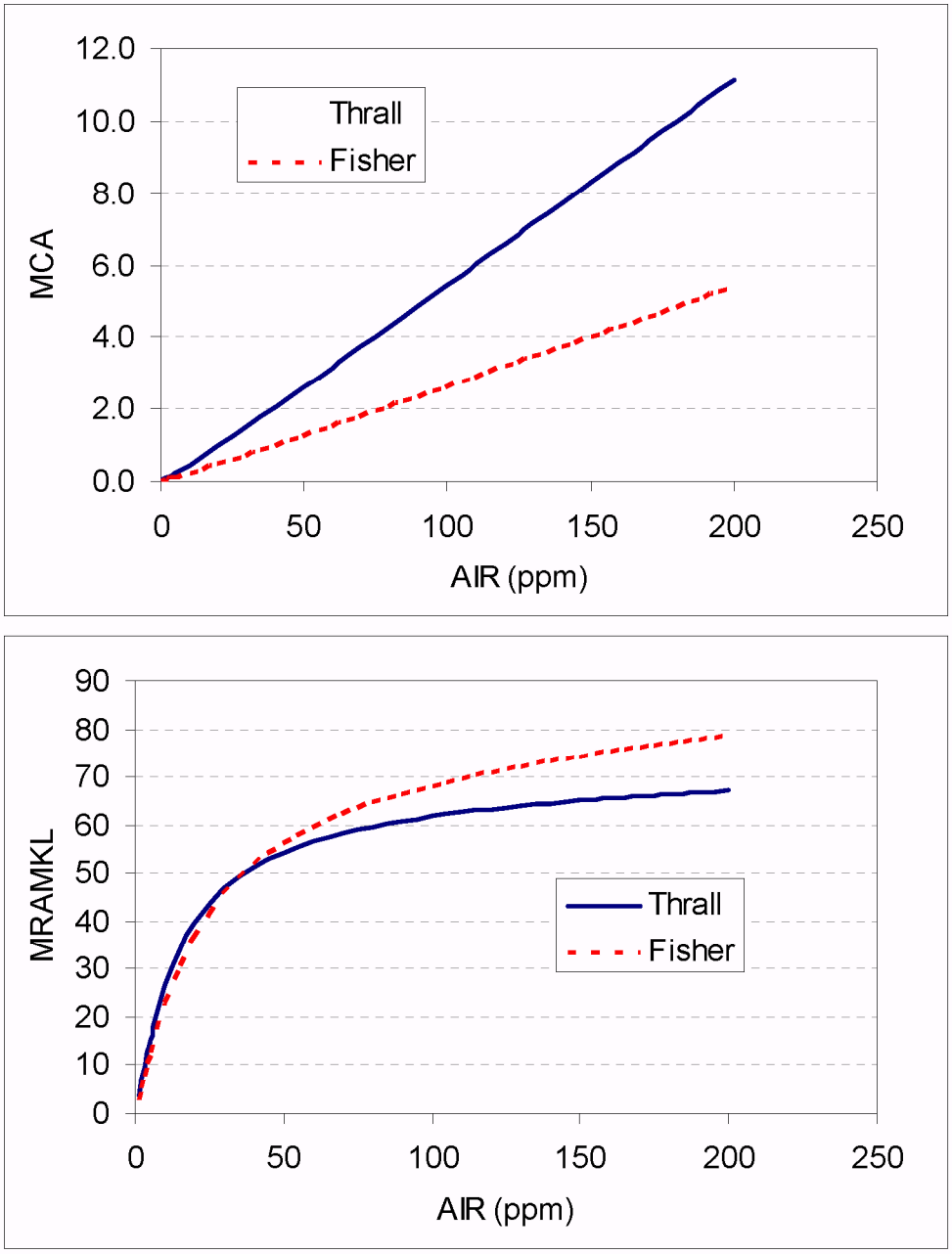


Figure 5-9. Internal dose metrics predicted from the Fisher et al. (2004) and Thrall et al. (2000) PBPK mouse models.

Dose metrics shown are time-averaged arterial concentration of carbon tetrachloride (MCA, :mol/L, upper panel), and time-averaged rate of metabolism of carbon tetrachloride (MRAMKL, µmol/hr/kg liver, lower panel). The dose metrics are plotted against exposure concentration (6 hours/day, 5 days/week, 2 years) for a 0.036 kg mouse.

5.4.2.3.3. ***Benchmark dose modeling of response data from animal bioassays***. BMD modeling methodology (U.S. EPA, 2000c, 1995) was used to analyze data on estimated internal doses (i.e., MCA, MRAMKL) and incidence data (i.e., liver tumors in rats, and liver tumors and adrenal pheochromocytomas in mice) from the 2-year rat and mouse inhalation bioassays (Nagano et al., 2007b; JBRC, 1998). The multistage model in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) was fit to the tumor incidence data for rats and mice. When adequate fit could not be achieved with the multistage model, other models from the BMDS suite of models were fit. The results of the BMD modeling are summarized below; detailed model outputs are provided in Appendix E.

Female F344 rat -- hepatocellular adenomas + carcinomas

Internal doses associated with a BMR of 5% extra risk of liver tumors were calculated. A BMR of 5% excess risk was in the low range of experimental data for the rat (see Appendix E). In addition, a BMR of 5% excess risk was preferred over a BMR of 10% in the interest of moving the POD further from the range where hepatocellular toxicity and a proliferative/regenerative response was observed and where tumor induction is more likely influenced by a cytotoxic-proliferative mode of action.

BMD modeling using the multistage model in BMDS was performed using the female rat liver tumor incidence data shown in Table 5-8 and internal doses shown in Table 5-5. A summary of the resulting BMD_5 and $BMDL_5$ values is presented in Table 5-11 (columns 2 and 3).

Table 5-11. BMD values for incidence data for liver tumors (adenoma plus carcinoma) in female F344 rats and corresponding HEC and HED values

Metric	BMD modeling[a]		$VMAXC_H$	HEC		HED	
	$VMAXC_R$ = 0.4	$VMAXC_R$ = 0.65		$VMAXC_R$ = 0.4	$VMAXC_R$ = 0.65	$VMAXC_R$ = 0.4	$VMAXC_R$ = 0.65
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA	BMD_5: 0.61 $BMDL_5$: 0.39	BMD_5: 0.59 $BMDL_5$: 0.35	0.40	26.083	23.922	3.65	3.37
			0.65	27.605	25.318	4.27	3.96
			1.49	27.605	28.203	6.35	5.95
			1.70	31.273	28.667	6.87	6.44
MRAMKL	BMD_5: 9.82 $BMDL_5$: 8.40	BMD_5: 14.6 $BMDL_5$: 12.3	0.40	107.759	236.171	5.10	11.19
			0.65	63.915	105.882	3.03	5.01
			1.49	39.635	59.326	1.88	2.81
			1.70	37.771	56.236	1.79	2.66

Rats were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Internal doses modeled correspond to exposure concentrations: 0, 5, 25, or 125 ppm.
HEC, human equivalent concentration, mg/m^3; HED, human equivalent dose, mg/kg-day; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; VMAXC, maximum rate of metabolism in humans (H) or rat (R), mg/hr/kg $BW^{0.70}$
[a] MCA, multistage (2-stage); MRAMKL, multistage (4-stage)
BMR (benchmark response) = 5%
[b] Number in parentheses indicates the column number.

A second analysis was performed to examine the effect on the cancer risk estimate of using only carbon tetrachloride cancer response data at exposure levels below those associated with evidence of cell replication. In the female F344 rat, the 3/50 hepatocellular carcinoma response at 25 ppm (an exposure concentration at which cytotoxicity occurred but below which regenerative proliferation was reported; see Table 4-17) is statistically significant (two-tailed p-value of 0.0002) when compared to the historical control incidence of 2/1797 for female rats for the same strain and research center (email data April 5, 2007, from Kasuke Nagano, JBRC, to Susan Rieth, U.S. EPA). A comparison to concurrent controls in the JBRC study did not yield a statistically significant difference in response; however, because the observed carcinomas in female rats at 25 ppm are part of a trend of increasing carcinoma incidences with increasing exposure, it is reasonable to consider the tumors to be biologically significant.

As noted above, cytotoxicity was reported in female rats at 25 ppm in the 104-week study, but regeneration and proliferation were not reported at this exposure level; additionally, regeneration and proliferation were not observed in 13-week studies at 30 ppm and below (Table 4-17). Thus, the tumor response at 25 ppm can be considered as potentially independent of, or at most minimally influenced by, regenerative proliferation.

A multistage POD model of the control, 5 ppm, and 25 ppm exposure groups (Table 5-12, columns 2 and 3) is provided for comparison with the results above for the full data set (see Table 5-11, columns 2 and 3).

Table 5-12. BMD values for incidence data for liver tumors (adenoma plus carcinoma) in female F344 rats (high dose dropped) and corresponding HEC and HED values

Metric	BMD modeling[a]		$VMAXC_H$	HEC		HED	
	$VMAXC_R$ = 0.4	$VMAXC_R$ = 0.65		$VMAXC_R$ = 0.4	$VMAXC_R$ = 0.65	$VMAXC_R$ = 0.4	$VMAXC_R$ = 0.65
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA	BMD_5: 0.65 $BMDL_5$: 0.35	BMD_5: 0.60 $BMDL_5$: 0.32	0.40	23.339	21.459	3.29	2.40
			0.65	24.701	22.713	3.88	3.61
			1.49	27.512	25.288	5.84	5.48
			1.70	27.965	25.701	6.33	5.95
MRAMKL	BMD_5: 11.6 $BMDL_5$: 6.92	BMD_5: 16.7 $BMDL_5$: 9.76	0.40	79.943	140.519	3.79	6.66
			0.65	50.626	77.275	3.05	3.66
			1.49	32.384	46.414	1.53	2.20
			1.70	30.918	44.157	1.46	2.09

Rats were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Internal doses modeled correspond to exposure concentrations: 0, 5, 25, or 125 ppm.
HEC, human equivalent concentration, mg/m^3; HED, human equivalent dose, mg/kg-day; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; VMAXC, maximum rate of metabolism in humans (H) or rat (R), mg/hr/kg $BW^{0.70}$
[a] MCA, multistage (2-stage); MRAMKL, multistage (2-stage)
BMR (benchmark response) = 5%
[b] Number in parentheses indicates the column number.

See Appendix E for the BMDS model outputs and graphs of the modeled data.

Female BDF1 mouse – hepatocellular adenomas + carcinomas

Internal doses associated with a BMR of 10% extra risk of liver tumors were calculated. As with the female rat liver tumor data, EPA considered a BMR of 5% excess risk in the interest of moving the POD further from the range where hepatocellular toxicity and a proliferative/regenerative response was observed and where tumor induction may more likely be influenced by a cytotoxic-proliferative mode of action. In the case of the female mouse liver tumor data, however, a BMR of 5% fell well below the experimental range; therefore, a BMR of 10% was used in the BMD modeling of female mouse liver tumor data.

BMD modeling using the multistage model in BMDS was performed using the female mouse liver tumor incidence data shown in Table 5-8 and internal doses shown in Table 5-10.

As noted in Section 5.4.2.2.1, the multistage model fits below were conducted without use of the highest exposure concentration data, an approach commonly used in BMD modeling when very high dose data are not compatible with model fits. A summary of the resulting BMD_{10} and $BMDL_{10}$ values is presented in Table 5-13 (columns 2 and 3).

Table 5-13. BMD values for incidence data for liver tumors (adenoma plus carcinoma) in female BDF1 mice (high dose dropped) and corresponding HEC and HED values

Metric	BMD modeling[a]		$VMAXC_H$	HEC		HED	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA	BMD_{10}: 0.10 $BMDL_{10}$: 0.047	BMD_{10}: 0.19 $BMDL_{10}$: 0.088	0.40	3.197	6.042	0.50	0.94
			0.65	3.385	6.396	0.61	1.14
			1.49	3.753	7.097	0.99	1.82
			1.70	3.811	7.208	1.08	2.00
MRAMKL	BMD_{10}: 9.71 $BMDL_{10}$: 6.32	BMD_{10}: 10.4 $BMDL_{10}$: 7.59	0.40	70.278	91.709	3.33	4.34
			0.65	45.526	56.492	2.16	2.68
			1.49	29.466	35.646	1.40	1.69
			1.70	28.152	34.005	1.33	1.61

Mice were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped)
HEC, human equivalent concentration, mg/m^3; HED, human equivalent dose, mg/kg-day; Fisher, Fisher et al. (2004) model; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; Thrall, Thrall et al. (2000) model; $VMAXC_H$, maximum rate of metabolism in humans, mg/hr/kg $BW^{0.70}$
[a] MCA, multistage (2-stage); MRAMKL, multistage (2-stage)
BMR (benchmark response) = 10%
[b] Number in parentheses indicates the column number.

As with the rat, a second analysis was performed with female mouse liver tumor data to examine the effect on the cancer risk estimate of using only carbon tetrachloride cancer response data at exposure levels below those associated with evidence of cell replication. A multistage model POD calculation using only the control and 5-ppm exposure group (Table 5-14, columns 2 and 3) is provided for comparison with the results above for the full data set (Table 5-13, columns 2 and 3).

Table 5-14. BMD values for incidence data for liver tumors (adenoma plus carcinoma) in female BDF1 mice (2 highest doses dropped) and corresponding HEC and HED values

Metric	BMD modeling[a]		$VMAXC_H$	HEC		HED	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA	BMD_{10}: 0.10 $BMDL_{10}$: 0.044	BMD_{10}: 0.20 $BMDL_{10}$: 0.085	0.40	3.025	5.792	0.48	0.91
			0.65	3.202	6.132	0.58	1.09
			1.49	3.550	6.804	0.94	1.75
			1.70	3.605	6.910	1.03	1.92
MRAMKL	BMD_{10}: 11.6 $BMDL_{10}$: 5.05	BMD_{10}: 14.2 $BMDL_{10}$: 6.16	0.40	52.187	67.796	2.47	3.21
			0.65	35.277	44.180	1.67	2.09
			1.49	23.367	28.683	1.11	1.36
			1.70	22.358	27.410	1.06	1.30

Mice were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped)
HEC, human equivalent concentration, mg/m^3; HED, human equivalent dose, mg/kg-day; Fisher, Fisher et al. (2004) model; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; Thrall, Thrall et al. (2000) model; $VMAXC_H$, maximum rate of metabolism in humans, mg/hr/kg $BW^{0.70}$
[a] MCA, multistage (2-stage); MRAMKL, multistage (2-stage)
BMR (benchmark response) = 10%
[b] Number in parentheses indicates the column number.

See Appendix E for the BMDS model outputs and graphs of the modeled data.

Male BDF1 mouse – hepatocellular adenomas + carcinomas

Internal doses associated with a BMR of 10% extra risk of liver tumors were calculated for the male mouse. As with the female mouse liver tumor data, a BMR of 10% was used in the BMD modeling.

Similar to the male rat data for liver adenomas and carcinomas, the male mouse data provided poor resolution of the dose-response relationship for liver tumors. Tumor incidence in 5-ppm male mice was below the control level, and was close to maximal response (49/50) at the mid- and high-exposure groups, without any intervening dose levels having submaximal responses. BMD modeling of this data set (shown in Table 5-8) and internal doses (shown in Table 5-10) revealed that none of the dichotomous models in BMDS provided an adequate fit of the liver tumor data. Therefore, multistage model fits were conducted without use of the highest exposure group (125-ppm) data. A marginal fit of the data was obtained when the multistage model was applied to this reduced data set. A summary of the resulting BMD_{10} and $BMDL_{10}$ values is presented in Table 5-15 (columns 2 and 3).

Table 5-15. BMD values for incidence data for liver tumors (adenoma plus carcinoma) in male BDF1 mice (high dose dropped) and corresponding HEC and HED values

Metric	BMD modeling[a]		$VMAXC_H$	HEC		HED	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA	BMD_{10}: 0.19 $BMDL_{10}$: 0.064	BMD_{10}: 0.39 $BMDL_{10}$: 0.12	0.40	4.33	8.26	0.68	1.28
			0.65	4.59	8.74	0.83	1.56
			1.49	5.09	9.72	1.33	2.48
			1.70	5.17	9.88	1.46	2.71
MRAMKL	BMD_{10}: 13.4 $BMDL_{10}$: 7.31	BMD_{10}: 14.2 $BMDL_{10}$: 8.82	0.40	86.55	116.95	4.10	5.54
			0.65	53.95	67.89	2.56	3.22
			1.49	34.25	41.70	1.62	1.98
			1.70	32.68	39.72	1.55	1.88

Mice were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped)
HEC, human equivalent concentration, mg/m^3; HED, human equivalent dose, mg/kg-day; Fisher, Fisher et al. (2004) model; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; Thrall, Thrall et al. (2000) model; $VMAXC_H$, maximum rate of metabolism in humans, mg/hr/kg $BW^{0.70}$
[a] MCA, multistage (3-stage); MRAMKL, multistage (3-stage)
BMR (benchmark response) = 10%
[b] Number in parentheses indicates the column number.

See Appendix E for the BMDS model outputs and graphs of the modeled data.

Female and male BDF1 mouse – pheochromocytomas

Internal doses associated with a BMR of 10% extra risk of pheochromocytomas were calculated. BMD modeling in BMDS was performed using the female and male mouse pheochromocytoma incidence data shown in Table 5-9 and internal doses shown in Table 5-10. The multistage model was used to fit female mouse pheochromocytoma data. The multistage model did not provide an adequate fit of the male mouse data for this tumor type; therefore, for this data set, other models for dichotomous data in BMDS were run. The log-probit model without restriction on the slope parameter provided the best fit of the male mouse pheochromocytoma data (based on χ^2 $p \geq 0.1$ and lowest AIC value). Bayesian analysis (see Appendix E) confirmed BMDS results and provided an explanation as to why the slope parameter of the log-probit model should not be constrained. Summaries of the resulting BMD_{10} and $BMDL_{10}$ values for the female and male mouse are presented in Table 5-16 (columns 2 and 3) and Table 5-17 (columns 2 and 3), respectively.

Table 5-16. BMD values for incidence data for pheochromocytomas in female BDF1 mice and corresponding HEC and HED values

Metric	BMD modeling[a]		$VMAXC_H$	HEC		HED	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA	BMD_{10}: 1.43 $BMDL_{10}$: 1.14	BMD_{10}: 2.95 $BMDL_{10}$: 2.34	0.4	74.551	149.096	9.66	18.54
			0.65	78.636	156.027	10.73	19.90
			1.49	88.173	174.686	14.20	24.34
			1.7	89.826	178.325	15.05	25.44

Mice were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped)
HEC, human equivalent concentration, mg/m^3; HED, human equivalent dose, mg/kg-day; Fisher, Fisher et al. (2004) model; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; Thrall, Thrall et al. (2000) model; $VMAXC_H$, maximum rate of metabolism in humans, mg/hr/kg $BW^{0.70}$
[a] Multistage (2-stage) model
BMR (benchmark response) = 10%
[b] Number in parentheses indicates the column number.

Table 5-17. BMD values for incidence data for pheochromocytomas in male BDF1 mice and corresponding HEC and HED values

Metric	BMD modeling[a]		$VMAXC_H$	HEC		HED	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA	BMD_{10}: 0.26 $BMDL_{10}$: 0.15	BMD_{10}: 0.53 $BMDL_{10}$: 0.30	0.40	10.19	19.96	1.56	2.87
			0.65	10.79	21.13	1.91	3.41
			1.49	12.00	23.56	3.04	5.21
			1.70	12.20	23.95	3.33	5.67

Mice were exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped)
HEC, human equivalent concentration, mg/m^3; HED, human equivalent dose, mg/kg-day; Fisher, Fisher et al. (2004) model; MCA, time-averaged arterial blood concentration, µmol/L; MRAMKL, time-averaged rate of metabolism per kg liver, µmol/hr/kg liver; Thrall, Thrall et al. (2000) model; $VMAXC_H$, maximum rate of metabolism in humans, mg/hr/kg $BW^{0.70}$
[a] log-probit model
BMR (benchmark response) = 10%
[b] Number in parentheses indicates the column number.

5.4.2.3.4. ***PBPK modeling of human equivalent exposure concentrations and doses***.

Interspecies extrapolation (i.e., rat-to-human, mouse-to-human) and route-to-route extrapolation of carbon tetrachloride inhalation dosimetry was accomplished using the human PBPK model

described in Paustenbach et al. (1988), Thrall et al. (2000), and Benson and Springer (1999). The human PBPK model was used to estimate human equivalent concentrations (HECs, in mg/m^3) or human equivalent doses (HEDs, i.e., daily ingested doses, in mg/kg-day) that would result in values for the internal dose metrics, MCA or MRAMKL, equal to the respective BMDLs for each toxicity endpoint (i.e., liver tumors in rats, liver tumors and adrenal pheochromocytomas in mice).

The approach used to derive the HECs and HEDs for each dose metric was as follows:
(1) The human PBPK model was used to calculate internal doses corresponding to a series of exposure concentrations (EC, continuous exposure, mg/m^3). For the dose metric MCA, the human PBPK model was run at intervals over the range from 0.1 to 100 ppm (0.63 to 629 mg/m^3); for MRAMKL, the human PBPK model was run at intervals from 1 to 300 ppm (6.3 to 1887 mg/m^3).
(2) For each of these internal doses, the human PBPK model was also used to calculate equivalent rates of uptake of carbon tetrachloride from the gastrointestinal tract to liver (RGIL) that yielded the same internal doses. Uptake was expressed in units of mg/kg-day. This simple approximation method assumed continuous infusion of carbon tetrachloride from the human gastrointestinal tract to the liver. It should be noted that doses extrapolated from inhalation to oral exposures in this analysis are approximations because they do not account for oral bioavailability or absorption kinetics, information that is not available for carbon tetrachloride.
(3) For each internal dose, conversion factors were calculated as the following corresponding ratios:

- EC/MCA (to relate a continuous chronic human inhalation exposure in mg/m^3 [EC] to an internal dose using MCA as the dose metric);
- RGIL/MCA (to relate the rate of uptake of carbon tetrachloride from the gastrointestinal tract to the liver (i.e., chronic daily ingested dose in mg/kg-day [RGIL] to an internal dose using MCA as the dose metric);
- EC/MRAMKL (to relate a continuous chronic human inhalation exposure in mg/m^3 [EC] to an internal dose using MRAMKL as the dose metric); and
- RGIL/MRAMKL (to relate the rate of uptake of carbon tetrachloride from the gastrointestinal tract to the liver in mg/kg-day [RGIL] to an internal dose using MRAMKL as the dose metric).

(4) Conversion factors were calculated for each of four assumed values of V_{maxC} in the human PBPK model: 0.40, 0.65, 1.49, or 1.70 mg/hr/kg $BW^{0.70}$. These conversion factors are provided in Appendix C. Trend equations were also developed to permit the calculation of EC or RGIL for any value of MCA or MRAMKL (see Appendix C).

Estimated values for inhalation HECs corresponding to BMDLs for the 2-year rat and mouse inhalation bioassays (Nagano et al., 2007b; JBRC, 1998) for different tumor types and alternative values of V_{maxC} are presented in Tables 5-11 to 5-17, columns 5 and 6. Estimated values for oral HEDs are presented in Tables 5-11 to 5-17, columns 7 and 8. As noted in the discussion of the RfC derivation, estimates of the dose metrics, MCA and MRAMKL, were sensitive to the value assigned to the V_{maxC} parameter (see Figures 5-5 and 5-9), and the inclusion of these alternative V_{maxC} values provides some indication of the uncertainty in the modeling. As in the derivation of the RfC, the human V_{maxC} estimated from in vitro human data (1.49 mg/hr/kg $BW^{0.70}$) was considered to yield the most appropriate estimate of the HEC and HED, and was used as the basis for cancer risk estimates. As discussed in Section 5.4.2.3.2, the dose metric MRAMKL was considered to be the most appropriate dose metric to represent internal doses in modeling liver tumors in rats and mice, and MCA was considered to be the appropriate dose metric to represent internal doses in modeling pheochromocytoma incidence in mice; these dose metrics were used as the basis for cancer risk estimates.

For the rat model, no information is available to establish whether a rat V_{maxC} of 0.4 or 0.65 mg/hr/kg $BW^{0.70}$ is the more scientifically defensible value for this parameter. Therefore, the cancer risk values derived using these two rat V_{maxC} values were averaged to derive the final cancer risk values for carbon tetrachloride. Similarly, for the mouse, it cannot be established whether the Fisher et al. (2004) or Thrall et al. (2000) model provides the more accurate prediction of the internal dose for the mouse. Therefore, the cancer risk values derived using these two mouse models were averaged to derive the final cancer risk values for carbon tetrachloride (see Section 5.4.2.4 below).

5.4.2.4. *Inhalation Unit Risk and Oral Slope Factor*

5.4.2.4.1. ***Inhalation unit risk***. Inhalation unit risk (IUR) estimates based on the five tumor data sets analyzed in Section 5.4.2.3.3 are provided in Table 5-18. The highest IUR was associated with pheochromocytomas in the male mouse [6×10^{-6} $(\mu g/m^3)^{-1}$]. Incidence of liver tumors was also increased in male mice. Because different internal dose metrics were used in the dose-response analysis of liver tumors (MRAMKL) and pheochromocytomas (MCA), the addition of individual tumor risks to obtain a composite risk for the male mouse could not be performed. Uncertainty in the estimate of the IUR associated with male mouse liver tumors also argues against risk addition. As noted in Section 5.4.2.3.3, data from the male mouse provided a poor resolution of the dose-response relationship for liver tumors. A marginal fit of this data set with the multistage model in BMDS was obtained only when the highest dose group was dropped.

Table 5-18. Summary of inhalation unit risk estimates using linear low-dose extrapolation approach

Tumor	Dose Groups Modeled	Model Parameters	HEC (mg/m^3)	Inhalation Unit Risk Estimate $(\mu g/m^3)^{-1}$	
Female rat hepatocellular adenoma + carcinoma	0, 5, 25, 125 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	39.63	1.3×10^{-6}	Average rounded to one signif. figure = 1×10^{-6}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	59.32	8.4×10^{-7}	
	0, 5, 25 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	32.33	1.5×10^{-6}	Average rounded to one signif. figure = 1×10^{-6}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	46.41	1.1×10^{-6}	
Female mouse hepatocellular adenoma + carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	29.46	3.4×10^{-6}	Average rounded to one signif. figure = 3×10^{-6}
		MRAMKL; Thrall model BMR = 10%	35.64	2.8×10^{-6}	
	0, 5 ppm	MRAMKL; Fisher model BMR = 10%	23.37	4.3×10^{-6}	Average rounded to one signif. figure = 4×10^{-6}
		MRAMKL; Thrall model BMR = 10%	28.68	3.5×10^{-6}	
Male mouse hepatocellular adenoma + carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	34.25	2.9×10^{-6}	Average rounded to one signif. figure = 3×10^{-6}
		MRAMKL; Thrall model BMR = 10%	41.70	2.4×10^{-6}	
Female mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	88.54	1.1×10^{-6}	Average rounded to one signif. figure = 8×10^{-7}
		MCA; Thrall model BMR = 10%	173.77	5.8×10^{-7}	
Male mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	12.00	8.3×10^{-6}	Average rounded to one signif. figure = 6×10^{-6}
		MCA; Thrall model BMR = 10%	23.56	4.2×10^{-6}	

Carbon tetrachloride also induced both liver tumors and pheochromocytomas in the female mouse. For the same reason as the male mouse (i.e., different internal dose metrics were used in the dose-response analysis), the risks associated with female liver tumors and pheochromocytomas could not be summed. To ensure that the composite tumor risk in female mouse did not exceed that associated with pheochromocytomas in the male mouse, a bounding exercise was performed by summing the IURs for female mouse liver tumors and pheochromocytomas [i.e., $3 \times 10^{-6} + 8 \times 10^{-7}$ $(\mu g/m^3)^{-1} = 4 \times 10^{-6}$ $(\mu g/m^3)^{-1}$], a procedure that results in an overestimation of composite risk. This bounding exercise confirms that the highest value of the IUR is derived from male mouse pheochromocytoma data.

Therefore, in consideration of the goal of providing an upper bound on risk[h], the IUR for carbon tetrachloride via the inhalation pathway is estimated as 6×10^{-6} $(\mu g/m^3)^{-1}$ based on pheochromocytomas in the male mouse. This data set was judged to be applicable, scientifically sound, and yielded the highest estimate of risk.

5.4.2.4.2. ***Oral slope factor***. Oral slope factor (SF) estimates based on the five inhalation tumor data sets analyzed in Section 5.4.2.3.3 and use of the human PBPK model of Paustenbach et al. (1988) and Thrall et al. (2000) to perform route-to-route extrapolation are provided in Table 5-19. The highest oral SF [7×10^{-2} $(mg/kg\text{-}day)^{-1}$] was associated with female mouse hepatocellular adenomas or carcinomas (using tumor data from the 0, 5, and 25-ppm exposure groups). An analysis of liver tumor data using only the 0 and 5 ppm groups yielded a higher SF, but because it is based on only two data points and thus provides a less informative characterization of the dose-response curve for female mouse liver tumors, the SF based on analysis of data from the 0, 5, and 25-ppm groups is considered more reliable. The analysis based on tumor response data using only the 0 and 5-ppm groups was performed to examine the effect on the liver cancer risk estimate of using only carbon tetrachloride response data at exposure levels below those associated with evidence of cell replication. This analysis reveals that dropping the 25-ppm group data had a relatively small impact on the SF [i.e., 7×10^{-2} vs 8×10^{-2} $(mg/kg\text{-}day)^{-1}$]. A similar analysis of female rat liver tumor data revealed a similarly negligible impact of performing a dose-response analysis on data points below those associated with evidence of cell replication (i.e., 2×10^{-2} vs 3×10^{-2} $(mg/kg\text{-}day)^{-1}$; see Table 5-19).

Carbon tetrachloride also induced pheochromocytomas in the female mouse. For the same reason provided for the male mouse tumor data used to derive the IUR, the estimated risks from the individual tumors could not be summed because different internal dose metrics were used in the dose-response/PBPK analysis. Because the SF associated with pheochromocytomas is an order of magnitude smaller than the SF associated with liver tumors in the female mouse, the pheochromocytoma data would be expected to contribute negligibly to the total cancer risk estimate.

Therefore, in consideration of the goal of providing an upper bound on risk[i], the oral slope factor for carbon tetrachloride is estimated as 7×10^{-2} $(mg/kg\text{-}day)^{-1}$ based on female mouse liver tumors. This data set was judged to be applicable, scientifically sound, and yielded the highest estimate of risk.

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[h] According to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,2005A), "[t]he use of upper bounds generally is considered to be a health-protective approach for covering the risk to susceptible individuals, although the calculation of upper bounds is not based on susceptibility data." Upper bound is defined in the IRIS glossary as a plausible upper limit to the true value of a quantity, and is usually not a true statistical confidence limit (<http://www.epa.gov/ncea/iris/help_gloss.htm>).

Table 5-19. Summary of oral slope factor estimates using linear low-dose extrapolation approach and route-to-route extrapolation

Tumor	Dose Groups Modeled	Model Parameters	HED (mg/kg-d)	Oral Slope Factor Estimate $(mg/kg\text{-}day)^{-1}$	
Female rat hepatocellular adenoma + carcinoma	0, 5, 25, 125 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	1.88	2.7×10^{-2}	Average rounded to one signif. figure = 2×10^{-2}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	2.81	1.8×10^{-2}	
	0, 5, 25 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	1.53	3.3×10^{-2}	Average rounded to one signif. figure = 3×10^{-2}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	2.20	2.3×10^{-2}	
Female mouse hepatocellular adenoma + carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	1.40	7.2×10^{-2}	Average rounded to one signif. figure = 7×10^{-2}
		MRAMKL; Thrall model BMR = 10%	1.69	5.9×10^{-2}	
	0, 5 ppm	MRAMKL; Fisher model BMR = 10%	1.11	9.0×10^{-2}	Average rounded to one signif. figure = 8×10^{-2}
		MRAMKL; Thrall model BMR = 10%	1.36	7.4×10^{-2}	
Male mouse hepatocellular adenoma + carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	1.62	6.2×10^{-2}	Average rounded to one signif. figure = 6×10^{-2}
		MRAMKL; Thrall model BMR = 10%	1.98	5.1×10^{-2}	
Female mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	14.2	7.0×10^{-3}	Average rounded to one signif. figure = 6×10^{-3}
		MCA; Thrall model BMR = 10%	24.34	4.1×10^{-3}	
Male mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	3.03	3.3×10^{-2}	Average rounded to one signif. figure = 3×10^{-2}
		MCA; Thrall model BMR = 10%	5.21	1.9×10^{-2}	

5.4.3. Choosing an Extrapolation Approach for Assessing Cancer Risk

According to EPA's (2005a) *Guidelines for Carcinogen Risk Assessment*, a nonlinear extrapolation approach should be selected for assessing cancer risk:

> "when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses. Special attention is important when the data support a nonlinear mode of action but there is also a suggestion of mutagenicity.

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[i] Ibid.

Depending on the strength of the suggestion of mutagenicity, the assessment may justify a conclusion that mutagenicity is not operative at low doses and focus on a nonlinear approach, or alternatively, the assessment may use both linear and nonlinear approaches."

A linear extrapolation approach is used as the default approach:

"[w]hen the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data,… because linear extrapolation generally is considered to be a health-protective approach."

Both linear and nonlinear approaches may be presented:

"[w]here alternative approaches with significant biological support are available for the same tumor response and no scientific consensus favors a single approach"
or
"when there are multiple modes of action."

The *Guidelines for Carcinogen Risk Assessment* also suggest that:

"[i]f there are multiple modes of action at a single tumor site, one linear and another nonlinear, that both approaches are used to decouple and consider the respective contributions of each mode of action in different dose ranges."

Alternative cancer assessment approaches are presented for carbon tetrachloride liver tumors in Sections 5.4.1 and 5.4.2. At high exposure levels, rodent bioassay data reveal a general correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors. Extensive mechanistic data support a hypothesized mode of action for carbon tetrachloride-induced liver tumors at relatively high exposure levels that includes the following key events: (1) metabolism to the trichloromethyl radical by CYP2E1 and subsequent formation of the trichloromethyl peroxy radical, (2) radical-induced mechanisms leading to hepatocellular cytotoxicity, and (3) sustained regenerative and proliferative changes in the liver in response to hepatotoxicity. A weight of evidence analysis of the genotoxicity literature suggests that carbon tetrachloride is more likely an indirect than direct mutagenic agent. As a whole, this empirical evidence provides significant biological support for the hypothesis that liver carcinogenicity by this mode of action occurs at carbon tetrachloride exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response, and that exposures that do not cause hepatotoxicity are not expected to result in liver cancer. This

hypothesis is consistent with a nonlinear approach to cancer assessment for liver tumors.

Several pieces of evidence suggest that carbon tetrachloride carcinogenicity may not be explained by a cytotoxic-proliferative mode of action alone. These pieces of evidence, described further in the paragraphs that follow, include: an increased incidence of liver tumors in the low-dose female mouse (Nagano et al., 2007b) in the absence of nonneoplastic liver toxicity; induction of pheochromocytomas in mice, a tumor for which the mode of action is unknown; fundamental reactivity of the chemical; and absence of data on low-dose genotoxicity.

At lower exposure levels the correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors is reveals inconsistencies. In particular, liver findings from the JBRC bioassay (Nagano et al., 2007b; JBRC, 1998) suggest that mouse hepatocarcinogenicity cannot be explained in terms of the cytotoxic-proliferative mode of action. An increased incidence of hepatocellular adenomas occurred in the low-dose (0.9-ppm adjusted) female mouse in the absence of nonneoplastic liver toxicity, raising the possibility of another mode of action operating in addition to or in conjunction with the cytotoxic-proliferative mode of action. It should be noted, however, that cytotoxicity and cellular regeneration are observed at comparable doses (5 ppm adjusted; see Table 4-15) even though they may not be directly observed at the dose level (0.9 ppm adjusted) inducing a significant increase in liver adenomas in the mouse model. These data add to the complexity of evaluating the weight of evidence for the hypothesized mode of action.

Carbon tetrachloride also induced pheochromocytomas in male and female mice by oral (NTP, 2007; Weisburger, 1977) and inhalation (Nagano et al., 2007b; JBRC, 1998) exposure. The mode of action for the induction of pheochromocytomas in the mouse is unknown. Where the mode of action for a tumor site is unknown, linear extrapolation is used as the default.

Other considerations suggest that the carbon tetrachloride database is insufficient for ruling out other modes of action at low exposure levels, in particular considerations related to the compound's genotoxicity and general reactivity. Carbon tetrachloride is metabolized to reactive species (trichloromethyl and trichloromethyl peroxy radical), and subsequent chemical reactions of carbon tetrachloride metabolites with cellular constituents lead to formation of reactive oxygen species that also can damage DNA and other macromolecules. A concern exists regarding the potential biological activity of carbon tetrachloride with macromolecules at low exposures (i.e., exposure levels below doses that are cytotoxic). Data to characterize this low-exposure activity is limited.

Results of extensive testing for genotoxic and mutagenic potential are largely negative, and a weight of evidence analysis of the genotoxicity literature suggests that carbon tetrachloride is more likely an indirect than direct mutagenic agent; however, the nature of the database does not characterize the role of genotoxicity at low doses of carbon tetrachloride There is little direct evidence that carbon tetrachloride induces intragenic or point mutations in mammalian systems. The mutagenicity studies that have been performed using transgenic mice have yielded

negative results, as have the vast majority of the mutagenesis studies that have been conducted in bacterial systems. Under highly cytotoxic conditions, bioactivated carbon tetrachloride can exert genotoxic effects. These tend to be modest in magnitude and are manifested primarily as DNA breakage and related sequelae. Chromosome loss leading to aneuploidy may also occur to a limited extent. The fact that carbon tetrachloride overall has not been found to be a potent mutagen and that positive genotoxic results are found only at high exposure levels and generally in concert with cytotoxic effects (see Tables 4-8 to 4-11) indicates that carbon tetrachloride does not likely induce genotoxic effects through direct binding or damage to DNA. The majority of genotoxicity studies, however, have been conducted at relatively high exposure levels such that the potential for genotoxic activity at low doses cannot be determined.

Thus, as summarized above and in Section 4.7.3.4, biological support exists for a hypothesized cytotoxicity-regenerative mode of action as a major mode of action driving the steep nonlinear increase in liver tumor dose-response at relatively high carbon tetrachloride exposures. Inconsistencies and uncertainties at the low end of the experimental exposure range (including bioassay evidence from the JBRC bioassay that indicates that female mouse liver tumors cannot simply be explained in terms of the cytotoxic-proliferative mode of action, the findings of pheochromocytomas in mouse by oral [NCI bioassay] and inhalation [JBRC bioassay] exposure for which the mode of action is unknown, and insufficient data at low doses to rule out the possibility of low-dose genotoxicity or other biological responses to a reactive chemical), suggest that other (or another) modes of action independent of cytotoxicity and regenerative cell proliferation may be operative in this range. It is the low end of the experimental range that best informs the choice of the low-dose extrapolation approach. Given an incomplete understanding of the cancer mode of action for carbon tetrachloride, linear low-dose extrapolation as a default approach is therefore recommended for assessing carbon tetrachloride cancer risk. The IUR provided in Section 5.4.2.4.1 of 6×10^{-6} $(\mu g/m^3)^{-1}$ and the oral SF provided in Section 5.4.2.4.2 of 7×10^{-2} $(mg/kg\text{-}day)^{-1}$ should be used for assessing cancer risk under a linear low-dose extrapolation approach.

5.4.4. Uncertainties in Cancer Risk Values

As in most risk assessments, extrapolation of the available experimental data for carbon tetrachloride to estimate potential cancer risk in human populations introduces uncertainty in the risk estimation. Several types of uncertainty may be considered quantitatively, whereas others can only be addressed qualitatively. Thus, an overall integrated quantitative uncertainty analysis cannot be developed. Major sources of uncertainty in the cancer assessment for carbon tetrachloride are summarized in this section and in Table 5-20 at the end of this section.

Relevance to humans. The relevance of the mode of action of liver tumor induction to humans was considered in Section 4.7.3.5. There is no evidence in humans for hepatic cancer associated with carbon tetrachloride exposure. The experimental animal literature, however, shows carbon tetrachloride to consistently induce liver tumors across species and routes of exposure. Further, there are similarities between experimental animals and humans in terms of carbon tetrachloride metabolism, antioxidant systems, and evidence for the liver as a sensitive target organ. Together, this evidence supports a conclusion that experimental evidence for liver cancer is relevant to humans.

Pheochromocytomas, on the other hand, were observed in only one species (the mouse). In humans, pheochromocytomas are rare catecholamine-producing neuroendocrine tumors that are usually benign, but may also present as or develop into a malignancy (Eisenhofer et al., 2004; Salmenkivi et al., 2004; Tischler et al., 1996). In humans, hereditary factors have been identified as important in the development of pheochromocytomas (Eisenhofer et al., 2004). In the mouse, few chemicals have been reported to cause mouse adrenal medullary tumors (Hill et al., 2003), and the mode of action for this tumor in mice is unknown. The relevance of mouse pheochromocytomas to humans is similarly unknown, although parallels between this tumor in the mouse and human led investigators to concluded that the mouse might be an appropriate model for human adrenal medullary tumors (Tischler et al., 1996). Like the human, pheochromocytomas in the mouse are relatively rare, as are metastases. Both the morphological variability of the mouse pheochromocytomas and the morphology of the predominant cells are comparable to those of human pheochromocytomas. An important characteristic of mouse pheochromocytomas is expression of immunoreactive phenylethanolamine-N-methyltransferase (PNMT); human pheochromocytomas are also usually PNMT-positive (Tischler et al., 1996). Overall, this evidence supports a conclusion that experimental evidence for pheochromocytomas is potentially relevant to humans.

Choice of low-dose extrapolation approach. The mode of action is a key determinant of which approach to apply for estimating low-dose cancer risk. For liver tumors, two approaches to low-dose cancer risk estimation were developed reflecting inconsistencies and uncertainty in the mode of action at low carbon tetrachloride exposures.

The mode of action of carbon tetrachloride liver carcinogenicity has been investigated extensively. Much of this research was conducted at relatively high exposure levels. The mode(s) of action at low exposure levels is not known. Presentation of a linear and nonlinear approach to cancer assessment is likely to bracket the risk of liver cancer associated with carbon tetrachloride exposure. Additional mode of action information in the low-dose region to

establish whether a linear or nonlinear approach applies to carbon tetrachloride liver tumors would significantly reduce the uncertainty associated with magnitude of liver tumor risk.

The nonlinear extrapolation approach for liver tumors assumes that the RfD and RfC can be used to assess the potential risk of liver cancer from carbon tetrachloride. This assumption is based upon the RfD and RfC which were both quantitatively derived from hepatotoxicity (cytotoxicity) as a noncancer endpoint. Hepatotoxicity is identified as a key event in the hypothesized nonlinear mode of action for liver tumors (see Section 4.7.1 and 5.4.1). Uncertainties in the derivation of the RfD and RfC are discussed in Section 5.3.

The effect on risk estimates derived using a linear extrapolation approach of using only data on carbon tetrachloride liver tumor response at levels below those associated with increased cell replication was examined. The risk calculations did not prove particularly sensitive to the limitation of data points to those below which increased cell replication was reported (see Tables 5-18 and 5-19). This consistency in cancer risk estimates provided some confidence that the IUR and SF estimates based on liver tumor data are not driven by high doses associated with significant hepatotoxicity.

In data sets where early mortality is observed, methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. Survival curves for female rats and mice from the JBRC bioassay (see Figures 4-1 and 4-2) show early mortality in some treated groups. Because liver tumors were the primary cause of early deaths in these groups, failure to apply a time to tumor analysis is not likely to significantly influence the inhalation unit risk for liver tumors. The impact on the unit risk from pheochromocytomas is unknown.

Cancer risk estimates were calculated by straight line extrapolation from the POD to zero, with the multistage model used to derive the POD. (The one exception is the male mouse pheochromocytoma data set, where the log-probit model was used.) It is unknown how well this extrapolation procedure predicts low-dose risks for carbon tetrachloride. The multistage model does not represent all possible models one might fit, and other models could conceivably be selected to yield different results consistent with the observed data, both higher and lower than those included in this assessment.

For pheochromocytomas, only a linear low-dose extrapolation approach was used to estimate human carcinogenic risk in the absence of any information on the mode of action for this tumor. Mode of action information to establish whether a linear or nonlinear approach applies to carbon tetrachloride-induced pheochromocytomas would significantly reduce the uncertainty associated with the magnitude of risk from exposure to this tumor type.

Cancer risk estimates for liver tumors and pheochromocytomas developed using a linear low-dose extrapolation approach were not combined because different dose metrics were used in

the dose-response/PBPK analysis of these two tumor types. Deriving the IUR or oral SF for data on one tumor site, however, may underestimate the carcinogenic potential of carbon tetrachloride. For the IUR based on male mouse pheochromocytomas, because of the poor resolution of the dose-response relationship for male rodent liver tumors, the magnitude of the potential risk underestimation cannot be characterized. Because the SF based on female mouse liver tumors was an order of magnitude greater that that for female mouse pheochromocytomas, any underestimation of the SF is expected to be small.

Interspecies extrapolation. Extrapolating dose-response data from animals to humans was accomplished using PBPK models in the rat, mouse, and human. Availability of a PBPK model generally reduces the pharmacokinetic component of uncertainty associated with animal to human extrapolation; however, any PBPK model has its own associated uncertainties. Specific uncertainties in the PBPK modeling for carbon tetrachloride were discussed previously in Section 5.3.

Route-to-route extrapolation for the oral SF. Studies of carbon tetrachloride carcinogenicity by the oral route were determined to be insufficient to derive a quantitative estimate of cancer risk. Therefore, a human PBPK model was used to extrapolate inhalation data to the oral route. A simple approximation method was used that assumed continuous infusion of carbon tetrachloride from the human gastrointestinal tract to the liver. Doses extrapolated from inhalation to oral exposures in this analysis were approximations because they did not account for oral bioavailability or absorption kinetics, information that is not available for carbon tetrachloride. The magnitude of uncertainty introduced by these assumptions cannot be quantified.

Statistical uncertainty at the point of departure. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-probit model applied to the male mouse pheochromocytoma data, there is a reasonably small degree of uncertainty at the 10% excess incidence level (the point of departure for linear low-dose extrapolation); the lower bound on the BMD (i.e., the $BMDL_{10}$) is 1.8-fold lower than the BMD. For the multistage model applied to the female mouse liver tumor data, there is similarly a reasonably small degree of uncertainty at the 10% excess incidence level; the lower bound on the BMD (i.e., the $BMDL_{10}$) is approximately 1.5-fold lower than the BMD.

Bioassay selection. The study by Nagano et al. (2007b; also reported as JBRC, 1998) was used for development of the inhalation unit risk. A full report of the bioassay findings was

published in 2007, although the study itself was conducted in the mid-1980s. Although not a recently conducted study, this bioassay was well-designed, and included both sexes in two species, an adequate number of animals per dose group, and an appropriate untreated control group. Examination of toxicological endpoints in both sexes of rats and mice was appropriate. No issues were identified with this bioassay that might have contributed to uncertainty in the cancer assessment. Alternative bioassays for developing an inhalation unit risk were unavailable.

Choice of species/gender. For liver tumors, modeling was performed using JBRC inhalation bioassay data for the female mouse and female rat. The male rat liver tumor data were not modeled because these data sets lacked the resolution desired for dose-response modeling; the male mouse liver data were modeled, but provided similarly poor dose-response curve resolution. Tumor frequencies jumped from control levels to close to maximal responses without any intervening dose levels having submaximal responses. In the female mice and rats, lower but biologically significant levels of response were seen at intermediate dose levels. Also, notably, increased levels of hepatocellular proliferation were not reported for rodents at these intermediate levels, increasing the likelihood that dose-response modeling may be relevant to lower (noncytotoxic) dose conditions. There is no indication that male rodents are more sensitive to carbon tetrachloride liver tumor induction and that use of female data only underestimated potential risk. For pheochromocytomas, JBRC inhalation data sets for both male and female mice were amenable to modeling, and the data set yielding the highest estimate of cancer risk could be selected.

Human population variability. Neither the extent of interindividual variability in carbon tetrachloride metabolism nor human variability in response to carbon tetrachloride has been fully characterized. Factors that could contribute to a range of human response to carbon tetrachloride include variations in CYP450 levels because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of carbon tetrachloride or antioxidant protection systems. Incomplete understanding of the potential differences in metabolism and susceptibility across exposed human populations represents a source of uncertainty.

