EPA/635/R-08/005F
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

CARBON TETRACHLORIDE

(CAS No. 56-23-5)

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

March 2010

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS —TOXICOLOGICAL REVIEW OF CARBON TETRACHLORIDE (CAS No. 56-23-5)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

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
BMD	benchmark dose
BMDL	benchmark dose, 95% lower bound
BMDS	benchmark dose software
BMR	benchmark response
BMRF	benchmark response factor
BrdU	5-bromo-2'-deoxyuridine
BRMF	benchmark response factor
BUN	blood urea nitrogen
BW	body weight
CASRN	Chemical Abstracts Service Registry Number
CBZ	N-benzyloxycarbonyl-valine-phenylalanine methyl ester
CCl4	carbon tetrachloride
CFC	chlorofluorocarbon
CHO	Chinese hamster ovary
CI	confidence interval
C_{max}	maximum tissue concentration
CPK	creatine phosphokinase
CPN	chronic progressive nephropathy
CV	coefficient of variation
CYP	cytochrome
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dpm	disintegrations per minute
FEL	frank effect level
G6Pase	glucose-6-phosphatase
GD	gestational day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GI	gastrointestinal
GSH	glutathione (reduced)
GST-P	glutathione S-transferase placental
HA	hemagglutinin
HEC	human equivalent concentration
HED	human equivalent dose
4-HNE	4-hydroxynonenal
4-HNE-dG	deoxyguanosine adducts of 4-HNE
4-HNE-dGp	1,N2-propanodeoxyguanosine adducts of 4-HNE
IFN-γ	interferon-γ
IgM	immunoglobulin

iNOS	inducible nitric oxide synthase
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
JBRC	Japan Bioassay Research Center
K_m	Michaelis-Menten constant
K_{oc}	organic carbon-water partition coefficient
K_{ow}	octanol-water partition coefficient
LAP	leucine aminopeptidase
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
MCA	mean arterial concentration
MCL	mean liver concentration
MCMC	Markov Chain Monte Carlo
MDA	malondialdehyde
MOA	mode of action
MRAMKL	mean rate of metabolism in the liver
mRNA	messenger RNA
MW	molecular weight
NADPH	nicotinamide adenine dinucleotide phosphate
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
NTP	National Toxicology Program
OCT	ornithine carbamoyl transferase
8-OHdG	8-hydroxy-2'-deoxyguanosine
OR	odds ratio
8-oxodG	8-oxo-7,8-dihydro-2'-deoxyguanosine
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PFC	plaque-forming cell
PH	partial hepatectomy
PND	postnatal day
PNMT	phenylethanolamine-N-methyltransferase
POD	point of departure
RfC	reference concentration
RfD	reference dose
RGIL	rate of uptake of carbon tetrachloride from the gastrointestinal tract to liver
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SEM	standard error of the mean

SF	slope factor
SMR	standardized mortality ratio
SOS	inducible DNA repair system
$t_{1/2}$	half-life
TBA	total bile acids
TBARS	thiobarbituric acid-reactive substances
TCPOBOP	1,4-bis[2-(3,5-dichloropyridyloxy)]benzene
TGF	tumor growth factor
T_{max}	time at which the maximum occurred
TNF-α	tumor necrosis factor α
TUNEL	terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling
UDS	unscheduled DNA synthesis
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
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 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).

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER/AUTHOR

Susan Rieth
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Reeder Sams
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

AUTHORS

Mary Manibusan
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, DC

Jennifer Jinot
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Leonid Kopylev
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Paul White
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Paul Schlosser
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

CONTRACTOR SUPPORT

Marc Odin
Environmental Science Center
Syracuse Research Corporation
Syracuse, NY

Gary Diamond
Environmental Science Center
Syracuse Research Corporation
Syracuse, NY

Margaret