Table 5-20. Summary of uncertainty in the carbon tetrachloride cancer risk assessment

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Human relevance of rodent tumor data	If rodent tumors proved not to be relevant to humans, unit risk would not apply, i.e., human risk would ↓	Liver tumors in rats and mice and pheochromocytomas in mice are relevant to human exposure	Liver: There is no evidence in humans for hepatic cancer associated with carbon tetrachloride exposure. The experimental animal literature, however, shows carbon tetrachloride to consistently induce liver tumors across species and routes of exposure. Further, there are similarities between experimental animals and humans in terms of carbon tetrachloride metabolism, antioxidant systems, and evidence for the liver as a sensitive target organ. Together, this evidence supports a conclusion that experimental evidence for liver cancer is relevant to humans. Pheochromocytomas: Pheochromocytomas were observed in the mouse only. In humans, pheochromocytomas are rare catecholamine-producing neuroendocrine tumors that are usually benign, but may also present as or develop into a malignancy. Hereditary factors have been identified as important in pheochromocytoma development. The mouse has been characterized as possibly an appropriate model for human adrenal medullary tumors.
Low-dose extrapolation approach	Departure from EPA's *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ unit risk an unknown extent	Liver: Nonlinear approach and linear approach presented. Under the linear extrapolation approach, a POD-based straight-line extrapolation was applied Pheochromocytoma: Linear approach, using a POD-based straight-line extrapolation	Liver: Biological support is available for a cytotoxic-proliferative mode of action (MOA) that is consistent with a nonlinear extrapolation approach; however, other evidence suggests that hepatocarcinogenicity may not be explained only in terms of this MOA. Where data are not strong enough to ascertain the MOA, EPA's 2005 *Guidelines for Carcinogen Risk Assessment* recommend application of a linear low-dose extrapolation approach in addition to a nonlinear approach. Pheochromocytoma: Application of a linear approach where the MOA has not been established is consistent with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*.
Interspecies extrapolation using PBPK model	↓ IUR	PBPK modeling used to extrapolate rodent tumor data to humans	PBPK modeling is considered to reduce the uncertainty in extrapolating rodent tumor data to humans.
Route-to-route extrapolation using PBPK model	The magnitude of uncertainty cannot be quantified.	A human PBPK model was used to extrapolate inhalation data to the oral route	Studies of carbon tetrachloride carcinogenicity by the oral route were determined insufficient to derive a quantitative estimate of cancer risk. A simple approximation method was used that assumed continuous infusion of carbon tetrachloride from the human gastrointestinal tract to the liver.

Table 5-20. Summary of uncertainty in the carbon tetrachloride cancer risk assessment

Consideration/ Approach	Impact on cancer risk estimate	Decision	Justification
Statistical uncertainty at POD	↓ IUR and SF by 1.5 to 1.8-fold if BMD used as the POD rather than lower bound on POD	BMDL (preferred approach for calculating reasonable upper bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on administered exposure.
Bioassay	Alternative bioassay, if available, could ↑ or ↓ slope factor by an unknown extent	JBRC bioassay	Alternative bioassays were unavailable.
Species/gender combination	Human risk could ↑ or ↓, depending on relative sensitivity	Female mouse and rat liver tumors Male and female mouse pheo-chromocytomas	It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. For liver tumors, female mouse and female rat data from the JBRC bioassay were considered more amenable for modeling and demonstrating a response that may be more relevant to lower dose conditions than males. For pheochromocytomas, JBRC inhalation data sets for both male and female mice were amenable to modeling, and the data set yielding the highest estimate of cancer risk could be selected.
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk could ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity. Factors that could contribute to a range of human response to carbon tetrachloride include variations in CYP450 levels, nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of carbon tetrachloride or antioxidant protection systems. On balance, available data do not indicate that children would necessarily be more sensitive.

5.4.5. Previous Cancer Assessment

The previous cancer assessment for carbon tetrachloride was posted on the IRIS database in 1987. At that time, carbon tetrachloride was classified as a B2 carcinogen (probable human carcinogen), based on the finding of treatment-related hepatocellular carcinomas in rats, mice and hamsters. In the previous assessment, an oral slope factor of 1.3×10^{-1} $(mg/kg\text{-}day)^{-1}$ was derived using linear extrapolation procedures and liver tumor data sets from the hamster (Della Porta et al., 1961), mouse (Edwards et al., 1942; NCI, 1977, 1976a, b), and rat (NCI, 1977, 1976a, b). In the current assessment, the available oral bioassay data were not considered adequate for dose-response analysis, and a SF was derived instead by application of a PBPK

model to extrapolate inhalation bioassay data to the oral route. The resulting SF [7×10^{-2} (mg/kg-day)$^{-1}$] is approximately 2-fold smaller than the previous SF.

An inhalation unit risk of 1.5×10^{-5} (μg/m^3)$^{-1}$ was derived previously from the oral slope factor by route-to-route extrapolation (assuming an air intake of 20 m^3/day, body weight of 70 kg, and 40% absorption rate by humans). The current IUR [6×10^{-6} (μg/m^3)$^{-1}$] was derived using a chronic inhalation bioassay (Nagano et al., 2007) that was not available at the time of the previous assessment and PBPK modeling for interspecies extrapolation.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Carbon tetrachloride is rapidly absorbed by any route of exposure. Once absorbed, it is widely distributed among tissues, especially those with high lipid content, reaching peak concentrations in less than 1–6 hours, depending on dose. It is efficiently metabolized by the liver, lung, and other tissues. The initial step in metabolism is reductive dehalogenation to trichloromethyl radical by CYP450. The fate of the trichloromethyl radical is dependent on the availability of oxygen and includes several alternative pathways for anaerobic or aerobic conditions. Unmetabolized parent compound is excreted in exhaled air. Volatile metabolites are also released in exhaled air, whereas nonvolatile metabolites are excreted in feces and, to a lesser degree, in urine.

Hepatic and renal toxicities are the primary noncancer effects of oral or inhalation exposure to carbon tetrachloride. In humans, damage to both the liver and kidney was observed in acute poisoning cases. Suggestive evidence of hepatotoxicity was also seen in workers exposed to carbon tetrachloride for an extended period of time in the workplace. Numerous animal studies confirmed the toxic effect of carbon tetrachloride to the liver by oral exposure and to both the liver and kidney by inhalation exposure. Exposure to high levels of carbon tetrachloride by the oral or inhalation routes can also produce effects on reproduction and development. Animal studies reported degeneration of the testes, reduced male fertility, delayed fetal growth, and whole litter resorption following high-level carbon tetrachloride exposure. Carbon tetrachloride was also carcinogenic in animal studies, inducing hepatocellular carcinomas in rats, mice, and hamsters in oral studies and in rats and mice by inhalation exposure. Pheochromocytomas were reported in mice in one oral and one inhalation bioassay.

The toxic effects of carbon tetrachloride are generally attributed to reactive products of metabolism. The first step of carbon tetrachloride metabolism results in the production of the trichloromethyl radical. In the presence of molecular oxygen, the trichloromethyl radical forms a transient, but more potent, trichloromethyl peroxy radical that can induce lipid peroxidation. The two reactive intermediates can also covalently bind to cellular components, causing disruption of the cellular membrane. Increased permeability of cellular membranes interferes with cellular processes dependent on calcium sequestration and also results in the release of hydrolytic enzymes that may attack adjacent cells.

Examination of rodent liver tumors reveals a general correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors, although at lower exposure levels this correspondence is less consistent. Studies of genotoxic

and mutagenic potential are largely negative. There is little direct evidence that carbon tetrachloride induces intragenic or point mutations in mammalian systems. Mutagenicity studies performed using transgenic mice have yielded negative results, as have the vast majority of the mutagenesis studies that have been conducted in bacterial systems. Under highly cytotoxic conditions, bioactivated carbon tetrachloride can exert genotoxic effects. These tend to be modest in magnitude and are manifested primarily as DNA breakage and related sequelae. Chromosome loss leading to aneuploidy may also occur to a limited extent. The fact that carbon tetrachloride overall has not been found to be a potent mutagen and that positive genotoxic results are found only at high exposure levels and generally in concert with cytotoxic effects indicates that carbon tetrachloride does not likely induce genotoxic effects through direct binding or damage to DNA. The nature of the genotoxicity database, however, poses distinct challenges to the evaluation of carbon tetrachloride genotoxicity, particularly at low exposure levels. Extensive mechanistic study data informs the mode(s) of action for carbon tetrachloride-induced liver tumors. The empirical evidence for carbon tetrachloride, particularly data from relatively high-exposure studies, provides support for the hypothesis that liver carcinogenicity is presumed to occur at exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response, and that exposures that do not cause hepatotoxicity are not expected to result in liver cancer. In the JBRC 2-year mouse bioassay (Nagano et al., 2007b; JBRC, 1998), however, an increased incidence of hepatocellular adenomas in the low-dose (5-ppm or 0.9-ppm adjusted) female mouse occurred in the absence of cytotoxicity, suggesting that mouse hepatocarcinogenicity cannot simply be explained in terms of a cytotoxic-proliferative mode of action. Information on the biological activity of carbon tetrachloride at low exposures is far less complete than at higher (cytotoxic) exposure levels. Considerable evidence points to the involvement of reactive metabolites and reaction products of carbon tetrachloride with cellular constituents in the induction of liver toxicity and carcinogenicity by carbon tetrachloride. In light of the fundamental reactivity of both direct and indirect products of carbon tetrachloride metabolism and uncertainties about genotoxic activity at low exposures, the mode(s) of action for carbon tetrachloride-induced liver tumors at low exposure levels cannot be characterized. The mode of action for pheochromocytomas induced by carbon tetrachloride is unknown. Carbon tetrachloride can be classified as *likely to be carcinogenic to humans* by all routes of exposure.

6.2. DOSE RESPONSE

6.2.1. Noncancer – Oral Exposure

The most sensitive endpoints identified for effects of carbon tetrachloride by oral exposure relate to liver toxicity in the subchronic corn oil gavage studies of Bruckner et al. (1986) in rats and Condie et al. (1986) in mice. The Bruckner et al. (1986) study identified serum enzyme changes and liver histopathology as the most sensitive endpoints for carbon tetrachloride. Serum SDH was the most sensitive serum chemistry endpoint and was considered a marker of histopathologic changes. Another target of carbon tetrachloride toxicity following oral exposure considered in the selection of the critical effect was the developing organism. Studies in experimental animals found that relatively high doses of carbon tetrachloride during gestation can produce prenatal loss; these doses also produced overt toxic effects in the dams. Carbon tetrachloride doses associated with liver toxicity were much lower than those associated with developmental toxicity.

BMD modeling methods were used to calculate the POD for deriving the RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$) for liver enzyme changes. An increase in SDH activity two times the control mean was used as the BMR. All of the models for continuous data in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to the 10-week SDH data. The power model, which provided the best fit to the data, estimated a BMD_{2X} of 7.32 mg/kg-day and a $BMDL_{2X}$ of 5.46 mg/kg-day.

Liver lesion incidence data from the Bruckner et al. (1986) study in rats and the Condie et al. (1986) study in mice do not provide adequate information in the response region of concern (i.e., 10% increase in extra risk over controls) to warrant BMD modeling of these endpoints (U.S. EPA, 2000c). The NOAEL of 1 and LOAEL of 10–12 mg/kg-day in these studies do, however, support the BMD_{2X} of 7.32 mg/kg-day and the $BMDL_{2X}$ of 5.46 mg/kg-day estimated from the increase in serum SDH observed in the Bruckner et al. (1986) study.

The $BMDL_{2X}$ of 5.46 mg/kg estimated from the increase in serum SDH activity in rats in the Bruckner et al. (1986) subchronic toxicity study was used as the POD for derivation of the RfD. Use of the modeled BMDL provides an inherent advantage over use of a NOAEL or LOAEL by making greater use of the available data. Because of the absence of a suitable PBPK model for oral exposure to carbon tetrachloride, one was not used for this assessment. Because the $BMDL_{2X}$ of 5.46 mg/kg was derived from a study (Bruckner et al., 1986) with an intermittent dosing schedule, it was adjusted to an average daily dose prior to application of UFs ($BMDL_{2X\text{-}ADJ}$ = 3.9 mg/kg-day). Applying a composite UF of 1000 to the $BMDL_{ADJ}$ of 3.9 mg/kg-day yields an RfD of 0.004 mg/kg-day for carbon tetrachloride. The composite UF of 1000 includes a factor of 10 to protect susceptible individuals, a factor of 10 to extrapolate from rats to humans, a factor of 3 ($10^{0.5}$) to extrapolate from a subchronic to a chronic duration of exposure, and a factor of 3 ($10^{0.5}$) to account for an incomplete database. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans

and the potential variability in human susceptibility (factors that could contribute to a range of human response include variations in CYP450 levels, nutritional status, alcohol consumption, or the presence of underlying disease); thus, the UF selected for uncertainties related to both interspecies and intraspecies was the default of 10. A UF of 3 for subchronic to chronic extrapolation was selected based on: (1) qualitative information demonstrating that the target of toxicity following chronic oral exposure as the liver; (2) knowledge of the relationship between effect levels in subchronic and chronic inhalation studies; and (3) early onset of liver toxicity. A database UF of 3 was selected to account for an incomplete database lacking an adequate multigeneration study of reproductive function.

To provide perspective on the RfD supported by Bruckner et al. (1986), PODs and oral RfDs based on other selected studies of carbon tetrachloride oral toxicity are arrayed in Figures 5-1 to 5-3 presented in Section 5. The predominant noncancer effect of subchronic and chronic oral exposure to carbon tetrachloride is hepatic toxicity. Figure 5-1 provides a graphical display of five studies that reported liver toxicity in experimental animals following subchronic oral exposure, including the PODs, applied uncertainty factors, and RfDs for comparison to that derived from the Bruckner et al. study. Studies in experimental animals have also reported developmental toxicity (prenatal loss) at relatively high doses of carbon tetrachloride during gestation. A graphical display of information from three developmental studies is provided in Figure 5-2. Figure 5-3 displays PODs for the major targets of toxicity associated with oral exposure to carbon tetrachloride. For the reasons discussed in Section 5.1.2, liver effects in the rat observed in the study by Bruckner et al. (1986) are considered the most appropriate basis for the carbon tetrachloride RfD. The text of Sections 5.1.1 and 5.1.2 should be consulted for a more complete understanding of the issues associated with each data set and the rationale for the selection of the critical effect and principal study used to derive the RfD.

Confidence in the principal study, Bruckner et al. (1986), is medium. The 12-week gavage study is a well-conducted, peer-reviewed study that used three dose groups plus a control and collected interim data at two-week intervals. The study is limited by relatively small group sizes (5 to 9 rats/group) and investigation of only two target organs (liver and kidney). Confidence in the oral database is medium. Two chronic oral animal studies were designed as cancer bioassays, and one of the two included only limited investigation of noncancer endpoints. The second chronic bioassay by NCI provided complete nonneoplastic incidence data; however, because of the marked hepatotoxicity in dosed rats at the lowest dose tested and the low survival in dosed mice as a result of the high incidence of liver tumors, the bioassay was not suitable for dose-response analysis. The toxicity of carbon tetrachloride has been more thoroughly investigated in a number of oral toxicity studies of subchronic duration, and a number of tests of immunotoxic potential are available. The oral database lacks an adequate multigeneration study of reproductive function. Testing for developmental toxicity has been performed in only one species. Overall confidence in the RfD is medium.

6.2.2. Noncancer – Inhalation Exposure

The most sensitive endpoint identified for effects of carbon tetrachloride by inhalation exposure was liver toxicity in the chronic rat study by JBRC (Nagano et al., 2007b; JBRC, 1998), manifested at an exposure concentration of 25 ppm by elevated serum enzymes, fatty change, fibrosis and cirrhosis. Other targets of carbon tetrachloride toxicity considered in the selection of the critical effect included the kidney, the adrenal gland, and the developing organism.

PBPK and BMD modeling methods were used to calculate the POD for deriving the RfC. Exposure levels studied in the 2-year JBRC rat bioassay were converted to estimates of internal dose metrics by application of PBPK models (Paustenbach et al., 1988; Thrall et al., 2000; Benson and Springer, 1999); rate of carbon tetrachloride metabolism in the liver was considered the most appropriate dose metric for liver toxicity. BMD modeling methodology (U.S. EPA, 2000c, 1995) was used to analyze the relationship between the estimated internal doses and response (i.e., fatty change of the liver) by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$). A 10% extra risk of fatty changes of the liver was used as the BMR. All of the models for dichotomous data in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to the incidence data for fatty liver in male and female rats. In the male rat, the logistic model provided the best fit of the data. For female rats, no models provided an adequate fit to the data when all dose groups were included, as assessed by the χ^2 goodness-of-fit test. After dropping the highest dose, the multistage model provided the best fit of the data. The resulting $BMDL_{10}$ values (expressed as internal doses) were converted to estimates of equivalent human exposure concentrations (HECs) by applying a human PBPK model and assuming a value for the human V_{maxC} estimated from in vitro human data. An HEC of 14.3 mg/m^3 is used as the POD for RfC derivation. An RfC of 0.1 mg/m^3 for carbon tetrachloride is derived by applying a composite UF of 100 to the HEC of 14.3 mg/m^3. The composite UF of 100 includes a factor of 10 to protect susceptible individuals, a factor of 3 ($10^{0.5}$) to extrapolate from rats to humans, and a factor of 3 ($10^{0.5}$) to account for an incomplete database. Information was unavailable to quantitatively assess the potential variability in human susceptibility (factors that could contribute to a range of human response include variations in CYP450 levels, nutritional status, alcohol consumption, or the presence of underlying disease); thus, a default UF of 10 was selected to account for the uncertainty in intraspecies variability. A pharmacokinetic model was used to adjust for pharmacokinetic differences across species. A UF of 3 was selected for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. A database UF of 3 was selected to account for an incomplete database lacking a multigeneration reproductive toxicity.

To provide perspective on the RfC derived using data from the JBRC inhalation bioassay in the rat, PODs and inhalation RfCs based on other selected studies of carbon tetrachloride

inhalation toxicity are arrayed in Figures 5-6 to 5-8 presented in Section 5. The liver and kidney are the predominant targets of carbon tetrachloride toxicity in subchronic and chronic inhalation studies in laboratory animals and in humans based on case reports and studies in exposed workers. Figures 5-6 and 5-7 provide graphical displays of information from studies that reported liver or kidney toxicity in experimental animals following subchronic oral exposure, including the PODs, applied uncertainty factors, and RfDs for comparison to that derived from JBRC liver data. Benign pheochromocytomas from the adrenal gland medulla, that could represent a potential noncancer health hazard, were observed following inhalation exposure only in mice in the JBRC chronic bioassay. A single study of developmental toxicity found significant reductions in fetal body weight and crown-rump length in rats at a carbon tetrachloride concentration that was also toxic to the dams. Figure 5-8 displays PODs for all major targets of carbon tetrachloride toxicity by the inhalation route. For the reasons discussed in Section 5.2.2, liver effects in the rat observed in the study by JBRC are considered the most appropriate basis for the carbon tetrachloride RfC. The text of Sections 5.2.1 and 5.2.2 should be consulted for a more complete understanding of the issues associated with each data set and the rationale for the selection of the critical effect and principal study used to derive the RfC.

Confidence in the principal study, the JBRC bioassay, is high. This chronic study was well conducted, using two species and adequate numbers of animals. The JBRC chronic study was preceded by a 13-week subchronic study, and an extensive set of endpoints was examined in both studies. Confidence in the database, which includes the JBRC two-year chronic inhalation bioassays in rats and mice, subchronic toxicity studies, and one study of immunotoxic potential, is medium. Testing for developmental toxicity by inhalation exposure found effects only at high, maternally toxic exposure concentrations but was limited to a single inhalation study in a single species that did not test an exposure concentration low enough to identify a NOAEL for maternal or fetal toxicity. The database lacks an adequate inhalation multigeneration study of reproductive function. Overall confidence in the RfC is medium.

6.2.3. Cancer

Two approaches to low-dose extrapolation were applied in the dose-response assessment for carbon tetrachloride carcinogenicity.

Nonlinear Approach. At high exposure levels, rodent bioassay data reveal a general correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors. Extensive mechanistic data support a hypothesized mode of action for carbon tetrachloride-induced liver tumors that includes the following key events: (1) metabolism to the trichloromethyl radical by CYP2E1 and subsequent formation of the trichloromethyl peroxy radical, (2) radical-induced mechanisms leading to hepatocellular cytotoxicity, and (3) sustained regenerative and proliferative changes in the liver in response to hepatotoxicity. A

weight of evidence analysis of the genotoxicity literature suggests that carbon tetrachloride is more likely an indirect than direct mutagenic agent. As a whole, this empirical evidence provides significant biological support for the hypothesis that liver carcinogenicity by this mode of action occurs at carbon tetrachloride exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response, and that exposures that do not cause hepatotoxicity are not expected to result in liver cancer. This hypothesis is consistent with a nonlinear approach to cancer assessment for liver tumors. The RfD and RfC were quantitatively derived based upon hepatotoxicity (cytotoxicity). Hepatotoxicity is a key event for the hypothesized nonlinear mode of action. Under an assumption of nonlinearity, the RfD of 0.004 mg/kg-day and RfC of 0.1 mg/m^3 can be used to assess the potential risk of liver cancer from carbon tetrachloride exposure for oral and inhalation exposures, respectively.

Linear Approach. Some bioassay data also reveal that at lower exposure levels the correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors is less consistent. In particular, liver findings from the JBRC bioassay (Nagano et al., 2007b; JBRC, 1998) suggest that mouse hepatocarcinogenicity cannot simply be explained in terms of the cytotoxic-proliferative mode of action. An increased incidence of hepatocellular adenomas occurred in the low-dose (5-ppm) female mouse in the absence of nonneoplastic liver toxicity, raising the possibility of another mode of action operating in addition to the cytotoxic-proliferative mode of action.

Carbon tetrachloride also induced pheochromocytomas in male and female mice by oral (NTP, 2007; Weisburger, 1977) and inhalation (Nagano et al., 2007b; JBRC, 1998) exposure. The mode of action for pheochromocytomas in the mouse is unknown. Where the mode of action for a tumor site is unknown, linear extrapolation is used as the default.

Other considerations suggest that the carbon tetrachloride database is insufficient for ruling out other modes of action at low exposure levels, in particular considerations related to the compound's genotoxicity and general reactivity. Carbon tetrachloride is metabolized to reactive species (trichloromethyl and trichloromethyl peroxy radical), and subsequent chemical reactions of carbon tetrachloride metabolites with cellular constituents lead to formation of reactive oxygen species that also can damage DNA and other macromolecules. A concern exists regarding the potential biological activity of carbon tetrachloride with macromolecules at low exposures (i.e., exposure levels below doses that are cytotoxic). Data to characterize this low-exposure activity is limited.

A weight of evidence analysis of the genotoxicity literature suggests that carbon tetrachloride is more likely an indirect than direct mutagenic agent; however, the nature of the genotoxicity database poses distinct challenges to the evaluation of carbon tetrachloride genotoxicity. Positive genotoxicity findings have generally been observed at exposures that induce cytotoxicity and regenerative cell proliferation. The majority of genotoxicity studies,

however, have been conducted at relatively high exposure levels such that the potential for genotoxic activity at low doses cannot be determined.

The above considerations provide support for the application of a low-dose linear extrapolation approach to carbon tetrachloride carcinogenicity.

The 104-week inhalation bioassay in rats and mice conducted by JBRC (Nagano et al., 2007b; JBRC, 1998) provided data adequate for dose-response modeling of the inhalation pathway and was used as the basis for the IUR. Exposure levels studied in the 2-year JBRC rat and mouse bioassay were converted to estimates of internal dose metrics by application of a PBPK model. BMD modeling methodology (U.S. EPA, 2000c, 1995) was used to analyze the relationship between the estimated internal doses and response (i.e., liver tumors in rats and mice and pheochromocytomas in mice). The resulting BMDL values were converted to estimates of equivalent human exposure concentrations (HECs) by applying a human PBPK model. Data for male mouse pheochromocytomas yielded the highest estimate of the IUR of those data sets modeled [i.e., 6×10^{-6} $(\mu g/m^3)^{-1}$].

Studies of carbon tetrachloride carcinogenicity in humans and experimental animals by the oral exposure route are not sufficient to derive a quantitative estimate of cancer risk using low-dose linear approaches. Therefore, PBPK modeling was applied to extrapolate inhalation tumor data to the oral route. Because liver tumors and pheochromocytomas have been observed in experimental animals following both inhalation and oral exposures, the data sets evaluated as the basis for the IUR were considered appropriate for estimation of an oral SF. Data for female mouse liver tumors yielded the highest estimate of the SF of those data sets modeled [i.e., 7×10^{-2} $(mg/kg\text{-}day)^{-1}$].

Choosing an Extrapolation Approach. A linear low-dose extrapolation approach is recommended for assessing carbon tetrachloride cancer risk for both liver tumors and pheochromocytomas. For liver tumors, this recommendation was reached in light of (1) evidence from the JBRC bioassay suggesting that mouse hepatocarcinogenicity cannot simply be explained in terms of a cytotoxic-proliferative mode of action alone; (2) considerable evidence that points to the involvement of highly reactive metabolites (with the capacity to chemically interact with DNA and other cellular macromolecules) in the processes of toxicity and carcinogenicity of carbon tetrachloride, and subsequent chemical reactions of carbon tetrachloride metabolites with cellular constituents that can lead to formation of reactive oxygen species that also can damage DNA and other macromolecules; and (3) a genotoxicity database that, while large, is complex and has various issues that make it difficult to reach a firm judgement about the potential for genotoxicity of carbon tetrachloride at doses below which there is overt toxicity. Linear extrapolation is supported for pheochromocytomas (observed in the male and female mouse by oral [NCI bioassay] and inhalation [JBRC bioassay] exposure) in the absence of any understanding of the cancer mode of action for this tumor. The IUR of

6×10^{-6} $(\mu g/m^3)^{-1}$ and oral SF of 7×10^{-2} $(mg/kg\text{-}day)^{-1}$ should be used for assessing cancer risk under a linear low-dose extrapolation approach.

Uncertainties in the Cancer Dose-Response Assessment. Major uncertainties in the cancer assessment are described below:

Relevance to humans. The relevance of the mode of action of liver tumor induction to humans was considered in Section 4.7.3.5. There is no evidence in humans for hepatic cancer associated with carbon tetrachloride exposure. The experimental animal literature, however, shows carbon tetrachloride to consistently induce liver tumors across species and routes of exposure. Further, there are similarities between experimental animals and humans in terms of carbon tetrachloride metabolism, antioxidant systems, and evidence for the liver as a sensitive target organ. Together, this evidence supports a conclusion that experimental evidence for liver cancer is relevant to humans.

Pheochromocytomas, on the other hand, were observed in only one species (the mouse). In humans, pheochromocytomas are rare catecholamine-producing neuroendocrine tumors that are usually benign, but may also present as or develop into a malignancy (Eisenhofer et al., 2004; Salmenkivi et al., 2004; Tischler et al., 1996). In humans, hereditary factors have been identified as important in the development of pheochromocytomas (Eisenhofer et al., 2004). In the mouse, few chemicals have been reported to cause mouse adrenal medullary tumors (Hill et al., 2003), and the mode of action for this tumor in mice is unknown. The relevance of mouse pheochromocytomas to humans is similarly unknown, although parallels between this tumor in the mouse and human led investigators to concluded that the mouse might be an appropriate model for human adrenal medullary tumors (Tischler et al., 1996). Like the human, pheochromocytomas in the mouse are relatively rare, as are metastases. Both the morphological variability of the mouse pheochromocytomas and the morphology of the predominant cells are comparable to those of human pheochromocytomas. An important characteristic of mouse pheochromocytomas is expression of immunoreactive phenylethanolamine-N-methyltransferase (PNMT); human pheochromocytomas are also usually PNMT-positive (Tischler et al., 1996). Overall, this evidence supports a conclusion that experimental evidence for pheochromocytomas is potentially relevant to humans.

Choice of low-dose extrapolation approach. The mode of action is a key determinant of the approach to apply for estimating low-dose cancer risk. For liver tumors, two

approaches to low-dose cancer risk were developed reflecting inconsistencies and uncertainty in the mode of action at low carbon tetrachloride exposures.

The mode of action of carbon tetrachloride liver carcinogenicity has been investigated extensively. Much of this research was conducted at relatively high exposure levels, such that the mode(s) of action at low exposure levels cannot be determined. Additional mode of action information in the low-dose region to establish whether a linear or nonlinear approach applies to carbon tetrachloride liver tumors would significantly reduce the uncertainty associated with magnitude of liver tumor risk.

The nonlinear extrapolation approach for liver tumors assumes that the RfD and RfC can be used to assess the potential risk of liver cancer from carbon tetrachloride. Uncertainties in the derivation of the RfD and RfC are discussed in Section 5.3. This assumption is based upon the RfD and RfC which were both quantitatively derived from hepatotoxicity (cytotoxicity) as a noncancer endpoint. Hepatotoxicity is identified as a key event in the hypothesized nonlinear mode of action for liver tumors (see Section 4.7.1 and 5.4.1).

The effect on risk estimates derived using a linear extrapolation approach of using only data on carbon tetrachloride liver cancer response at levels below those associated with increased cell replication was examined. The risk calculations did not prove particularly sensitive to the limitation of data points to below which increased cell replication was reported. This consistency in cancer risk estimates provided some confidence that the IUR and SF estimates based on liver tumor data are not driven by high doses associated with significant hepatotoxicity.

In data sets where early mortality is observed, methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. Survival curves for female rats and mice from the JBRC bioassay (see Figures 4-1 and 4-2) show early mortality in some treated groups. Because liver tumors were the primary cause of early deaths in these groups, failure to apply a time to tumor analysis is not likely to significantly influence the inhalation unit risk for liver tumors. The impact on the unit risk from pheochromocytomas is unknown.

Cancer risk estimates were calculated by straight line extrapolation from the POD to zero, with the multistage model used to derive the POD. (The one exception is the male mouse pheochromocytoma data set, where the log-probit model was used.) It is unknown how well this extrapolation procedure predicts low-dose risks for carbon tetrachloride. The multistage model does not represent all possible models one might fit, and other models could conceivably be selected to yield more extreme results consistent with the observed data, both higher and lower than those included in this assessment.

For pheochromocytomas, only a linear low-dose extrapolation approach was used to estimate human carcinogenic risk in the absence of any information on the mode of action for this tumor. Mode of action information to establish whether a linear or nonlinear approach applies to carbon tetrachloride-induced pheochromocytomas would significantly reduce the uncertainty associated with the magnitude of risk from exposure to this tumor type.

Cancer risk estimates for liver tumors and pheochromocytomas developed using a linear low-dose extrapolation approach were not combined because different internal dose metrics were used in the dose-response/PBPK analysis of these two tumor types. Deriving the IUR or SF for data on one tumor site, however, may underestimate the carcinogenic potential of carbon tetrachloride. For the IUR based on male mouse pheochromocytomas, because of the poor resolution of the dose-response relationship for male mouse liver tumors, the magnitude of the potential risk underestimation cannot be characterized. Because the SF based on female mouse liver tumors was an order of magnitude greater that that for female mouse pheochromocytomas, any underestimation of the SF is expected to be small.

Interspecies extrapolation. Extrapolating dose-response data from animals to humans was accomplished using PBPK models in the rat, mouse, and human. Availability of a PBPK model generally reduces the pharmacokinetic component of uncertainty associated with animal to human extrapolation; however, any PBPK model has its own associated uncertainties. Specific uncertainties in the PBPK modeling for carbon tetrachloride are discussed in Section 5.3.

Route-to-route extrapolation for the oral SF. Studies of carbon tetrachloride carcinogenicity by the oral route were determined to be insufficient to derive a quantitative estimate of cancer risk. Therefore, a human PBPK model was used to extrapolate inhalation data to the oral route. A simple approximation method was used that assumed continuous infusion of carbon tetrachloride from the human gastrointestinal tract to the liver. Doses extrapolated from inhalation to oral exposures in this analysis were approximations because they did not account for oral bioavailability or absorption kinetics, information that is not available for carbon tetrachloride. The magnitude of uncertainty introduced by these assumptions cannot be quantified.

Statistical uncertainty at the point of departure. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-probit model applied to the male mouse pheochromocytoma data, there is a reasonably small degree of uncertainty at the 10% excess incidence level (the point of departure for linear low-dose extrapolation); the lower bound on the BMD (i.e., the $BMDL_{10}$) is 1.8-fold lower than the BMD. For the multistage model applied to the female mouse liver tumor data, there is similarly a reasonably small degree of uncertainty at the 10% excess incidence level; the lower bound on the BMD (i.e., the $BMDL_{10}$) is approximately 1.5-fold lower than the BMD.

Bioassay selection. The study by Nagano et al. (2007b; also reported as JBRC, 1998) was used for development of the inhalation unit risk. A full report of the bioassay findings was published in 2007, although the study itself was conducted in the mid-1980s. Although not a recently conducted study, this bioassay was well-designed, and included both sexes in two species, an adequate number of animals per dose group, and an appropriate untreated control group. Examination of toxicological endpoints in both sexes of rats and mice was appropriate. No issues were identified with this bioassay that might have contributed to uncertainty in the cancer assessment. Alternative bioassays for developing an inhalation unit risk were unavailable.

Choice of species/gender. For liver tumors, modeling was performed using JBRC inhalation bioassay from the female mouse and female rat. The male rat liver tumor data were not modeled because these data sets lacked the resolution desired for dose-response modeling; The male mouse liver data were modeled, but provided similarly poor dose-response curve resolution. Tumor frequencies jumped from control levels to close to maximal responses without any intervening dose levels having submaximal responses. In the female mice and rats, lower but biologically significant levels of response were seen at intermediate dose levels. Also, notably, increased levels of hepatocellular proliferation were not reported for rodents at these intermediate levels, increasing the likelihood that dose-response modeling may be relevant to lower dose conditions. There is no indication that male rodents are more sensitive to carbon tetrachloride liver tumor induction and that use of female data only underestimated potential risk. For pheochromocytomas, JBRC inhalation data sets for both male and female mice were amenable to modeling, and the data set yielding the highest estimate of cancer risk could be selected.

Human population variability. Neither the extent of interindividual variability in carbon tetrachloride metabolism nor human variability in response to carbon tetrachloride has been fully characterized. Factors that could contribute to a range of human response to carbon tetrachloride include variations in CYP450 levels because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of carbon tetrachloride or antioxidant protection systems. Incomplete understanding of the potential differences in metabolism and susceptibility across exposed human populations represents a source of uncertainty.

7. REFERENCES

Abbas, R; Fisher, JW. (1997) A physiologically based pharmacokinetic model for trichloroethylene and its metabolites, chloral hydrate, trichloroacetate, dichloroacetate, trichloroethanol, and trichloroethanol glucuronide in B6C3F1 mice. Toxicol Appl Pharmacol 147:15-30.

Abraham, P; Wilfred, G; Catherine, SP; et al. (1999) Oxidative damage to the lipids and proteins of the lungs, testis and kidney of rats during carbon tetrachloride intoxication. Clin Chim Acta 289:177-179.

ACGIH (American Conference of Governmental Industrial Hygienists). (2001) Documentation of threshold limit values and biological exposure indices for chemical substances in the workroom air. 7th edition. Supplement. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Adams, EM; Spencer, HC; Rowe, VK; et al. (1952) Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. Arch Ind Hyg Occup Med 6:50-66.

Agarwal, AK; Mehendale, HM. (1984) Excessive hepatic accumulation of intracellular Ca2+ in chlordecone potentiated CCl4 toxicity. Toxicology 30:17-24.

Agarwal, AK; Mehendale, HM. (1986) Effect of chlordecone on carbon tetrachloride-induced increase in calcium uptake in isolated perfused rat liver. Toxicol Appl Pharmacol 83:342-348.

Ahn, YK; Kim, JH. (1993) Preventive effects of diphenyl dimethyl dicarboxylate on the immunotoxicity of carbon tetrachloride in ICR mice. J Toxicol Sci 18(3):185-195.

Ahr, HJ; King, LJ; Nastainczyk, W; et al. (1980) The mechanism of chloroform and carbon monoxide formation from carbon tetrachloride by microsomal cytochrome P-450. Biochem Pharmacol 29:2855-2861.

Allis, JW; Ward, TR; Seely, JC; et al. (1990) Assessment of hepatic indicators of subchronic carbon tetrachloride injury and recovery in rats. Fundam Appl Toxicol 15:558-570.

Allis, JW; Brown, BL; Simmons, JE; et al. (1996) Methanol potentiation of carbon tetrachloride hepatotoxicity: the central role of cytochrome P450. Toxicology 112:131-140.

Alric, L; Orfila, C; Carrere, N; et al. (2000) Reactive oxygen intermediates and eicosanoid production by Kupffer cells and infiltrated macrophages in acute and chronic liver injury induced in rats by CCl4. Inflamm Res 49:700-707.

Alumot, E; Nachtomi, E; Mandel, E; et al. (1976) Tolerance and acceptable daily intake of chlorinated fumigants in the rat diet. Food Cosmet Toxicol 14:105-111.

Amacher, DE; Zelljadt, I. (1983) The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to *Salmonella typhimurium*. Carcinogenesis 4(3):291-296.

Andersen, ME; Clewell, HJ, III; Gargas, ML. (1987) Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 87:185-205.

Anderson, MW; Reynolds, SH; You, M; et al. (1992) Role of proto-oncogene activation in carcinogenesis. Environ Health Perspect 98:13-24.

Andersen, NJ; Waller, CL; Adamovic, JB; et al. (1996) A pharmacokinetic model of anaerobic in vitro carbon tetrachloride metabolism. Chem Biol Interact 101:13-31.

Andervont, HB. (1958) Induction of hepatomas in strain C3II mice with 4-o-tolylazo-o-toluidine and carbon tetrachloride. J Natl Cancer Inst 20:431-438.

Ansari, GA; Moslen, MT; Reynolds, ES. (1982) Evidence for in vivo covalent binding of CCl3 derived from CCl4 to cholesterol of rat liver. Biochem Pharmacol 31:3509-3510.

Araki, A; Kamigaitao, N; Sasaki, T; et al. (2004) Mutagenicity of carbon tetrachloride and chloroform in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2uvrA/pKM101 and WP2/pKM101, using a gas exposure method. Environ Mol Mutagen 43:128-133.

Arms, AD; Travis, CC (1988) Reference physiological parameters in pharmacokinetic modeling. Oak Ridge National Laboratory, Oak Ridge, TN; EPA/600/6-88/004. Available from: National Technical Information Service, Springfield, VA; PB88-196019.

Atkinson, R. (1989) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds. Monograph No. 1. New York, NY: American Chemical Society and the American Institute of Physics; p. 66.

ATSDR (Agency for Toxic Substances and Disease Registry). (2003) Toxicological profile for carbon tetrachloride (CAS# 56-23-5); Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available from: National Technical Information Service, Springfield, VA; PB/95/100103/AS, and <http://www.atsdr.cdc.gov/toxprofiles>.

Avasarala, S; Yang, L; Sun, Y; et al. (2006) A temporal study on the histopathological, biochemical and molecular responses of CCl(4)-induced hepatotoxicity in Cyp2e1-null mice. Toxicology 228(2-3):310-22.

Azri, S; Mata, HP; Gandolfi, AJ; et al. (1991) CCl4-induced cytochrome P-450 loss and lipid peroxidation in rat liver slices. In: Witmer, CM; ed. Biological reactive intermediates IV: molecular and cellular effects and their impact on human health. New York, NY: Plenum Press; pp. 669-674.

Badger, DA; Kuester, RK; Sauer, JM; et al. (1997) Gadolinium chloride reduces cytochrome P450: Relevance to chemical-induced hepatotoxicity. Toxicology 121:143-153.

Badger, DA; Sauer, J-M; Hoglen, NC; et al. (1996) The role of inflammatory cells and cytochrome P450 in the potentiation of CCl4-induced liver injury by a single dose of retinol. Toxicol Appl Pharmacol 141:507-519.

Ban, M; Hettich, D; Bonnet, P. (2003) Effect of inhaled industrial chemicals on systemic and local immune response. Toxicology 184(1):41-50.

Barber, ED; Donish, WH; Mueller, KR. (1981) A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames Salmonella/microsome assay. Mutat Res 90:31-48.

Barbin, A; Beresiat, JC; Bartsch, H. (1983) Evaluation of DNA damage by the alkaline elution technique in liver, kidneys and lungs of rats and hamsters treated with N-nitrosodialkylamines. Carcinogenesis 4:541-545.

Barros, L; Stutzin, A; Calixto, A; et al. (2001) Nonselective cation channels as effectors of free radical-induced rat liver cell necrosis. Hepatology 33:114-122.

Barrows, LR; Shank, RC (1981)Aberrant methylation of liver DNA in rats during hepatotoxicity. Toxicol Appl Pharmacol 60:334-345.

Bartosiewicz, MJ; Jenkins, D; Penn, S; et al. (2001) Unique gene expression patterns in liver and kidney associated with exposure to chemical toxicants. Pharmacol Exp Ther 297:895-905.

Bechtold, MM; Gee, DL; Bruenner, U; et al. (1982) Carbon tetrachloride-mediated expiration of pentane and chloroform by the intact rat: the effects of pretreatment with diethyl maleate, SKF-525A and phenobarbital. Toxicol Lett 11:165-171.

Beddowes, EJ; Fau, SP; Chipman, JK. (2003) Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. Toxicology 187:101-115.

Bedossa, P; Houglum, K; Trautwein, C; et al. (1994) Stimulation of collagen alpha 1(I) gene expression is associated with lipid peroxidation in hepatocellular injury: a link to tissue fibrosis? Hepatology 19:1262-1271.

Bell, AN; Mehendale, HM. (1985) The effect of dietary exposure to a mirex plus chlordecone combination on CCl4 hepatotoxicity. Fundam Appl Toxicol 5:679-687.

Bell, AN; Mehendale, HM. (1987) Comparative changes in hepatic DNA, RNA, protein, lipid, and glycogen induced by a subtoxic dose of CCl4 in chlordecone, mirex, and phenobarbital pretreated rats. Toxicol Lett 35:191-200.

Benigni, R; Andreoli, C; Conti, L; et al. (1993) Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in *Aspergillus nidulans*. Mutagenesis 8:301-305.

Benson, JM; Springer DL. (1999) Improved risk estimates for carbon tetrachloride. Final report. U.S. Department of Energy, Albuquerque, New Mexico; Report DE-FC04-96AL76406. Project No. 54940.

Benson, JM; Tibbetts, BM; Thrall, KD; et al. (2001) Uptake, tissue distribution, and fate of inhaled carbon tetrachloride: comparison of rat, mouse, and hamster. Inhal Toxicol 13:207-217.

Bergman, K; Muller, L; and Teigen, SW. (1996) Series: current issues in mutagenesis and carcinogenesis, No. 65. The genotoxicity and carcinogenicity of paracetamol: a regulatory (re)view. Mutat Res 349:63-288.

Bergman, K. (1983) Application and results of whole-body autoradiography in distribution studies of organic solvents. Crit Rev Toxicol 12:59-118.

Bermudez, E; Mirsalis, JC; Eales, HC. (1982) Detection of DNA damage in primary cultures of rat hepatocytes following in vivo and in vitro exposure to genotoxic agents. Environ Mutagen 4:667-679.

Bignami, M; Conti, G; Crebelli, R; et al. (1981) Growth-mediated metabolic activation of promutagens in Aspergillus nidulans. Mutat Res 80:265-272.

Blair, A; Decoufle, P; Grauman, D. (1979) Causes of death among laundry and dry cleaning workers. Am J Public Health 69:508-511.

Blair, A; Stewart, PA; Tolbert, TE. (1990) Cancer and other causes of death among a cohort of dry cleaners. Br J Ind Med 47:162-168.

Blair, PC; Thompson, MB; Wilson, RE; et al. (1991) Correlation of changes in serum analytes and hepatic histopathology in rats exposed to carbon tetrachloride. Toxicol Lett 55:149-159.

Blair, A; Hartge, P; Stewart, PA; et al. (1998) Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: extended follow-up. Occup Environ Med 55:161-171.

Bogers, M; Appelman, LM; Feron, VJ; et al. (1987) Effects of the exposure profile on the inhalation toxicity of carbon tetrachloride in male rats. J Appl Toxicol 7:185-191.

Boll, M; Weber, LWD; Becker, E; et al. (2001a) Pathogenesis of carbon tetrachloride-induced hepatocyte injury: bioactivation of CCl4 by cytochrome P450 and effects on lipid homeostasis. Z Naturforsch [C] 56:111-121.

Boll, M; Weber, LWD; Becker, E; et al. (2001b) Hepatocyte damage induced by carbon tetrachloride: inhibited lipoprotein secretion and changed lipoprotein composition. Z Naturforsch [C] 56:283-290.

Bond, GG; Flores, GH; Shellenberger, RJ; et al. (1986) Nested case-control study of lung cancer among chemical workers. Am J Epidemiol 124:53-66.

Boone, L; Meyer, D.; Cusick, P; et al. (2005) Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies. Vet Clin Pathol 34(3):182-188. <http://www.toxpath.org/Position_Papers/ASVCP_IndicatorInjury.pdf>

Boublik, T; Fried, V; Hala, E. (1984) The vapor pressures of pure substances: selected values of the temperature dependence of the vapor pressures of some pure substances in the normal and low pressure region. 2nd revised edition. Vol. 17. New York, NY: Elsevier Science Publishers.

Boutelet-Bochan, H; Huang, Y; Juchau, MR. (1997) Expression of CYP2E1 during embryogenesis and fetogenesis in human cephalic tissues: implications for the fetal alcohol syndrome. Biochem Biophys Res Commun 238:443-447.

Bove, FJ; Fulcomer, MC; Klotz, JB; et al. (1992a) Population-based surveillance and etiological research of adverse reproductive outcomes and toxic wastes. Report on phase IV-A: public drinking water contamination and birth weight, fetal deaths, and birth defects. A cross-sectional study. New Jersey Department of Health, Trenton, New Jersey.

Bove, FJ; Fulcomer, MC; Klotz, JB; et al. (1992b) Population-based surveillance and etiologic research of adverse reproductive outcomes and toxic wastes. Report on phase IV-B: public drinking water contamination and birth weight, fetal deaths, and birth defects. A case-control study. New Jersey Department of Health, Trenton, New Jersey.

Bove, FJ; Fulcomer, MC; Klotz, JB; et al. (1995) Public drinking water contamination and birth outcomes. Am J Epidemiol 141:850-862.

Boyd, MR; Statham, CN; Longo, NS. (1980) The pulmonary Clara cell as a target for toxic chemicals requiring metabolic activation: studies with carbon tetrachloride. J Pharmacol Exp Ther 212:109-114.

Brambilla, G; Carlo, P; Finollo, R; et al. (1983) Viscometric detection of liver DNA fragmentation in rats treated with minimal doses of chemical carcinogens. Cancer Res 43:202-209.

Brams, A; Buchet, JP; Crutzen-Fayt, MC; et al. (1987) A comparative study, with 40 chemicals, of the efficiency of the salmonella assay and the SOS chromotest (kit procedure). Toxicol Lett 38:123-133.

Braun, R; Schoneich, J. (1975) The influence of ethanol and carbon tetrachloride on the mutagenic effectivity of cyclophosphamide in the host-mediated assay with Salmonella typhimurium. Mutat Res 31:191-194.

Brennan, RJ; Schiestl, RH. (1998) Chloroform and carbon tetrachloride induce intrachromosomal recombination and oxidative free radicals in *Saccharomyces cerevisiae*. Mutat Res 397:271-278.

Brondeau, MT; Bonnet, P; Guenier, JP; et al. (1983) Short-term inhalation test for evaluating industrial hepatotoxicants in rats. Toxicol Lett 19:139-146.

Brooks, S. (1998) Markov chain Monte Carlo method and its application. The Statistician 47(1):69-100.

Brown, RP; Delp, MD; Lindstedt, SL; et al. (1997) Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 13:407-484.

Bruckner, JV; MacKenzie, WF; Muralidhara, S; et al. (1986) Oral toxicity of carbon tetrachloride: acute, subacute and subchronic studies in rats. Fundam Appl Toxicol 6:16-34.

Bruckner, JV; Kim, HJ; Muralidhara, S; et al. (1990) Influence of route and pattern exposure on the

pharmacokinetics and hepatotoxicity of carbon tetrachloride. In: Gerrity, TR; Henry, CJ, eds. Principle of route to route extrapolation for risk assessment. New York, NY: Elsevier Science Publishing Co., Inc.; pp. 271-284.

Bruckner, JV; Ramanathan, R; Lee, KM; et al. (2002) Mechanisms of circadian rhythmicity of carbon tetrachloride hepatotoxicity. J Pharmacol Exp Ther 300:273-281.

Brzezinski, MR; Boutelet-Bochan, H; Person, RE; et al. (1999) Catalytic activity and quantitation cytochrome P-450 2E1 in prenatal human brain. J Pharmacol Exp Ther 289:1648-1653.

Burke, DA; Wedd, DJ; Herriott, D; et al. (1994) Evaluation of pyrazole and ethanol induced S9 fraction in bacterial mutagenicity testing. Mutagenesis 9:3-29.

Butterworth, BE; Smith-Oliver, T; Earle, L, Loury; et al. (1989) Use of primary cultures of human hepatocytes in toxicology studies. Cancer Res 49:1075-1084.

Cabre, M; Camps, J; Paternain, JL; et al. (2000) Time-course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis. Clin Exp Pharmacol Physiol 27:694-699.

Cabre, M; Ferre, N, Folch, J; et al. (1999) Inhibition of hepatic cell nuclear DNA fragmentation by zinc in carbon tetrachloride-treated rats. J Hepatol 31:228-234.

Calabrese, EJ; Baldwin, LA; Leonard, DA; et al. (1995) Decrease in hepatotoxicity by lead exposure is not explained by its mitogenic response. J Appl Toxicol 15:129-132.

Callen, DF; Wolf, CR; Philpot, RM. (1980) Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in *Saccharomyces cerevisiae*. Mutat Res 77:55-63.

Cambon-Gros, C; Deltour, P; Boigegrain, RA; et al. (1986) Short communications: radical activation of carbon tetrachloride in foetal and maternal rat liver microsomes. Biochem Pharmacol 35:2041-2044.

Cantor, KP; Stewart, PA; Brinton, LA; et al. (1995) Occupational exposures and female breast cancer mortality in the United States. J Occup Environ Med 37:336-348.

Carpenter, SP; Lasker, JM; Raucy, JL. (1996) Expression, induction, and catalytic activity of the ethanol-inducible cytochrome P450 (CYP2E1) in human fetal liver and hepatocytes. Mol Pharmacol 49:260-268.

Casella, G; George, E. (1992) Explaining the Gibbs sampler. Amer. Statistician 46(3):167-174.

Castillo, T; Koop, DR; Kamimura, S; et al. (1992) Role of cytochrome P-450 2E1 in ethanol-, carbon tetrachloride- and iron-dependent microsomal lipid peroxidation. Hepatology 16:992-996.

Castro, GD; Simpson, JT; Castro, JA. (1994) Interaction of trichloromethyl free radicals with thymine in a model system: a mass spectrometric study. Chem Biol Interact 90:3-22.

Castro, JA; Diaz Gomez, MI. (1972) Studies on the irreversible binding of 14C-CCl4 to microsomal lipids in rats under varying experimental conditions. Toxicol Appl Pharmacol 23:541-552.

Castro, JA; de Castro, CR; de Fenos, OM; et al. (1972) Effect of cystamine on the mixed function oxygenase system from rat liver microsomes and its preventive effect on the destruction of cytochrome P450 by carbon tetrachloride. Pharmacol Res Commun 4:185-190.

Castro, JA; de Ferreyra, EC; de Castro, CR; et al. (1973) Studies on the mechanism of cystamine prevention of several liver structural and biochemical alterations caused by carbon tetrachloride. Toxicol Appl Pharmacol 24:1-19.

Castro, GD; Diaz Gomez, MI; Castro, JA. (1989) Species differences in the interaction between CCl_4 reactive metabolites and liver DNA or nuclear protein fractions. Carcinogenesis 10:289-294.

Chandra, M; Frith, CH. (1993/94) Non-neoplastic renal lesions in Sprague-Dawley and Fischer-344 rats. Exp Toxic Pathol 45:439-447.

Charbonneau, M; Oleskevich, S; Brodeur, J; et al. (1986) Acetone potentiation of rat liver injury induced by trichloroethylene-carbon tetrachloride mixtures. Fundam Appl Toxicol 6:654-661.

Chatterjee, A. (1966) Testicular degeneration in rats by carbon tetrachloride intoxication. Experientia 22:394-396.

Chaudhary, AK; Nokubo, M; Reddy, GR; et al. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science 265:1580-1582.

Checkoway, H; Wilcosky, T; Wolf, P; et al. (1984) An evaluation of the associations of leukemia and rubber industry solvent exposures. Am J Ind Med 5:239-249.

Chib, S; Greenberg, E. (1995) Understanding the Metropolis-Hastings algorithm. Amer. Statistician 49(4):327-335.

Chien, KC; Sherman, SC; Mittnacht, S, Jr.; et al. (1980) Membrane structure and function subsequent to calcium activation of an endogenous phospholipase. Arch Biochem Biophys 205:614-622.

Chiu, PY; Tang, MH; Mak, DH; et al. (2003) Hepatoprotective mechanism of schisandrin B: role of mitochondrial glutathione antioxidant status and heat shock proteins. Free Radic Biol Med 35:368-380.

Chung, F-L; Nath, RG; Ocando, J; et al. (2000) Deoxyguanosine adducts of t-4-hydroxy-2-nonal are endogenous DNA lesions in rodents and humans: detection and potential sources. Cancer Res 60:1507-1511.

CITI (Chemicals Inspection and Testing Institute). (1992) Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan. Chemicals Inspection and Testing Institute, Chemical Industry Ecology-Toxicology and Information Center, Tokyo, Japan; pp. 2-9.

Clawson, GA. (1989) Mechanisms of tetrachloride hepatotoxicity. Pathol Immunopathol Res 8:104-112.

Columbano, A; Ledda-Columbano, GM; Pibiri, M; et al. (1997) Increased expression of c-fos, c-jun and LRF-1 is not required for in vivo priming of hepatocytes by the mitogen TCPOBOP. Oncogene 14:857-63.

Comporti, M. (1985) Biology of disease: lipid peroxidation and cellular damage in toxic liver injury. Lab Invest 53:599-623.

Comporti, M; Benedetti, A; Ferrali, M; et al. (1984) Reactive aldehydes (4-hydroxyalkenals) originating from the peroxidation of liver microsomal lipids: biological effects and evidence for their binding to microsomal protein in CCl4 or BrCCl3 intoxication. Front Gastrointest Res 8:46-62.

Condie, LW; Laurie, RD; Mills, T; et al. (1986) Effect of gavage vehicle on hepatotoxicity of carbon tetrachloride in CD-l mice: corn oil versus Tween-60 aqueous emulsion. Fundam Appl Toxicol 7:199-206.

Cornish, HH; Ling, BP; Barth ML. (1973) Phenobarbital and organic solvent toxicity. Am Ind Hyg Assoc J 34:487-492.

Conner, HD; Thurman, RG; Galizi, MD; et al. (1986) The formation of a novel free radical metabolite from CCl4 in the perfused rat liver and in vivo. J Biol Chem 261:4542-48.

Correa, P. (1996) Morphology and natural history of cancer precursors. In: Schottenfield, D; Fraumeni, JF; eds. Cancer epidemiology and prevention. New York: Oxford University Press.

Coutino, RR. (1979) Analysis of anaphase in cell culture: an adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus. Environ Health Perspect

31:131-136.

Craddock, VM; Henderson, AR. (1978) De novo and repair replication of DNA in liver of carcinogen-treated animals. Cancer Res 38:2135-2143.

Crebelli, R; Andreoli, C; Carere, A; et al. (1992) The induction of mitotic chromosome malsegregation in Aspergillus nidulans. Quantitative structure activity relationship (OSAR) analysis with chlorinated aliphatic hydrocarbons. Mutat Res 266:117-134.

Crebelli, R; Benigni, R; Franekic, J; et al. (1988) Induction of chromosome malsegregation by halogenated organic solvents in Aspergillus nidulans: unspecific or specific mechanism? Mutat Res 201:401-411.

Crebelli, R; Carere, A; Leopardi, P. (1999) Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test. Mutagenesis 14:207-215.

Croen, LA; Shaw, GM; Sanbonmatsu, L; et al. (1997) Maternal residential proximity to hazardous waste sites and risk for selected congenital malformations. Epidemiology 8:347-354.

Crump, KS; Hoel, DG; Langley, CH; Peto. R. (1976) Fundamental carcinogenic processes and their implications for low dose4 risk assessment. Cancer Res 36:2973-2979.

Curren, RD; Yang, LL; Conklin, PM; et al. (1988) Mutagenesis of xeroderma pigmentosum fibroblasts by acrolein. Mutat Res 209:17-22.

Curtis, HJ; and Tilley, J. (1968) Chromosome aberrations in liver forced to regenerate by chemical or surgical methods. J Gerontol 23:140-141.

Curtis, LR; Williams, WL; Mehendale, HM. (1979) Potentiation of the hepatotoxicity of carbon tetrachloride following preexposure to chlordecone (kepone) in the male rat. Toxicol Appl Pharmacol 51:283-293.

Dai, Y; Cederbaum, AI. (1995) Inactivation and degradation of human cytochrome P4502D1 by CCl4 in a transfected HepG2 cell line. J Pharmacol Exp Ther 275:1614-1622.

Dambrauskas, T; Cornish, HH. (1970) Effect of pretreatment of rats with carbon tetrachloride on tolerance development. Toxicol Appl Pharmacol 17:83-97.

Damment, SJ; Beevers, C; and Gatehouse, DG. (2005) Evaluation of the potential genotoxicity of the phosphate binder lanthanum carbonate. Mutagenesis 20:29-37.

Daubert, TE; Danner, RP. (1995) Physical and thermodynamic properties of pure chemicals: data compilation. Supplement 5. Washington, DC: Taylor and Francis.

Delp, MD; Manning, RO; Bruckner, JV; Armstrong, RB. (1991) Distribution of cardiac output during diurnal changes of activity in rats. Am J Physiol 261:H1487-H1493.

de Ferreyra, EC; Castro, JA; Diaz Gomez, MI; et al. (1974) Prevention and treatment of carbon tetrachloride hepatotoxicity by cysteine: studies about its mechanism. Toxicol Appl Pharmacol 27:558-568.

de Flora, S. (1981) Study of 106 organic and inorganic compounds in the salmonella/microsome test. Carcinogenesis 2:283-298.

de Flora, S; Zanacchi, P; Camoirano, A; et al. (1984) Genotoxicity activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat Res 133:161-198.

de Groot, H; Haas, W. (1981) Self-catalysed, O2-independent inactivation of NADPH- or dithionite-reduced microsomal cytochrome P-450 by carbon tetrachloride. Biochem Pharmacol 30:2343-2347.

de Toranzo, EG; Diaz Gomez, MI; Castro, JA. (1978) Carbon tetrachloride activation, lipid peroxidation and liver necrosis in different strains of mice. Res Commun Chem Pathol Pharmacol 19:347-352.

de Zwart, LL; Venhorst, J; Groot, M; et al. (1997) Simultaneous determination of eight lipid peroxidation degradation products in urine of rats treated with carbon tetrachloride using gas chromatography with electron-capture detection. J Chromatogr B Biomed Sci Appl 694:277-287.

Dean, BJ; Hodson-Walker, G. (1979) An in vitro chromosome assay using cultured rat-liver cells. Mutat Res 64:329-337.

De Bont, R; van Larebeke, N. (2004) Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis 19:169-185.

Delaney, B; Kaminski, NE. (1993) Induction of serum-borne immunomodulatory factors in B6C3F1 mice by carbon tetrachloride. I. Carbon tetrachloride-induced suppression of helper T-lymphocyte function is mediated by a serum borne factor. Toxicology 85(1):67-84.

Delaney, B; Kaminski, NE. (1994) Induction of serum borne immunomodulatory factors in B6C3F1 mice by carbon tetrachloride. Exposure to carbon tetrachloride produces an increase in B-cell number and function. Toxicology 88(1-3):201-212.

Delaney, B; Strom, SC; Collins, S; et al. (1994) Carbon tetrachloride suppresses T-cell-dependent immune responses by induction of transforming growth factor-beta 1. Toxicol Appl Pharmacol 126(1):98-107.

Della Porta, GD; Terracini, B; Shubik, P. (1961) Induction with carbon tetrachloride of liver cell carcinomas in hamsters. J Natl Cancer Inst 26:855-863.

Dianzani, MU. (1984) Lipid peroxidation and haloalkylation: two distinct mechanisms for CCl4-induced liver damage. Calandra, S., Carulli, N., Salvioli, G. (Eds.) Liver and Lipid Metabolism, Elsevier Exerpta Medica, Amsterdam, 39-50

Diaz Gomez, MI; and Castro, JA. (1981) Reaction of trichloromethyl free radicals with deoxyribonucleic acid bases. Res Commun Chem Pathol Pharmacol 32:147-153.

Diaz Gomez, MI; Castro, JA. (1980a) Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins and lipids. Toxicol Appl Pharmacol 56:199-206.

Diaz Gomez, MI; Castro, JA. (1980b) Nuclear activation of carbon tetrachloride and chloroform. Res Commun Chem Pathol Pharmacol 27:191-194.

DiRenzo, AB; Gandolfi, AJ; Sipes, IG. (1982) Microsomal bioactivation and covalent binding of aliphatic halides to DNA. Toxicol Lett 11:243-252.

DiSilvestro, RA; Carlson, GP. (1994) Effects of mild zinc deficiency, plus or minus acute phase response, on CCl4 hepatotoxicity. Free Radic Biol Med 16:57-61.

Docherty, JF; Burgess, E. (1922) The action of carbon tetrachloride on the liver. Br Med J 2:907-908.

Docherty, JF; Nicholls, L. (1923) Report of three autopsies following carbon tetrachloride treatment. Br Med J 2:753.

Dogukan, A; Akpolat, N; Celiker, H; et al. (2003) Protective effect of interferon-alpha on carbon tetrachloride-induced nephrotoxicity. J Nephrol 16:81-84.

Doherty, AT; Ellard, S; Parry, EM; et al. (1996) An investigation into the activation and deactivation of chlorinated

hydrocarbons to genotoxins in metabolically competent human cells. Mutagenesis 11:247-274.

Dolak, JA; Waller, RL; Glende, EA; et al. (1988) Liver cell calcium homeostasis in carbon tetrachloride liver cell injury: new data with Fura2. J Biochem Toxicol 3:329-42.

Doolittle, DJ; Muller, G; Scribner, HE. (1987) Relationship between hepatotoxicity and induction of replicative DNA synthesis following single or multiple doses of carbon tetrachloride. J Toxicol Environ Health 22:63-78.

Dosemeci, M; Cocco, P; Chow, W-H. (1999) Gender differences in risk of renal cell carcinoma and occupational exposures to chlorinated aliphatic hydrocarbons. Am J Ind Med 36:54-59.

Draper, HH; Agarwal, S; Nelson, DE; et al. (1995) Effects of peroxidative stress and age on the concentration of a deoxyguanosine-malondialdehyde adduct in rat DNA. Lipids 30:959-961.

Dumas, S; Parent, ME; Siemiatycki, J; et al. (2000) Rectal cancer and occupational risk factors: a hypothesis-generating, exposure-based case-control study. Int J Cancer 87:874-879.

Edgren, M; Revesz, L. (1987) Compartmentalized depletion of glutathione in cells treated with buthionine sulphoximine. Br J Radiol 60:723-724.

Edwards, JE. (1941) Hepatomas in mice induced with carbon tetrachloride. J Natl Cancer Inst 2:197-199.

Edwards, JE; Dalton, AJ. (1942) Induction of cirrhosis of the liver and of hepatomas in mice with carbon tetrachloride. J Natl Cancer Inst 3:19-41.

Edwards, J; Heston, WE; Dalton, AJ. (1942) Induction of the carbon tetrachloride hepatoma in strain L mice. J Natl Cancer Inst 3:297-301.

Eisenhofer, G; Bornstein, SR; Brouwers, FM; et al. (2004) Malignant pheochromocytoma: current status and initiatives for future progress. Endocrine-Related Cancer 11:423-436.

El Masri, HA; Thomas, RS; Sabados, GR; Phillips, JK; Constan, AA; Benjamin, SA; Andersen, ME; Mehendale, HM; Yang, RSH. (1996) Physiologically based pharmacokinetic/pharmacodynamic modeling of the toxicologic interaction between carbon tetrachloride and kepone. Arch Toxicol 70:704-713.

Elkins, HB. (1942) Maximal allowable concentrations. II. Carbon tetrachloride. J Ind Hyg Toxicol 24:233-235.

Elia, MC; Storer, RD; McKelvey, TW; et al. (1994) Rapid DNA degradation in primary rat hepatocytes treated with diverse cytotoxic chemicals: analysis by pulsed field gel electrophoresis and implications for alkaline elution assays. Environ Mol Mutagen 24:181-191.

El Sisi, AD; Earnest, DL; Sipes, IG. (1993a) Vitamin A potentiation of carbon tetrachloride hepatotoxicity: role of liver macrophages and active oxygen species. Toxicol Appl Pharmacol 119:295-301.

El Sisi, AD; Hall, P; Sim, W-L W; et al. (1993b) Characterization of vitamin A potentiation of carbon tetrachloride-induced liver injury. Toxicol Appl Pharmacol 119:280-288.

Eschenbrenner, AB; Miller, E. (1946) Liver necrosis and the induction of carbon tetrachloride hepatomas in strain A mice. J Natl Cancer Inst 6:325-341.

Esterbauer, H; Schaur, RJ; Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radic Biol Med 11:81-128.

EMEA (European Medicines Agency). (2006) Draft guidelines on detection of early signals of drug-induced hepatotoxicity in non-clinical studies. Committee for Medicinal Products for Human Use (CHMP), London.

Adoption by CHMP for release for consultation 28 June 2006. Doc. Ref. EMEA/CHMP/SWP/150115/2006. <http://www.emea.eu.int/pdfs/human/swp/15011506en.pdf>

Evans, MV; Simmons, JE. (1996) Physiologically based pharmacokinetic estimated metabolic constants and hepatotoxicity of carbon tetrachloride after methanol pretreatment in rats. Toxicol Appl Pharmacol 140:245-253.

Evans, MV; Crank, WD; Yang, HM; Simmons, JE. (1994) Application of sensitivity analysis to a physiologically based pharmacokinetic model for carbon tetrachloride in rats. Toxicol Appl Pharmacol 128:36-44.

Fanelli, SL; Castro, JA. (1995) Covalent binding of carbon tetrachloride reactive metabolites to liver microsomal and nuclear lipid and phospholipid classes from Sprague Dawley and Osborne Mendel male rats. Teratog Carcinog Mutagen 15:155-166.

Farber, JL. (1981) The role of calcium in cell death. Life Sci 29:1289-1295.

Farrell, CL; Senseman, LA. (1944) Carbon tetrachloride polyneuritis. A case report. RI Med J 27:334-346.

FDA Working Group. (2000) Nonclincial assessment of potential hepatotoxicity in man (a concept paper meant to provide a framework for discussion at a February 12-13, 2001 public workshop on drug-induced hepatotoxicity). <http://www.fda.gov/cder/livertox/preclinical.pdf>

Feng, L; Pereira, B; Kraus-Friedmann, N. (1992) Different localization of inositol 1,4,5-trisphosphate and ryanodine binding sites in rat liver. Cell Calcium 13:79-87.

Fernández, G; Villarruel, MC; de Toranzo, EGD; et al. (1982) Covalent binding of carbon tetrachloride metabolites to the heme moiety of cytochrome P-450 and its degradation products. Res Commun Chem Pathol Pharmacol 35:283-290.

Fisher, J; Mahle, D; Bankston, L; et al. (1997) Lactational transfer of volatile chemicals in breast milk. Am Ind Hyg Assoc J 58:425-431.

Fisher, J; Lumpkin, M; Boyd, J; et al. (2004) PBPK modeling of the metabolic interactions of carbon tetrachloride and tetrachloroethylene in B6C3F1 mice. Environ Toxicol Pharmacol 16:93-105.

Folland, DS; Schaffner, W; Ginn, HE; et al. (1976) Carbon tetrachloride toxicity potentiated by isopropyl alcohol. Investigation of an industrial outbreak. J Am Med Assoc 236:1853-1856.

Fountoulakis, M; de Vera, MC; Crameri, F; et al. (2002) Modulation of gene and protein expression by carbon tetrachloride in the rat liver. Toxicol Appl Pharmacol 183:71-80.

Foureman, P; Mason, JM; Valencia, R; et al. (1994) Chemical mutagenesis testing in drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ Mol Mutagen 23:208-277.

Fowler, JSL. (1969) Carbon tetrachloride metabolism in the rabbit. Br J Pharmacol 37:733-737.

Fraga, CG; Zamora, R; Tappel, AL. (1987) Damage to protein syntheses concurrent with lipid peroxidation in rat liver slices: effect of halogenated compounds, peroxides, and vitamin E. Arch Biochem Biophys 270:84-91.

Frantik, R; Hornychova, M; Horvath, M. (1994) Relative acute neurotoxicity of solvents: isoeffective air concentrations of 48 compounds evaluated in rats and mice. Environ Res 66:173-185.

Galelli, ME; Castro, JA. (1998) Effect of trichloromethyl and trichloromethyl peroxyl free radicals on protein sulfhydryl content studies in model and enzymatic carbon tetrachloride activation systems. Res Commun Mol Pathol Pharmacol 100:227-238.

Galli, A; Schiestl, RH. (1995) Salmonella test positive and negative carcinogens show different effects on

intrachromosomal recombination in G_2 cell cycle arrested yeast cells. Carcinogenesis 16:659-663.

Galli, A; Schiestl, RH. (1996) Effects of salmonella assay negative and positive carcinogens on intrachromosomal recombination in G_1-arrested yeast cells. Mutat Res 370:109-221.

Galli, A; Schiestl, RH. (1998) Effect of salmonella assay negative and positive carcinogens on intrachromosomal recombination in S-phase arrested yeast cells. Mutat Res 419:53-68.

Gallo, JM; Cheung, LL; Kim, JJ; et al. (1993) A physiological and system analysis hybrid pharmacokinetic model to characterize carbon tetrachloride blood concentrations following administration in different oral vehicles. J Pharmacokinet Biopharm 21:551-567.

Galloway, SM. (2000) Cytotoxicity and chromosome aberrations in vitro: experience in industry and the case for an upper limit on toxicity in the aberration assay. Environ Mol Mutagen 35:191-201.

Galloway, SM; Deasy, DA; Bean, CL.; et al. (1987) Effects of high osmotic strength on chromosome aberrations, sister-chromatid exchanges and DNA strand breaks, and the relation to toxicity. Mutat Res 189:15-25.

Gans, JH; Korson, R. (1984) Liver nuclear DNA synthesis in mice following carbon tetrachloride administration or partial hepatectomy. Proc Soc Exper Biol Med 175:237-242.

Garberg, P; Akerblom, EL; Bolcsfoldi, G. (1988) Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. Mutat Res 203:155-176.

Gardner, GH; Gove, RC; Gustafson, RK; et al. (1925) Studies on the pathological histology of experimental carbon tetrachloride poisoning. Bull Johns Hopkins Hosp 36:107-133.

Gargas, ML; Andersen, ME; Clewell, HJ, III. (1986) A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol Appl Pharmacol 86:341-352.

Gargas, ML; Burgess, RJ; Voisard, DE; et al. (1989) Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 98:87-99.

Garner, RC; McLean, AEM. (1969) Increased susceptibility to carbon tetrachloride poisoning in the rat after pretreatment with oral phenobarbitone. Biochem Pharmacol 18:645-650.

Garrett, RH; Grisham, CM. (1999) Biochemistry: 2nd edition. New York, NY: Saunders College Publishing.

Garry, VF; Nelson, RL; Griffith, J; et al. (1990) Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants. Teratog Carcinog Mutagen 10:21-29.

Gasso, M; Rubio, M; Varela, G; et al. (1996) Effect of S-adenosylmethionine on lipid peroxidation and liver fibrogenesis in carbon tetrachloride-induced cirrhosis. J Hepatol 25:200-205.

Gatehouse, D; Haworth, S; Cebula, T; et al. (1994) Recommendations for the performance of bacterial mutation assays. Mutat Res 312:217-233.

Gaynes, BI; Watkins, JB, III. (1989) Carbon tetrachloride and the sorbitol pathway in the diabetic mouse. Comp Biochem Physiol B 94:213-217.

Gearhart, JM; Mahle, DA; Greene, RJ; Seckel, CS; Flemming, CD; Fisher, JW; Clewel III, HJ. (1993) Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). Toxicol Lett 68:131-144.

Gee, DL; Bechtold, MM; Tappel, AL. (1981) Carbon tetrachloride-induced lipid peroxidation: simultaneous in vivo

measurements of pentane and chloroform exhaled by the rat. Toxicol Lett 8:299-306.

Gilks, W; Richardson, S; Spiegelhalter, D. (1998) Markov Chain Monte Carlo in practice. New York: Chapman & Hall/CRC.

Gillespie, WR; Cheung, LL; Kim, HJ; et al. (1990) Application of system analysis to toxicology: characterization of carbon tetrachloride oral absorption kinetics. In: Gentry TR; Henry CJ, eds. Principles of route-to-route extrapolations for risk assessment. New York, NY: Elsevier Science Publishing Company; pp. 285-295.

Gillette, JR. (1973) Factors that affect the covalent binding and toxicity of drugs. In: Pharmacology and the future of man: proceedings of the 5th international congress on pharmacology; July 1972; San Francisco, CA. Vol. 2. Basel, Switzerland: S. Karger AG; pp.187-202.

Gilman, MR. (1971) A preliminary study of the teratogenic effects of inhaled carbon tetrachloride and ethyl alcohol consumption in the rat [dissertation]. Drexel University, Philadelphia, PA.

Ginsberg, G; Hattis, D; Sonawane, B; et al. (2002) Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Tox Sci 66:185-200.

Glende, EA. (1972) Carbon tetrachloride-induced protection against carbon tetrachloride toxicity. The role of the liver microsomal drug-metabolizing system. Biochem Pharmacol 21:1697-1702.

Glende, EA; Pushpendran CK. (1986) Activation of phospholipase A2 by carbon tetrachloride in isolated hepatocytes. Biochem Pharmacol 35:3301-3307.

Glende, EA; Recknagel, RO. (1992) Phospholipase A2 activation and cell injury in isolated rat hepatocytes exposed to bromotrichloromethane, chloroform, and 1,1-dichloroethylene as compared to effects of carbon tetrachloride. Toxicol Appl Pharmacol 113:159-162.

Glende, EA, Jr.; Hruszkewycz, AM; Recknagel, RO. (1976) Critical role of lipid peroxidation in carbon tetrachloride-induced loss of aminopyrine demethylase, cytochrome P-450 and glucose 6-phosphatase. Biochem Pharmacol 25:2163-2170.

Gonzalez Padron, A; de Toranzo, EG; Castro, JA. (1993) Late preventive effects of quinacrine on carbon tetrachloride induced liver necrosis. Arch Toxicol 67:386-391.

Gonzalez-Reimers, E; Lopez-Lirola, A; Olivera, RM; et al. (2003) Effects of protein deficiency on liver trace elements and antioxidant activity in carbon tetrachloride-induced liver cirrhosis. Biol Trace Elem Res 93:127-139.

Gordis, E. (1969) Lipid metabolites of carbon tetrachloride. J Clin Invest 48:203-209.

Gorla, N; de Ferreyra, EC; Villarruel, MC; et al. (1983) Studies on the mechanism of glutathione prevention of carbon tetrachloride-induced liver injury. Br J Exp Pathol 64:388-395.

Gosselin, RE; Hodge, HC; Smith, RP; et al. (1976) Clinical toxicology of commercial products. In: Acute poisoning. 4th edition. Baltimore, MD: The Williams and Wilkins Co.; pp. 13, 92-97, 110.

Gould, VE; Smuckler, EA. (1971) Alveolar injury in acute carbon tetrachloride intoxication. Arch Intern Med 128:109-117.

Grasl-Kraupp, B; Ruttkay-Nedecky, B; Koudelka, H; et al. (1995) In situ detection of fragmented DNA (TUNEL assay) fails to discriminate among apoptosis, necrosis, and autolytic cell death: a cautionary note. Hepatology 21:1465-1468.

Gruebele, A; Zawaski, K; Kaplan, D. (1996) Effects on signal transduction as demonstrated by altered immediate-early (c-Fos and c-Jun) gene expression and nuclear AP-1 and NK-kB transcription factor levels. Drug Metab

Dispos 24:15-22.

Gualandi, G. (1984) Genotoxicity of the free-radical producers CCl_4 and lipoperoxide in Aspergillus nidulans. Mutat Res 136:109-114.

Gubskii, IuI; Kurskii, MD; Zadorina, OV; et al. (1990) Calcium transport in endoplasmic reticulum of the rat liver during lipid peroxidation. Biokhimiia 55:12-22.

Guo, TL; McCay, JA; Brown, RD; et al. (2000) Carbon tetrachloride is immunosuppressive and decreases host resistance to Listeria monocytogenes and Streptococcus pneumoniae in female B6C3F1 mice. Toxicology 154:85-101.

Hachiya, N; Motohashi, Y. (2000) Examination of lacZ mutant induction in the liver and testis of Muta Mouse following injection of halogenated aliphatic hydrocarbons classified as human carcinogens. Ind Health 38:213-220.

Hadley, M; Draper, HH. (1990) Isolation of a guanine-malondialdehyde adduct from rat and human urine. Lipids 25:82-85.

Haehner, BD; Gorski, JC; Vandenbranden, M; et al. (1996) Bimodal distribution of renal cytochrome P450 3A activity in humans. Molec Pharmacol 50:52-59.

Hafeman, DG; Hoekstra, WG. (1977) Protection against carbon tetrachloride-induced lipid peroxidation in the rat by dietary vitamin E, selenium and methionine as measured by ethane evolution. J Nutr 107:656-665.

Hagmar, L; Bonassi, S; Stromberg, U; et al. (1998) Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer Res 58:4117-4121.

Hagmar, L; Stromberg, U; Bonassi, S; et al. (2004) Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res 64:2258-2263.

Hakkola, J; Raunio, H; Purkunen, R; et al. (1996) Detection of cytochrome P450 gene expression in human placenta in first trimester of pregnancy. Biochem Pharmacol 52:379-383.

Hall, PM; Plummer, JL; Ilsley, AH; et al. (1991) Hepatic fibrosis and cirrhosis after chronic administration of alcohol and "low-dose" carbon tetrachloride vapor in the rat. Hepatology 13:815-819.

Halliwell, B; and Gutteridge, J. (1999) Free Radicals in Biology and Medicine. Oxford University Press, New York

Hamlin, G.P., Kholkute, SD; Dukelow, WR. (1993) Toxicology of maternally ingested carbon tetrachloride (CCl4) on embryonal and fetal development and in vitro fertilization in mice. Zool Sci 10:111-116.

Hanasono, GK; Cote, MG; Plaa, GL. (1975) Potentiation of carbon tetrachloride-induced hepatotoxicity in alloxan- or streptozotocin-diabetic rats. J Pharmacol Exp Ther 192:592-604.

Hansch, C; Leo, A; Hoekman, D. (1995) Exploring QSAR: Hydrophobic, electronic, and steric constants. ACS professional reference book. Vol. 2. In: Heller, SR; ed. Washington, DC: American Chemistry Society; p. 3.

Hansen, M. (1998) Disorders of somatic and motor autonomic function. In: Pathophysiology: foundations of disease and clinical intervention. Philadelphia, PA: W.B. Saunders Company; pp. 644-645.

Hansen, MF; Cavenee, WK. (1987) Genetics of cancer predisposition. Cancer Res 47:5518-5527.

Hard, GC; Seely, JC. (2005) Recommendations for the interpretation of renal tubule proliferative lesions occurring in rat kidneys with advanced chronic progressive nephropathy (CPN). Toxicol Path 33:641-649.

Harris, CC. (1991) Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res 51:5023s-5044s.

Harris, RN; Anders, MW. (1980) Effect of fasting, diethyl maleate and alcohols on carbon tetrachloride-induced hepatotoxicity. Toxicol Appl Pharmacol 56:191-198.

Hartley, DP; Kolaja, KL; Reichard, J; et al. (1999) 4-Hydroxynonenal and malondialdehyde hepatic protein adducts in rats treated with carbon tetrachloride: immunochemical detection and lobular localization. Toxicol Appl Pharmacol 161:23-33.

Hayes, JR; Condie, LW; Borzelleca, JF. (1986) Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-l mice. Fundam Appl Toxicol 7:454-463.

Heddle, JA; Dean, S; Nohmi, T; et al. (2000) In vivo transgenic mutation assays. Environ Mol Mutagen 35:253-259.

Heineman, EF; Cocco, P; Gomez, MR; et al. (1994) Occupational exposure to chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. Am J Ind Med 26:155-169.

Hellmer, L; Bolcsfoldi, G. (1992) An evaluation of the *E. coli* K-12 uvrB/recA DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds. Mutat Res 272:145-160.

Hemmings, SJ; Pulga, VB; Tran ST; et al. (2002) Differential inhibitory effects of carbon tetrachloride on the hepatic plasma membrane, mitochondrial and endoplasmic reticular calcium transport systems: implications to hepatotoxicity. Cell Biochem Funct 20:47-59.

Hewitt, WR; Miyajima, H; Cote, MG; et al. (1980) Modification of haloalkane-induced hepatotoxicity by exogenous ketones and metabolic ketosis. Federation Proc 39:3118-3123.

Higami, Y; Tsuchiya, T; To, K; et al. (2004) Expression of DNase gamma during Fas-independent apoptotic DNA fragmentation in rodent hepatocytes. Cell Tissue Res 316:403-407.

Hill, GD; Pace, V; Persohn, E; et al. (2003) A comparative immunohistochemical study of spontaneous and chemically induced pheochromocytomas in B6C3F1 mice. Endocrine Path 14:81-91.

Hocher, B; Zart, R; Diekmann, F; et al. (1996) Paracrine renal endothelin system in rats with liver cirrhosis. Br J Pharmacol 118:220-227.

Holbrook, MT. (1993) Carbon tetrachloride. In: Kroschwitz, JI; Howe-Grant, M, eds. Kirk-Othmer encyclopedia of chemical technology. 4th edition. Vol. 5. New York, NY: John Wiley and Sons; pp. 1062-1072.

Holden, PR; James, NH; Brooks, AN; et al. (2000) Identification of a possible association between carbon tetrachloride-induced hepatotoxicity and interleukin-8 expression. J Biochem Mol Toxicol 14:283-290.

Holsapple, MP; Pitot, HC; Cohen, SM; et al. (2006) Mode of action relevance of rodent liver tumors to human risk. Toxicol Sci 89(1):51-6.

Horvath, AL. (1982) Halogenated hydrocarbons: solubility-miscibility with water. New York, NY: Marcel Dekker, Inc.; p. 889.

Hyland, R; Gescher, A; Thummel, K; et al. (1992) Metabolic oxidation and toxification of N-methylformamide catalyzed by the cytochrome P450 isoenzyme CYP2E1. Mol Pharmacol 41:259-266.

Ichinose, T; Miller, MG; Shibamoto, T. (1994) Determination of free malonaldehyde formed in liver microsomes upon CCl_4 oxidation. J Appl Toxicol 14:453-455.

Ikatsu, H; Okino, T; Nakajima, T. (1991) Ethanol and food deprivation induced enhancement of hepatotoxicity in

rats given carbon tetrachloride at low concentration. Br J Ind Med 48:636-642.

Ikegwuonu, FI; Mehendale, HM. (1991) Biochemical assessment of the genotoxicity of the in vitro interaction between chlordecone and carbon tetrachloride in rat hepatocytes. J Appl Toxicol 11:303-310.

Iwai, S; Karim, R; Kitano, M; et al. (2002) Role of oxidative DNA damage caused by carbon tetrachloride-induced liver injury -- enhancement of MeIQ-induced glutathione S-transferase placental form-positive foci in rats. Cancer Lett 179:15-24.

Jaeger, RJ; Conolly, RB; Murphy, SD. (1975) Short-term inhalation toxicity of halogenated hydrocarbons. Effects on fasting rats. Arch Environ Health 30:26-31.

Jaeschke, H; Gores, GJ; Cederbaum, AI; et al. (2002) Mechanisms in hepatotoxicity. Toxicol Sci 65:166-176.

Jakobson, I; Wahlberg, JE; Holmberg, B; et al. (1982) Uptake via the blood and elimination of 10 organic solvents following epicutaneous exposure of anesthetized guinea pigs. Toxicol Appl Pharmacol 63:181-187.

JBRC (Japan Bioassay Research Center). (1998) Subchronic inhalation toxicity and carcinogenicity studies of carbon tetrachloride in F344 rats and BDF1 mice (Studies Nos. 0020, 0021, 0043, and 0044). Kanagawa, Japan Industrial Safety and Health Association, Japan Bioassay Research Center, Kanagawa, Japan. Unpublished report to the Ministry of Labor. Hirasawa Hadano Kanagawa, 257 Japan.

Jeon, YJ; Han, SH; Yang, KH; et al. (1997) Induction of liver-associated transforming growth factor beta 1 (TGF-beta 1) mRNA expression by carbon tetrachloride leads to the inhibition of T helper 2 cell-associated lymphokines. Toxicol Appl Pharmacol 144(1):27-35.

Jessen, BA; Mullins, JS; De Peyster, A; et al. (2003) Assessment of hepatocytes and liver slices as in vitro test systems to predict in vivo gene expression. Toxicol Sci 75:208-222.

Johnstone RT. 1948. Occupational medicine and industrial hygiene. St. Louis, MO: CV Mosby Co.; pp. 148-158.

Kadiiska, MB; Gladen, BC; Baird, DD; et al. (2005) Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 38:698-710.

Kalla, NR; Bansal, MP. (1975) Effect of carbon tetrachloride on gonadal physiology in male rats. Acta Anat 91:380-385.

Kaminski, NE; Jordan, SD; Holsapple, MP. (1989) Suppression of humoral and cell-mediated immune responses by carbon tetrachloride. Fundam Appl Toxicol 12:117-128.

Kaminski, NE; Barnes, DW; Jordan, SD; et al. (1990) The role of metabolism in carbon tetrachloride-mediated immunosuppression: in vivo studies. Toxicol Appl Pharmacol 102:9-20.

Karpinets, TV; Foy, BD. (2005) Tumorigenesis: the adaptation of mammalian cells to sustained stress environment by epigenetic alterations and succeeding matched mutations. Carcinogenesis 26(8):1323-34.

Kauppinen, T; Pukkala, E; Saalo, A; et al. (2003) Exposure to chemical carcinogens and risk of cancer among Finnish laboratory workers. Am J Ind Med 44:343-350.

Kazantzis, G; Bomford, RR. (1960) Dyspepsia due to inhalation of carbon tetrachloride vapor. Lancet 1:360-362.

Kefalus, V; Stacey, NH. (1989) Potentiation of carbon tetrachloride-induced lipid peroxidation by trichloroethylene in isolated rat hepatocytes: no role in enhanced toxicity. Toxicol Appl Pharmacol 101(1):158-69.

Kerckaert, GA; Isfort, RJ; Carr, GJ; et al. (1996) A comprehensive protocol for conducting the Syrian hamster embryo cell transformation assay at pH 6.70. Mutat Res 356:65-84.

Kernan, GJ; Ji, B-T; Dosemeci, M; et al. (1999) Occupational risk factors for pancreatic cancer: a case-control study based on death certificates from 24 U.S. states. Am J Ind Med 36:260-270.

Kim, HJ; Bruckner, JV; Dallas, CE; et al. (1990a) Effect of dosing vehicles on the pharmacokinetics of orally administered carbon tetrachloride in rats. Toxicol Appl Pharmacol 102:50-60.

Kim, HJ; Odendhal, S; Bruckner, JV. (1990b) Effect of oral dosing vehicles on the acute hepatotoxicity of carbon tetrachloride in rats. Toxicol Appl Pharmacol 102:34-49.

Kiplinger, GF; Kensler, CJ. (1963) Failure of phenoxybenzamine to prevent formation of hepatomas after chronic carbon tetrachloride administration. J Natl Cancer Inst 30:837-843.

Kitchin, KT; Brown, JL. (1989) Biochemical effects of three carcinogenic chlorinated methanes in rat liver. Teratog Carcinog Mutagen 9:61-69.

Kitta, D; Schwarz, M; Tennekes, HA; et al. (1982) Covalent binding of CCl_4-intermediates to reduced pyridine nucleotides in mouse liver. In Biological Reactive Intermediates-II: Chemical Mechanisms and Biological Effects (R. Snyder, D. V. Parke, J. J. Kocsis, D. J. Jollow, C. G. Gibson, and C. M. Witmer, Eds.), pp. 769-777. Plenum Press, New York.

Klaassen, CD; ed.. (1996) Casarett and Doull's toxicology: the basic science of poisons. 5th edition. New York, NY: McGraw-Hill, Health Professions Division.

Kluwe, WM; Herrmann, CL; Hook JB. (1979) Effects of dietary polychlorinated biphenyls and polybrominated biphenyls on the renal and hepatic toxicities of several chlorinated hydrocarbon solvents in mice. J Toxicol Environ Health 5:605-615.

Kniepert, E; Siegemund, A; Gorisch, V. (1990) Influence of ethanol pretreatment of differing duration on toxic effects of carbon tetrachloride in rats. Biomed Biochim Acta 49:1097-1102.

Koch, I.; Weil, R; Wolbold, R; et al. (2002) Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. Drug Metab Dispos 30:1108-1114.

Kodavanti, PR; Kodavanti, UP; Faroon, OM; et al. (1992) Pivotal role of hepatocellular regeneration in the ultimate hepatotoxicity of CCl4 in chlordecone-, mirex-, or phenobarbital-pretreated rats. Toxicol Pathol 20:556-569.

Kohno, H; Hoshino, Y; Katoh, S; et al. (1992) Effect of retinoic acid on liver transglutaminase activity and carbon tetrachloride-induced liver damage in mice. Experientia 48:386-388.

Koporec, KP; Kim, HJ; MacKenzie, WF; et al. (1995) Effect of oral dosing vehicles on the subchronic hepatotoxicity of carbon tetrachloride in the rat. J Toxicol Environ Health 44:13-27.

Kopylev, L., Chen, C., White, P. (2007). Towards quantitative uncertainty assessment for cancer risks: central estimates and probability distributions of risk in dose-response modeling. Reg Tox Pharm 49:203-207.

Korsrud, GO; Grice, HC; McLaughlan, JM. (1972) Sensitivity of several serum enzymes in detecting carbon tetrachloride-induced liver damage in rats. Toxicol Appl Pharmacol 22:474-483.

Krokan, H; Grafstrom, RC; Sundqvist, K; et al. (1985) Cytotoxicity, thiol depletion and inhibition of O6-methylguanine-DNA methyltransferase by various aldehydes in cultured human bronchial fibroblasts. Carcinogenesis 6:1755-1759.

Kroner, H. (1982) The intracellular distribution of liver cell calcium in normal rats and one hour after administration of CCl4. Biochem Pharmacol 31:1069-1073.

Kubale, TL; Daniels, RD; Yiin, JH; et al. (2005) A nested case-control study of leukemia mortality and ionizing radiation at the Portsmouth Naval Shipyard. Radiation Research 164:810-819.

Kurtz, AJ; and Lloyd, RS. (2003) 1,N2-deoxyguanosine adducts of acrolein, crotonaldehyde, and trans-4-hydroxynonenal cross-link to peptides via Schiff base linkage. J Biol Chem 278:5970-5976.

Kwon, YH; Jovanovic, A; Serfas, MS; et al. (2003) The Cdk inhibitor p21 is required for necrosis, but it inhibits apoptosis following toxin-induced liver injury. J Biol Chem 278:30348-30355.

Ladics, GS; Smith, C; Elliott, GS; et al. (1998) Further evaluation of the incorporation of an immunotoxicological functional assay for assessing humoral immunity for hazard identification purposes in rats in a standard toxicology study. Toxicology 126(2):137-152.

Lai, EK; McCay, PB; Noguchi, T; et al. (1979) In vivo spin-trapping of trichloromethyl radicals formed from carbon tetrachloride. Biochem Pharmacol 28:2231-2235.

Lambert, IB; Singer, TM; Boucher, SE; et al. (2005) Detailed review of transgenic rodent mutation assays. Mutat Res 590:1-280.

LeBoeuf, RA; Kerckaert, GA; Aardema, MJ; et al. (1996) The pH 6.7 Syrian hamster embryo cell transformation assay for assessing the carcinogenic potential of chemicals. Mutat Res 356:85-127.

Lee, PY; McCay, PB; Hornbrook, KR. (1982) Evidence for carbon tetrachloride-induced lipid peroxidation in mouse liver. Biochem Pharmacol 31:405-409.

Lee, VM; Cameron, RG; Archer, MC. (1998) Zonal location of compensatory hepatocyte proliferation following chemically induced hepatotoxicity in rats and humans. Toxicol Pathol 26:621-627.

Lehmann, KB; Schmidt-Kehl, L. (1936) The thirteen most important chlorinated aliphatic hydrocarbons from the standpoint of industrial hygiene. Arch Hygiene 116:132-200.

Leighton, DT, Jr.; Calo, JM. (1981) Distribution coefficients of chlorinated hydrocarbons in dilute air-water systems for groundwater contamination applications. J Chem Eng 26:382-585.

Letteron, P; Labbe, G; Degott, C; et al. (1990) Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice. Evidence that silymarin acts both as an inhibitor of metabolic activation and as a chain-breaking antioxidant. Biochem Pharmacol 39:2027-2034.

Levy, GN; and Brabec, MJ. (1984) Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA. Toxicol Lett 22:229-234.

Lewis, RJ, Sr; ed. (1997) Hawley's condensed chemical dictionary. 13th edition. New York, NY: John Wiley and Sons, Inc.; p. 213.

Lide, DR; ed. (2000) CRC handbook of chemistry and physics. 81st Edition. Boca Raton, FL: CRC Press LLC, pp. 3-207.

Lil'p, IG. (1982) [Chromosome instability in 101/H and C57BL/6 strain mice during aging]. Genetika 18:1976-1982.

Limaye, PB; Apte, UM; Shankar, K; et al. (2003) Calpain released from dying hepatocytes mediates progression of acute liver injury induced by model hepatotoxicants. Toxicol Appl Pharmacol 191:211-226.

Litchfield, MH; Gartland, CJ. (1974) Plasma enzyme activity and hepatocellular changes in the beagle dog after single or repeated administration of carbon tetrachloride. Toxicol Appl Pharmacol 30:117-128.

Lockard, VG; Mehendale, HM; O'Neal, RM. (1983) Chlordecone-induced potentiation of carbon tetrachloride

hepatotoxicity: a morphometric and biochemical study. Exp Mol Pathol 39:246-255.

Long, RM; Moore, L. (1986) Elevated cytosolic calcium in rat hepatocytes exposed to carbon tetrachloride. J Pharmacol Exp Ther 238:186-191.

Lopez-Diazguerrero, NE.; Luna-Lopez, A; Gutierrez-Ruiz, MC; et al. (2005) Susceptibility of DNA to oxidative stressors in young and aging mice. Life Sci 77:2840-2854.

Loveday, KS; Anderson, BE; Resnick, MA; et al. (1990) Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: results with 46 chemicals. Environ Mol Mutagen 16:272-303.

Lowrey, KE; Glende, A, Jr.; Recknagel, RO. (1981) Destruction of liver microsomal calcium pump activity by carbon tetrachloride and bromotrichloromethane. Biochem Pharmacol 30:135-140.

Luckey, SW; Petersen, DR. (2001) Activation of Kupffer cells during the course of carbon tetrachloride-induced liver injury and fibrosis in rats. Exp Mol Pathol 71:226-240.

Luster, MI; Simeonova, PP; Gallucci, RM; et al. (2000) Immunotoxicology: role of inflammation in chemical-induced hepatotoxicity. Int J Immunopharmacol 22(12):1143-1147.

Lutz, W.K. (1979) In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat Res 65:289-356.

Lutz, WK. (1986) Quantitative evaluation of DNA binding data for risk estimation and for classification of direct and indirect carcinogens. J Cancer Res Clin Oncol 112:85-91.

Lutz, WK; Gaylor, DW; Conolly, RB; et al. (2005) Nonlinearity and thresholds in dose–response relationships for carcinogenicity due to sampling variation, logarithmic dose scaling, or small differences in individual susceptibility. Toxicology and Applied Pharmacology 207:S565-S569.

Madle, S; Dean, SW; Andrae, U; et al. (1994) Recommendation for the performance of UDS tests in vitro and in vivo. Mutat Res 312(3):263-85.

Magos, L; Snowden, R; White, INH; et al. (1982) Isotoxic oral and inhalation exposure of carbon tetrachloride in Porton-Wistar and Fischer rats. J Appl Toxicol 2:238-240.

Manno, M; deMatteis, F; King, LJ. (1988) The mechanism of the suicidal, reductive inactivation of microsomal cytochrome P-450 by carbon tetrachloride. Biochem Pharmacol 37:1981-1990.

Manno, M; Ferrara, R; Cazzaro, S; et al. (1992) Suicidal inactivation of human cytochrome P-450 by carbon tetrachloride and halothane in vitro. Pharmacol Toxicol 70:13-18.

Manno, M; Rezzadore, M; Grossi, M; et al. (1996) Potentiation of occupational carbon tetrachloride toxicity by ethanol abuse. Hum Exp Toxicol 15:294-300.

Marchand, C; McLean, S; Plaa, GL. (1970) The effect of SKF 525A on the distribution of carbon tetrachloride in rats. J Pharmacol Exp Ther 174:232-238.

Maron, DM; Ames, BN. (1983) Revised methods for Salmonella mutagenicity testing. Mutat Res 113(3-4):173-215.

Marra, F; Arrighi, MC; Fazi, M; et al. (1999) Extracellular signal-regulated kinase activation differentially regulates platelet-derived growth factor's actions in hepatic stellate cells, and is induced by in vivo liver injury in the rat. Hepatology 30:951-958.

Martin, C; Dutertre-Catella, H; Radionoff, M; et al. (2003) Effect of age and photoperiodic conditions on metabolism and oxidative stress related markers at different circadian stages in rat liver and kidney. Life Sci 73:327-

335.

Martinez, A; Urios, A; Blanco, M. (2000) Mutagenicity of 80 chemicals in Escherichia coli tester strains IC203, deficient in OxyR, and its oxyR(+) parent WP2 uvrA/pKM101: detection of 31 oxidative mutagens. Mutat Res 467:41-53.

Martinez, M.; Mourelle, M.; Muriel, P. (1995) Protective effect of colchicine on acute liver damage induced by CCl_4. Role of cytochrome P-450. J Appl Toxicol 15:49-52.

McCann, J; Choi, E; Yamasaki, E; et al. (1975) Detection of carcinogens as mutagens in the salmonella/microsome test: assay of 300 chemicals. Proc Nat Acad Sci 72:5135-5139.

McCay, PB; Lai, EK; Poyer, JL; et al. (1984) Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. Observations of lipid radicals in vivo and in vitro. J Biol Chem 259:2135-2143.

McCollister, DD; Beamer, WH; Atchison, GJ; et al. (1951) The absorption, distribution and elimination of radioactive carbon tetrachloride by monkeys upon exposure to low vapor concentrations. J Pharmacol Exp Ther 102:112-124.

McLean, AEM; McLean, EK. (1966) The effect of diet and l,l,l-trichloro-2,2-bis-(p-chlorophenyl)ethane (DDT) on microsomal hydroxylating enzymes and on sensitivity of rats to carbon tetrachloride poisoning. Biochem J 100:564-571.

McLean, AJ; Le Couteur, DG. (2004) Aging biology and geriatric clinical pharmacology. Pharmacol Rev 56:163-184.

Mehendale, HM. (1990) Potentiation of halomethane hepatotoxicity by chlordecone: a hypothesis for the mechanism. Med Hypotheses 33:289-299.

Mehendale, HM. (1991) Commentary: role of hepatocellular regeneration and hepatolobular healing in the final outcome of liver injury. A two-stage model of toxicity. Biochem Pharmacol 42:1155-1162.

Mehendale, HM. (1992) Biochemical mechanisms of biphasic dose-response relationships: role of hormesis. In: Calabrese EJ; ed. Biological effects of low level exposures to chemicals and radiation. Chelsea, MI: Lewis Publishers; pp. 59-94.

Menegazzi, M; Carcereri-De Prati, A; Suzuki, H; et al. (1997) Liver cell proliferation induced by nafenopin and cyproterone acetate is not associated with increases in activation of transcription factors NF-kappaB and AP-1 or with expression of tumor necrosis factor alpha. Hepatology 25:585-92.

Mico, BA; Pohl, LR. (1983) Reductive oxygenation of carbon tetrachloride. Trichloromethylperoxyl radical as a possible intermediate in the conversion of carbon tetrachloride to electrophilic chlorine. Arch Biochem Biophys 225:596-609.

Mirsalis, JC. (1987) In vivo measurement of unscheduled DNA synthesis and S-phase synthesis as an indicator of hepatocarcinogenesis in rodents. Cell Biol Toxicol 3:165-173.

Mirsalis, JC; Butterworth, BE. (1980) Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an in vivo-in vitro assay for potential carcinogens and mutagens. Carcinogenesis 1:621-625.

Mirsalis, JC; Tyson, CK; Butterworth, BE. (1982) Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay. Environ Mutagen 4:553-562.

Mirsalis, JC. (1995) Transgenic models for detection of mutations in tumors and normal tissues of rodents. Toxicol Lett 82-83:131-134.

Mirsalis, JC; Monforte, JA; and Winegar, RA. (1994) Transgenic animal models for measuring mutations in vivo. Crit Rev Toxicol 24:255-280.

Moghaddam, AP; Eggers, JS; Calabrese, EJ. (1998) Evaluation of sex difference in tissue repair following acute carbon tetrachloride toxicity in male and female Sprague-Dawley rats. Toxicology 130:95-105.

Molina, MD; Rowland, FS. (1974) Predicted present stratospheric abundances of chlorine species from photodissociation of carbon tetrachloride. Geophys Res Lett 1:309-312.

Moore, L. (1980) Inhibition of liver-microsome calcium pump by in vivo administration of CCl4, CHCl3, and 1,1-dichloroethylene (vinylidene chloride). Biochem Pharmacol 29:2505-2511.

Moore, L; Rodman-Daveport, G; Landon, EJ. (1976) Calcium uptake of a liver microsomal subcellular fraction in response to in vivo administration of carbon tetrachloride. J Biol Chem 251:1197-1201.

Morgan, A; Black, A; Belcher, DR. (1970) The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. Ann Occup Hyg 13:219-233.

Morgan, DL; Copper, SW; Carlock, DL; et al. (1991) Dermal absorption of neat and aqueous volatile organic chemicals in the Fischer 344 rat. Environ Res 55:51-63.

Morita, T; Asano, N; Awogi, T; et al. (1997) Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test. Mammalian Mutagenicity Study Group. Mutat Res 389:3-122.

Morly, AA; Turner, DR. (1999) The contribution of exogenous and endogenous mutagens to in vivo mutations. Mutat Res 428(1-2):11-5.

Mourelle, M; Villalon, C; Amezcua, JL. (1988) Protective effect of colchicine on acute liver damage induced by carbon tetrachloride. Hepatology 6:337-342.

Muller, L; Kikuchi, Y; Probst, G; et al. (1999) ICH-harmonised guidances on genotoxicity testing of pharmaceuticals: evolution, reasoning and impact. Mutat Res 436:195-225.

Muller, L; Sofuni, T. (2000) Appropriate levels of cytotoxicity for genotoxicity tests using mammalian cells in vitro. Environ Mol Mutagen 35:202-205.

Muriel, P.; Escobar, Y. (2003) Kupffer cells are responsible for liver cirrhosis induced by carbon tetrachloride. J Appl Toxicol 23:103-108.

Muriel, P; Alba, N; Perez-Alvarez, VM; et al. (2001) Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp Biochem Physiol C Toxicol Pharmacol Endrocrinol 130:219-226.

Nagano, K. (2004a). Letter dated March 8, 2004. From Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA.

Nagano, K. (2004b). Letter dated March 9, 2004. From Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA.

Nagano, K. (2004c). Email dated March 9, 2004. Subject: Carbon tetrachloride 2-year chronic bioassay. From Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA.

Nagano, K. (2005). Email dated October 15, 2005. Subject: Carbon tetrachloride 1998 inhalation study. From Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA.

Nagano, K. (2007). Email dated April 5, 2007. Subject: Historical control data. From Kasuke Nagano, JBRC, to Susan Rieth, U.S. EPA.

Nagano, K; Umeda, Y; Saito, M; et al. (2007a) Thirteen-week inhalation toxicity of carbon tetrachloride in rats and mice. J Occup Health 49:249-259.

Nagano, K; Sasaki, T; Umeda, Y; et al. (2007b) Inhalation carcinogenicity and chronic toxicity of carbon tetrachloride in rats and mice. Inhalation Toxicology 19:1089-1103.

Nakajima, T; Sato, A. (1979) Enhanced activity of liver drug-metabolizing enzymes for aromatic and chlorinated hydrocarbons following food deprivation. Toxicol Appl Pharmacol 50:549-556.

Nakamura, T; Hotchi, M. (1992) Changes in DNA strand breaks in non-parenchymal cells following hepatocyte regeneration in CCl4-induced rat liver injury. Virchows Arch B Cell Pathol Incl Mol Pathol 63:11-16.

Nakamura, S; Oda, Y; Shimada, T; et al. (1987) SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: examination with 151 chemicals. Mutat Res 192:239-246.

Nakata, R; Tsukamoto, I; Miyoshi, M; et al. (1975) Liver regeneration after carbon tetrachloride intoxication in the rat. Biochem Pharmacol 34:586-588.

Narotsky, MG; Kavlock, RJ. (1995) A multidisciplinary approach to toxicological screening: II. Developmental toxicity. J Toxicol Environ Health 45:145-171.

Narotsky, MG; Hamby, BT; Best, DS; et al. (1995) Carbon tetrachloride (CCl4)-induced pregnancy loss in F-344 rats: luteinizing hormone (LH) levels and rescue by human chorionic gonadotropin (hCG). Biol Reprod 52(Suppl 1):172.

Narotsky, MG; Brownie, CF; Kavlock, RJ; et al. (1997a) Critical period of carbon tetrachloride-induced pregnancy loss in Fischer 344 rats, with insights into the detection of resorption sites by ammonium sulfide staining. Teratology 56:252-261.

Narotsky, MG; Pegram, RA; Kavlock, RJ. (1997b) Effect of dosing vehicle on the developmental toxicity of bromodichloromethane and carbon tetrachloride in rats. Fundam Appl Toxicol 40:30-36.

Nath, RG; Li, DH; Randerath, K. (1990) Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compounds) in male mouse liver. Chem Biol Interact 76:343-357.

NCI (National Cancer Institute). (1976a) Report on the carcinogenesis bioassay of chloroform. National Institutes of Health, Bethesda, MD.

Natarajan, SK; Basivireddy, J; Ramachandran, A; et al. (2006) Renal damage in experimentally-induced cirrhosis in rats: role of oxygen free radicals. Hepatology 43:1248-56.

NCI (National Cancer Institute). (1976a) Report on the carcinogenesis bioassay of chloroform. National Institutes of Health, Bethesda, MD.

NCI. (1976b) Carcinogenesis bioassay of trichloroethylene. National Cancer Institute carcinogenesis technical report series, No. 2. National Institutes of Health, Bethesda, MD; NCI-CG-TR-2.

NCI. (1977) Bioassay of 1,1,1-trichloroethane for possible carcinogenicity. National Cancer Institute carcinogenesis technical report series, No. 3. National Institutes of Health, Bethesda, MD; NCI-CG-TR-3.

New, PS; Lubash, GD; Scherr, L; et al. (1962) Acute renal failure associated with carbon tetrachloride intoxication. J Am Med Assoc 181:903-906.

Niederberger, M; Gines, P; Martin PY; et al. (1998) Increased renal and vascular cytosolic phospholipase A2 activity in rats with cirrhosis and ascites. Hepatology 27:42-47.

Niedernhofer, LJ; Daniels, JS; Rouzer, CA; et al. (2003) Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J Biol Chem 278:31426-31433.

Nikula, K; Benson, J; Barr, E; et al. (1998) Comparative interspecies metabolism of carbon tetrachloride, cytolethality and regenerative cell proliferation. Toxicol Sci 42:368.

NLM (National Library of Medicine). (2003) Carbon tetrachloride. Hazardous Substances Data Bank (HSDB). National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available from: <http://toxnet.nlm.nih.gov>.

Noguchi, T; Fong, K-L; Lai, EK; et al. (1982a) Selective early loss of polypeptides in liver microsomes of CCl4-treated rats. Relationship to cytochrome P-450 content. Biochem Pharmacol 31:609-614.

Noguchi, T; Fong, K-L; Lai, EK; et al. (1982b) Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of carbon tetrachloride to the trichloromethyl radical. Biochem Pharmacol 31:615-624.

Norpoth, K; Reisch, A; Heinecke, A. (1980) Biostatistics of Ames-test data. In: Norpoth, K; Garner, RC, eds. Short-term test systems for detecting carcinogens. New York, NY: Springer-Verlag; pp. 312-322.

Norwood, WD; Fuqua, PA; Scudder, BC. (1950) Carbon tetrachloride poisoning. Arch Ind Hyg Occup Med 1:90-100.

NRC (National Research Council). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NTP (National Toxicology Program). (2007) National Toxicology database search application. Standard Toxicology & Carcinogenesis Studies. Search results for carbon tetrachloride (CAS No. 56-23-5). Available at: <http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchresults&searchterm=56-23-5>.

Okamoto, T. (2000) Suppression of cytochrome P450 gene expression in the livers of mice with concanacalin A-induced hepatitis. Eur J Pharmacol 394:157-161.

Omura, M; Katsumata, T; Misawa, H; et al. (1999) Decrease in protein kinase and phosphatase activities in the liver nuclei of rats exposed to carbon tetrachloride. Toxicol Appl Pharmacol 160:192-197.

O'Neil, MJ; Smith, A; eds. (2001) The Merck index: an encyclopedia of chemicals, drugs, and biologicals. 13th edition. Whitehouse Station, NJ: Merck and Co., Inc.; pp. 305-306.

Onfelt, A. (1987) Spindle disturbances in mammalian cells. III. Toxicity, c-mitosis and aneuploidy with 22 different compounds. Specific and unspecific mechanisms. Mutat Res 182:135-154.

Oruambo, IF; Van Duuren, BL. (1987) Distribution of carbon tetrachloride-metabolite(s) to DNase I-sensitive and -resistant chromatin. Cancer Lett 37:311-316.

Oshimura, M; Barrett, JC. (1986) Chemically induced aneuploidy in mammalian cells: mechanisms and biological significance in cancer. Environ Mutagen 8:129-159.

Ozturk, F; Ucar, M; Ozturk, IC; et al. (2003) Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology 62:353-356.

Packer, JE; Slater, TF; Willson, RL. (1978) Reactions of the carbon tetrachloride-related peroxy free radical (CCl3O.2) with amino acids: Pulse radiolysis evidence. Life Sci 23:2617-2620.

Page, DA; Carlson, GP. (1994) The role of the intestinal tract in the elimination of carbon tetrachloride. Toxicol Appl Pharmacol 124:268-274.

Parola, M; Leonarduzzi, G; Biasi, F; et al. (1992) Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis. Hepatology 16:1014-1021.

Patki, KC; von Moltke, LL; Harmatz, JS; et al. (2004) Effect of age on in vitro triazolam biotransformation in male human liver microsomes. J Pharmacol Exp Ther 308:874-879.

Paul, BB; Rubinstein, D. (1963) Metabolism of carbon tetrachloride and chloroform by the rat. J Pharmacol Exp Ther 141:141-148.

Paustenbach, DJ; Carlson, GP; Christian, JE; et al. (1986a) A comparative study of the pharmacokinetics of carbon tetrachloride in the rat following repeated inhalation exposures of 8 and 11.5 hr/day. Fundam Appl Toxicol 6:484-497.

Paustenbach, DJ; Christian, JE; Carlson, GP; et al. (1986b) The effect of an 11.5-hr/day exposure schedule on the distribution and toxicity of inhaled carbon tetrachloride in the rat. Fundam Appl Toxicol 6:472-483.

Paustenbach, DJ; Clewell, HJ, III; Gargas, ML; et al. (1987) Development of a physiologically based pharmacokinetic model for multiday inhalation of carbon tetrachloride. In: Pharmacokinetics in risk assessment. Vol. 8. Washington, DC: National Academies Press; pp. 312-326.

Paustenbach, DJ; Clewell, HJ, III; Gargas, ML; et al. (1988) A physiologically based pharmacokinetic model for inhaled carbon tetrachloride. Toxicol Appl Pharmacol 96:191-211.

Pedersen-Bjergaard, J; Andersen, MK; Christiansen, DH; et al. (2002) Genetic pathways in therapy-related myelodysplasia and acute myeloid leukemia. Blood 99:1909-1912.

Pentz, R; Strubelt, O. (1983) Fasting increases the concentrations of carbon tetrachloride and of its metabolite chloroform in the liver of mice. Toxicol Lett 16:231-234.

Perocco, P; Prodi, G. (1981) DNA damage by haloalkanes in human lymphocytes cultured in vitro. Cancer Lett 13:213-218.

Peter, CP; Burek, JD; van Zwieten, MJ. (1986) Spontaneous nephropathies in rats. Toxicol Pathol 14:91-100,

Peters, HA; Levine, RL; Matthews, CG; et al. (1987) Synergistic neurotoxicity of carbon tetrachloride/carbon disulfide (80/20 fumigants) and other pesticides in grain storage workers. Acta Pharmacol Toxicol (Copenhagen) 59:535-546.

Phillips, DH; Farmer, PB; Beland, FA; et al. (2000) Methods of DNA adduct determination and their application to testing compounds for genotoxicity. Environ Mol Mutagen 35:222-233.

Pilon, D; Brodeur, J; Plaa, GL. (1986) 1,3-Butanediol-induced increases in ketone bodies and potentiation of CCl4 hepatotoxicity. Toxicology 40:165-180.

Plaa, GL. (2000) Chlorinated methanes and liver injury: highlights of the past 50 years. Ann Rev Pharmacol Toxicol 40:42-65.

Plaa, GL; Traiger, GJ. (1972) Mechanism of potentiation of CCl4-induced hepatotoxicity. In: Loomis, TA; ed. Pharmacology and the future of man. Vol. 3: Toxicological problems. Basel, Switzerland: Larger; pp 100-113.

Plummer, JL; Hall, PM; Ilsley, AH; et al. (1990) Influence of enzyme induction and exposure profile on liver injury due to chlorinated hydrocarbon inhalation. Pharmacol Toxicol 67:329-335.

Plummer, JL; Hall, PM; Ilsley, AH; et al. (1994) Dose-response relationships in hepatic injury produced by alcohol and carbon tetrachloride. Alcohol Clin Exper Res 18:1523-1526.

Pohl, LR; Schulick, RD; Highet, RJ; et al. (1984) Reductive-oxygenation mechanism of metabolism of carbon tetrachloride to phosgene by cytochrome P-450. Mol Pharmacol 25:318-321.

Poyer, JL; Floyd, RA; McCay, PB; et al. (1978) Spin-trapping of the trichloromethyl radical produced during enzymic NADPH oxidation in the presence of carbon tetrachloride or bromotrichloromethane. Biochim Biophys Acta 539:402-409.

Poyer, JL; McCay, PB; Lai, EK; et al. (1980) Confirmation of assignment of the trichloromethyl radical spin adduct detected by spin trapping during 13C-carbon tetrachloride metabolism in vitro and in vivo. Biochem Biophys Res Commun 94:1154-1160.

Prendergast, JA; Jones, RA; Jenkins, LJ, Jr; et al. (1967) Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol Appl Pharmacol 10:270-289.

Qin, L-Q; Wang, Y; Xu, J-Y; et al. (2007) One-day dietary restriction changes hepatic metabolism and potentiates the hepatotoxicity of carbon tetrachloride and chloroform in rats. Tohoku J Experimental Med 212:379-387.

Racay, P; Kaplan, P; Mezesova, V; et al. (1997) Lipid peroxidation both inhibits Ca(2+)-ATPase and increases Ca2+ permeability of endoplasmic reticulum membrane. Biochem Mol Biol Int 41:647-655.

Ramsey, JC; Andersen, ME. (1984) A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73:159-175.

Rao, KS; Recknagel, RO. (1969) Early incorporation of carbon-labeled carbon tetrachloride into rat liver particulate lipids and proteins. Exp Mol Pathol 10:219-228.

Rao, PS; Dalu, A; Kulkarni, SG; et al. (1996) Stimulated tissue repair prevents lethality in isopropanol-induced potentiation of carbon tetrachloride hepatotoxicity. Toxicol Appl Pharmacol 140:235-244.

Rasheed, A; Hines, RN; McCarver-May, DG. (1997) Variation in induction of human placental CYP2E1: possible role in susceptibility to fetal alcohol syndrome? Toxicol Appl Pharmacol 144:396-400.

Raucy, JL; Kraner, JC; Lasker, JM. (1993) Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. Crit Rev Toxicol 23:1-20.

Ray, SD; Mehendale, HM. (1990) Potentiation of CCl4 and CHCl3 hepatotoxicity and lethality by various alcohols. Fundam Appl Toxicol 15:429-440.

Raymond, P; Plaa, GL. (1995) Ketone potentiation of haloalkane-induced hepato- and nephrotoxicity. I. Dose-response relationships. J Toxicol Environ Health 45:465-480.

Raymond, P; Plaa, GL. (1997) Effect of dosing vehicle on the hepatotoxicity of CCl4 and hepatotoxicity of CHCl3 in rats. J Toxicol Environ Health 51:463-476.

Recknagel, RO; Glende, EA. (1973) Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. Crit Rev Toxicol 2:263-297.

Recknagel, RO; Glende, EA; Dolak, JA; et al. (1989) Mechanisms of carbon tetrachloride toxicity. Pharmac Ther 43:139-154.

Recknagel, RO; Glende, EA; Dolak, JA; et al. (1989) Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 43:139-154.

Reinke, LA; Janzen, EG. (1991) Detection of spin adducts in blood after administration of carbon tetrachloride to

rats. Chem Biol Interact 78:155-165.

Reinke, LA; Lai, EK; McCay, PB. (1988) Ethanol feeding stimulates trichloromethyl radical formation from carbon tetrachloride in liver. Xenobiotica 18:1311-1318.

Reitz, RH; Gargas, ML; Mendrala, AL; et al. (1996) In vivo and in vitro studies of perchloroethylene metabolism for physiologically based pharmacokinetic modeling in rats, mice, and humans. Toxicol Appl Pharmacol 136:289-306.

Reuber, MD; Glover EL. (1967a) Cholangiofibrosis in the liver of buffalo strain rats injected with carbon tetrachloride. Br J Exp Pathol 48:319-322.

Reuber, MD; Glover EL. (1967b) Hyperplastic and early neoplastic lesions of the liver in buffalo strain rats of various ages given subcutaneous carbon tetrachloride. J Natl Cancer Inst 38:891-899.

Reuber, MD; Glover EL. (1970) Cirrhosis and carcinoma of the liver in male rats given subcutaneous carbon tetrachloride. J Natl Cancer Inst 44:419-427.

Reynolds, ES; Treinen, RJ; Farrish, HH. (1984) Metabolism of [14C]carbon tetrachloride to exhaled, excreted and bound metabolites. Biochem Pharmacol 33:3363-3374.

Rocchi, P; Prodi, G; Grilli, S; et al. (1973) In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver. Int J Cancer 11:419-425.

Roldan-Arjona, T; Pueyo, C. (1993) Mutagenic and lethal effects of halogenated methanes in the Ara test of *Salmonella typhimurium*: quantitative relationship with chemical reactivity. Mutagenesis 8:127-131.

Roldan-Arjona, T; Garcia-Pedrajas, MD; Luque-Romero, FL; et al. (1991) An association between mutagenicity of the Ara test of *Salmonella typhimurium* and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons. Mutagenesis 6:199-205.

Rose, ML; Bradford, BU; Germolec, DR; et al. (2001) Gadolinium chloride-induced hepatocyte proliferation is prevented by antibodies to tumor necrosis factor α. Toxicol and Appl Pharmacol 170:39-45.

Rosengren, RJ; Sauer, J-M; Hooser, SB; et al. (1995) The interactions between retinol and five different hepatotoxicants in the Swiss Webster mouse. Fundam Appl Toxicol 25:281-292.

Rossberg, M. (2002) Chlorinated hydrocarbons. In: Gerhartz, W; Yamamoto, YS; Campbell, FT; eds. Ullmann's encyclopedia of industrial chemistry. 5th edition. Vol. A6. New York, NY: VCH Publishers; pp. 370-371.

Rossi, AM; Zaccaro, L; Rosselli, F; et al. (1988) Clastogenic effects induced in mice and rats by 1,4-bis[2-(3,5-dichloropyridyloxy)]-benzene, a phenobarbital-like enzyme inducer and liver tumour promoter. Carcinogenesis 9:1147-1151.

Ruprah, M; Mant, TGK; Flanagan, RJ. (1985) Acute carbon tetrachloride poisoning in 19 patients: implications for diagnosis and treatment. Lancet 1:1027-1029.

Sagai, M; Tappel, AL. (1978) Effect of vitamin E on carbon tetrachloride-induced lipid peroxidation as demonstrated by in vivo pentane production. Toxicol Lett 2:149-155.

Salmenkivi, K; Heikkila, P; Haglund, C; et al. (2004) Malignancy in pheochromocytomas. APMIS 112:551-559.

Sandy, MS; Di Monte, D; Smith, MT. (1988) Relationships between intracellular vitamin E, lipid peroxidation, and chemical toxicity in hepatocytes. Toxicol Appl Pharmacol 93(2):288-97.

Sanzgiri, UY; Bruckner, JV. (1997) Effect of Emulphor, an emulsifier, on the pharmacokinetic and hepatotoxicity of

oral carbon tetrachloride in the rat. Fund Appl Toxicol 36:54-61.

Sanzgiri, UY; Kim, HJ; Muralidhara, S; et al. (1995) Effect of route and pattern of exposure on the pharmacokinetics and acute hepatotoxicity of carbon tetrachloride. Toxicol Appl Pharmacol 134:148-154.

Sanzgiri, UY; Srivattsan, V; Muralidhara, S; et al. (1997) Uptake, distribution, and elimination of carbon tetrachloride in rat tissue following inhalation and ingestion exposures. Toxicol Appl Pharmacol 143:120-129.

Sarkar, A; Pradhan, S; Mukhopadhyay, I; et al. (1999) Inhibition of early DNA-damage and chromosomal aberrations by Trianthema portulacastruml. In carbon tetrachloride-induced mouse liver damage. Cell Biol Int 23:703-708.

Sasaki, YF; Saga, A; Akasaka, M; et al. (1998) Detection of in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs. Mutat Res 419:13-20.

Sato, A; Nakajima, T. (1985) Enhanced metabolism of volatile hydrocarbons in rat liver following food deprivation, restricted carbohydrate intake, and administration of ethanol, phenobarbital, polychlorinated biphenyl and 3-methylcholanthrene: a comparative study. Xenobiotica 15:67-75.

Sato, A; Nakajima, T. (1987) Pharmacokinetics of organic solvent vapors in relation to their toxicity. Scand J Work Environ Health 13:81-93.

Sato, A; Nakajima, T; Koyama, Y. (1980) Effects of chronic ethanol consumption on hepatic metabolism of aromatic and chlorinated hydrocarbons in rats. Br J Ind Med 37:382-286.

Sawada, S; Yamanaka, T; Yamatsu, K; et al. (1991) Chromosome aberrations, micronuclei and sister-chromatid exchanges (SCEs) in rat liver induced in vivo by hepatocarcinogens including heterocyclic amines. Mutat Res 251:59-69.

Sawant, SP; Dnyanmote, AV; Shankar, K; et al. (2004) Potentiation of carbon tetrachloride hepatotoxicity and lethality in Type 2 diabetic rats. J Pharmacol Exp Ther 308:694-704.

Sawant, SP; Dnyanmote, AV; Mehendale, HM. (2007) Mechanisms of inhibited liver tissue repair in toxicant challenged type 2 diabetic rats. Toxicology 232:200-215.

Schiestl, RH; Gietz, RD; Mehta, RD; et al. (1989) Carcinogens induce intrachromosomal recombination in yeast. Carcinogenesis 10:1445-1455.

Schwarz, M; Hummel, J; Appel, KE; et al. (1979) DNA damage induced in vivo evaluated with a non-radioactive alkaline elution technique. Cancer Lett 6:221-226.

Schwetz, BA; Leong, BKJ; Gehring, PJ. (1974) Embryo- and fetotoxicity of inhaled carbon tetrachloride, 1,1-dichloroethane and methyl ethyl ketone in rats. Toxicol Appl Pharmacol 28:452-464.

Seawright, AA; Wilkie, IW; Costigan, P; et al. (1980) The effect of an equimolar mixture of carbon tetrachloride and carbon disulphide on the liver of the rat. Biochem Pharmacol 29:1007-1014.

Seidler, A; Raum, E; Arabin, B; et al. (1999) Maternal occupational exposure to chemical substances and the risk of infants small-for-gestational-age. Am J Ind Med 36:213-222.

Seidler, A; Mohner, M; Berger, J; et al. (2007) Solvent exposure and malignant lymphoma: a population-based case-control study in Germany. J Occup Med Toxicol 2:2.

Seki, M; Kasama, K; Imai, K. (2000) Effect of food restriction on hepatotoxicity of carbon tetrachloride in rats. J Toxicol Sci 25:33-40.

Selden, JR; Dolbeare, F; Miller, JE; et al. (1994) Validation of a flow cytometric in vitro DNA repair (UDS) assay in rat hepatocytes. Mutat Res 315:147-167.

Semino, G; Lilly, P; Andersen, ME. (1997) A pharmacokinetic model describing pulsatile uptake of orally-administered carbon tetrachloride. Toxicology 117:25-33.

Semprini, L. (1995) In situ bioremediation of chlorinated solvents. Environ Health Perspect 103:101-105.

Shah, H; Hartman, SP; Weinhouse, S. (1979) Formation of carbonyl chloride in carbon tetrachloride metabolism by rat liver in vitro. Cancer Res 39:3942-3947.

Shamberger, RJ; Andreone, TL; Willis, CE. (1974) Antioxidants and cancer. IV. Initiating activity of malonaldehyde as a carcinogen. J Nat Cancer Inst 53:1771-1773.

Shankar, K; Vaidya, VS; Apte, UM; et al. (2003) Type 1 diabetic mice are protected from acetaminophen hepatotoxicity. Toxicol Sci 73:220-234.

Shertzer, HG; Reitman, FA; Tabor, MW. (1988) Influence of diet on the expression of hepatotoxicity from carbon tetrachloride in ICR mice. Drug Nutr Interact 5:275-282.

Siegers, CP; Horn, W; Younes, M. (1985) Effect of hypoxia on the metabolism and hepatotoxicity of carbon tetrachloride and vinylidene chloride in rats. Acta Pharmacol Toxicol 56:81-86.

Simmon, VF; Tardiff, RG. (1978) The mutagenic activity of halogenated compounds found in chlorinated drinking water. Water Chlorination Impact Health Effects 2:417-431.

Simmon, VF; Kauhanen, K; Tardiff, R. (1977) Mutagenic activity of chemicals identified in drinking water. In: Scott, D; Bridges, BA; Sobels, FH; eds. Progress in genetic toxicology: proceedings of the 2nd international conference on environmental mutagens; July 1977; Edinburgh, Scotland. Amsterdam, Holland: Elsevier/North-Holland Biomedical Press; pp. 249-258.

Sina, JF; Bean, CL; Dysart, GR; et al. (1983) Evaluation of the alkaline elution/rate hepatocyte assay as predictor of carcinogenic/mutagenic potential. Mutat Res 113:357-391.

Sipes, IG; Krishna, G; Gillette, JR. (1977) Bioactivation of carbon tetrachloride, chloroform and bromotrichloromethane: role of cytochrome P-450. Life Sci 20:1541-1548.

Sivikova, K; Piesova, E; Dianovsky, J. (2001) The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes. Mutat Res 494:135-142.

Slater, TF. (1981) Free radicals as reactive intermediates in tissue injury. Adv Exp Med Biol 136:575-589.

Slater, TF. (1982) Activation of carbon tetrachloride: chemical principles and biological significance. In: McBrien, DCH; Slater, TF; eds. Free radicals, lipid peroxidation and cancer. New York, NY: Academic Press; pp. 243-274.

Smialowicz, RJ; Simmons, JE; Luebke, RW; et al. (1991) Immunotoxicologic assessment of subacute exposure of rats to carbon tetrachloride with comparison to hepatotoxicity and nephrotoxicity. Fundam Appl Toxicol 17:186-196.

Smith, A; Gelfand, A. (1992) Bayesian statistics without tears: A sampling-resampling perspective. The American Statistician 46(2):84-89.

Smyth, HF; Smyth, HF, Jr.; Carpenter, CP. (1936) The chronic toxicity of carbon tetrachloride; animal exposure and field studies. J Ind Hyg Toxicol 18:277-298.

Solomon, E; Borrow, J; Goddard, AD. (1991) Chromosome aberrations and cancer. Science 254:1153-1160.

Soni, MG; Mehendale, HM. (1993) Hepatic failure leads to lethality of chlordecone-amplified hepatotoxicity of carbon tetrachloride. Fundam Appl Toxicol 21:442-450.

Sorsa, M; Wilbourn, J; Vainio, H. (1992) Human cytogenetic damage as a predictor of cancer risk. Iarc Scientific Publications:543-554.

Spiegelhalter, D; Thomas, A; Best, N. (2003) WinBugs Version 1.4 User Manual. http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/manual14.pdf.

Spirtas, R; Stewart, PA; Lee, JS; et al. (1991) Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. Br J Ind Med 48:515-530.

Srivastava, SP; Chen, NQ; Holtzman, JL. (1990) The in vitro NADPH-dependent inhibition by CCl4 of the ATP-dependent calcium uptake of hepatic microsomes from male rats. J Biol Chem 265:8392-8399.

Stacey, NH; Klaassen, CD. (1981) Inhibition of lipid peroxidation without prevention of cellular injury in isolated rat hepatocytes. Toxicol Appl Pharmacol 58(1) 8-18.

Stanley, LA. (1995) Molecular aspects of chemical carcinogenesis: the roles of oncogenes and tumour suppressor genes. Toxicology 96:173-194.

Steup, DR; Hall, P; McMillan, DA; et al. (1993) Time course of hepatic injury and recovery following coadministration of carbon tetrachloride and trichloroethylene in Fischer 344 rats. Toxicol Pathol 21:327-334.

Stewart, BW. (1981) Generation and persistence of carcinogen-induced repair intermediates in rat liver DNA in vivo. Cancer Res 41:3228-3243.

Stewart, RD; Dodd, HC. (1964) Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride and l,l,l-trichloroethane through the human skin. Am Ind Hyg Assoc J 25:439-446.

Stewart, RD; Gay, HH; Erley, DS; et al. (1961) Human exposure to carbon tetrachloride vapor. J Occup Expos 3:586-590.

Stewart, RD; Boettner, EA; Southworth, RR; et al. (1963) Acute carbon tetrachloride intoxication. J Am Med Assoc 183:94-97.

Stewart, RD; Dodd, HC; Erley, DS; et al. (1965) Diagnosis of solvent poisoning. J Am Med Assoc 193:115-118.

Stoyanovsky, DA; Cederbaum, AL. (1999) Metabolism of carbon tetrachloride to trichloromethyl radical: An ESR and HPLC-EC study. Chem Res Toxicol 12(8):730-6.

Stoyanovsky, DA; Cederbaum, AI. (1996) Thiol oxidation and cytochrome P450-dependent metabolism of CCl4 triggers Ca2+ release from liver microsomes. Biochemistry 35:15839-15845.

Strubelt, O. (1984) Alcohol potentiation of liver injury. Fundam Appl Toxicol 4:144-151.

Suh; JH; Shenvi, SV; Dixon, BM; et al. (2004) Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proc Natl Acad Sci USA 101:3381-3386.

Sundari, PN; Wilfred, G; Ramakrishna, B. (1997) Does oxidative protein damage play a role in the pathogenesis of carbon tetrachloride-induced liver injury in the rat? Biochim Biophys Acta 1362(2-3):169-176.

Suzuki, H; Hirano, N; Watanabe, C; et al. (1997) Carbon tetrachloride does not induce micronucleus in either mouse bone marrow or peripheral blood. Mutat Res 394:77-80.

Tafazoli, M; Baeten, A; Geerlings, P; et al. (1998) In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential. Mutagenesis 13:115-126.

Takahashi, S; Hirose, M; Tamano, S; et al. (1998) Immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment. Toxicol Pathol 26:247-252.

Takahashi, S; Takahashi, T; Mizobuchi, S; et al. (2002) Increased cytotoxicity of carbon tetrachloride in a human hepatoma cell line overexpressing cytochrome P450 2E1. J Int Med Res 30:400-405.

Tanaka, E. (1998) In vivo age-related changes in hepatic drug-oxidizing capacity in humans. J Clin Pharmacol Therapeut 23:247-255.

Taylor, SL; Tappel, AL. (1976) Effect of dietary antioxidants and phenobarbital pretreatment on microsomal lipid peroxidation and activation by carbon tetrachloride. J Life Sci 19:1151-1160.

Teitz, NW, ed. (1976) Fundamental of Clinical Chemistry. Philadelphia: W.B. Saunders Company.

Teschke, R; Vierke, W; Gellert, J. (1984) Effect of ethanol on carbon tetrachloride levels and hepatotoxicity after acute carbon tetrachloride poisoning. Arch Toxicol 56:78-82.

Thrall, KD; Kenny, DV. (1996) Evaluation of a carbon tetrachloride physiologically based pharmacokinetic model using real-time breath-analysis monitoring of the rat. Inhal Toxicol 8:251-261.

Thrall, KD; Vucelick, ME; Gies, RA; et al. (2000) Comparative metabolism of carbon tetrachloride in rats, mice, and hamsters using gas uptake and PBPK modeling. J Toxicol Environ Health A 60:531-548.

Thrall, K. (2006). Email dated 9/5/2006, RE: a final follow-up to your 2000 paper on carbon tetrachloride comparative metabolism, to Susan Rieth, U.S. EPA.

Tischler, AS; Sheldon, W; Gray, R. (1996) Immunohistochemical and morphological characterization of spontaneously occurring pheochromocytomas in the aging mouse. Vet Pathol 33:512-520.

Tian, L; Cai, Q; Wei, H. (1998) Alterations of antioxidant enzymes and oxidative damage to macromolecules in different organs of rats during aging. Free Radic Biol Med 29:1477-1484.

Tomasi, A; Albano, E; Banni, S; et al. (1987) Free-radical metabolism of carbon tetrachloride in rat liver mitochondria. A study of the mechanism of action. Biochem J 246:313-317.

Tombolan, F; Renault, D; Brault, D; et al. (1999) Effect of mitogenic or regenerative cell proliferation on lacz mutant frequency in the liver of MutaTMMice treated with 5, 9-dimethyldibenzo[c,g]carbazole. Carcinogenesis 20:1357-1362.

Tomenson, JA; Baron, CE; O'Sullivan, JJ; et al. (1995) Hepatic function in workers occupationally exposed to carbon tetrachloride. Occup Environ Med 52:508-514.

Topham, JC. (1980) Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogenesis? Mutat Res 74:379-387.

Towner, RA; Reinke, LA; Janzen, EG; et al. (1994) In vivo magnetic resonance imaging study of Kupffer cell involvement in CCl4-induced hepatotoxicity in rats. Can J Physiol Pharmacol 72(5):441-446.

Tracey JP, Sherlock P. (1968) Hepatoma following carbon tetrachloride poisoning. NY J Med 68:2202-2204.

Traiger, GJ; Plaa, GL. (1971) Differences in the potentiation of carbon tetrachloride in rats by ethanol and isopropanol pretreatment. Toxicol Appl Pharmacol 20:105-112.

Traiger, GJ; Bruckner, JV. (1976) The participation of 2-butanone in 2-butanol-induced potentiation of carbon tetrachloride hepatotoxicity. J Pharmacol Exp Therap 196:493-500.

Travlos GS; Mirris RW; Elwell MR; et al. (1996) Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13-week toxicity studies in rats. Toxicology 107:17-29.

Tribble, DL; Aw, TY; Jones, DP. (1987) The pathophysiological significance of lipid peroxidation in oxidative cell injury. Hepatology 7:377-386.

Tsuruta, H. (1975) Percutaneous absorption of organic solvents. Comparative study of the in vivo percutaneous absorption of chlorinated solvents in mice. Ind Health 13:227-236.

Uehleke, H; Hellmer, KH; Tabarelli, S. (1973) Binding of 14C-carbon tetrachloride to microsomal proteins in vitro and formation of CHC13 by reduced liver microsomes. Xenobiotica 3:1-11.

Uehleke, H; Werner, T; Greim, H; et al. (1977) Metabolic activation of haloalkanes and tests in vitro for mutagenicity. Xenobiotica 7:393-400.

Umiker, W; Pearce J. (1953) Nature and genesis of pulmonary alterations in carbon tetrachloride poisoning. Arch Pathol 55:203-217.

Uryvaeva, IV; Delone, GV. (1995) An improved method of mouse liver micronucleus analysis: an application to age-related genetic alteration and polyploidy study. Mutat Res 334:71-80.

U.S. Coast Guard. (1999) Carbon tetrachloride. Chemical Hazards Response Information System (CHRIS) Hazardous Chemical Data. Department of Transportation, Washington, DC; Available from: <http://www.chrismanual.com/findform.htm>.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014-34025.

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006-34012.

U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk assessment. Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC; EPA 600/6-87/008. Available from: National Technical Information Service, Springfield, VA; PB88-179874/AS.

U.S. EPA. (1989) Interim methods for development of inhalation reference doses. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Research Triangle Park, NC; EPA/600/8-88/066F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798-63826.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington, DC; EPA/600/8-90/066F. Available from: National Technical Information Service, Springfield, VA; PB2000-500023, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R-94/007. Available from: National Technical Information Service, Springfield, VA;

PB95-213765, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1996a) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274-56322.

U.S. EPA. (1996b) Symposium on natural attenuation of chlorinated organics in groundwater. Office of Research and Development, Washington, DC; EPA-540/R-96/509. Available from: National Technical Information Service, Springfield, VA; AD-A319 114/5.

U.S. EPA. (1998a) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926-26954.

U.S. EPA. (1998b) Science policy council handbook: peer review. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC; EPA 100-B-98-001. Available from: National Technical Information Service, Springfield, VA; PB98-140726, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000a) Science policy council handbook: peer review. 2nd edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC; EPA 100-B-00-001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000b) Science policy council handbook: risk characterization. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC; EPA 100-B-00-002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000c) Benchmark dose technical guidance document [external review draft]. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000d) Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC; EPA/630/R-00/002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA (2000e) Toxicological review of vinyl chloride (CAS No. 75-01-4): In support of summary information on the integrated risk information system (IRIS), Washington, DC; EPA/635R-00/004. Available from: <http://www.epa.gov/iris>.

U.S. EPA. (2001a) Toxicological review of chloroform. Integrated Risk Information System (IRIS), National Center for Environmental Assessment, Washington, DC; EPA/635/R-01/001. Available from: <http://www.epa.gov/iris>.

U.S. EPA. (2001b) Exploration of aging and toxic response issues [final report]. Prepared by Versar, Inc. for U.S. Environmental Protection Agency. Risk Assessment Forum, Washington, DC; EPA/630/R-01/003.

U.S. EPA. (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001b. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/P-03/003F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2006a) Science Policy Council Handbook: Peer review. 3rd edition. Science Policy Council, Washington, DC. Available from: <http://www.epa.gov/ncea/iris/Peer_Review_Handbook_2006_3rd_edition.pdf>

U.S. EPA. (2006b) A Framework for Assessing Health Risk of Environmental Exposures to Children. National Center for Environmental Assessment, Washington, DC, EPA/600/R-05/093F. Available from: <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363>.

U.S. EPA. (2006c) Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-05/043F.

U.S. EPA. (2007) Benchmark dose software (BMDS) version 1.4.1. (last modified February 2007). Available from: <http://www.epa.gov/ncea/bmds.htm>.

Van Goethem, F; Ghahroudi, MA; Castelain, P; et al. (1993) Frequency and DNA content of micronuclei in rat parenchymal liver cells during experimental hepatocarcinogenesis. Carcinogenesis 14:2397-2406.

Van Kuijk, FJ; Holte, LL; Dratz, EA. (1990) 4-Hydroxyhexenal: a lipid peroxidation product derived from oxidized docosahexaenoic acid. Biochim Biophys Acta 1043:116-118.

Varela-Moreiras, G; Alonso-Aperte, E; Rubio, M; et al. (1995) Carbon tetrachloride-induced hepatic injury is associated with global DNA hypomethylation and homocysteinemia: effect of S-adenosylmethionine treatment. Hepatology 22:1310-1315.

Varma, MM; Ampy, FR; Verma, K; et al. (1988) In vitro mutagenicity of water contaminants in complex mixtures. J Appl Toxicol 8:243-248.

Veng-Pedersen, P; Paustenbach, DJ; Carlson, GP; et al. (1987) A linear systems approach to analyzing the pharmacokinetics of carbon tetrachloride in the rat following repeated exposures of 8 and 11.5 h/day. Arch Toxicol 60:355-364.

Vieira, I; Sonnier, M; Cresteil, T. (1996) Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur J Biochem 238:476-483.

Villarruel, M; de Toranzo, EGD; Castro, JA. (1977) Carbon tetrachloride activation, lipid peroxidation and the mixed function oxygenase activity of various rat tissues. Toxicol Appl Pharmacol 41:337-344.

von Oettingen, WF. (1964) The halogenated hydrocarbons of industrial and toxicological importance. In: Browning, E; ed.. Elsevier monographs on toxic agents. New York, NY: Elsevier Publishing Co.

von Oettingen, WF; Powell, CC; Sharpless, NE; et al. (1950) Comparative studies of the toxicity and pharmacodynamic action of chlorinated methanes with special reference to their physical and chemical characteristics. Arch Int Pharmacodyn 81:17-34.

Wacker, M; Wanek, P; Eder, E. (2001) Detection of 1, N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats. Chem Biol Interact 137:269-283.

Waller, RL; Glende, EA, Jr.; Recknagel, RO. (1983) Carbon tetrachloride and bromotrichloromethane toxicity. Biochem Pharmacol 32:1613-1617.

Wang, MY; Liehr, JG. (1995) Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis. Arch Biochem Biophys 316:38-46.

Wang, P-Y; Kaneko, T; Tsukada, H; et al. (1997) Time courses of hepatic injuries induced by chloroform and by carbon tetrachloride: comparison of biochemical and histopathological changes. Arch Toxicol 71:638-645.

Wangenheim, J; Bolcsfoldi, G. (1988) Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis 3:193-205.

Warrington, JS; Poku, JW, von Moltke, LL; et al. (2000) Effects of age on in vitro midazolam biotransformation in male CD-1 mouse liver microsomes. J Pharmacol Exp Ther 292:1024-1031.

Warrington, JS; Von Moltke, LL; Greenblatt, DJ. (2004) Age-related differences in CYP3A expression and activity in the rat liver, intestine and kidney. J Pharmacol Exp Ther 309:720-729.

Watanabe, K; Sakamoto, K; Sasaki, T. (1998) Comparisons on chemically-induced mutation among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: collaborative study II. Mutat Res 412:17-31.

Watkins, JB, III; Sanders, RA; Beck, LV. (1988) The effect of long-term streptozotocin-induced diabetes on the hepatotoxicity of bromobenzene and carbon tetrachloride and hepatic biotransformation in rats. Toxicol Appl Pharmacol 93:329-338.

Wauthier, V; Verbeeck, RK; Caulderon, PB. (2004) Age-related changes in the protein and mRNA levels of CYP2E1 and CYP3A isoforms as well as in their hepatic activities in Wistar rats. What role for oxidative stress? Arch Toxicol 78:131-138.

Weber, LW; Boll, M; Stampfl, A. (2003) Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 33:105-136.

Weddle, CE; Hornbrook, KR; McCay, PB. (1976) Lipid peroxidation and alteration of membrane lipids in isolated hepatocytes exposed to carbon tetrachloride. J Biol Chem 251:4973-4978.

Weisburger, EK. (1977) Carcinogenicity studies on halogenated hydrocarbons. Environ Health Perspect 21:7-16.

Whittaker, SG; Zimmerman, FK; Dicus, B; et al. (1989) Detection of induced mitotic chromosome loss in *Saccharomyces cerevisiae*—an interlaboratory study. Mutat Res 224:31-78.

Wilcosky, TC; Checkoway, H; Marshall, EG; et al. (1984) Cancer mortality and solvent exposures in the rubber industry. Am Ind Hyg Assoc J 45:809-811.

Will, O; Mahler, HC; Arrigo, AP; et al. (1999) Influence of glutathione levels and heat shock on the steady state levels of oxidative DNA base modifications in mammalian cells. Carcinogenesis 20:333-337.

Wilson, JG. (1954) Influence of the offspring of altered physiologic states during pregnancy in the rat. Ann NY Acad Sci 57:517-525.

Wolf, CR; Mansuy, D; Nastainczyk, W; et al. (1977) The reduction of polyhalogenated methanes by liver microsomal cytochrome P-450. Mol Pharmacol 13:698-705.

Wong, FW; Chan, W; Lee, SS. (1998) Resistance to carbon tetrachloride-induced hepatotoxicity in mice which lack CYP2E1 expression. Toxicol Appl Pharmacol 153:109-118.

Yasuda, M; Okabe, T; Itoh, J; et al. (2000) Differentiation of necrotic cell death with or without lysosomal activation: application of acute liver injury models induced by carbon tetrachloride (CCL4) and dimethylnitrosamine (DMN). J Histochem Cytochem 48:1331-1339.

Yoon, M; Madden, MC; Barton, HA. (2007) Extrahepatic metabolism by CYP2E1 in PBPK modeling of lipophilic volatile organic chemicals: Impacts on metabolic parameter estimation and prediction of dose metrics. J Toxicol Environ Health, Part A 70:1527-1541.

Yoshida, T; Andoh, K; Fukuhara, M. (1999) Estimation of absorption of trihalomethanes and carbon tetrachloride in low-level exposure by inhalation pharmacokinetic analysis in rats. Arch Environ Contam Toxicol 36:347-354.

Yoshimine, K; Takagi, M. (1982) Effects of starvation and protein deficiency on the acute carbon tetrachloride-induced hepatotoxicity. Bull Tokyo Med Dent Univ 29:37-46.

Younes, M; Siegers, CP. (1985) The role of iron in the paracetamol- and CCl4-induced lipid peroxidation and

hepatotoxicity. Chem Biol Interact 55:327-334.

Young, RA; Mehendale, HM. (1989) Carbon tetrachloride metabolism in partially hepatectomized and sham-operated rats pre-exposed to chlordecone (kepone). J Biochem Toxicol 4:211-219.

Yuen, ST; Gogo, AR; Luk, ISC; et al. (1995) The effect of nicotine and its interaction with carbon tetrachloride in the rat liver. Pharmacol Toxicol 77:225-230.

Yunis, J.J. (1983) The chromosomal basis of human neoplasia. Science 221:227-236.

Zangar, RC; Benson, JM; Burnett, VL; et al. (2000) Cytochrome P450 2E1 is the primary enzyme responsible for low-dose carbon tetrachloride metabolism in human liver microsomes. Chem Biol Interact 125:233-243.

Zeiger, E; Anderson, B; Haworth, S; et al. (1988) Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 11(Suppl. 12):1-158.

Zeise, L; Wilson, R; Crouch, EAC. (1987) Dose-response relationships for carcinogens: a review. Environ Health Persp 73:259-308.

APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

[to be added after external peer review and public comment period]

APPENDIX B. DOSE-RESPONSE MODELING FOR DERIVING THE RfD

Serum enzyme data (indicators of liver toxicity) from Bruckner et al. (1986) are summarized in Table B-1.

Table B-1. Serum enzyme data in male rats after 10- or 12-week exposure to carbon tetrachloride

Daily dose (mg/kg-day)	SDH (IU/mL)[a]		OCT (nmol CO_2/mL)[a]		ALT (IU/mL)[a]	
	10 weeks	12 weeks	10 weeks	12 weeks	10 weeks	12 weeks
0	3.5 ± 0.4	3.2 ± 0.4	28 ± 8	45 ± 4	18 ± 1	20 ± 0.3
1	2.3 ± 0.6	1.9 ± 0.1	23 ± 3	61 ± 12	20 ± 1	19 ± 1
10	7.6 ± 2.5[b]	8.7 ± 2.0[b]	55 ± 10	69 ± 16	23 ± 1	27 ± 2[b]
33	134.8 ± 15.0[b]	145.7 ± 57.9[b]	148 ± 48[b]	247 ± 31[b]	617 ± 334	502 ± 135[b]

[a]Values presented are mean standard error for groups of five rats at 10 weeks and seven to nine rats at 12 weeks.
[b]$p<0.05$

Source: Bruckner et al., 1986.

B.1. BMD Modeling of SDH

SDH data for the 10-week time point were used for BMD analysis. Although serum enzyme data for the 10- and 12-week time points are similar, the 10-week data were modeled because the precise group sizes were not known for the 12-week data (a range of 7–9 rats per group was reported), and these data are needed to run the BMD model.

All of the models for continuous data in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to the 10-week serum SDH data from Bruckner et al. (1986), which are shown in Table B-1, column 2. Because of the nonhomogeneous variances in the SDH data, a nonhomogeneous variance model was used in running each of the models in BMDS. A twofold increase in mean control SDH was used as the BMR (see Section 5.1.2. for the rationale for using this BMR), with "relative deviation" selected as the BMR type. As stated in U.S. EPA's benchmark dose technical guidance (U.S. EPA, 2000c), relative deviation means the BMR will be the background estimate (P0) plus (or minus) the product of the background estimate times the BMR Factor (BRMF) entered by the user, or

$$BMR = P0 \pm (BMRF*P0)$$

To achieve a doubling of the control mean, a BMRF of one was used. Thus, the BMR was calculated as P0 + (1 x P0) or 2 x P0. It should be noted that BMDS uses the fitted, or estimated, value for the mean and standard deviation to calculate the BMR and BMD. The value estimated by BMDS for the control SDH mean is 2.71 IU/mL (see detailed model run; a box appears around the estimated mean). Thus the BMR using relative deviation (as the BMR type) and a BMRF of 1 was calculated as BMR = 2.71 + (1 x 2.71) = 5.42.

Modeling results are summarized in Table B-2. The 3rd degree polynomial and power models provided adequate fits of the 10-week SDH data (based on a goodness-of-fit p-value ≥0.1); with both models, the modeling of the variance (test 3 in BMDS output) was marginally adequate (p-value = 0.07515). The power model provided the better fit of the data (based on the lowest AIC value) and therefore was selected as the basis for deriving the RfD; this model estimated a BMD_{2X} of 7.32 mg/kg-day and $BMDL_{2X}$ of 5.46 mg/kg-day. Figure B-1 shows the power model fit to the SDH data and the associated BMD_{2X} and $BMDL_{2X}$; the detailed model run is provided at the end of this section.

Table B-2. Model predictions for changes in serum SDH levels (IU/mL) in male rats exposed to carbon tetrachloride for 10 weeks

Model	*p*-value[a]	AIC[f] for fitted model	BMD_{2X} (mg/kg-day)	$BMDL_{2X}$ (mg/kg-day)
Linear[b]	<0.0001	138.26	5×10^{-8}	4.5×10^{-8}
Polynomial (3rd degree)[b,c]	0.253	85.95	7.15	4.29
Power[d]	**0.264**	**85.88**	**7.32**	**5.46**
Hill[d]	NA[e]	87.84	8.88	5.49

[a] p-value for Test 4: Does the model fit? Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b] Betas restricted to 0.
[c] Insufficient degrees of freedom to fit higher degree polynomials.
[d] Power restricted to 1.
[e] Insufficient degrees of freedom.
[f] AIC = Akaike's Information Criterion.

For purposes of comparison across chemicals, the BMD and BMDL corresponding to a change in the mean response equal to one control standard deviation (SD) from the control mean were also calculated, consistent with BMD guidance (U.S. EPA, 2000c):

BMD_{1SD}: 5.5 mg/mg-day

$BMDL_{1SD}$: 3.8 mg/kg-day

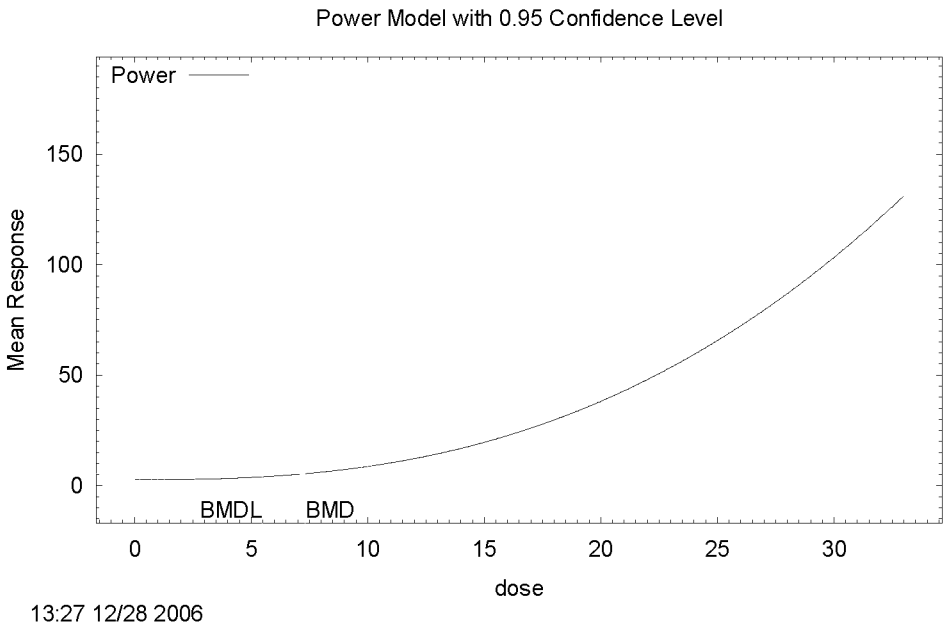


Figure B-1. Power model fit to the SDH data of Bruckner et al. (1986)

```
 BMDS MODEL RUN - Power Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power

   Dependent variable = MEAN
   Independent variable = Dose(mg/kg-d)
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 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

                  Default Initial Parameter Values
                          alpha =      289.698
                            rho =            0
                        control =          2.3
                          slope =    0.0106715
                          power =      2.69605

           Asymptotic Correlation Matrix of Parameter Estimates

                  alpha          rho      control        slope        power

     alpha            1        -0.87        -0.45        -0.17         0.19
       rho        -0.87            1         0.32         0.14        -0.18
   control        -0.45         0.32            1        -0.12          0.1
     slope        -0.17         0.14        -0.12            1        -0.99
     power         0.19        -0.18          0.1        -0.99            1


                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha         0.393849         0.284596          -0.163949            0.951647
            rho          1.64633         0.261152            1.13449             2.15818
        control          2.70501         0.432245            1.85783              3.5522
          slope        0.0161484        0.0130984         -0.00952409           0.0418208
          power          2.57243         0.243917            2.09436              3.0505


     Table of Data and Estimated Values of Interest

 Dose      N      Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------    ---     --------     --------   -----------  -----------   ----------
    0      5        3.5         [2.71]         0.9         1.42           1.25
    1      5        2.3          2.72          1.3         1.43         -0.658
   10      5        7.6          8.74          5.6         3.74         -0.681
   33      5        135           133         33.5         35.1          0.125


 Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = alpha*(Mu(i))^rho
```

```
Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-64.456951	5	138.913902
A2	-34.731110	8	85.462220
A3	-37.319331	6	86.638662
fitted	-37.942951	5	85.885902
R	-91.888765	2	187.777530

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	114.315	6	<.0001
Test 2	59.4517	3	<.0001
Test 3	5.17644	2	0.07515
Test 4	1.24724	1	0.2641

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data.

Benchmark Dose Computation

Specified effect = 1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 7.32096

BMDL = 5.46287

B.2. BMD Modeling of OCT and ALT

BMD modeling was also conducted for OCT and ALT. Available continuous-variable models in the EPA BMDS (linear, polynomial, power, and Hill models; BMDS version 1.4.1; U.S. EPA, 2007) were fit to the data shown in Table B-1 for changes in serum OCT and ALT in male rats exposed to carbon tetrachloride for 10 weeks (Bruckner et al., 1986). For each of these endpoints, a twofold increase in mean enzyme level was used as the BMR (see Section 5.1.2.), with relative deviation as the BMR type and a BMRF of one (see Section B.1). A nonhomogeneous variance model was used in running each of the models in BMDS.

Modeling results are summarized in Tables B-3 and B-4. None of the models for continuous data provided an adequate fit to the 10-week OCT data (based on a goodness-of-fit p-value ≥0.1). The power model provided an adequate fit of the 10-week ALT data; however, as shown in Table B-1, the standard error of the mean ALT for the high-dose (33 mg/kg-day) rats was extremely large (617 ± 334). Bruckner et al. (1986) noted: "There was a pronounced rise in GPT [ALT] at 10 and 12 weeks. Scrutiny of values of individual animals revealed that dramatic increases in two rats at each time point were largely responsible for the late increase in GPT [ALT] activity." In light of the large variation in response at 33 mg/kg-day, using this data set for quantitative analysis was not considered appropriate.

Table B-3. Model predictions for changes in serum OCT levels (nmol CO_2/mL) in male rats exposed to carbon tetrachloride for 10 weeks

Model	*p* value[a]	AIC[e] for fitted model	BMD_{2X} (mg/kg-day)	$BMDL_{2X}$ (mg/kg-day)
Linear[b]	0.0449	157.57	8.04	4.44
Polynomial (2nd degree)[b,c]	0.0427	157.47	11.4	5.86
Power[d]	0.0553	157.04	11.04	6.19
Hill[d]	NA[f]	158.60	10.12	6.52

[a] Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b] Betas restricted to ≥0.
[c] Insufficient degrees of freedom to fit higher degree polynomials.
[d] Power restricted to≥1.
[e] AIC = Akaike's Information Criterion.
[f] NA = not available; insufficient degrees of freedom.

Source: Bruckner et al., 1986.

Table B-4. Model predictions for changes in serum ALT levels (IU/mL) in male rats exposed to carbon tetrachloride for 10 weeks

Model	*p* value[a]	AIC[f] for fitted model	BMD_{2X} (mg/kg-day)	$BMDL_{2X}$ (mg/kg-day)
Linear[b]	<0.0001	291.27	33.05	0.0071
Polynomial (3rd degree)[b,c]	0.01022	123.31	13.66	12.71
Power[d]	0.1145	118.70	14.66	13.21
Hill[d]	NA[f]	120.70	NA[f]	NA[f]

[a] Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b] Betas restricted to ≥0.
[c] Insufficient degrees of freedom to fit higher degree polynomials.
[d] Power restricted to ≥1.
[e] AIC = Akaike's Information Criterion.
[f] NA = not available; insufficient degrees of freedom (BMD software could not generate a model output).

Source: Bruckner et al., 1986.

APPENDIX C. PBPK MODELING

C.1. Paustenbach et al. (1988) and Thrall et al. (2000) PBPK Models (rat, mouse, human)

Detailed summaries of the Paustenbach et al. (1988) and Thrall et al. (2000) PBPK models appear in Section 3.5. Source code for the rat, mouse, and hamster models (reported in Thrall et al., 2000) in Advanced Continuous Simulation Language (ACSL) was graciously provided to Syracuse Research Corporation (SRC) by Dr. Karla Thrall. Included with the code were data collected from gas uptake studies conducted in these species (also reported in Thrall et al., 2000). Accuracy of the implementation of the rat and mouse models in ACSL (version 11.8.4) was verified by comparing model predictions to observations from the closed chamber studies. These simulations are shown in Figures C-1 and C-2. The comparisons of observed and predicted closed chamber CCl_4 concentrations as a function of exposure times match those reported in Figure 2 of Thrall et al. (2000).

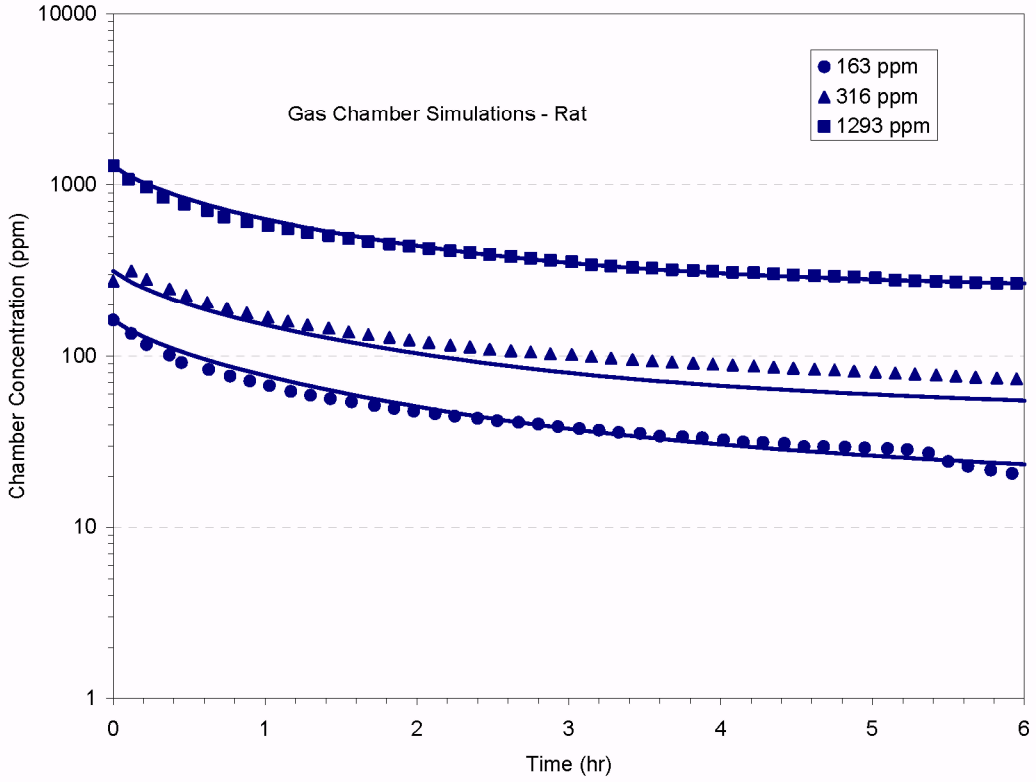


Figure C-1. Comparison of observed and predicted chamber carbon tetrachloride concentrations in closed chamber studies conducted in rats.
Data points are observations (provided by Thrall) for exposures for 3 rats per chamber (body weight, 0.24 kg); lines are simulations. The non-specific loss rate of carbon tetrachloride from the chamber was assumed to be 0.05 hr^{-1} (from Thrall). Partition coefficients were from Thrall source code.

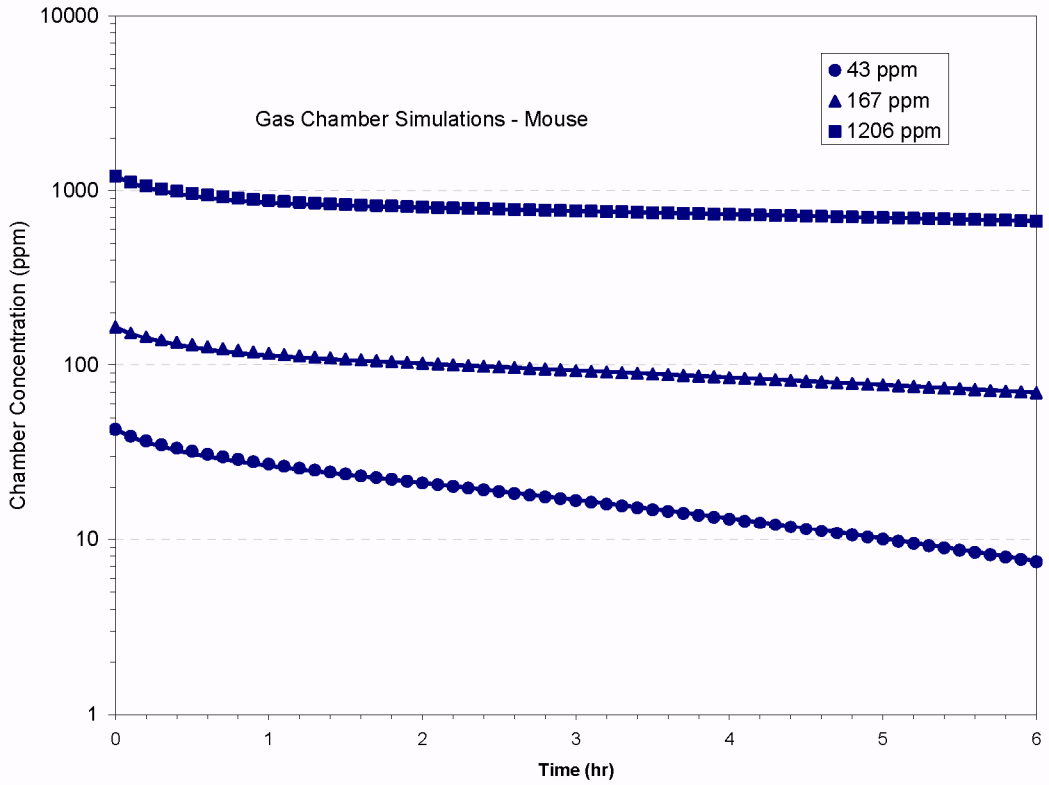


Figure C-2. Comparison of observed and predicted chamber carbon tetrachloride concentrations in closed chamber studies conducted in mice. Data points are observations (provided by Thrall) for exposures for 7 mice per chamber (body weight, 0.024 kg); lines are simulations. The non-specific loss rate of CCl_4 from the chamber was assumed to be 0.05 hr^{-1} (from Thrall source code). Partition coefficients were from Thrall source code.

As noted above, Thrall et al. (2000) compared model predictions for the rat and mouse with experimental data collected over a 48-hour period following a 4-hour nose-only inhalation exposure to 20 ppm of [^{14}C]-carbon tetrachloride (data from a personal communication and not presented in Thrall et al. (2000)). This comparison of PBPK model-predicted and experimentally-observed values for selected parameters is provided in Table C-1. Thrall et al. (2000) also compared the model simulation for humans with human data of Stewart et al. (1961) (see Figure C-3). As this figure shows, the model simulation of expired carbon tetrachloride levels provided good agreement with the experimental data, particularly at longer periods postexposure.

Table C-1. Comparison of predicted and observed values for selected parameters from toxicokinetic data collected from rats and mice 48 hours post exposure to a 4-hour nose-only inhalation exposure (20 ppm carbon tetrachloride)

Species	Parameter	Model (µmol)	Data (µmol equivalents of $CCl_4 \pm SD$)[a]	Ratio (predicted/observed)
Rat	Initial body burden	7.8	11.7 ± 0.54	0.7
	Total amount trapped by KOH[b]	2.8	2.7 ± 0.25	1.0
	Total amount trapped on charcoal[c]	4.1	7.4 ± 0.44	0.6
	Total amount metabolized[d]	3.7	3.7 ± 0.22	1.0
Mouse	Initial body burden	2.2	2.0 ± 0.48	1.1
	Total amount trapped by KOH[b]	0.95	0.69 ± 0.11	1.4
	Total amount trapped on charcoal[c]	0.94	0.76 ± 0.37	1.2
	Total amount metabolized[d]	1.3	1.2 ± 0.11	1.1

[a] n = 3-4 animals.
[b] $^{14}CO_2$ measured using a KOH trap.
[c] Parent compound ($^{14}CCl_4$) measured using a charcoal trap.
[d] Represents the sum of radioactivity (in µmol equivalents) in urine, feces, and trapped on KOH (CO_2).

Source: Thrall et al. (2000); Benson and Springer (1999).

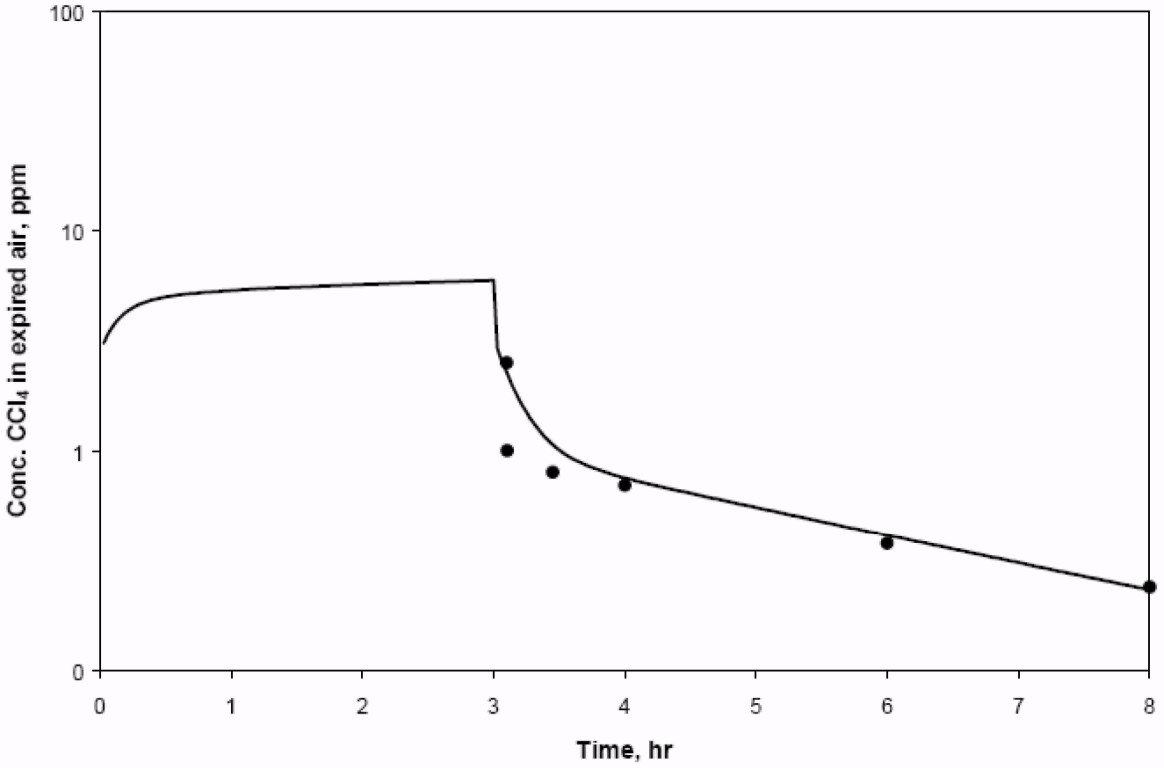


Figure C-3. Comparison of the actual versus predicted concentration of carbon tetrachloride in the expired breath of humans exposed to 10 ppm of carbon tetrachloride for 180 minutes (data from Stewart et al., 1961).

Source: Thrall et al. (2000); Benson and Springer (1999)

Parameter values for the rat and human models used in the Paustenbach et al. (1988) and Thrall et al. (2000) models are summarized in Table C-2. Parameter values for the mouse are shown in Table C-3.

Table C-2. Parameter values for rat and human models[a]

Parameter	Definition	Rat model	Human model
BW	Body weight (kg)	0.452[b]	70
VLC	Liver volume (fraction of body)	0.04[c,d]	0.04[c]
VFC	Fat volume (fraction of body)	0.08[c,d]	0.2[g]
VSC	Slowly-perfused tissue volume (fraction of body)	0.74[c,d]	0.62[c]
VRC	Rapidly-perfused tissue volume (fraction of body)	0.05[c,d]	0.05[c]
QCC	Cardiac output (L/hour-kg BW)	15[c,d]	15[c]
QPC	Alveolar ventilation rate (L/hour-kg BW)	15[c,d]	15[c]
QLC	Liver blood flow (fraction of cardiac output)	0.25[c,d]	0.25[c]
QFC	Fat blood flow (fraction of cardiac output)	0.04[c,d]	0.06[c]
QSC	Slowly-perfused blood flow (fraction of cardiac output)	0.2[c,d]	0.18[c]
QRC	Rapidly-perfused blood flow (fraction of cardiac output)	0.51[c,d]	0.51[c]
PB	Blood:air partition coefficient	4.52[e]	2.64[c]
PL	Liver:blood partition coefficient	3.14[e]	3.14[e]
PF	Fat:blood partition coefficient	79.42[e]	79.42[e]
PS	Slowly-perfused partition coefficient	1[e]	1[e]
PR	Readily-perfused partition coefficient	3.14[f]	3.14[f]
V_{maxC}	Maximum rate of metabolism (mg/hour-kg BW)	0.4[e],0.65[c]	0.4[e], 0.65[c], 1.49[d], 1.7[d]
K_{mX}	Michaelis-Menten coefficient for metabolism (mg/L)	0.25[c,d]	0.25[c,d]

[a]See summary of the Paustenbach et al. (1988) and Thrall et al. (2000) models in Section 3.5 for discussion the source of parameter values.
[b]Time-weighted mean body weight for the exposure group of interest (0.452 kg, male rats) and an exposure of 3 ppm, 6 hours/day, 5 days/week (based on Nagano et al., 2007b; JBRC, 1998).
[c]Paustenbach et al., 1988.
[d]Thrall et al., 2000.
[e]Gargas et al., 1986.
[f]Partition coefficient for readily-perfused is assumed to be equal to that of liver.
[g]Adjusted from reported value of 0.1 in Paustenbach et al., 1988.

Table C-3. Parameter values for mouse models[a]

Parameter	Definition	Thrall et al. (2000)	Fisher et al. (2004)
BW	Body weight (kg)	0.036[b]	--
VLC	Liver volume (fraction of body)	0.04[c]	0.04[g]
VFC	Fat volume (fraction of body)	0.04[c]	0.04[g]
VSC	Slowly-perfused tissue volume (fraction of body)	0.78[c]	0.69[g]
VRC	Richly-perfused tissue volume (fraction of body)	0.05[c]	0.14[g]
QCC	Cardiac output (L/hour-kg BW^{SF})[d,h]	28[c,d]	30[g,.h]
QPC	Alveolar ventilation rate (L/hour-kg BW^{SF})[d,h]	28[c,d]	30[g,h]
QLC	Liver blood flow (fraction of cardiac output)	0.24[c]	0.24[g]
QFC	Fat blood flow (fraction of cardiac output)	0.05[c]	0.05[g]
QSC	Slowly-perfused blood flow (fraction of cardiac output)	0.19[c]	0.17[g]
QRC	Richly-perfused blood flow (fraction of cardiac output)	0.52[c]	0.54[g]
PB	Blood:air partition coefficient	7.83[c]	3.8[h]
PL	Liver:blood partition coefficient	2.08[e]	4.8[h]
PF	Fat:blood partition coefficient	23.0[e]	91.4[h]
PS	Slowly-perfused partition coefficient	0.61[e]	2.5[h]
PR	Richly-perfused partition coefficient	2.08[e]	4.8[h]
V_{maxC}	Maximum rate of metabolism (mg/hour-kg BW^{SF})[f,j]	0.79[e,f]	1[i,j]
K_{mX}	Michaelis-Menten coefficient for metabolism (mg/L)	0.46[e]	0.3[i]
K1	GI absorption rate coefficient C1-liver ($hour^{-1}$)	--	0.4, 10[k]
K2	GI absorption rate coefficient C1-C2 ($hour^{-1}$)	--	2[k]
K2	GI absorption rate coefficient C2-liver ($hour^{-1}$)	--	0.05[l]

[a]See Paustenbach et al. (1988) and Thrall et al. (2000) for discussion the source of parameter values.
[b]Reference value for mouse body weight in a chronic study (0.036 kg; U.S. EPA, 1988)
[c]Andersen et al., 1987
[d] SF, scaling factor; QC (L/hour)=QCC*$BW^{0.74}$; QP (L/hour)=QPC*$BW^{0.74}$
[e]Thrall source code (CARBON TETRACHLORIDE PBPK MODEL KD THRALL 3/98 ITRICCL4.ACSL). Thrall et al. (2000) reported the tissue:blood partition coefficients for the mouse were based on values for blood:air for the mouse (7.83) from Thrall et al. (2000) and tissue:air values (liver:air=14.2; muscle:air=4.54; fat:air=359) from Gargas et al. (1986). The corresponding tissue:blood values would be: PL=1.81; PF=45.85; PS=0.58; PR=1.81.
[f] SF, scaling factor; VMAX=VBMAXC*$BW^{0.70}$
[g]Brown et al., 1997
[h] SF, scaling factor; QC (L/hour)=QCC*$BW^{0.75}$; QP (L/hour)=QPC*$BW^{0.75}$
[i]Fisher et al. (2004) vial equilibrium measurements
[j] VMAX=VBMAXC*$BW^{0.75}$
[k]Fisher et al. (2004) fit to closed chamber data.
[l]Fisher et al. (2004) fit to oral gavage blood data. K1 values are 0.4 hr^{-1} for 20 mg/kg dose and 10 hr^{-1} for 50 and 100 mg/kg dose.

C.2. Fisher PBPK Model (mouse)

A detailed summary of the mouse PBPK model developed by Fisher et al. (2004) is provided in Section 3.5. This model was reconstructed from the information provided in their paper.

Fisher et al. (2004) performed gas uptake experiments with mice at four concentrations of carbon tetrachloride to estimate metabolic constants. As shown in Figure C-3, metabolic constants provided a good fit between model predictions and observations for the gas uptake study.

Parameter values for the mouse used in the Fisher et al. (2004) model are summarized in Table C-3 and are compared with the mouse parameter values from the Thrall et al. (2000) model. Values for K_m and V_{maxC} used in the two models are similar: 0.3 mg/L, 1 mg/hr/kg$^{0.75}$ (Fisher et al., 2004) compared to 0.46 mg/L, 0.79 mg/hr/kg$^{0.70}$ (Thrall et al., 2000); although different allometric scaling factors were used to scale V_{max} to body weight. The corresponding V_{max} values for a 0.036-kg mouse are 0.077 mg/hr (Thrall et al., 2000) and 0.082 mg/hr (Fisher et al., 2004). Tissue partition coefficients used in the Fisher et al. (2004) model were 2-4 times higher than in the Thrall et al. (2000) model.

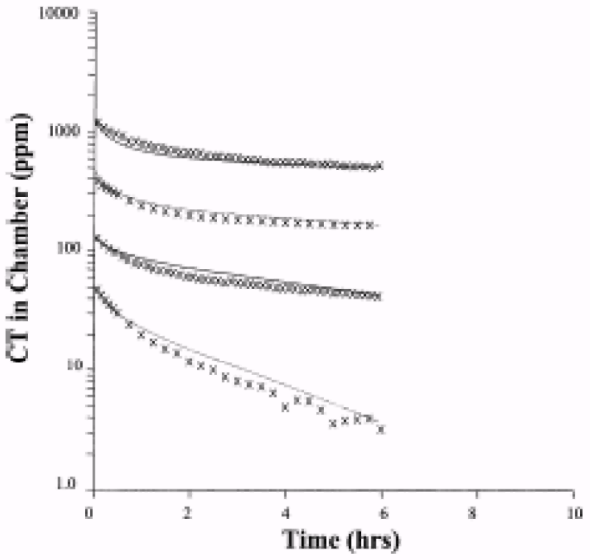


Figure C-4. Atmospheric clearance of carbon tetrachloride from gas uptake chambers containing mice (initial concentrations about 50, 130, 450, or 1250 ppm). At initial concentrations of 50, 450 and 1250 ppm: 3 mice in a 2-liter chamber; at 130 ppm: 7 mice in a 9-liter chamber. Metabolic constants for carbon tetrachloride (Vmaxc (mg/h/kg) and Km (mg/l)) were estimated by fitting the gas uptake data with the carbon tetrachloride PBPK model.

Source: Fisher et al. (2004)

C.3. PBPK Modeling of Human Equivalent Concentrations and Doses

Interspecies extrapolation (i.e., rat-to-human, mouse-to-human) and route-to-route extrapolation of carbon tetrachloride inhalation dosimetry was accomplished using a human PBPK model described in Paustenbach et al. (1988), Thrall et al. (2000), and Benson and Springer (1999). The human PBPK model was used to estimate the continuous chronic human inhalation exposure in mg/m^3 (abbreviated as EC in the following tables) or the rate of uptake of carbon tetrachloride from the GI tract to the liver (i.e., chronic daily ingested dose) in mg/kg-day (abbreviated RGIL in the following tables) that would result in values for the internal dose metrics, MCA or MRAMKL, equal to the respective BMDLs for each toxicity endpoint (i.e., RfC: fatty liver degeneration; cancer: liver tumors in rats, liver tumors and adrenal pheochromocytomas in mice). This procedure is described in Section 5.4.2.3.4.

Conversion factors that relate EC or RGIL to the two dose metrics (MCA and MRAMKL) for each of the assumed values of human V_{maxC} (0.40, 0.65, 1.49, or 1.70 mg/hr/kg $BW^{0.70}$) are provided in Tables C-4 to C-11. Figures C-5 to C-12 display plots of MCA and corresponding values of EC or RGIL predicted from the human PBPK model, with trend equations developed to permit the calculation of EC or RGIL for any value of MCA. Trend equations shown on the plots are power functions fit to each data set using the method of least squares (Microsoft Excel). The corresponding fit to the PBPK model predictions were evaluated by R^2 (shown on the trend plots) and the magnitude of the difference between PBPK model predictions and the trend function predictions (i.e., shown in the plots of % delta, where % delta = 100*[Trend-PBPK]/PBPK). If values for % delta uniformly ≤5% could not be achieved with single trend functions applied to the full ranges of internal dose metric values presented in Tables C-4 to C-11, trend functions were developed for subsets of the full MCA range that yielded achieved % delta values ≤5%. Similar plots were developed for the dose metric MRAMKL (see Figures C-13 and C-14).

Table C-4. Interspecies conversion factors based on MCA dose metric (VMAXC=0.40)

EC (ppm)	EC (mg/m^3)	MCA (µmol/L)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg/day/ µmol/L)
0.1	0.6290	0.009182	0.1016	0.1614	68.51	11.06
0.2	1.258	0.01837	0.2021	0.1607	68.48	11.00
0.3	1.887	0.02757	0.3019	0.1600	68.45	10.95
0.4	2.516	0.03678	0.4007	0.1592	68.42	10.89
0.5	3.145	0.04599	0.4987	0.1586	68.38	10.84
0.6	3.774	0.05522	0.5959	0.1579	68.35	10.79
0.7	4.403	0.06445	0.6923	0.1572	68.32	10.74
0.8	5.032	0.07369	0.7880	0.1566	68.29	10.69
0.9	5.661	0.08293	0.8829	0.1560	68.26	10.65
1	6.290	0.09219	0.9772	0.1554	68.23	10.60
2	12.58	0.1852	1.887	0.1500	67.94	10.19
3	18.87	0.2790	2.752	0.1458	67.65	9.864
4	25.16	0.3735	3.584	0.1424	67.37	9.595
5	31.45	0.4687	4.392	0.1396	67.11	9.370
6	37.74	0.5646	5.183	0.1373	66.85	9.180
7	44.03	0.6611	5.961	0.1354	66.60	9.016
8	50.32	0.7583	6.729	0.1337	66.36	8.874
9	56.61	0.8560	7.490	0.1323	66.14	8.749
10	62.90	0.9543	8.245	0.1311	65.92	8.640
20	125.8	1.961	15.67	0.1245	64.17	7.992
30	188.7	2.995	23.06	0.1222	63.01	7.699
40	251.6	4.045	30.47	0.1211	62.21	7.534
50	314.5	5.103	37.91	0.1205	61.63	7.428
60	377.4	6.167	45.36	0.1202	61.20	7.355
70	440.3	7.234	52.82	0.1200	60.87	7.302
80	503.2	8.304	60.29	0.1198	60.60	7.261
90	566.1	9.375	67.77	0.1197	60.39	7.229
100	629.0	10.447	75.25	0.1196	60.21	7.203

EC, air exposure concentration; MCA, time-averaged arterial concentration of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

Table C-5. Interspecies conversion factors based on MCA dose metric (VMAXC=0.65)

EC (ppm)	EC (mg/m^3)	MCA (µmol/L)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg/day/ µmol/L)
0.1	0.6290	0.008674	0.1182	0.1879	72.52	13.63
0.2	1.258	0.01735	0.2350	0.1868	72.49	13.54
0.3	1.887	0.02604	0.3504	0.1857	72.46	13.45
0.4	2.516	0.03474	0.4645	0.1846	72.43	13.37
0.5	3.145	0.04344	0.5774	0.1836	72.40	13.29
0.6	3.774	0.05215	0.6890	0.1826	72.37	13.21
0.7	4.403	0.06087	0.7995	0.1816	72.34	13.14
0.8	5.032	0.06959	0.9088	0.1806	72.31	13.06
0.9	5.661	0.07832	1.0171	0.1797	72.28	12.99
1	6.290	0.08706	1.1243	0.1787	72.25	12.91
2	12.58	0.1748	2.147	0.1706	71.96	12.28
3	18.87	0.2633	3.097	0.1641	71.66	11.760
4	25.16	0.3525	3.994	0.1588	71.37	11.331
5	31.45	0.4424	4.853	0.1543	71.09	10.969
6	37.74	0.5330	5.683	0.1506	70.81	10.661
7	44.03	0.6243	6.489	0.1474	70.54	10.395
8	50.32	0.7162	7.279	0.1447	70.27	10.164
9	56.61	0.8087	8.055	0.1423	70.00	9.961
10	62.90	0.9019	8.821	0.1402	69.75	9.780
20	125.8	1.864	16.21	0.1289	67.51	8.699
30	188.7	2.866	23.51	0.1246	65.85	8.203
40	251.6	3.893	30.85	0.1226	64.63	7.923
50	314.5	4.936	38.22	0.1215	63.72	7.743
60	377.4	5.988	45.63	0.1209	63.03	7.619
70	440.3	7.047	53.05	0.1205	62.48	7.529
80	503.2	8.110	60.50	0.1202	62.05	7.460
90	566.1	9.176	67.95	0.1200	61.70	7.406
100	629.0	10.244	75.41	0.1199	61.41	7.362

EC, air exposure concentration; MCA, time-averaged arterial concentration of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

Table C-6. Interspecies conversion factors based on MCA dose metric (VMAXC=1.49)

EC (ppm)	EC (mg/m^3)	MCA (µmol/L)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg/day/ µmol/L)
0.1	0.6290	0.007827	0.1742	0.2770	80.37	22.26
0.2	1.258	0.01566	0.3457	0.2748	80.35	22.08
0.3	1.887	0.02349	0.5146	0.2727	80.33	21.90
0.4	2.516	0.03133	0.6808	0.2706	80.31	21.73
0.5	3.145	0.03917	0.8447	0.2686	80.29	21.56
0.6	3.774	0.04702	1.0060	0.2665	80.27	21.40
0.7	4.403	0.05487	1.1651	0.2646	80.25	21.23
0.8	5.032	0.06272	1.3219	0.2627	80.23	21.07
0.9	5.661	0.07058	1.4766	0.2608	80.21	20.92
1	6.290	0.07844	1.6291	0.2590	80.19	20.77
2	12.58	0.1573	3.053	0.2427	79.99	19.41
3	18.87	0.2365	4.326	0.2293	79.80	18.294
4	25.16	0.3161	5.487	0.2181	79.60	17.358
5	31.45	0.3962	6.559	0.2085	79.39	16.557
6	37.74	0.4766	7.564	0.2004	79.19	15.871
7	44.03	0.5575	8.514	0.1934	78.98	15.272
8	50.32	0.6388	9.419	0.1872	78.78	14.744
9	56.61	0.7205	10.288	0.1817	78.57	14.278
10	62.90	0.8027	11.130	0.1769	78.36	13.864
20	125.8	1.650	18.67	0.1484	76.24	11.316
30	188.7	2.545	25.69	0.1361	74.16	10.095
40	251.6	3.482	32.67	0.1299	72.26	9.384
50	314.5	4.454	39.74	0.1263	70.61	8.922
60	377.4	5.453	46.90	0.1243	69.22	8.601
70	440.3	6.470	54.13	0.1229	68.06	8.367
80	503.2	7.501	61.42	0.1221	67.09	8.188
90	566.1	8.542	68.76	0.1215	66.28	8.049
100	629.0	9.590	76.13	0.1210	65.59	7.938

EC, air exposure concentration; MCA, time-averaged arterial concentration of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

Table C-7. Interspecies conversion factors based on MCA dose metric (VMAXC=1.70)

EC (ppm)	EC (mg/m^3)	MCA (µmol/L)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg/day/ µmol/L)
0.1	0.6290	0.007709	0.1882	0.2993	81.60	24.42
0.2	1.258	0.01542	0.3735	0.2969	81.58	24.22
0.3	1.887	0.02314	0.5557	0.2945	81.56	24.02
0.4	2.516	0.03086	0.7351	0.2922	81.54	23.82
0.5	3.145	0.03858	0.9118	0.2899	81.53	23.63
0.6	3.774	0.04630	1.0857	0.2877	81.51	23.45
0.7	4.403	0.05403	1.2571	0.2855	81.49	23.26
0.8	5.032	0.06177	1.4259	0.2834	81.47	23.09
0.9	5.661	0.06950	1.5924	0.2813	81.46	22.91
1	6.290	0.07724	1.7565	0.2792	81.44	22.74
2	12.58	0.1548	3.284	0.2610	81.26	21.21
3	18.87	0.2327	4.642	0.2460	81.09	19.948
4	25.16	0.3110	5.873	0.2334	80.91	18.885
5	31.45	0.3896	7.005	0.2227	80.73	17.978
6	37.74	0.4686	8.060	0.2135	80.54	17.200
7	44.03	0.5480	9.051	0.2055	80.36	16.517
8	50.32	0.6277	9.993	0.1986	80.17	15.920
9	56.61	0.7078	10.893	0.1924	79.98	15.390
10	62.90	0.7883	11.758	0.1869	79.79	14.915
20	125.8	1.616	19.40	0.1542	77.83	12.003
30	188.7	2.488	26.38	0.1398	75.84	10.602
40	251.6	3.403	33.28	0.1323	73.94	9.780
50	314.5	4.355	40.25	0.1280	72.22	9.242
60	377.4	5.336	47.32	0.1254	70.73	8.868
70	440.3	6.340	54.49	0.1238	69.45	8.595
80	503.2	7.361	61.73	0.1227	68.36	8.386
90	566.1	8.394	69.03	0.1219	67.45	8.224
100	629.0	9.435	76.36	0.1214	66.67	8.093

EC, air exposure concentration; MCA, time-averaged arterial concentration of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

Table C-8. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=0.40)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MRAMKL (mg/m^3/ µmol/hr/kg liver)	RGIL/MRAMKL (mg/kg/day/ µmol/hr/kg liver)
1	6.290	0.7352	0.2980	0.04737	8.556	0.4053
2	12.58	1.433	0.5960	0.04737	8.782	0.4161
3	18.87	2.093	0.8940	0.04737	9.015	0.4271
4	25.16	2.719	1.192	0.04737	9.254	0.4384
5	31.45	3.311	1.490	0.04737	9.498	0.4500
6	37.74	3.872	1.788	0.04737	9.749	0.4618
7	44.03	4.402	2.086	0.04737	10.004	0.4739
8	50.32	4.903	2.384	0.04737	10.264	0.4862
9	56.61	5.377	2.682	0.04737	10.529	0.4987
10	62.90	5.826	2.980	0.04737	10.798	0.5115
20	125.8	9.196	5.959	0.04737	13.681	0.6480
30	188.7	11.24	8.938	0.04736	16.792	0.7953
40	251.6	12.57	11.92	0.04736	20.025	0.9483
50	314.5	13.48	14.89	0.04735	23.329	1.105
60	377.4	14.15	17.87	0.04735	26.675	1.263
70	440.3	14.65	20.85	0.04735	30.049	1.423
80	503.2	15.05	23.83	0.04734	33.442	1.583
90	566.1	15.36	26.80	0.04734	36.849	1.744
100	629.0	15.62	29.78	0.04734	40.265	1.906
110	691.9	15.84	32.75	0.04733	43.689	2.068
120	754.8	16.02	35.73	0.04733	47.119	2.230
130	817.8	16.18	38.70	0.04733	50.553	2.393
140	880.7	16.31	41.68	0.04732	53.990	2.555
150	943.6	16.43	44.65	0.04732	57.430	2.718
160	1006	16.53	47.63	0.04732	60.873	2.880
170	1069	16.63	50.60	0.04732	64.318	3.043
180	1132	16.71	53.57	0.04731	67.765	3.206
190	1195	16.78	56.54	0.04731	71.213	3.369
200	1258	16.85	59.52	0.04731	74.662	3.532
210	1321	16.91	62.49	0.04730	78.112	3.695
220	1384	16.97	65.46	0.04730	81.563	3.858
230	1447	17.02	68.43	0.04730	85.015	4.021
240	1510	17.06	71.40	0.04730	88.468	4.184
250	1573	17.11	74.37	0.04729	91.921	4.347
260	1636	17.15	77.34	0.04729	95.375	4.510
270	1698	17.19	80.31	0.04728	98.830	4.673

Table C-8. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=0.40)

280	1761	17.22	83.28	0.04728	102.284	4.836
290	1824	17.25	86.24	0.04728	105.740	4.999
300	1887	17.28	89.21	0.04728	109.195	5.162

EC, air exposure concentration; MRAMKL, time-averaged rate of metabolism of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

Table C-9. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=0.65)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MRAMKL (mg/m^3/ µmol/hr/kg liver)	RGIL/MRAMKL (mg/kg/day/ µmol/hr/kg liver)
1	6.290	0.9770	0.2980	0.04737	6.438	0.3050
2	12.58	1.920	0.5960	0.04737	6.552	0.3104
3	18.87	2.830	0.8940	0.04737	6.669	0.3159
4	25.16	3.706	1.192	0.04737	6.789	0.3216
5	31.45	4.550	1.490	0.04737	6.913	0.3275
6	37.74	5.361	1.788	0.04737	7.041	0.3335
7	44.03	6.140	2.086	0.04737	7.171	0.3397
8	50.32	6.888	2.384	0.04737	7.305	0.3461
9	56.61	7.607	2.682	0.04737	7.443	0.3525
10	62.90	8.296	2.980	0.04737	7.583	0.3592
20	125.8	13.772	5.959	0.04737	9.135	0.4327
30	188.7	17.33	8.938	0.04736	10.889	0.5157
40	251.6	19.71	11.92	0.04736	12.768	0.6047
50	314.5	21.36	14.89	0.04736	14.723	0.697
60	377.4	22.57	17.87	0.04735	16.726	0.792
70	440.3	23.47	20.85	0.04735	18.760	0.888
80	503.2	24.18	23.83	0.04735	20.815	0.986
90	566.1	24.74	26.80	0.04734	22.886	1.084
100	629.0	25.19	29.78	0.04734	24.967	1.182
110	691.9	25.57	32.75	0.04734	27.057	1.281
120	754.8	25.89	35.73	0.04733	29.153	1.380
130	817.8	26.16	38.71	0.04733	31.254	1.479
140	880.7	26.40	41.68	0.04733	33.359	1.579
150	943.6	26.60	44.66	0.04733	35.467	1.679
160	1006	26.78	47.63	0.04732	37.578	1.778
170	1069	26.94	50.60	0.04732	39.691	1.878
180	1132	27.08	53.57	0.04731	41.805	1.978
190	1195	27.21	56.55	0.04731	43.922	2.078
200	1258	27.33	59.52	0.04731	46.039	2.178
210	1321	27.43	62.49	0.04730	48.158	2.278
220	1384	27.52	65.46	0.04730	50.278	2.378
230	1447	27.61	68.44	0.04730	52.398	2.479
240	1510	27.69	71.40	0.04730	54.519	2.579
250	1573	27.76	74.38	0.04730	56.641	2.679
260	1636	27.83	77.34	0.04729	58.764	2.779
270	1698	27.89	80.32	0.04729	60.887	2.879

Table C-9. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=0.65)

280	1761	27.95	83.28	0.04728	63.010	2.979
290	1824	28.01	86.25	0.04728	65.134	3.080
300	1887	28.06	89.22	0.04728	67.259	3.180

EC, air exposure concentration; MRAMKL, time-averaged rate of metabolism of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

Table C-10. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=1.49)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MRAMKL (mg/m^3/ µmol/hr/kg liver)	RGIL/MRAMKL (mg/kg/day/ µmol/hr/kg liver)
1	6.290	1.3834	0.2980	0.04737	4.547	0.2154
2	12.58	2.749	0.5960	0.04738	4.577	0.2168
3	18.87	4.095	0.8940	0.04737	4.608	0.2183
4	25.16	5.423	1.192	0.04737	4.640	0.2198
5	31.45	6.731	1.490	0.04737	4.672	0.2213
6	37.74	8.020	1.788	0.04737	4.706	0.2229
7	44.03	9.289	2.086	0.04737	4.740	0.2246
8	50.32	10.537	2.384	0.04737	4.776	0.2263
9	56.61	11.764	2.682	0.04737	4.812	0.2280
10	62.90	12.971	2.980	0.04737	4.850	0.2297
20	125.8	23.832	5.960	0.04737	5.279	0.2501
30	188.7	32.48	8.940	0.04737	5.810	0.2752
40	251.6	39.11	11.92	0.04737	6.434	0.3048
50	314.5	44.09	14.90	0.04736	7.134	0.338
60	377.4	47.83	17.87	0.04736	7.891	0.374
70	440.3	50.68	20.85	0.04736	8.689	0.411
80	503.2	52.88	23.83	0.04736	9.516	0.451
90	566.1	54.62	26.81	0.04735	10.365	0.491
100	629.0	56.01	29.79	0.04735	11.230	0.532
110	691.9	57.15	32.76	0.04735	12.107	0.573
120	754.8	58.10	35.74	0.04734	12.992	0.615
130	817.8	58.90	38.71	0.04734	13.885	0.657
140	880.7	59.57	41.69	0.04734	14.783	0.700
150	943.6	60.16	44.66	0.04733	15.685	0.742
160	1006	60.67	47.64	0.04733	16.590	0.785
170	1069	61.11	50.61	0.04733	17.499	0.828
180	1132	61.50	53.59	0.04733	18.410	0.871
190	1195	61.85	56.56	0.04732	19.323	0.914
200	1258	62.17	59.53	0.04732	20.238	0.958
210	1321	62.45	62.51	0.04732	21.154	1.001
220	1384	62.70	65.47	0.04731	22.071	1.044
230	1447	62.93	68.45	0.04731	22.989	1.088
240	1510	63.14	71.42	0.04730	23.909	1.131
250	1573	63.34	74.39	0.04730	24.829	1.175
260	1636	63.52	77.36	0.04730	25.750	1.218
270	1698	63.68	80.33	0.04730	26.671	1.261

Table C-10. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=1.49)

280	1761	63.83	83.30	0.04729	27.593	1.305
290	1824	63.97	86.27	0.04729	28.516	1.349
300	1887	64.10	89.24	0.04729	29.439	1.392

EC, air exposure concentration; MRAMKL, time-averaged rate of metabolism of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

Table C-11. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=1.70)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg/day)	RGIL/EC (mg/kg/day/ mg/m^3)	EC/MRAMKL (mg/m^3/ µmol/hr/kg liver)	RGIL/MRAMKL (mg/kg/day/ µmol/hr/kg liver)
1	6.290	1.4401	0.2980	0.04737	4.368	0.2069
2	12.58	2.865	0.5960	0.04738	4.392	0.2081
3	18.87	4.273	0.8940	0.04737	4.417	0.2092
4	25.16	5.665	1.192	0.04737	4.442	0.2104
5	31.45	7.040	1.490	0.04737	4.468	0.2117
6	37.74	8.398	1.788	0.04737	4.494	0.2129
7	44.03	9.738	2.086	0.04737	4.522	0.2142
8	50.32	11.060	2.384	0.04737	4.550	0.2155
9	56.61	12.365	2.682	0.04737	4.579	0.2169
10	62.90	13.650	2.980	0.04737	4.608	0.2183
20	125.8	25.429	5.960	0.04737	4.947	0.2344
30	188.7	35.14	8.939	0.04737	5.370	0.2544
40	251.6	42.84	11.92	0.04737	5.874	0.2782
50	314.5	48.77	14.90	0.04737	6.448	0.305
60	377.4	53.31	17.88	0.04736	7.080	0.335
70	440.3	56.79	20.85	0.04736	7.754	0.367
80	503.2	59.49	23.83	0.04736	8.459	0.401
90	566.1	61.62	26.81	0.04735	9.187	0.435
100	629.0	63.33	29.79	0.04735	9.933	0.470
110	691.9	64.72	32.76	0.04735	10.691	0.506
120	754.8	65.88	35.74	0.04735	11.459	0.543
130	817.8	66.84	38.71	0.04734	12.234	0.579
140	880.7	67.66	41.69	0.04734	13.015	0.616
150	943.6	68.37	44.66	0.04734	13.801	0.653
160	1006	68.98	47.64	0.04733	14.591	0.691
170	1069	69.51	50.61	0.04733	15.383	0.728
180	1132	69.99	53.59	0.04733	16.179	0.766
190	1195	70.40	56.56	0.04733	16.976	0.803
200	1258	70.78	59.53	0.04732	17.775	0.841
210	1321	71.11	62.51	0.04732	18.576	0.879
220	1384	71.42	65.48	0.04731	19.378	0.917
230	1447	71.69	68.45	0.04731	20.181	0.955
240	1510	71.94	71.42	0.04731	20.985	0.993
250	1573	72.17	74.39	0.04731	21.790	1.031
260	1636	72.38	77.36	0.04730	22.596	1.069
270	1698	72.57	80.34	0.04730	23.403	1.107

Table C-11. Interspecies conversion factors based on MRAMKL dose metric (VMAXC=1.70)

280	1761	72.75	83.30	0.04730	24.210	1.145
290	1824	72.92	86.28	0.04730	25.017	1.183
300	1887	73.07	89.24	0.04729	25.825	1.221

EC, air exposure concentration; MRAMKL, time-averaged rate of metabolism of carbon tetrachloride; RGIL, rate of uptake of carbon tetrachloride from GI-tract to liver; VMAXC, maximum rate of metabolism of carbon tetrachloride (mg/hr/kg $BW^{0.70}$).

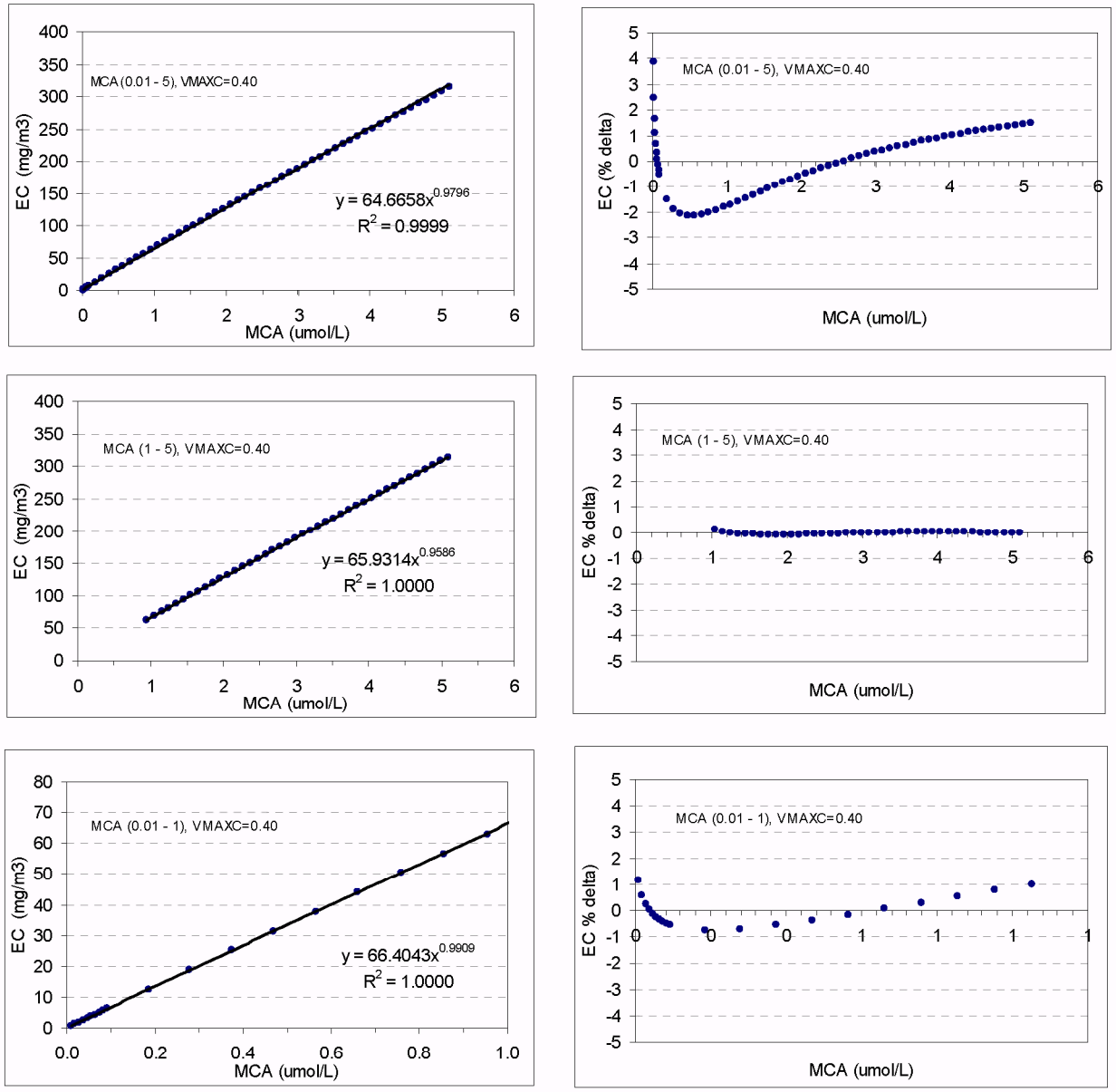


Figure C-5. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent exposure concentration (EC, left panel) and values for % delta for trend lines (right panel). VMAXC=0.40 mg/hr/kg $BW^{0.70}$.

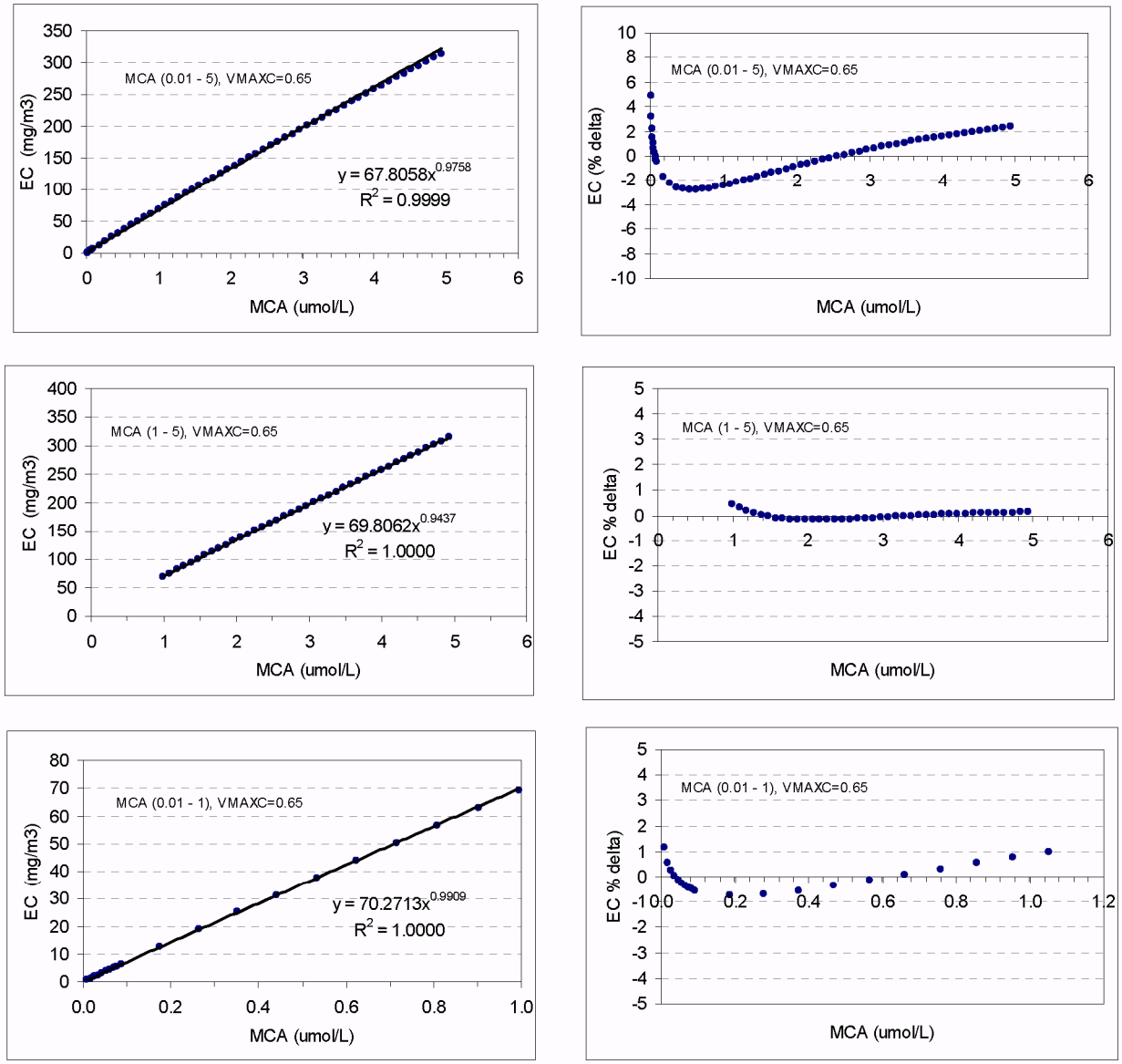


Figure C-6. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent exposure concentration (EC, left panel) and values for % delta for trend lines (right panel). VMAXC=0.65 mg/hr/kg $BW^{0.70}$.

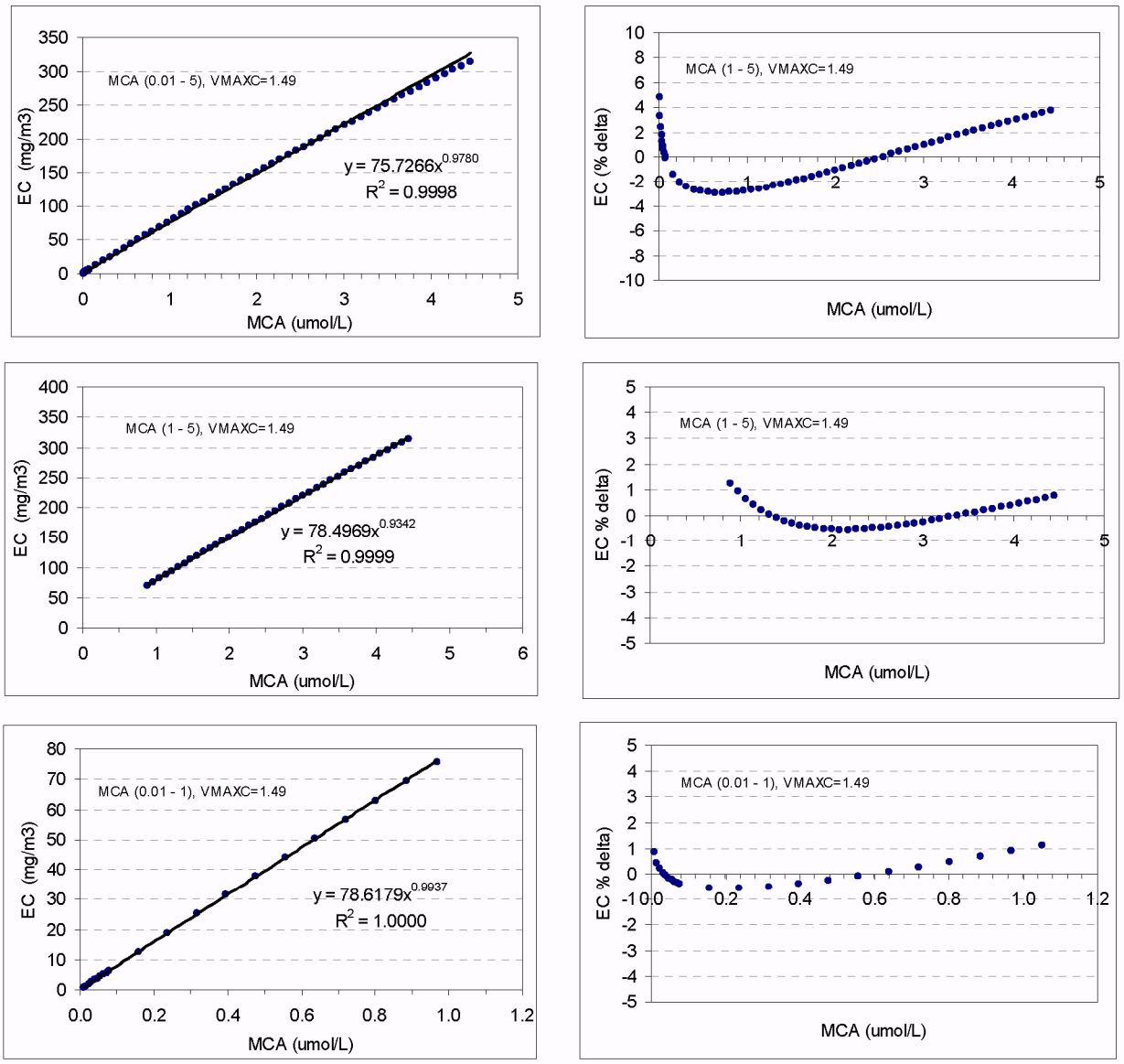


Figure C-7. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent exposure concentration (EC, left panel) and values for % delta for trend lines (right panel). VMAXC=1.49 mg/hr/kg $BW^{0.70}$.

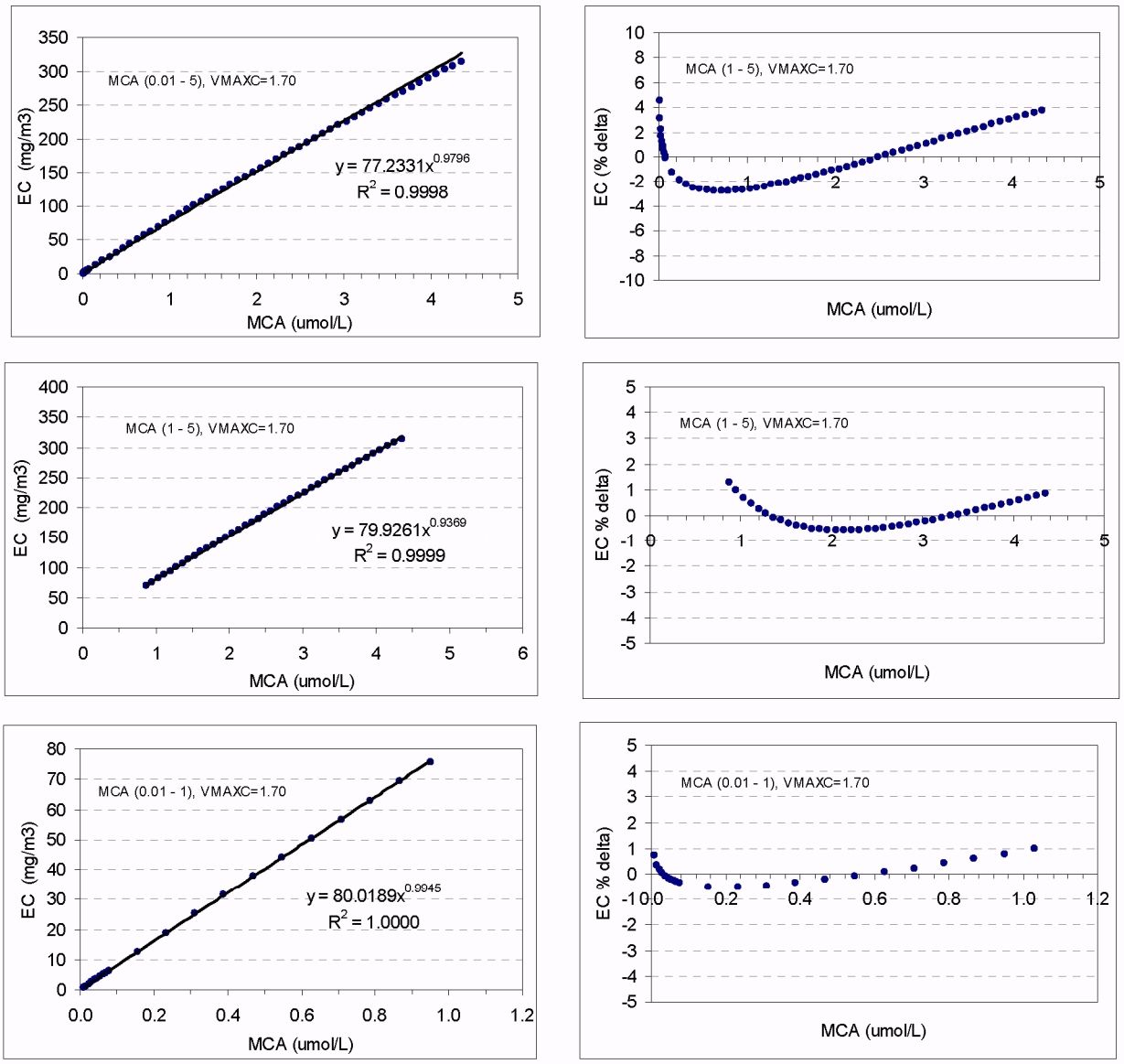


Figure C-8. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent exposure concentration (EC, left panel) and values for % delta for trend lines (right panel). VMAXC=1.70 mg/hr/kg $BW^{0.70}$.

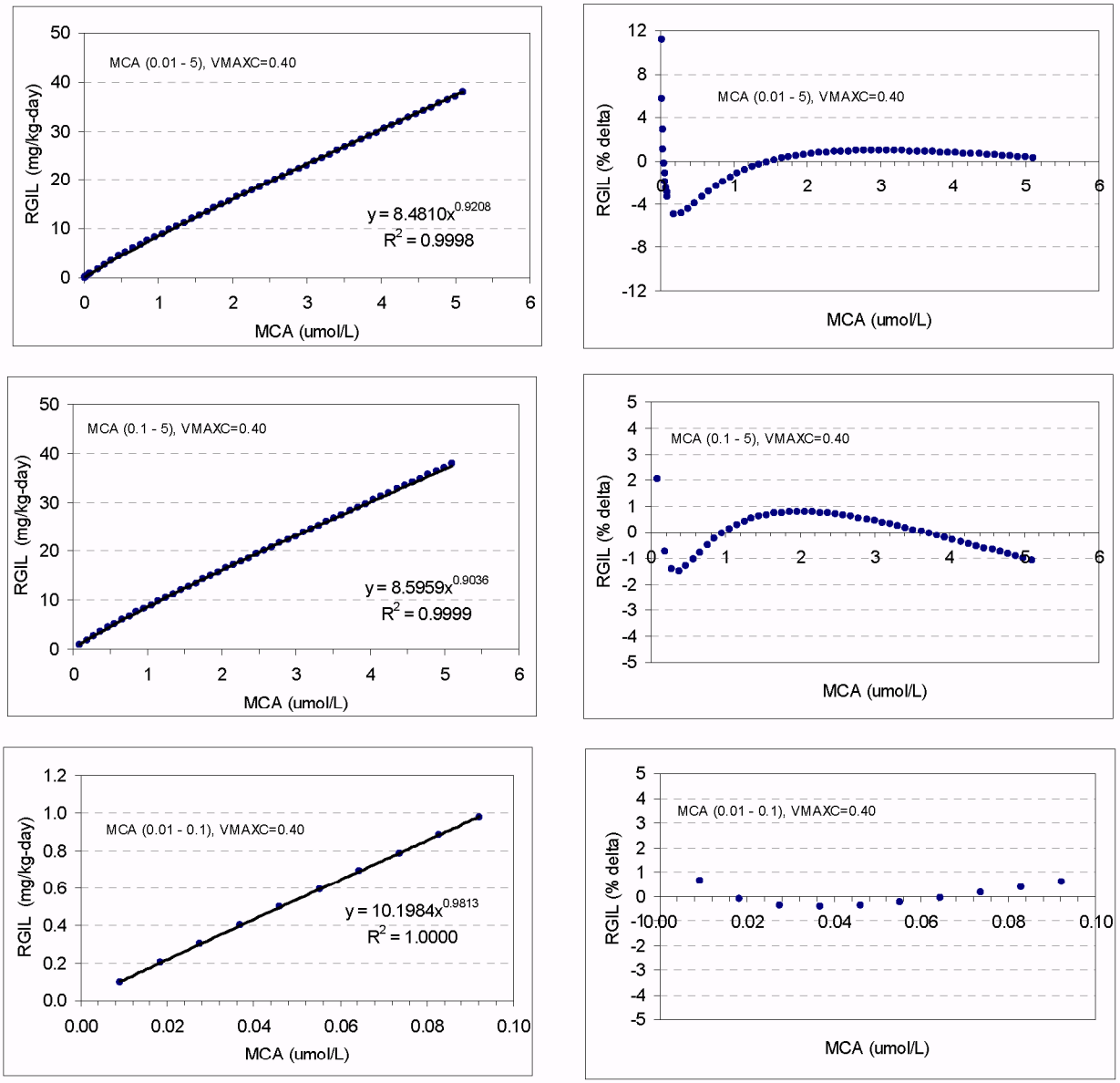


Figure C-9. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent rate of uptake from GI tract to liver (RGIL, left panel) and values for % delta for trend lines (right panel). VMAXC=0.40 mg/hr/kg $BW^{0.70}$.

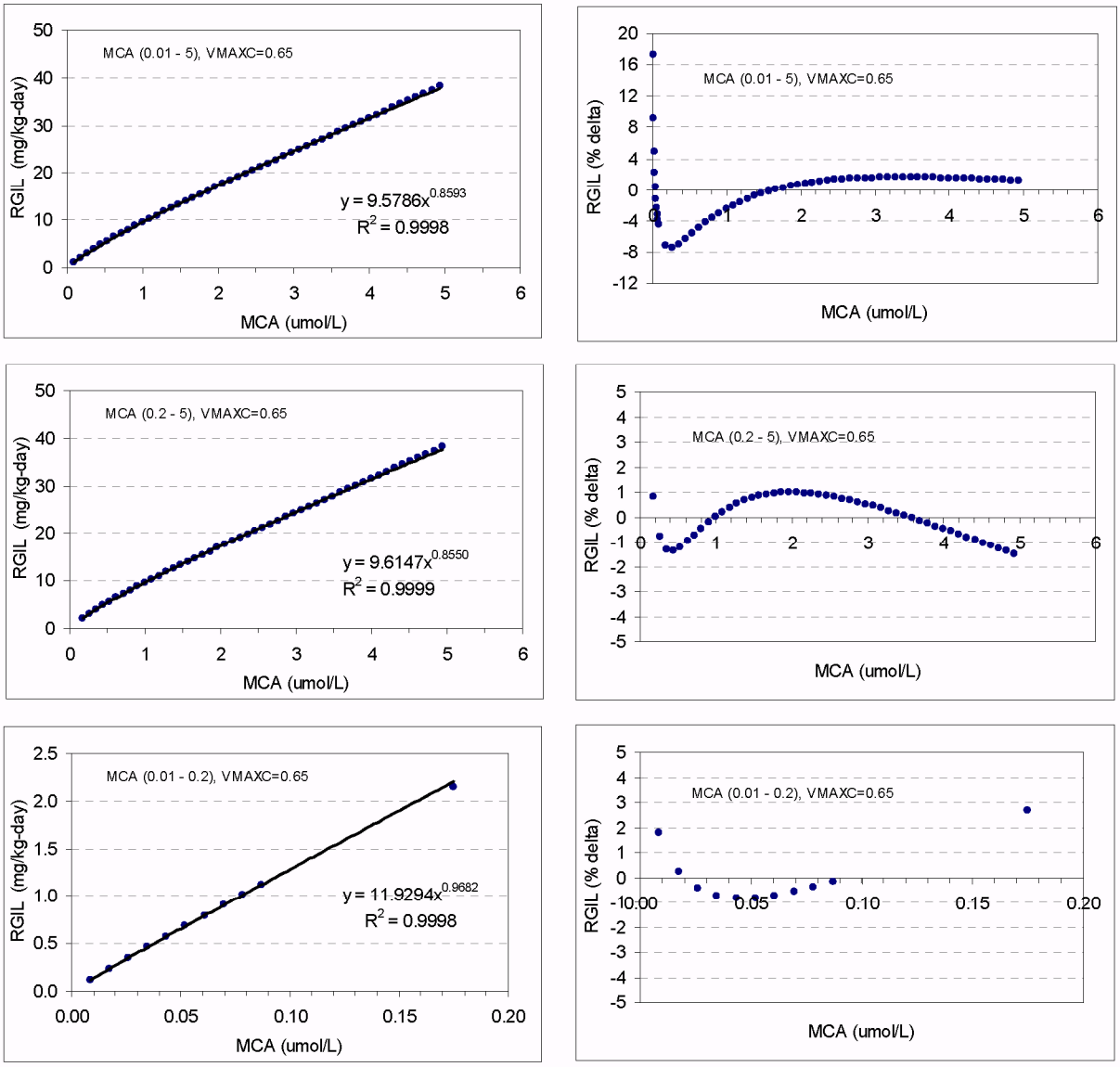


Figure C-10. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent rate of uptake from GI tract to liver (RGIL, left panel) and values for % delta for trend lines (right panel). VMAXC=0.65 mg/hr/kg $BW^{0.70}$.

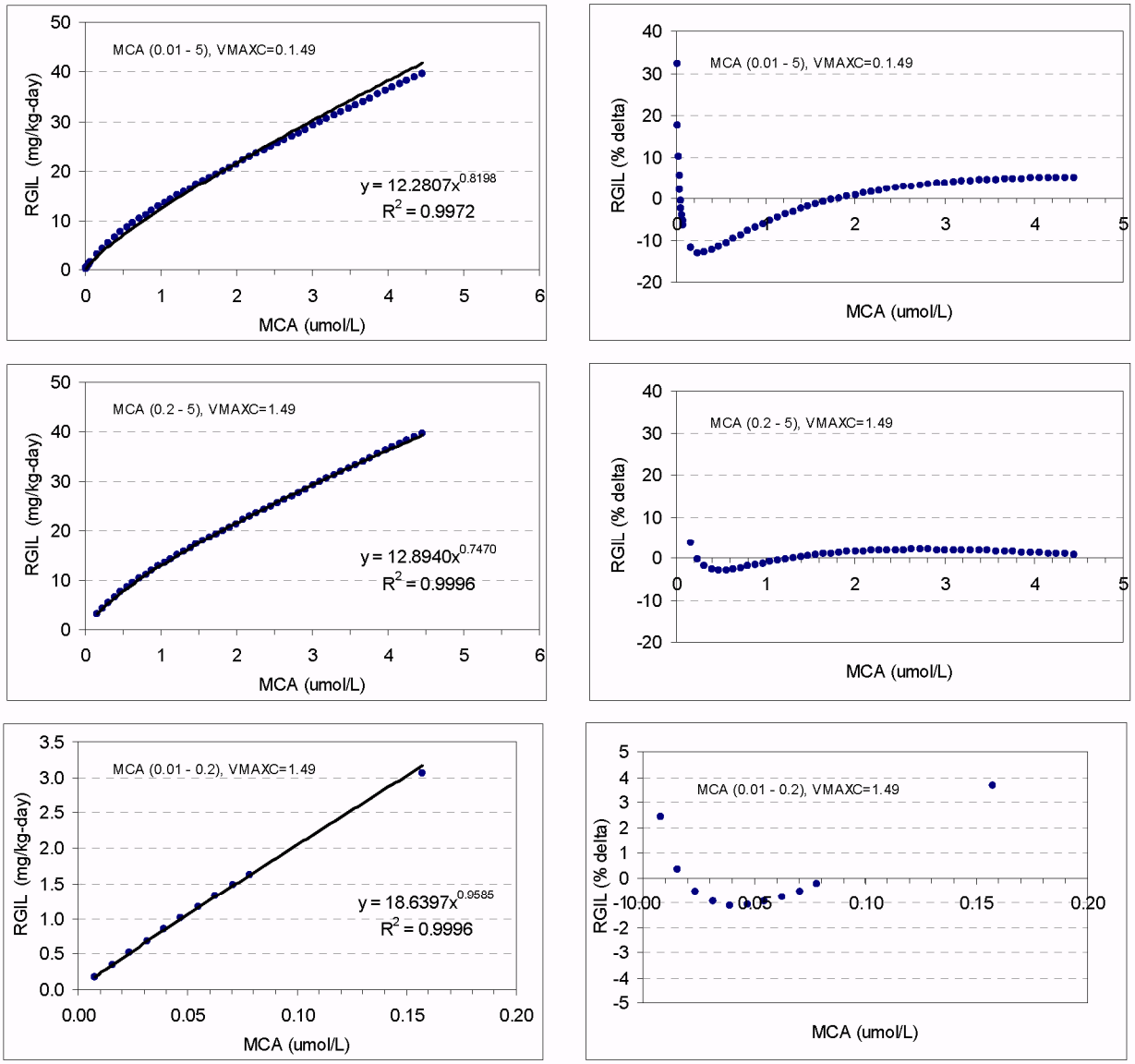


Figure C-11. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent rate of uptake from GI tract to liver (RGIL, left panel) and values for % delta for trend lines (right panel). VMAXC=1.49 mg/hr/kg $BW^{0.70}$.

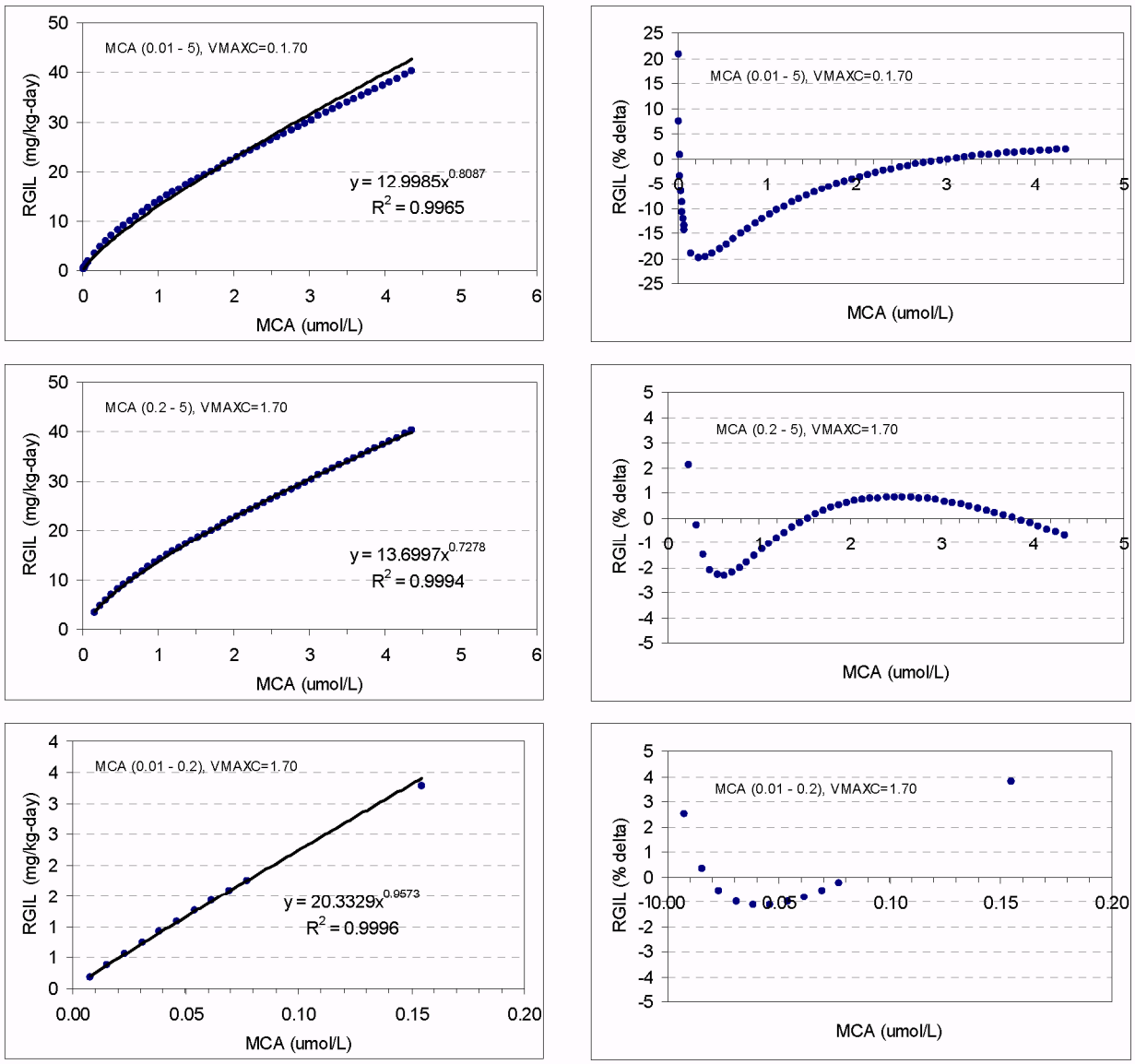


Figure C-12. Relationship between internal dose metric MCA (time-averaged arterial blood concentration of carbon tetrachloride) and equivalent rate of uptake from GI tract to liver (RGIL, left panel) and values for % delta for trend lines (right panel). VMAXC=1.70 mg/hr/kg $BW^{0.70}$.

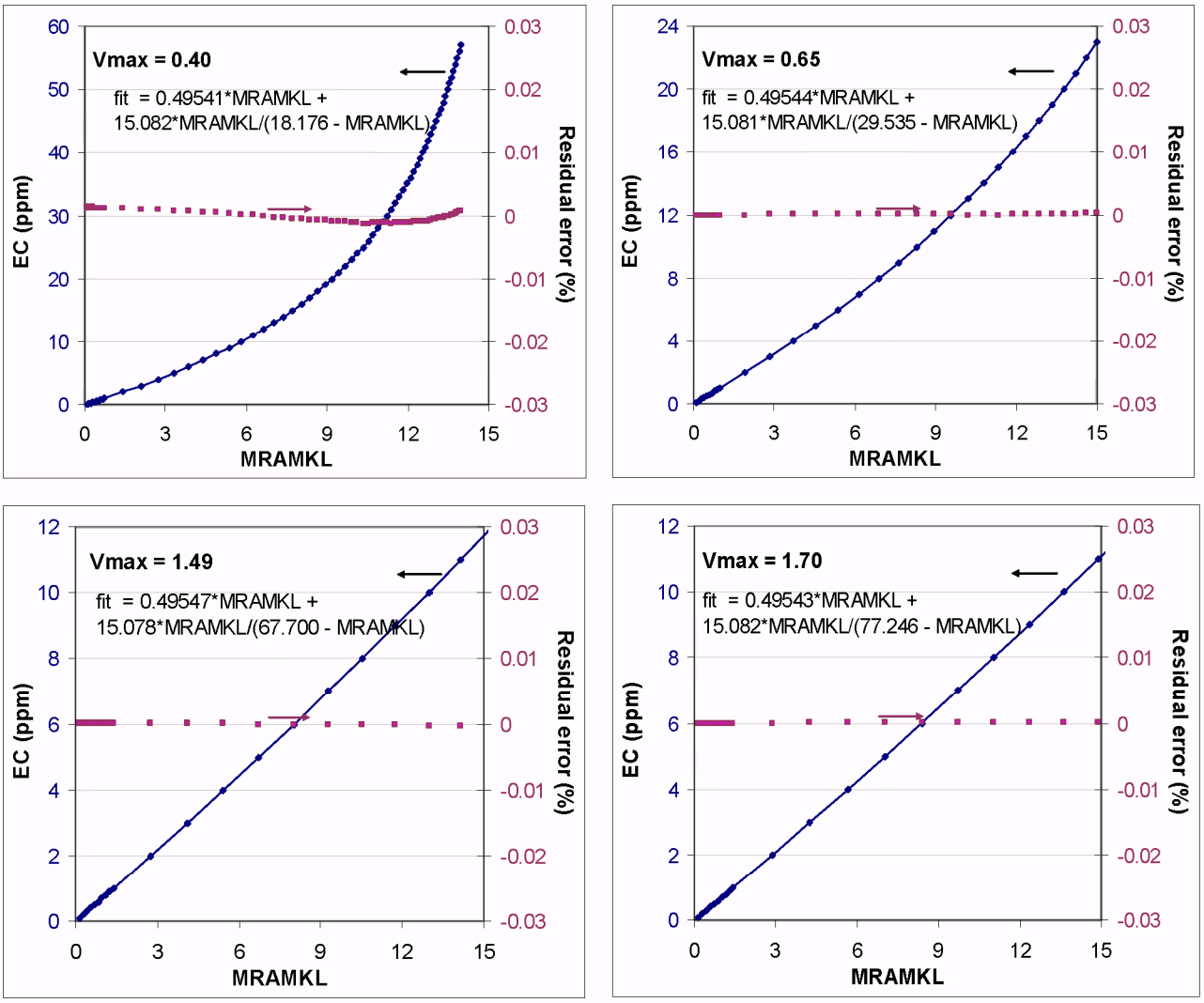


Figure C-13. Relationship between internal dose metric MRAMKL (mean rate of carbon tetrachloride metabolism in the liver) and equivalent exposure concentration (EC) and values for % delta for trend lines.

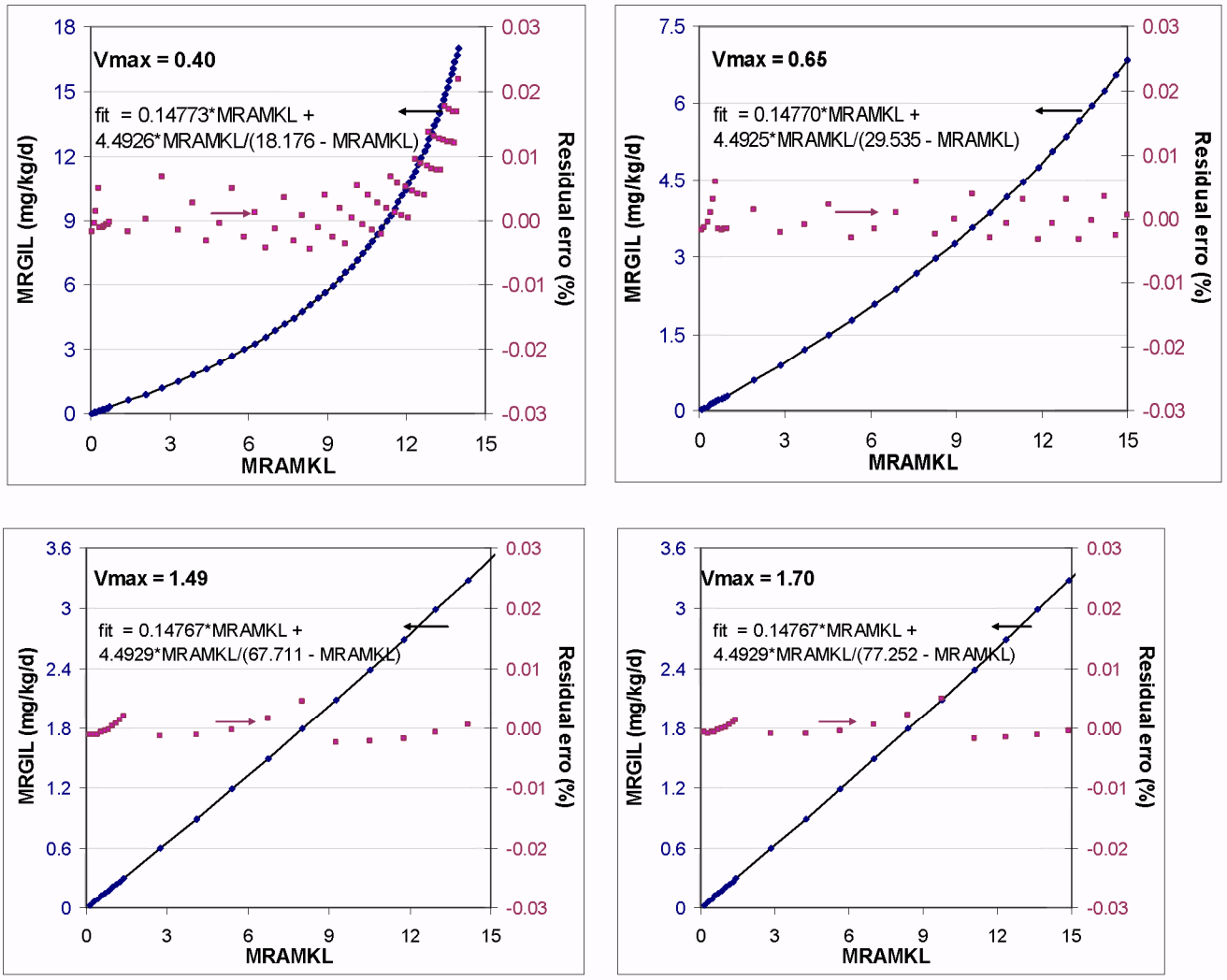


Figure C-14. Relationship between internal dose metric MRAMKL (mean rate of carbon tetrachloride metabolism in the liver) and equivalent rate of uptake from GI tract to liver (RGIL) and values for % delta for trend lines.

C.4. Sensitivity Analysis

Univariate sensitivity analysis consisted of running the model after perturbing values for single parameters by a factor of 0.01, in the up and down directions. Parameter sensitivities were assessed from comparison of standardized sensitivity coefficients:

$$SC = f'(x) = \frac{f(x+\Delta x) - f(x-\Delta)}{2\Delta x} \cdot \frac{x}{f(x)} \qquad \text{Eq. (1)}$$

where SC is the standardized sensitivity coefficient, f(x) is the output variable at parameter value x, and) is the perturbation of x (i.e., 0.01x). Figures C-15 and C-16 show sensitivity coefficients for each internal dose metric (i.e., MCA, MRAMKB) for the human model. Absolute values of sensitivity coefficients that were ≥0.01 are shown in these figures. Conversion to absolute value removes information on the direction of the change in the output variable, allowing the magnitudes of the influence of each parameter on the output variable to be directly compared. Parameters having sensitivity coefficients ≥0.1 can be considered to be highly influential parameters. Chemical parameters in this category (i.e., sensitivity coefficient ≥0.1) are shown in Table C-12 (indicated with +). The mouse and rat models yielded the same rank order of sensitivity coefficients as the human model.

Table C-12. Sensitive parameters (indicated with +) in the human model

Parameter	Definition	Internal Dose Metric	
		MCA	MRAMKB
PB	Blood:air partition coefficient	+	
PL	Liver:blood partition coefficient		+
PF	Fat:blood partition coefficient		
PS	Slowly-perfused:blood partition coefficient		+
PR	Readily-perfused:blood partition coefficient		+
V_{maxC}	Maximum rate of metabolism (mg/hour-kg BW)	+	+
K_m	Michaelis-Menten coefficient for metabolism (mg/L)	+	

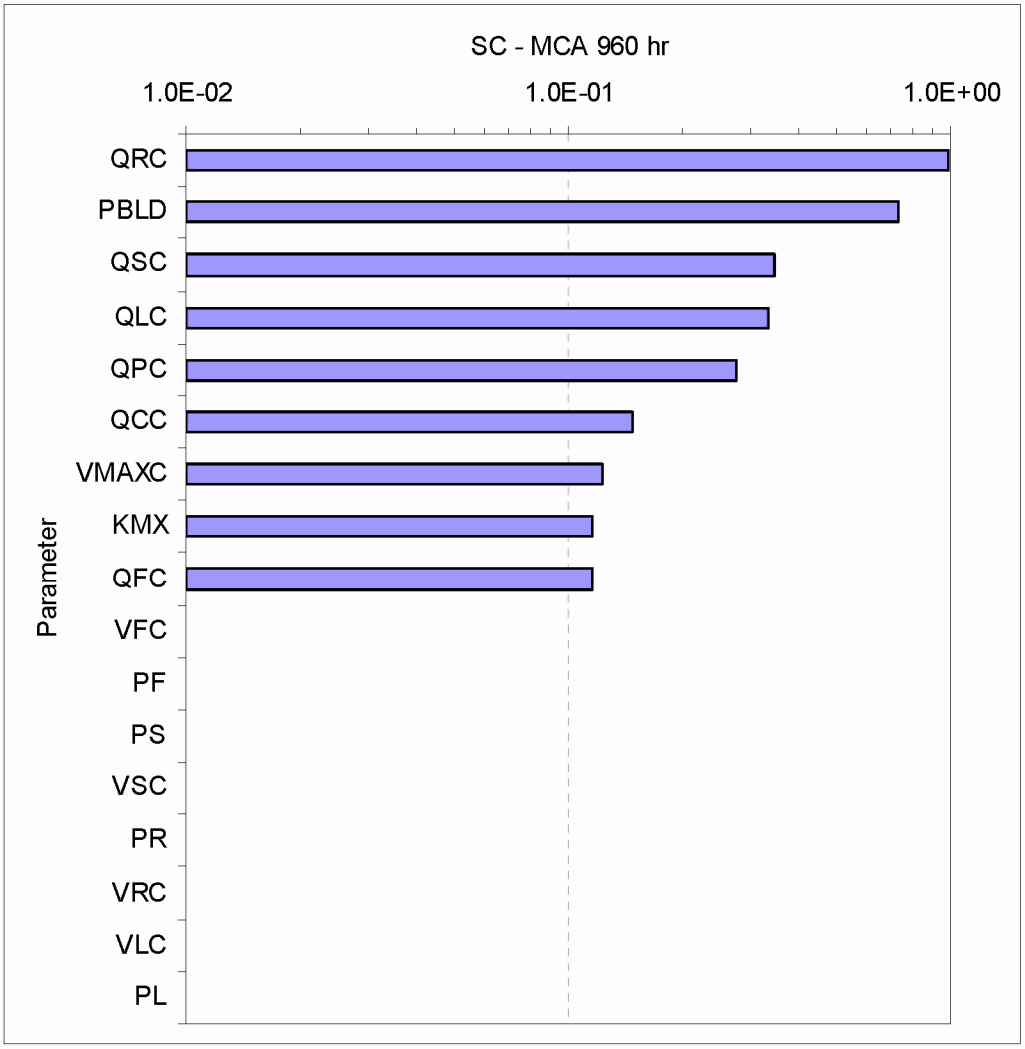


Figure C-15. Standardized sensitivity coefficients for the MCA dose metric (average concentration of carbon tetrachloride in blood, µmol/L) simulated with the human carbon tetrachloride PBPK model.
Absolute values of coefficients ≥0.01 are shown. The simulation was of a continuous exposure to 2.5 ppm for 980 hours (rank order of sensitivity coefficients was not dependent on exposure time).

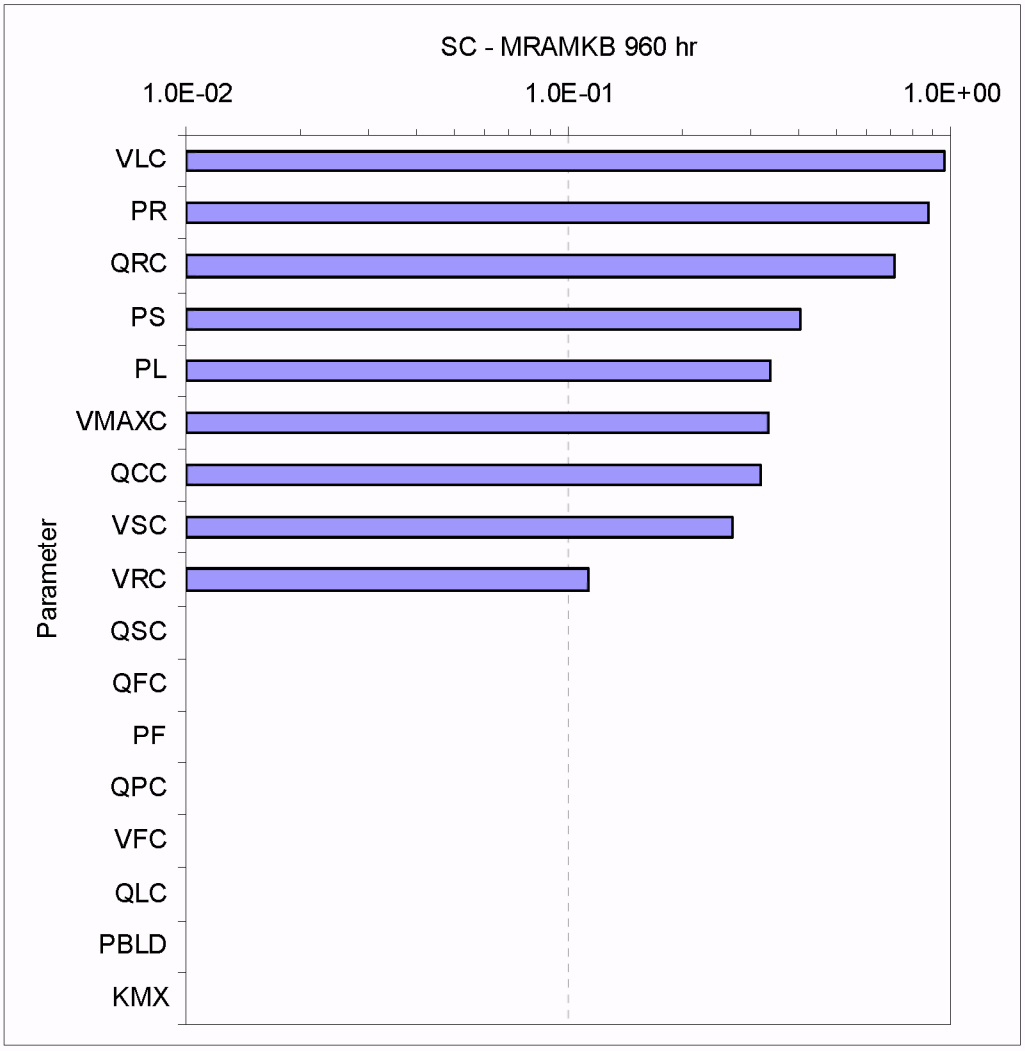


Figure C-16. Standardized sensitivity coefficients for the MRAMKB dose metric (average rate of metabolism of carbon tetrachloride µmol/hr/kg body weight) simulated with the human carbon tetrachloride PBPK model. Absolute values of coefficients ≥0.01 are shown. The simulation was of a continuous exposure to 2.5 ppm for 980 hours (rank order of sensitivity coefficients was not dependent on exposure time).

APPENDIX D. BENCHMARK DOSE MODELING FOR DERIVING THE RfC

MALE RAT:

Incidence data for fatty changes of the liver
Male F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25, 125 ppm***
BMR = 10%

Model	V_{max} = 0.4				V_{max} = 0.65			
	AIC	χ^2 *p* value[a]	BMC_{10}	$BMCL_{10}$	AIC	χ^2 *p* value[a]	BMC_{10}	$BMCL_{10}$
MCA (µmol/L)								
Gamma[b]	144.336	0.0007	0.0793248	0.0551873	144.772	0.0005	0.0689847	0.051179
Logistic[c]	155.104	0.0000	0.170834	0.137191	156.51	0.0000	0.157857	0.126743
Log-Logistic[c]	**137.403**	**0.4355**	**0.136715**	**0.0790319**	**137.463**	**0.4087**	**0.123076**	**0.0707077**
Multistage 1-degree[d, e]	142.388	0.0074	0.0714015	0.0550523	142.778	0.0031	0.0665234	0.0511645
Probit[c]	169.521	0.0000	0.22329	0.17626	171.234	0.0000	0.21463	0.168317
Log-probit[c]	138.408	0.1761	0.124953	0.0755939	138.529	0.1581	0.112257	0.0803264
Quantal-linear	142.388	0.0074	0.0714017	0.0550523	142.778	0.0031	0.0665234	0.0511645
Weibull[b]	142.388	0.0074	0.0714016	0.0550523	142.778	0.0031	0.0665235	0.0511645
MRAMKL (µmol/hr-kg liver)								
Gamma[b]	137.468	0.4177	3.98707	2.6343	137.338	0.4760	5.31098	3.35649
Logistic[c]	**136.747**	**0.3444**	**3.25675**	**2.58557**	**136.513**	**0.3671**	**4.60057**	**3.65284**
Log-Logistic[c]	136.933	0.8012	4.56744	3.08461	136.996	0.7246	6.20422	4.00273
Multistage 2-degree[e, f]	137.073	0.2702	3.55184	2.02617	138.991	0.0944	4.99656	2.5022
Probit[c]	138.891	0.0826	2.97807	2.41619	138.712	0.0728	4.23817	3.44383
Log-probit[c]	136.871	0.9538	4.27176	3.06539	136.872	0.9470	5.73628	3.97844
Quantal-linear	151.674	0.0008	1.01942	0.831472	148.898	0.0025	1.45532	1.18412
Weibull[b]	138.997	0.1316	3.34831	2.18252	138.601	0.1751	4.4781	2.81908

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Used smallest degree polynomial available with an adequate fit; the 2- and 3-degree polynomials provided the same fit as the 1-degree.
[d]Betas restricted to >0.
[f]Used smallest degree polynomial available with an adequate fit; the 3-degree polynomial provided the same fit as the 2-degree.

FEMALE RAT:

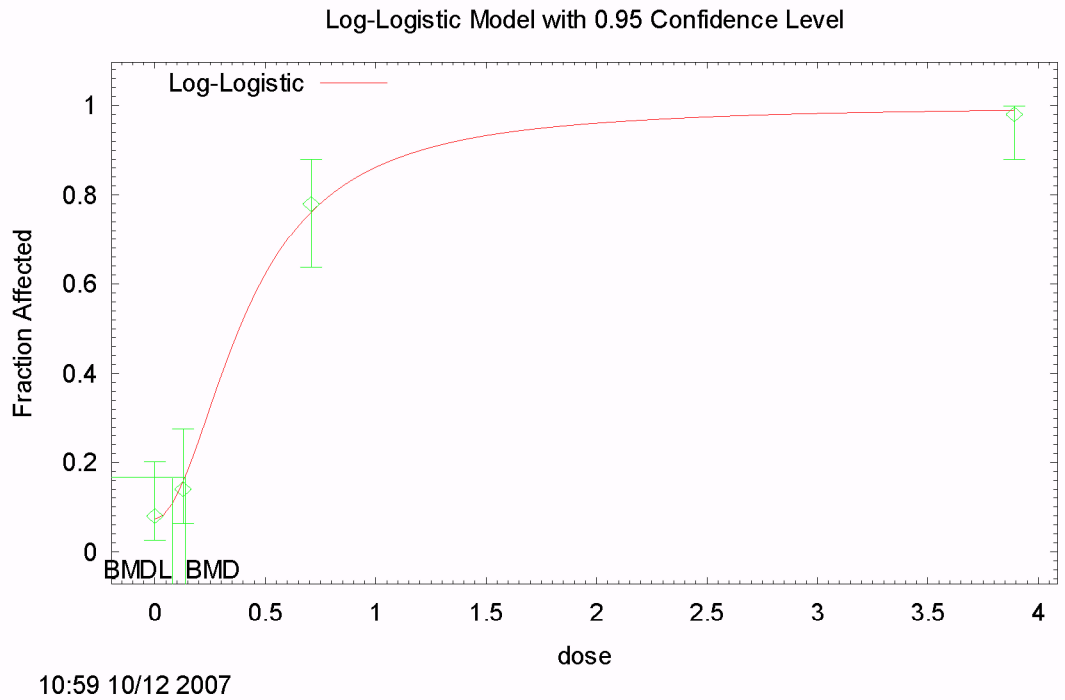
Incidence data for fatty changes of the liver ***Female F344 rats*** exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week) Doses modeled: ***0, 5, 25, 125 ppm*** BMR = 10%
None of the models in BMDS provided an adequate fit of the female rat data.

Incidence data for fatty changes of the liver
Female F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25 ppm*** [high dose dropped]
BMR = 10%

Model	**V_{max} = 0.4**				**V_{max} = 0.65**			
	AIC	**χ^2 *p* value[a]**	**BMC_{10}**	**$BMCL_{10}$**	**AIC**	**χ^2 *p* value[a]**	**BMC_{10}**	**$BMCL_{10}$**
MCA (µmol/L)								
Gamma[b]	92.9928	NA	0.187771	0.107455	92.9928	NA	0.170979	0.0971536
Logistic[c]	93.4185	0.1121	0.106984	0.0803379	93.3172	0.1201	0.0979754	0.0734707
Log-Logistic[c]	92.9928	NA	0.182663	0.111838	92.9928	NA	0.166144	0.101213
Multistage[d, e]	**2nd degree** **92.4089**	**0.2442**	**0.123631**	**0.0851972**	**2nd degree** **92.3049**	**0.2617**	**0.113721**	**0.0775873**
	3rd degree 94.9928	NA	0.213915	0.090506	3rd degree 92.9928	NA	0.195194	0.08177
Probit[c]	93.6833	0.0968	0.100288	0.0779817	93.5689	0.1043	0.0919928	0.0714911
Log-probit[c]	92.9928	NA	0.174053	0.112578	92.9928	NA	0.158234	0.101889
Quantal-linear	111.424	0.0000	0.0363563	0.0277405	111.025	0.0001	0.0332712	0.0253689
Weibull[b]	92.9928	NA	0.213201	0.102923	92.9928	NA	0.194228	0.0930656
MRAMKL (µmol/hr-kg liver)								
Gamma[b]	92.9928	NA	4.85516	3.42634	92.9928	NA	6.52318	4.43018
Logistic[c]	99.7262	0.0020	2.45785	1.90371	97.8675	0.0064	3.34536	2.58247
Log-Logistic[c]	92.9928	NA	4.84705	3.48106	92.9928	NA	6.48806	4.51798
Multistage[d, e]	2nd degree 100.7	0.0039	2.43344	1.99357	2nd degree 98.1134	0.0124	3.42266	2.75565
	3rd degree **92.2866**	**0.2650**	**3.76974**	**2.82488**	**3rd degree** **91.5964**	**0.4421**	**5.42354**	**3.74923**
Probit[c]	100.988	0.0013	2.16088	1.70134	98.8142	0.0044	2.98448	2.34695
Log-probit[c]	92.9928	NA	4.69168	3.49658	92.9928	NA	6.26103	4.54001
Quantal-linear	127.034	0.0000	0.817323	0.634088	123.548	0.0000	1.12472	0.870515
Weibull[b]	92.9928	NA	5.3798	3.29131	92.9928	NA	7.27174	4.24944

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Used smallest degree polynomial available with an adequate fit.
[e]Betas restricted to >0.

Male Rat
Dose metric: MCA
Vmax = 0.4 mg/hr/kg BW$^{0.07}$

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                                                    Fri Oct 12 10:59:34 2007
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 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = FattyLiver
   Independent variable = umol/L
   Slope parameter is not restricted

   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 Parameter Values
                     background =         0.08
                      intercept =      1.42536
                          slope =      1.89476
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Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.077	0.34
intercept	-0.077	1	0.54
slope	0.34	0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.073606	*	*	*
intercept	1.74202	*	*	*
slope	1.97967	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-65.434	4			
Fitted model	-65.7017	3	0.535433	1	0.4643
Reduced model	-138.619	1	146.371	3	<.0001

AIC: 137.403

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0736	3.680	4	50	0.173
0.1280	0.1559	7.796	7	50	-0.310
0.7080	0.7614	38.068	39	50	0.309
3.8920	0.9891	49.456	49	50	-0.621

Chi^2 = 0.61 d.f. = 1 P-value = 0.4355

Benchmark Dose Computation

Specified effect = 0.1

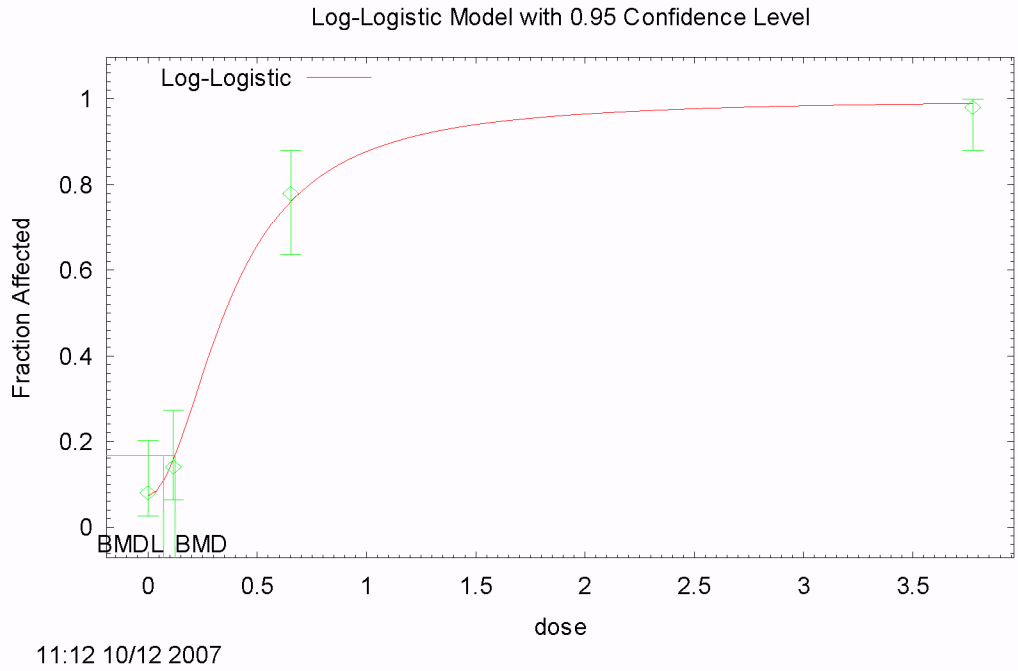
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.136715

BMDL = 0.0790319

Male Rat
Dose metric: MCA
Vmax = 0.65 mg/hr/kg BW$^{0.07}$




```
=====================================================================
          Logistic Model. (Version: 2.9; Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\MALE RAT\MCA-
VMAX=0.65\RAT-FATTYLIVER-MCA-65.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\MALE
RAT\MCA-VMAX=0.65\RAT-FATTYLIVER-MCA-65.plt
                                                        Fri Oct 12 11:12:25 2007
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = FattyLiver
   Independent variable = umol/L
   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 Parameter Values
                     background =         0.08
                      intercept =      1.54201
                          slope =      1.85672
```

Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.05	0.33
intercept	-0.05	1	0.6
slope	0.33	0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0733292	*	*	*
intercept	1.88323	*	*	*
slope	1.94775	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-65.434	4			
Fitted model	-65.7316	3	0.595159	1	0.4404
Reduced model	-138.619	1	146.371	3	<.0001

AIC: 137.463

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0733	3.666	4	50	0.181
0.1160	0.1568	7.841	7	50	-0.327
0.6530	0.7603	38.017	39	50	0.326
3.7750	0.9895	49.476	49	50	-0.661

Chi^2 = 0.68 d.f. = 1 P-value = 0.4087

Benchmark Dose Computation

Specified effect = 0.1

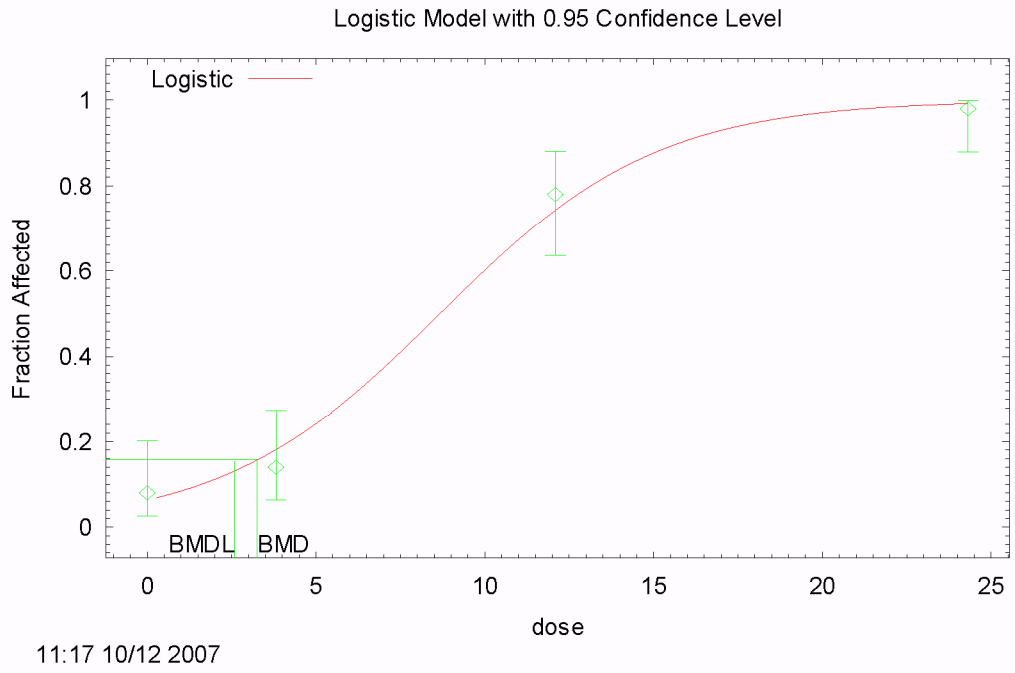
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.123076

BMDL = 0.0707077

Male Rat
Dose metric: MRAMKL
Vmax = 0.4 mg/hr/kg BW$^{0.07}$




```
=====================================================================
          Logistic Model. (Version: 2.9; Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\MALE RAT\MRAMKL-
VMAX=0.4\FATTY_LIVER_MRAMKL-4.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\MALE
RAT\MRAMKL-VMAX=0.4\FATTY_LIVER_MRAMKL-4.plt
                                                    Fri Oct 12 11:17:49 2007
 =====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]

   Dependent variable = FattyLiver
   Independent variable = umol/hr-kgL
   Slope parameter is not restricted

   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


                  Default Initial Parameter Values
                     background =            0   Specified
                      intercept =     -2.35241
                          slope =     0.249767


           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	-0.82
slope	-0.82	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-2.68587	0.383165	-3.43685	-1.93488
slope	0.309634	0.0415113	0.228273	0.390994

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-65.434	4			
Fitted model	-66.3737	2	1.87944	2	0.3907
Reduced model	-138.619	1	146.371	3	<.0001

AIC: 136.747

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0638	3.191	4	50	0.468
3.8130	0.1816	9.082	7	50	-0.764
12.0920	0.7424	37.118	39	50	0.609
24.3200	0.9922	49.609	49	50	-0.979

Chi^2 = 2.13 d.f. = 2 P-value = 0.3444

Benchmark Dose Computation

Specified effect = 0.1

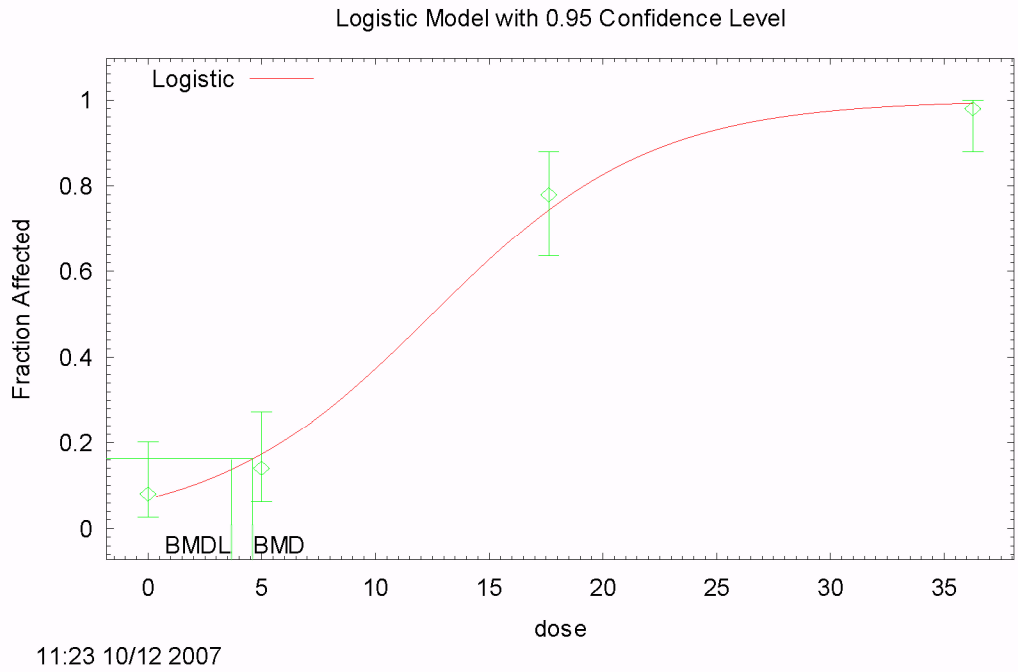
Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.25675

BMDL = 2.58557

Male Rat
Dose metric: MRAMKL
Vmax = 0.65 mg/hr/kg $BW^{0.07}$




```
=====================================================================
          Logistic Model. (Version: 2.9; Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\MALE RAT\MRAMKL-
VMAX=0.65\MRAT_FATTY_LIVER_MRAMKL-65.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\MALE
RAT\MRAMKL-VMAX=0.65\MRAT_FATTY_LIVER_MRAMKL-65.plt
                                                        Fri Oct 12 11:23:29 2007
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]


   Dependent variable = FattyLiver
   Independent variable = umol/hr-kgL
   Slope parameter is not restricted

   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


                  Default Initial Parameter Values
                     background =            0   Specified
                      intercept =     -2.28912
                          slope =     0.166325


            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         -0.8

     slope        -0.8            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-2.59592	0.370821	-3.32272	-1.86913
slope	0.207777	0.0278282	0.153235	0.26232

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-65.434	4			
Fitted model	-66.2567	2	1.64536	2	0.4393
Reduced model	-138.619	1	146.371	3	<.0001

AIC: 136.513

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0694	3.470	4	50	0.295
4.9910	0.1738	8.690	7	50	-0.631
17.6260	0.7439	37.195	39	50	0.585
36.2660	0.9929	49.645	49	50	-1.085

Chi^2 = 2.00 d.f. = 2 P-value = 0.3671

Benchmark Dose Computation

Specified effect = 0.1

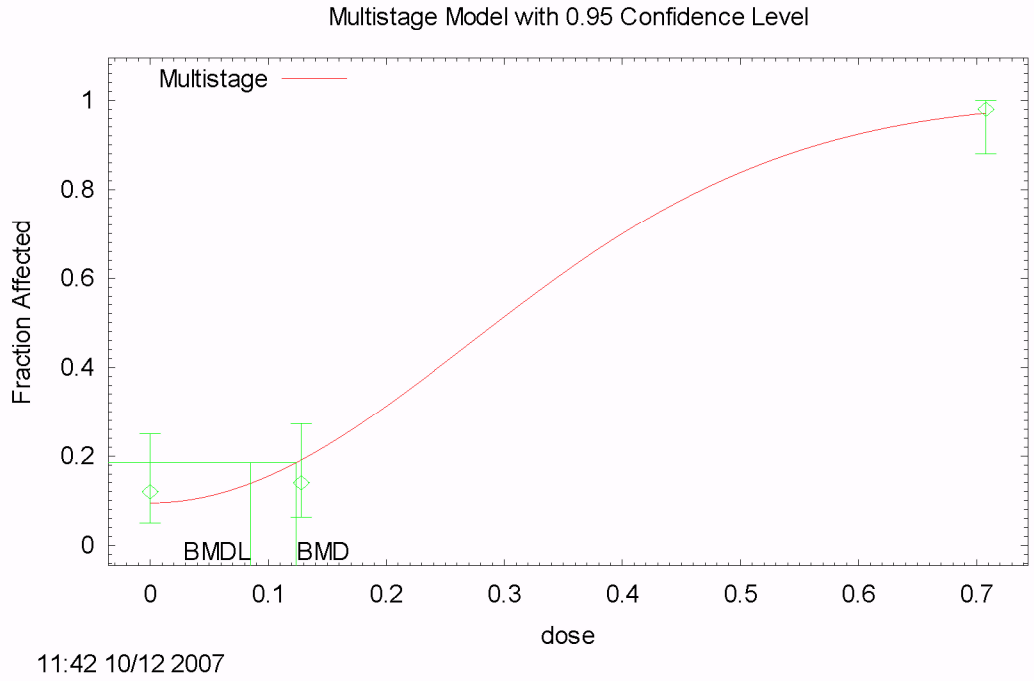
Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.60057

BMDL = 3.65284

Female Rat
Dose metric: MCA
Vmax = 0.4 mg/hr/kg BW$^{0.07}$




```
=====================================================================
          Multistage Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE RAT\MCA-
VMAX=0.4\FRAT-FATTYLIVER-MCA-4.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE
RAT\MCA-VMAX=0.4\FRAT-FATTYLIVER-MCA-4.plt
                                                  Fri Oct 12 11:42:22 2007
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = FattyLiver
   Independent variable = umol/L

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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 =    0.0746099
                        Beta(1) =            0
                        Beta(2) =      7.64624
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.21
Beta(2)	-0.21	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0951491	*	*	*
Beta(1)	0	*	*	*
Beta(2)	6.89319	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-43.4964	3			
Fitted model	-44.2044	2	1.41613	1	0.234
Reduced model	-101.707	1	116.422	2	<.0001

AIC: 92.4089

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0951	4.757	6	50	0.599
0.1280	0.1918	9.589	7	50	-0.930
0.7080	0.9714	48.571	49	50	0.364

Chi^2 = 1.36 d.f. = 1 P-value = 0.2442

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

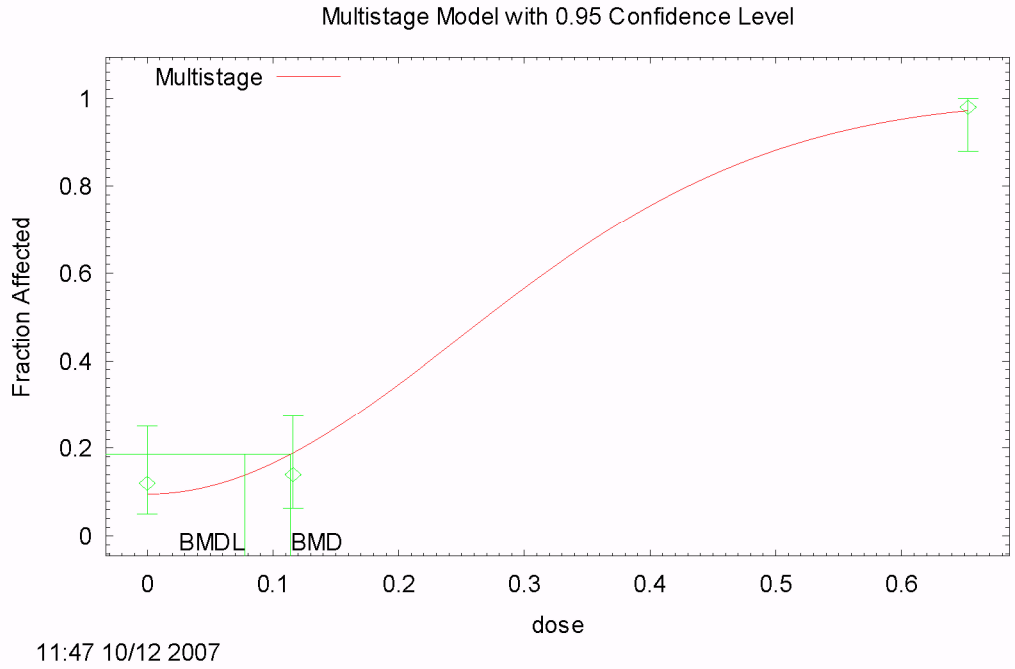
BMD = 0.123631

BMDL = 0.0851972

BMDU = 0.148857

Taken together, (0.0851972, 0.148857) is a 90 % two-sided confidence interval for the BMD

Female Rat
Dose metric: MCA
Vmax = 0.65 mg/hr/kg BW$^{0.07}$




```
=================================================================
          Multistage Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE RAT\MCA-
VMAX=0.65\FRAT-FATTYLIVER-MCA-65.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE
RAT\MCA-VMAX=0.65\FRAT-FATTYLIVER-MCA-65.plt
                                                    Fri Oct 12 11:47:23 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = FattyLiver
   Independent variable = umol/L

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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 =    0.0765787
                        Beta(1) =            0
                        Beta(2) =      8.98383
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.21
Beta(2)	-0.21	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.095736	*	*	*
Beta(1)	0	*	*	*
Beta(2)	8.14699	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-43.4964	3			
Fitted model	-44.1525	2	1.31215	1	0.252
Reduced model	-101.707	1	116.422	2	<.0001

AIC: 92.3049

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0957	4.787	6	50	0.583
0.1160	0.1896	9.481	7	50	-0.895
0.6530	0.9720	48.599	49	50	0.344

Chi^2 = 1.26 d.f. = 1 P-value = 0.2617

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

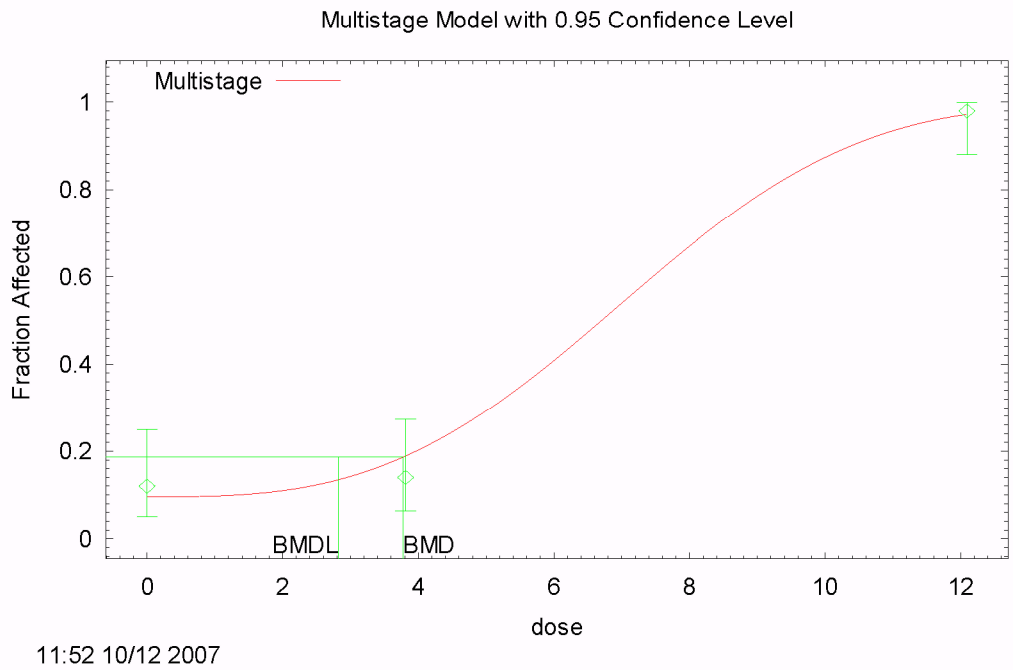
BMD = 0.113721

BMDL = 0.0775873

BMDU = 0.137047

Taken together, (0.0775873, 0.137047) is a 90 % two-sided confidence interval for the BMD

Female Rat
Dose metric: MRAMKL
Vmax = 0.4 mg/hr/kg BW$^{0.07}$




```
=====================================================================
          Multistage Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE
RAT\MRAMKL-VMAX=0.4\FRAT_FATTY_LIVER_MRAMKL-4.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE
RAT\MRAMKL-VMAX=0.4\FRAT_FATTY_LIVER_MRAMKL-4.plt
                                                  Fri Oct 12 11:52:42 2007
 ====================================================================

 BMDS MODEL RUN
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Observation # < parameter # for Multistage model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = FattyLiver
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 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 =    0.0769299
                         Beta(1) =            0
```

```
                    Beta(2) =            0
                    Beta(3) =   0.00216647


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Beta(1)    -Beta(2)
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            Background      Beta(3)

Background           1        -0.21

   Beta(3)       -0.21            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background        0.0958436            *               *                   *
       Beta(1)                0            *               *                   *
       Beta(2)                0            *               *                   *
       Beta(3)       0.00196673            *               *                   *

* - Indicates that this value is not calculated.


                      Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -43.4964         3
  Fitted model        -44.1433         2       1.29386      1          0.2553
 Reduced model        -101.707         1       116.422      2         <.0001

          AIC:         92.2866


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------
   0.0000     0.0958         4.792         6          50         0.580
   3.8130     0.1892         9.462         7          50        -0.889
  12.0920     0.9721        48.603        49          50         0.340

 Chi^2 = 1.24      d.f. = 1        P-value = 0.2650


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

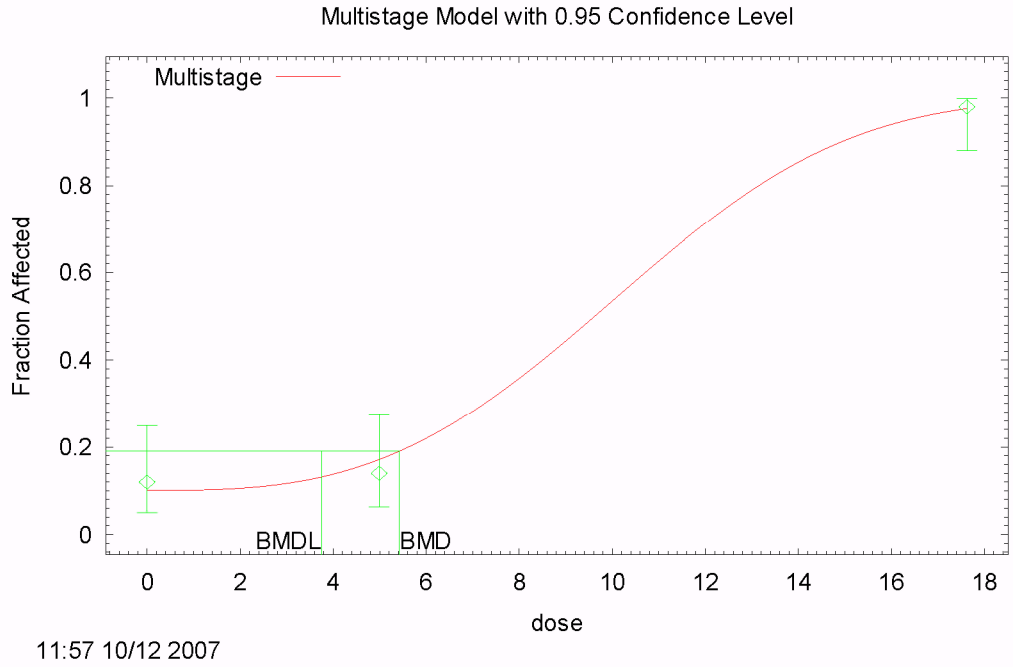
             BMD =        3.76974

            BMDL =        2.82488

            BMDU =        4.26949

Taken together, (2.82488, 4.26949) is a 90     % two-sided confidence
interval for the BMD
```

Female Rat
Dose metric: MRAMKL
Vmax = 0.65 mg/hr/kg BW$^{0.07}$




```
=====================================================================
          Multistage Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE
RAT\MRAMKL-VMAX=0.65\FRAT_FATTY_LIVER_MRAMKL-65.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\FEMALE
RAT\MRAMKL-VMAX=0.65\FRAT_FATTY_LIVER_MRAMKL-65.plt
                                                    Fri Oct 12 11:57:06 2007
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Observation # < parameter # for Multistage model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = FattyLiver
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3

 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
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) =  0.000714264
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(3)
Background	1	-0.19
Beta(3)	-0.19	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.101433	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0.000660435	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-43.4964	3			
Fitted model	-43.7982	2	0.603632	1	0.4372
Reduced model	-101.707	1	116.422	2	<.0001

AIC: 91.5964

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1014	5.072	6	50	0.435
4.9910	0.1723	8.613	7	50	-0.604
17.6260	0.9759	48.793	49	50	0.191

Chi^2 = 0.59 d.f. = 1 P-value = 0.4421

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 5.42354

BMDL = 3.74923

BMDU = 6.17189

Taken together, (3.74923, 6.17189) is a 90 % two-sided confidence
interval for the BMD

APPENDIX E. CANCER ASSESSMENT: BMD MODELING OUTPUTS FOR LOW-DOSE LINEAR EXTRAPOLATION APPROACH

E.1. Benchmark Dose Analysis

Liver tumors (adenoma + carcinoma)
Female F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25, 125 ppm***
Multistage; MCA: 2-stage model MRAMKL: 4-stage model

BMR (extra risk)	V_{max} = 0.4					V_{max} = 0.65				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
0.05	61.6602	0.9842	0.609955	0.387377	0.129	61.5904	0.9916	0.588686	0.354766	0.141
MRAMKL (µmol/hr-kg liver)										
0.05	63.3399	0.6503	9.8151	8.40334	0.00595	62.8343	0.7440	14.582	12.2867	0.00407

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the Π^2 test.

Liver tumors (adenoma + carcinoma)
Female F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25 ppm***
Multistage; 2-stage model

BMR (extra risk)	V_{max} = 0.4					V_{max} = 0.65				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
0.05	24.8957	0.9507	0.655398	0.345984	0.144	24.8889	0.9523	0.604144	0.317726	0.157
MRAMKL (µmol/hr-kg liver)										
0.05	25.2825	0.8571	11.5604	6.92352	0.00722	25.1734	0.8831	16.6986	9.76339	0.00512

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.
Note: 3-stage model did not provide a sufficiently improved model fit.

Liver tumors (adenoma + carcinoma)
Female BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25 ppm***
Multistage; MCA: 2-stage model MRAMKL: 2-stage model

BMR (extra risk)	Fisher					Thrall				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
0.1	117.307	NA	0.10186	0.0467576	2.14	117.307	NA	0.194624	0.0885305	1.13
MRAMKL (µmol/hr-kg liver)										
0.1	115.912	0.4437	9.70893	6.3204	0.0158	117.341	0.1654	10.4557	7.59255	0.0132

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the Π^2 test.
Note: 3-stage model did not provide a sufficiently improved model fit.

Liver tumors (adenoma + carcinoma)
Female BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5 ppm***
Multistage; 2-stage model

BMR (extra risk)	Fisher					Thrall				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
0.1	80.6149	NA	0.101967	0.044224	2.26	80.6149	NA	0.195666	0.0848621	1.18
MRAMKL (µmol/hr-kg liver)										
0.1	80.6149	NA	11.6352	5.04631	0.0198	80.6149	NA	14.1982	6.15788	0.0162

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.

Liver tumors (adenoma + carcinoma)
Male BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25 ppm***
Note: models could not fit data with all 4 dose groups; highest dose group dropped
BMR = 0.1
Multistage; 3-stage model

BMR (extra risk)	Fisher					Thrall				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
0.1	151.192	0.3562	0.191106	0.063650	1.57	151.158	0.3660	0.388392	0.122027	0.819
MRAMKL (µmol/hr-kg liver)										
0.1	152.089	0.1864	13.3804	7.30705	0.0137	152.924	0.1086	14.185	8.82145	0.0113

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.

Pheochromocytomas
Female BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25, 125 ppm***
Multistage; 2-stage model
BMR = 10%

BMR (extra risk)	Fisher					Thrall				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
0.1	71.4077	0.7947	1.42662	1.13753	0.0879	71.3358	0.8039	2.94801	2.34113	0.0427

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.
Note: 3-stage model did not provide a sufficiently improved model fit.

Pheochromocytomas
Male BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25, 125 ppm***
Cancer Multistage
BMR = 10%
Cancer Multistage (restricted mode) model did not provide an adequate fit of the male pheochromocytoma data (1, 2, and 3 stage models provided the same outputs); therefore other models in BMDS were used (see table below).

BMR (extra risk)	Fisher					Thrall				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
1st, 2nd & 3rd 0.1	139.129	0.0513	0.292123	0.230102	0.435	139.077	0.0488	0.600117	0.472644	0.212

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.

Pheochromocytomas
Male BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Doses modeled: ***0, 5, 25, 125 ppm***
Models other than Multistage
BMR = 0.1

Model	Fisher					Thrall				
	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL	AIC	χ^2 *p* value[a]	BMC	BMCL	BMR/ BMCL
MCA (µmol/L)										
Gamma[b]	139.129	0.0513	0.292124	0.230102	0.435	139.077	0.0488	0.600118	0.472644	0.212
Gamma -- unrestricted	140.755	0.0401	0.238028	0.10463	0.956	140.587	0.0428	0.473653	0.204957	0.488
Logistic[c]	161.228	0.0000	0.929566	0.75614	0.132	161.353	0.0000	1.9184	1.56019	0.064
Logistic -- unrestricted	161.228	0.0000	0.929566	0.75614	0.132	161.353	0.0000	1.9184	1.56019	0.064
Log-logistic[c]	138.661	0.0978	0.24731	0.147398	0.678	138.467	0.1050	0.492945	0.297393	0.336
Log-logistic – unrestricted	138.661	0.0978	0.247311	0.130943	0.764	138.467	0.1050	0.492945	0.257935	0.388
Probit[c]	159.808	0.0000	0.851235	0.702221	0.142	159.949	0.0000	1.75643	1.44878	0.069
Probit – unrestricted	159.808	0.0000	0.851235	0.702221	0.142	159.949	0.0000	1.75643	1.44878	0.069
Log-probit[c]	141.637	0.0044	0.423924	0.340228	0.294	141.988	0.0035	0.867906	0.696011	0.144
Log-probit – unrestricted	**137.136**	**0.1533**	**0.264859**	**0.150882**	**0.663**	**136.945**	**0.1648**	**0.527758**	**0.297349**	**0.336**
Quantal-linear	139.129	0.0513	0.292124	0.230102	0.435	139.077	0.0488	0.60012	0.472644	0.212
Weibull[b]	139.129	0.0513	0.292124	0.230102	0.435	139.077	0.0488	0.60012	0.472644	0.212
Weibull -- unrestricted	140.513	0.0497	0.226525	0.10562	0.947	140.316	0.0535	0.45102	0.207636	0.482

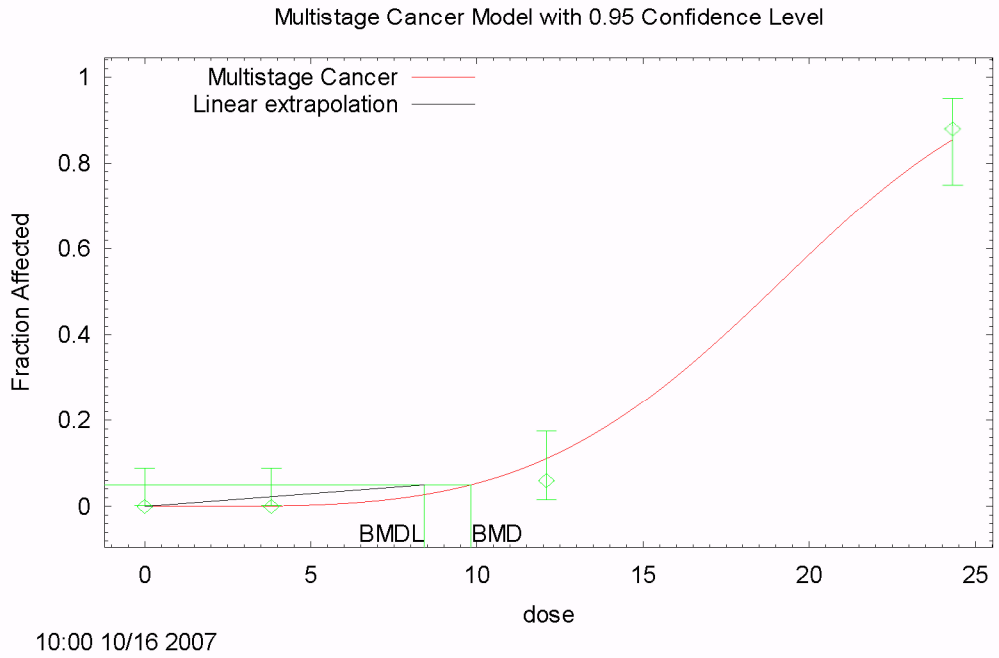
Pheochromocytomas ***Male BDF1 mouse*** exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week) Doses modeled: ***0, 5, 25, 125 ppm*** Models other than Multistage BMR = 0.1										
Model	**Fisher**					**Thrall**				
	AIC	**χ^2 *p* value[a]**	**BMC**	**BMCL**	**BMR/ BMCL**	**AIC**	**χ^2 *p* value[a]**	**BMC**	**BMCL**	**BMR/ BMCL**

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria; *p* value from the χ^2 test.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.

Female F344 rat -- hepatocellular adenomas + carcinomas (0, 5, 25, 125 ppm dose groups)

Dose metric: MRAMKL

Vmax = 0.4 mg/hr/kg $BW^{0.07}$




```
=====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT LIVER\MRAMKL-
VMAX=0.4\FRAT_LIVER_ADCAR_MRAMKL-4.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT
LIVER\MRAMKL-VMAX=0.4\FRAT_LIVER_ADCAR_MRAMKL-4.plt
                                                    Tue Oct 16 10:00:27 2007
 ====================================================================

 BMDS MODEL RUN
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Observation # < parameter # for Multistage Cancer model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4)]

   The parameter betas are restricted to be positive


   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 0
 Degree of polynomial = 4


 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
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) =            0
```

Beta(4) = 6.11699e-006

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) -Beta(2) -Beta(3)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(4)
Beta(4)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0	*	*	*
Beta(4)	5.52689e-006	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-29.6946	4			
Fitted model	-30.67	1	1.95065	3	0.5827
Reduced model	-109.05	1	158.71	3	<.0001

AIC: 63.3399

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	50	0.000
3.8130	0.0012	0.058	0	50	-0.242
12.0920	0.1114	5.572	3	50	-1.156
24.3200	0.8554	42.768	44	50	0.495

Chi^2 = 1.64 d.f. = 3 P-value = 0.6503

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 9.8151

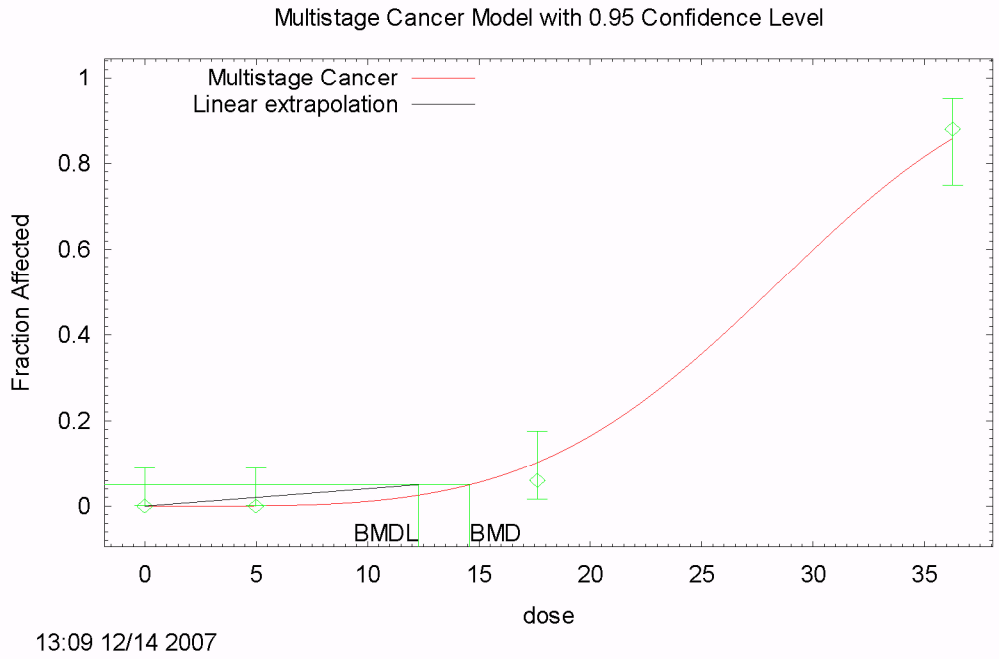
BMDL = 8.40334

BMDU = 10.5331

Taken together, (8.40334, 10.5331) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00595002

Dose metric: MRAMKL
Vmax = 0.65 mg/hr/kg $BW^{0.07}$




```
=====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT LIVER\MRAMKL-
VMAX=0.65\FRAT_LIVER_ADCAR_MRAMKL-65.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT
LIVER\MRAMKL-VMAX=0.65\FRAT_LIVER_ADCAR_MRAMKL-65.plt
                                                    Tue Oct 16 10:05:09 2007
 =====================================================================

 BMDS MODEL RUN
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Observation # < parameter # for Multistage Cancer model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4)]

   The parameter betas are restricted to be positive

   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 0
 Degree of polynomial = 4


 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
                          Beta(1) =            0
```

```
                    Beta(2) =            0
                    Beta(3) =            0
                    Beta(4) = 1.23526e-006


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)    -Beta(3)
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

               Beta(4)

   Beta(4)           1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background                0            *                *                   *
       Beta(1)                0            *                *                   *
       Beta(2)                0            *                *                   *
       Beta(3)                0            *                *                   *
       Beta(4)     1.13446e-006            *                *                   *

* - Indicates that this value is not calculated.


                      Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -29.6946         4
  Fitted model        -30.4171         1       1.44504      3           0.695
 Reduced model         -109.05         1        158.71      3         <.0001

          AIC:         62.8343


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0000         0.000         0          50        0.000
    4.9910     0.0007         0.035         0          50       -0.188
   17.6260     0.1037         5.186         3          50       -1.014
   36.2660     0.8595        42.974        44          50        0.418

 Chi^2 = 1.24      d.f. = 3        P-value = 0.7440


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         14.582

            BMDL =        12.2867

            BMDU =        15.6526

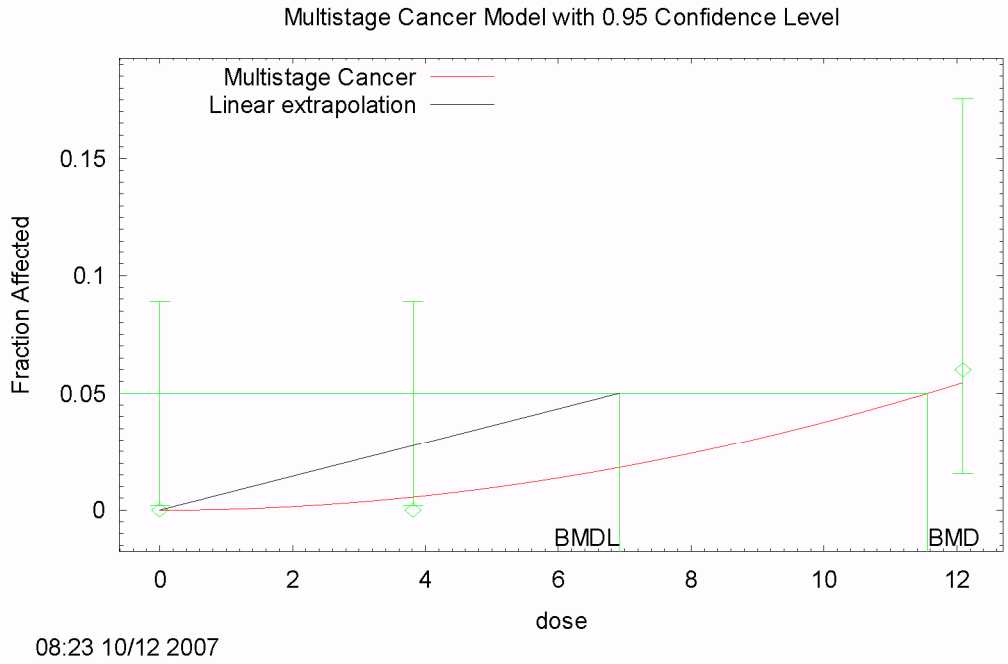
Taken together, (12.2867, 15.6526) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00406945
```

Female F344 rat -- hepatocellular adenomas + carcinomas (0, 5, 25 ppm dose groups)

Dose metric: MRAMKL

Vmax = 0.4 mg/hr/kg $BW^{0.07}$




```
 ====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT LIVER\MRAMKL-
VMAX=0.4\FRAT_LIVER_ADCAR_MRAMKL-4.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT
LIVER\MRAMKL-VMAX=0.4\FRAT_LIVER_ADCAR_MRAMKL-4.plt
                                                    Fri Oct 12 08:23:17 2007
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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
                        Beta(1) =            0
                        Beta(2) =   0.00044169
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.000383811	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.3484	3			
Fitted model	-11.6412	1	0.585705	2	0.7461
Reduced model	-14.7059	1	6.71498	2	0.03482

AIC: 25.2825

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	50	0.000
3.8130	0.0056	0.278	0	50	-0.529
12.0920	0.0546	2.729	3	50	0.169

Chi^2 = 0.31 d.f. = 2 P-value = 0.8571

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 11.5604

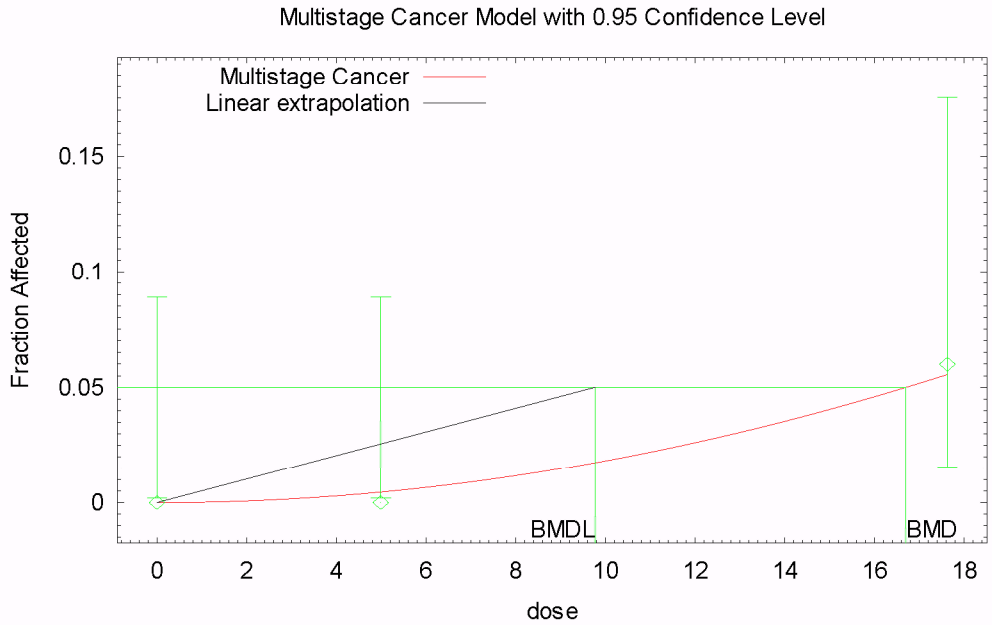
BMDL = 6.92352

BMDU = 30.5183

Taken together, (6.92352, 30.5183) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00722176

Dose metric: MRAMKL
Vmax = 0.65 mg/hr/kg BW$^{0.07}$



08:35 10/12 2007

```
 ====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT LIVER\MRAMKL-
VMAX=0.65\FRAT_LIVER_ADCAR_MRAMKL-65.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE RAT
LIVER\MRAMKL-VMAX=0.65\FRAT_LIVER_ADCAR_MRAMKL-65.plt
                                                    Fri Oct 12 08:35:44 2007
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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
                          Beta(1) =            0
                          Beta(2) =  0.000206402


            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Background    -Beta(1)
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.000183949	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.3484	3			
Fitted model	-11.5867	1	0.476667	2	0.7879
Reduced model	-14.7059	1	6.71498	2	0.03482

AIC: 25.1734

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	50	0.000
4.9910	0.0046	0.229	0	50	-0.479
17.6260	0.0555	2.777	3	50	0.137

Chi^2 = 0.25 d.f. = 2 P-value = 0.8831

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 16.6986

BMDL = 9.76339

BMDU = 43.9237

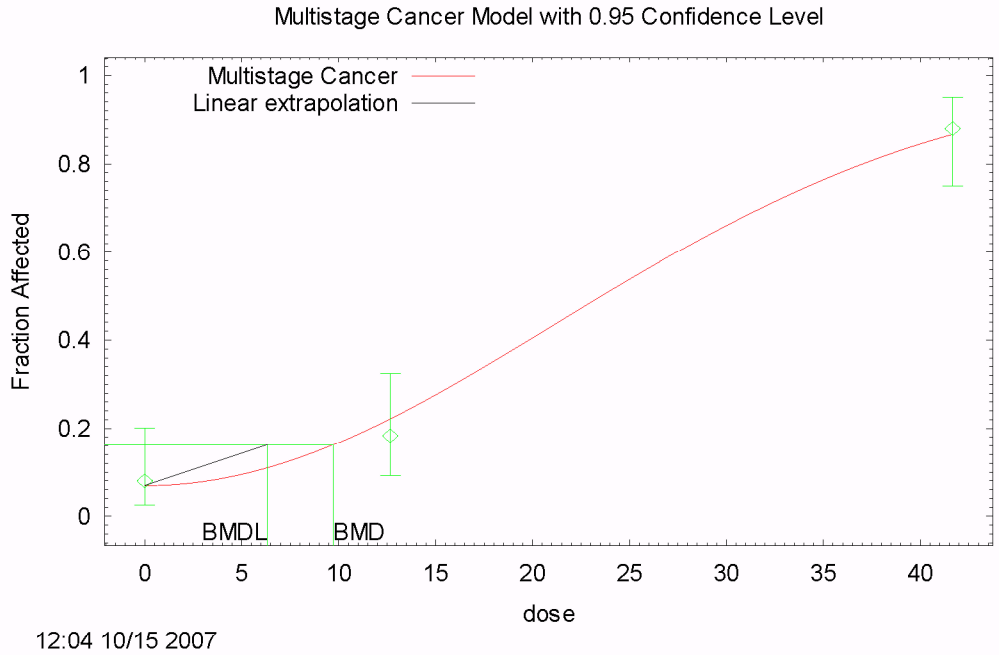
Taken together, (9.76339, 43.9237) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00512117

Female BDF1 mouse – hepatocellular adenomas + carcinomas (0, 5, 25 ppm dose groups)

Dose metric: MRAMKL

Fisher model

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=================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE MOUSE
LIVER\MRAMKL-FISHER\FMOUSE_LIVER_ADCAR_MRAMKL-FISHER.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE MOUSE
LIVER\MRAMKL-FISHER\FMOUSE_LIVER_ADCAR_MRAMKL-FISHER.plt
                                                    Fri Oct 12 08:54:44 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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 =    0.0482072
                        Beta(1) =            0
                        Beta(2) =   0.00119035
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.38
Beta(2)	-0.38	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0693295	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.00111772	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-55.6537	3			
Fitted model	-55.9559	2	0.604318	1	0.4369
Reduced model	-99.1295	1	86.9516	2	<.0001

AIC: 115.912

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0693	3.466	4	50	0.297
12.6660	0.2221	10.883	9	49	-0.647
41.6750	0.8664	43.321	44	50	0.282

Chi^2 = 0.59 d.f. = 1 P-value = 0.4437

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 9.70893

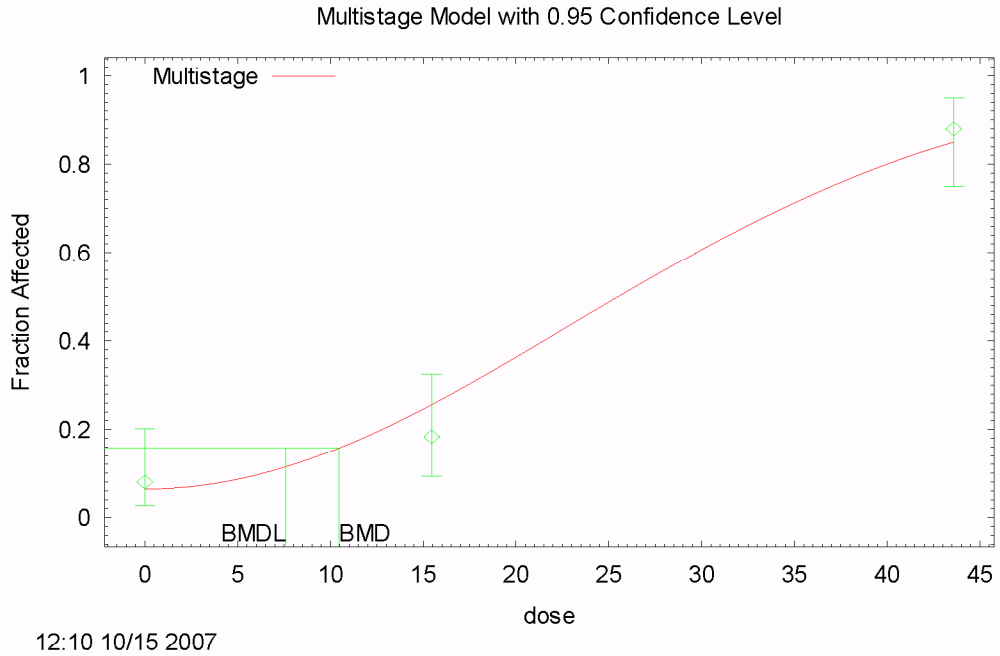
BMDL = 6.3204

BMDU = 11.2942

Taken together, (6.3204 , 11.2942) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0158218

Dose metric: MRAMKL
Thrall model




```
 ==================================================================
                 Multistage Model. (Version: 2.8;  Date: 02/20/2007)
                 Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE
MOUSE LIVER\MRAMKL-THRALL\FMOUSE_LIVER_ADCAR_MRAMKL-THRALL.(d)
                 Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS
FEMALE MOUSE LIVER\MRAMKL-THRALL\FMOUSE_LIVER_ADCAR_MRAMKL-THRALL.plt
                                                        Fri Oct 12 09:01:03 2007
 ==================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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 =    0.0162478
                        Beta(1) =            0
                        Beta(2) =   0.00110173


           Asymptotic Correlation Matrix of Parameter Estimates
```

```
          ( *** The model parameter(s)  -Beta(1)
                have been estimated at a boundary point, or have been specified by the
user,
                and do not appear in the correlation matrix )

            Background      Beta(2)

Background           1         -0.4

   Beta(2)        -0.4            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0643165            *                *                  *
        Beta(1)                0            *                *                  *
        Beta(2)      0.000963757            *                *                  *

* - Indicates that this value is not calculated.


                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -55.6537         3
  Fitted model        -56.6705         2       2.03362      1          0.1539
 Reduced model        -99.1295         1       86.9516      2         <.0001

          AIC:         117.341


                                Goodness  of  Fit
                                                                     Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
   0.0000     0.0643         3.216         4          50        0.452
  15.4560     0.2567        12.580         9          49       -1.171
  43.5990     0.8502        42.510        44          50        0.590

 Chi^2 = 1.92      d.f. = 1        P-value = 0.1654


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        10.4557

            BMDL =        7.59255

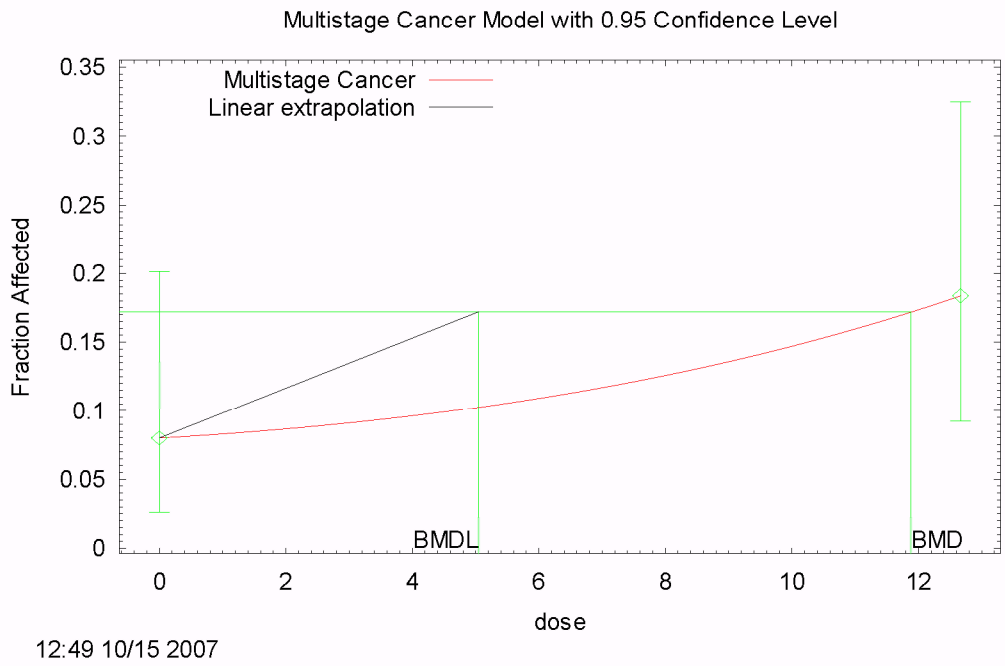
            BMDU =         12.107

Taken together, (7.59255, 12.107 ) is a 90     % two-sided confidence
interval for the BMD
```

Female BDF1 mouse – hepatocellular adenomas + carcinomas (0, 5 ppm dose groups)

Dose metric: MRAMKL

Fisher model




```
=====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE MOUSE
LIVER\MRAMKL-FISHER\FMOUSE_LIVER_ADCAR_MRAMKL-FISHER.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE MOUSE
LIVER\MRAMKL-FISHER\FMOUSE_LIVER_ADCAR_MRAMKL-FISHER.plt
                                                        Fri Oct 12 09:15:17 2007
 =====================================================================

 BMDS MODEL RUN
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Observation # < parameter # for Multistage Cancer model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 2
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 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 =      0.24898
```

```
                        Beta(1) =    0.0160225
                        Beta(2) =     0.001265


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)      Beta(2)

Background            1    -2.2e-008     8.3e-009

   Beta(1)      -6e-009            1           -1

   Beta(2)    -3.2e-009           -1            1


                                 Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background             0.08            *               *                   *
        Beta(1)       0.00471969           *               *                   *
        Beta(2)      0.000372627           *               *                   *

* - Indicates that this value is not calculated.

Error in computing chi-square; returning 2

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -37.3075         2
   Fitted model        -37.3075         3  2.84217e-014     -1         NA
  Reduced model        -38.4987         1       2.38238      1          0.1227

           AIC:         80.6149

                                 Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0800         4.000         4          50       -0.000
   12.6660     0.1837         9.000         9          49        0.000

 Chi^2 = 0.00      d.f. = -1        P-value =     NA


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

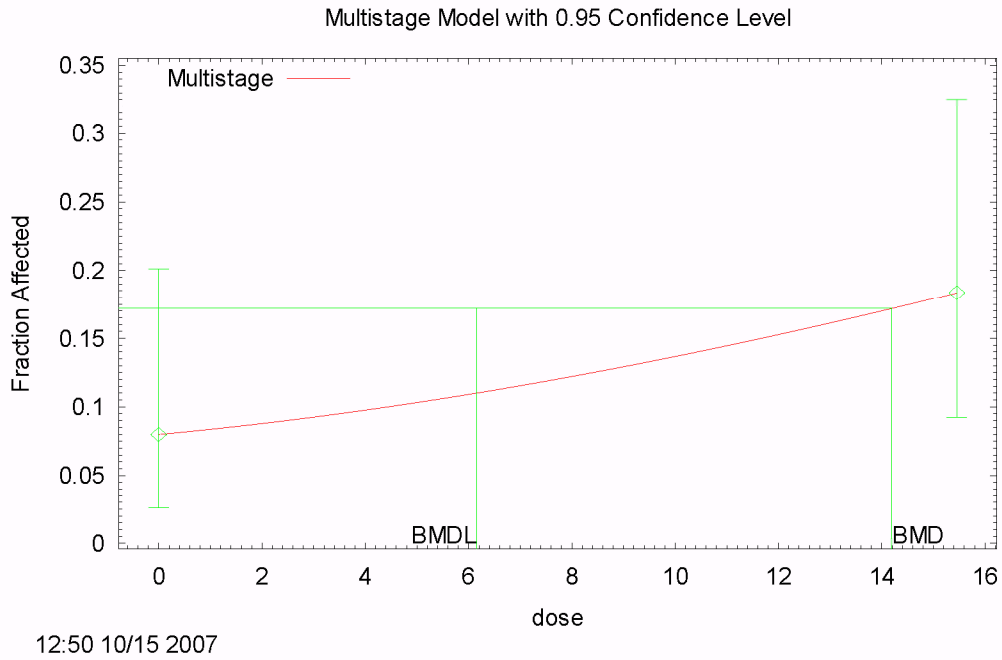
             BMD =        11.6352

            BMDL =        5.04631


BMDU did not converge for BMR = 0.100000
BMDU calculation failed
            BMDU =   3.56605e+007

Multistage Cancer Slope Factor =     0.0198165
```

Dose metric: MRAMKL
Thrall model




```
 ====================================================================
          Multistage Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE MOUSE
LIVER\MRAMKL-THRALL\FMOUSE_LIVER_ADCAR_MRAMKL-THRALL.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE MOUSE
LIVER\MRAMKL-THRALL\FMOUSE_LIVER_ADCAR_MRAMKL-THRALL.plt
                                                     Fri Oct 12 09:17:46 2007
 ====================================================================

 BMDS MODEL RUN
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Observation # < parameter # for Multistage model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 2
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 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 =      0.24898
                        Beta(1) =    0.0131302
                        Beta(2) =  0.000849523
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)	Beta(2)
Background	1	NA	NA
Beta(1)	NA	NA	NA
Beta(2)	NA	NA	NA

NA - This parameter's variance has been estimated as zero or less.
THE MODEL HAS PROBABLY NOT CONVERGED!!!

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.08	*	*	*
Beta(1)	0.00386773	*	*	*
Beta(2)	0.000250241	*	*	*

* - Indicates that this value is not calculated.

At least some variance estimates are negative.
THIS USUALLY MEANS THE MODEL HAS NOT CONVERGED!
Try again from another starting point.

Error in computing chi-square; returning 2

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-37.3075	2			
Fitted model	-37.3075	3	2.84217e-014	-1	NA
Reduced model	-38.4987	1	2.38238	1	0.1227

AIC: 80.6149

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0800	4.000	4	50	-0.000
15.4560	0.1837	9.000	9	49	0.000

Chi^2 = 0.00 d.f. = -1 P-value = NA

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 14.1982

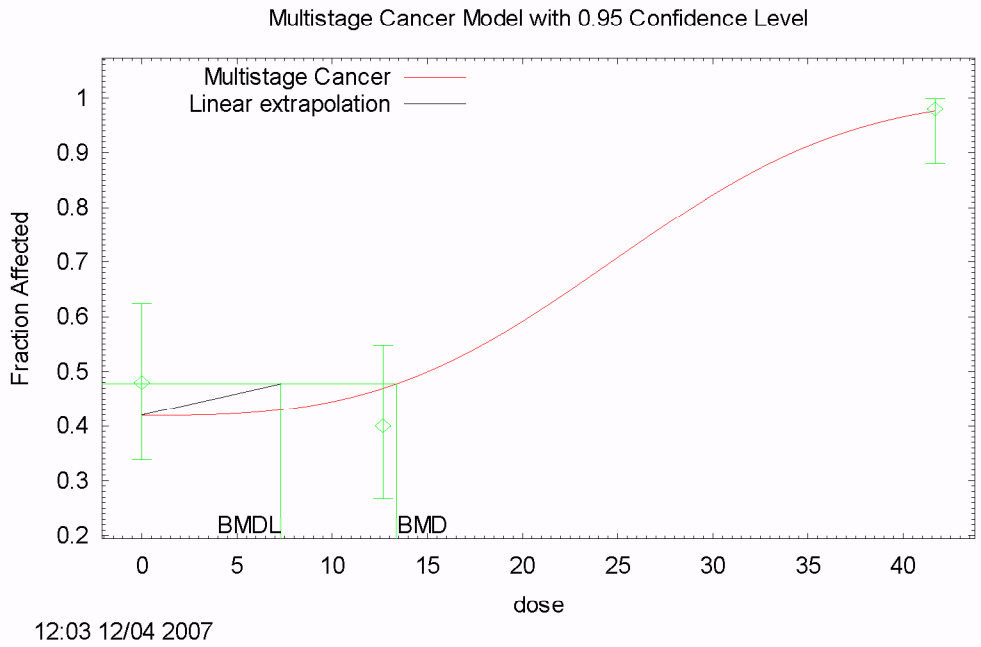
BMDL = 6.15788

BMDU = 2.64632e+014

Taken together, (6.15788, 2.64632e+014) is a 90 % two-sided confidence interval for the BMD

Male BDF1 mouse – hepatocellular adenomas + carcinomas (0, 5, 25 ppm)

Dose metric: MRAMKL
Fisher model




```
 ====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE MOUSE LIVER\MRAMKL-
FISHER\MMOUSE_LIVER_ADCAR_MRAMKL-FISHER.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE MOUSE
LIVER\MRAMKL-FISHER\MMOUSE_LIVER_ADCAR_MRAMKL-FISHER.plt
                                                    Tue Dec 04 12:03:25 2007
 ====================================================================

 BMDS MODEL RUN
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Observation # < parameter # for Multistage Cancer model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3

 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 =     0.352068
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 4.77425e-005
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(3)
Background	1	-0.22
Beta(3)	-0.22	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.41973	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	4.39818e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-73.1699	3			
Fitted model	-74.0443	2	1.74874	1	0.186
Reduced model	-99.6096	1	52.8795	2	<.0001

AIC: 152.089

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.4197	20.987	24	50	0.864
12.6660	0.4693	23.467	20	50	-0.982
41.6750	0.9760	48.798	49	50	0.187

Chi^2 = 1.75 d.f. = 1 P-value = 0.1864

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 13.3804

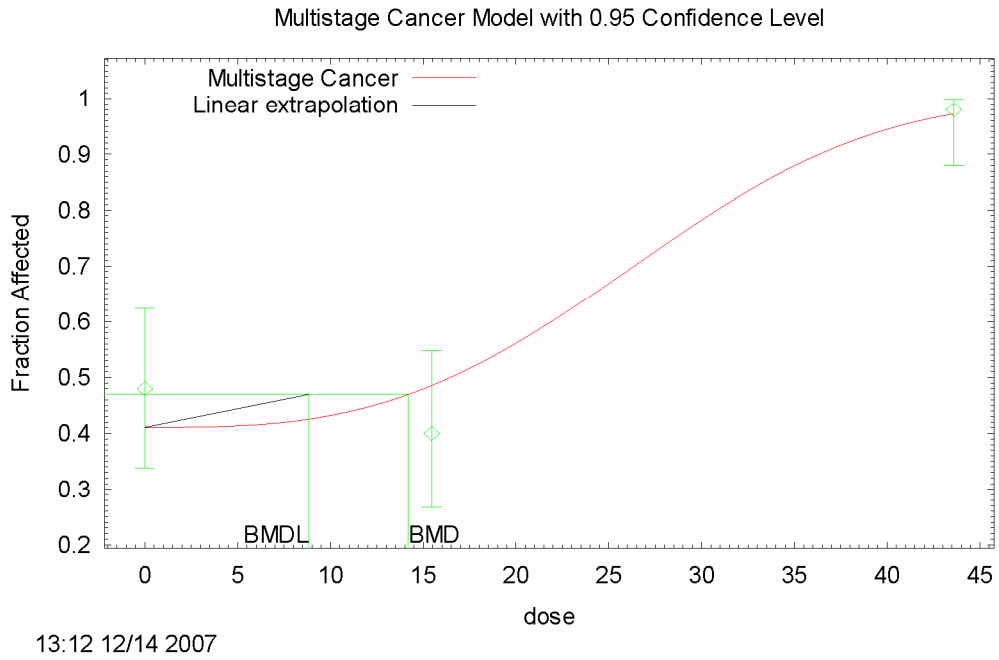
BMDL = 7.30705

BMDU = 15.6428

Taken together, (7.30705, 15.6428) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0136854

Dose metric: MRAMKL
Thrall model




```
=====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE MOUSE LIVER\MRAMKL-
THRALL\MMOUSE_LIVER_ADCAR_MRAMKL-THRALL.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE MOUSE
LIVER\MRAMKL-THRALL\MMOUSE_LIVER_ADCAR_MRAMKL-THRALL.plt
                                                    Tue Dec 04 12:19:57 2007
 ====================================================================

 BMDS MODEL RUN
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Observation # < parameter # for Multistage Cancer model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = IncLiverTumor
   Independent variable = umol/hr-kgL

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 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 =     0.317881
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 4.21166e-005
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(3)
Background	1	-0.26
Beta(3)	-0.26	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.410703	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	3.69143e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-73.1699	3			
Fitted model	-74.462	2	2.58426	1	0.1079
Reduced model	-99.6096	1	52.8795	2	<.0001

AIC: 152.924

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.4107	20.535	24	50	0.996
15.4560	0.4858	24.289	20	50	-1.214
43.5990	0.9724	48.618	49	50	0.330

Chi^2 = 2.57 d.f. = 1 P-value = 0.1086

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 14.185

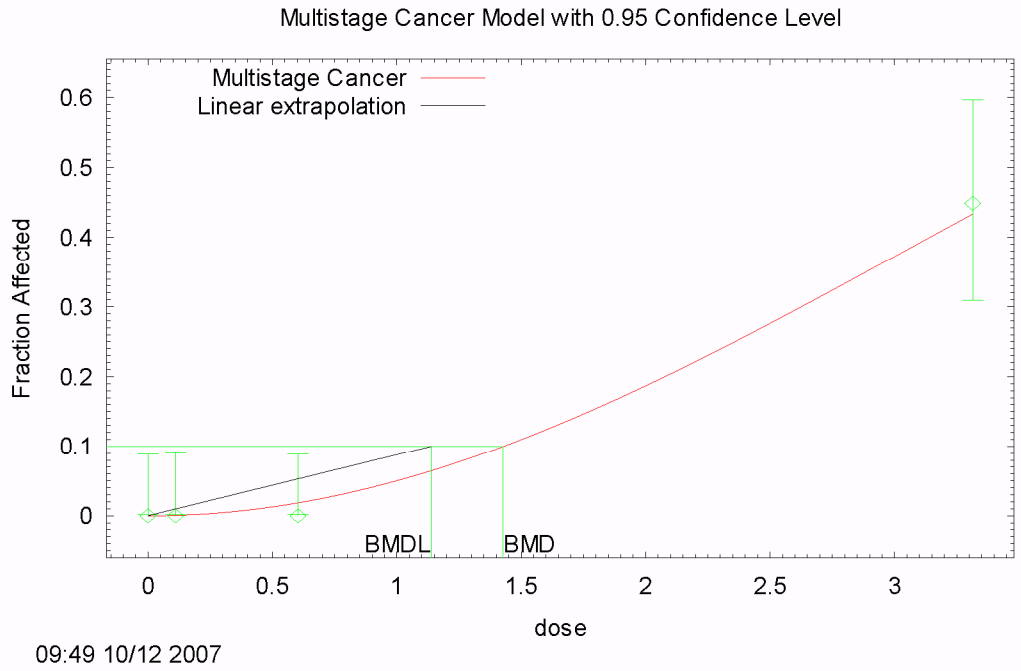
BMDL = 8.82145

BMDU = 16.5171

Taken together, (8.82145, 16.5171) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.011336

BDF1 mouse (female) – pheochromocytomas
Dose metric: MCA
Fisher model




```
 ====================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE
PHEOCHROMOCYTOMAS\FISHER\FMOUSE_PHEOCHROMOCYTOMA_MCA-FISHER.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE
PHEOCHROMOCYTOMAS\FISHER\FMOUSE_PHEOCHROMOCYTOMA_MCA-FISHER.plt
                                                     Fri Oct 12 09:49:11 2007
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = Pheochrom
   Independent variable = umol/L

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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
```

```
                    Beta(1) =            0
                    Beta(2) =    0.0548062


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Background    -Beta(1)
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

               Beta(2)

   Beta(2)           1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background               0            *               *                   *
       Beta(1)               0            *               *                   *
       Beta(2)        0.0517683           *               *                   *

* - Indicates that this value is not calculated.


                      Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -33.7087         4
   Fitted model       -34.7039         1       1.99041      3          0.5744
  Reduced model       -69.0688         1       70.7202      3         <.0001

          AIC:         71.4077


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000         0          50        0.000
    0.1110     0.0006         0.031         0          49       -0.177
    0.6030     0.0186         0.932         0          50       -0.975
    3.3150     0.4338        21.259        22          49        0.214

 Chi^2 = 1.03      d.f. = 3        P-value = 0.7947


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.42662

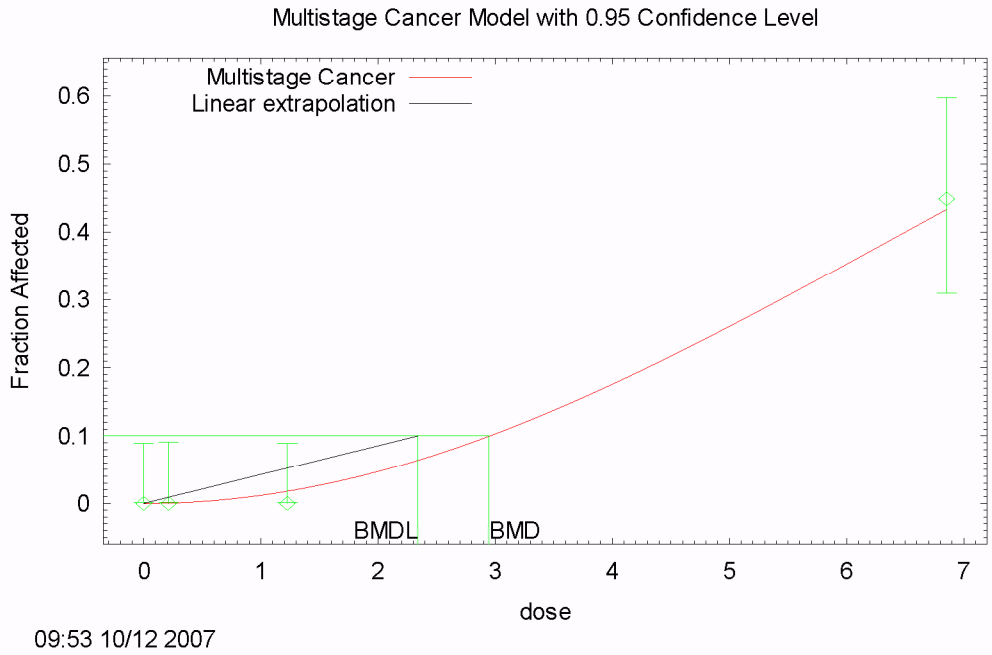
            BMDL =        1.13753

            BMDU =        1.72224

Taken together, (1.13753, 1.72224) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =       0.08791
```

Dose metric: MCA
Thrall model




```
 ======================================================================
          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE
PHEOCHROMOCYTOMAS\THRALL\FMOUSE_PHEOCHROMOCYTOMA-MCA-THRALL.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE
PHEOCHROMOCYTOMAS\THRALL\FMOUSE_PHEOCHROMOCYTOMA-MCA-THRALL.plt
                                                     Fri Oct 12 09:53:23 2007
 ======================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = Pheochrom
   Independent variable = umol/L

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 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
                         Beta(1) =            0
                         Beta(2) =    0.0128084
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.0121232	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-33.7087	4			
Fitted model	-34.6679	1	1.91847	3	0.5895
Reduced model	-69.0688	1	70.7202	3	<.0001

AIC: 71.3358

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	50	0.000
0.2130	0.0005	0.027	0	49	-0.164
1.2260	0.0181	0.903	0	50	-0.959
6.8560	0.4344	21.285	22	49	0.206

Chi^2 = 0.99 d.f. = 3 P-value = 0.8039

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.94801

BMDL = 2.34113

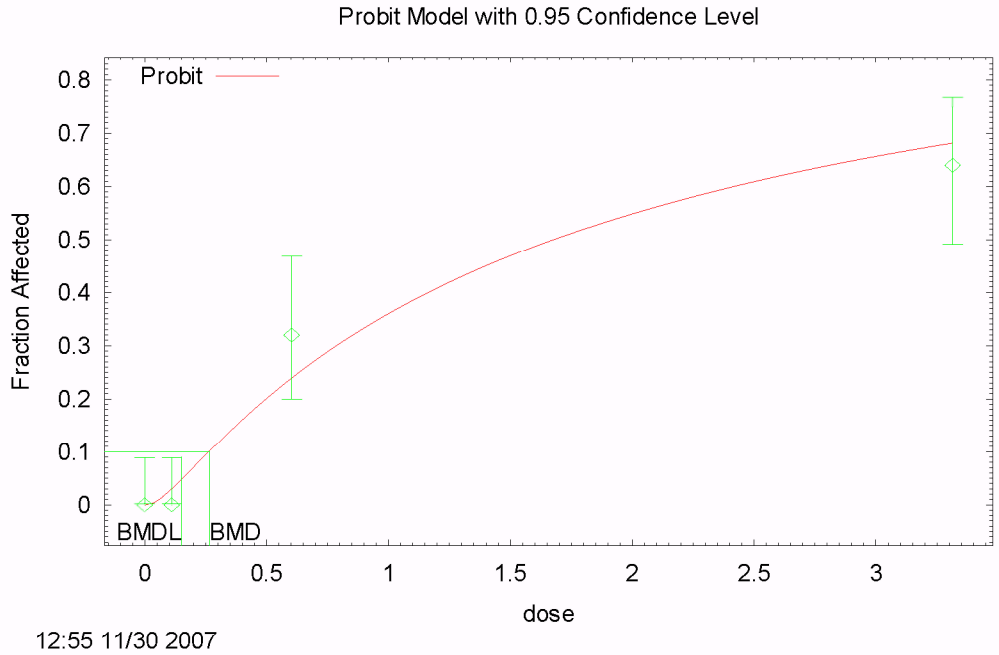
BMDU = 3.55893

Taken together, (2.34113, 3.55893) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0427144

BDF1 mouse (male) – pheochromocytomas

Dose metric: MCA
Fisher model




```
=====================================================================
          Probit Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE
PHEOCHROMOCYTOMAS\FISHER\MMOUSE_PHEOCHROMOCYTOMA_MCA-FISHER.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE
PHEOCHROMOCYTOMAS\FISHER\MMOUSE_PHEOCHROMOCYTOMA_MCA-FISHER.plt
                                                     Fri Nov 30 12:55:04 2007
 ====================================================================

 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 = Pheochrom
   Independent variable = umol/L
   Slope parameter is not restricted

   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
                      intercept =    -0.416734
                          slope =     0.792244
```

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	-0.092
slope	-0.092	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-0.358995	0.125298	-0.604574	-0.113416
slope	0.694404	0.110458	0.47791	0.910899

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	-64.0144	4			
Fitted model	-66.5682	2	5.10756	2	0.07779
Reduced model	-110.216	1	92.4032	3	<.0001

AIC: 137.136

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	50	0.000
0.1110	0.0297	1.484	0	50	-1.237
0.6030	0.2388	11.939	16	50	1.347
3.3150	0.6820	34.099	32	50	-0.637

Chi^2 = 3.75 d.f. = 2 P-value = 0.1533

Benchmark Dose Computation

Specified effect = 0.1

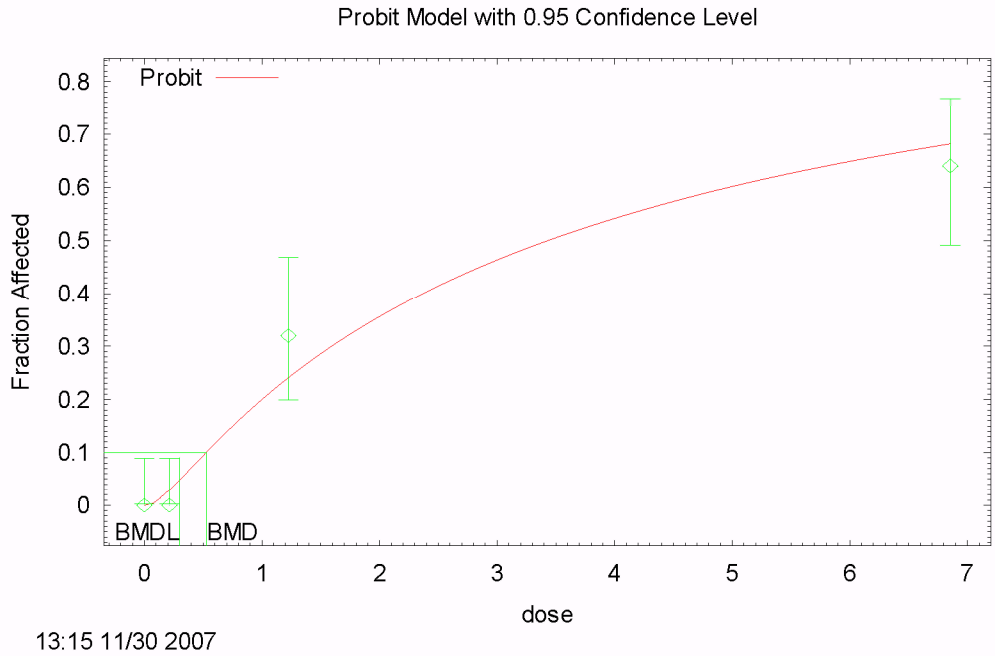
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.264859

BMDL = 0.150882

Dose metric: MCA
Thrall model




```
=====================================================================
          Probit Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE
PHEOCHROMOCYTOMAS\THRALL\MMOUSE_PHEOCHROMOCYTOMA_MCA-THRALL.(d)
          Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS MALE
PHEOCHROMOCYTOMAS\THRALL\MMOUSE_PHEOCHROMOCYTOMA_MCA-THRALL.plt
                                                    Fri Nov 30 13:15:12 2007
 ====================================================================

 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 = Pheochrom
   Independent variable = umol/L
   Slope parameter is not restricted

   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
                      intercept =    -0.965049
                          slope =     0.776315


            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        -0.58

     slope        -0.58            1


                              Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background               0               NA
      intercept        -0.844448         0.153761            -1.14581           -0.543082
          slope         0.683918         0.109119            0.470048            0.897787

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        -64.0144         4
   Fitted model        -66.4723         2       4.91585      2         0.08561
  Reduced model        -110.216         1       92.4032      3         <.0001

           AIC:         136.945


                              Goodness  of  Fit
                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000         0          50        0.000
    0.2130     0.0286         1.429         0          50       -1.213
    1.2260     0.2404        12.019        16          50        1.318
    6.8560     0.6816        34.080        32          50       -0.631

 Chi^2 = 3.61      d.f. = 2        P-value = 0.1648


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.527758

            BMDL =       0.297349
```

E.2. A Bayesian Approach to Modeling Pheochromocytoma Incidence in Male Mice

A Bayesian analysis was conducted utilizing the log-probit model in order to: 1) provide an alternative to modeling the pheochromocytoma incidence data in male mice using the profile likelihood method implemented in BMDS; and 2) investigate the distribution of the slope parameter in the log-probit model.

This Bayesian approach was used to generate a probability distribution of risk estimates. This formal application of Bayesian methods to the evaluation of uncertainty in dose-response modeling, although conceptually simple, relies on recent computational advances that allow use of Markov Chain Monte Carlo (MCMC) methods. The analysis here takes advantage of the computational power of WinBugs 1.4.1, free software (Spiegelhalter et al., 2003) for the Bayesian analysis of statistical models using MCMC methods (e.g., Brooks, 1998; Gilks et al., 1998; Chib and Greenberg, 1995; Casella and George, 1992; Smith and Gelfand, 1992).

More specifically, the use of MCMC methods (via WinBugs) to derive a distribution of BMDs for the multistage model in BMDS has been recently described by Kopylev et al. (2007). This same methodology can be straightforwardly generalized to derive a distribution of BMDs for the log-probit model. For this analysis, diffuse (high variance) Gaussian prior distributions for both the intercept and slope parameters were used, truncated at zero to exclude negative parameter values. A uniform (0,1) prior was used for the background parameter. The posterior distributions of parameters and BMDs are based on three Markov chains of 550,000 simulations each with a burn-in of 50,000 and thinning rate 10 so that 150,000 total simulations were used for deriving the posterior distributions of the parameters and the BMDs. Standard practices of MCMC analysis were followed for verifying convergence using multiple chains and for checking sensitivity to initial values. The mean and 5th percentile of the posterior distribution provide estimates of the BMD and the BMDL ("lower bound"), respectively.

Using outputs from the Thrall model and MCA as the dose metric, the BMD_{10} and $BMDL_{10}$ calculated by this analysis were 0.57568 μmol/L and 0.3177 μmol/L, respectively; these values are very close to the modeling results generated in BMDS for the log-probit model (BMD_{10} = 0.5278 μmol/L and $BMDL_{10}$ = 0.2973 μmol/L), thus confirming the results of the BMDS analysis. Additionally, Figure E-1 below shows the posterior distribution of the slope or shape parameter for the log-probit model generated by the Bayesian analysis. This graph shows that more than 99% of the posterior distribution for the shape parameter is below 1; whereas in BMDS the slope parameter for the log-probit model is typically constrained to be greater than 1. Clearly, constraining the slope parameter in this situation leads to misspecifying the statistical model and should be avoided.

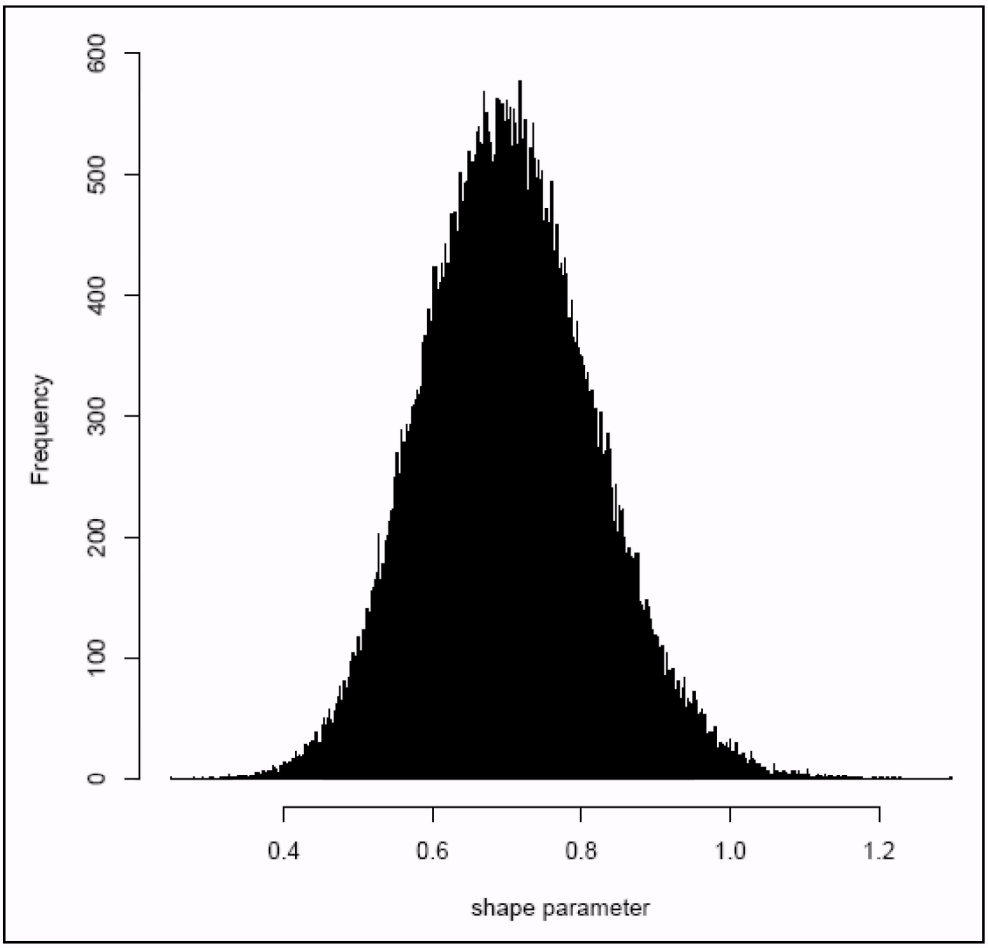

Figure E-1. Histogram of the shape parameter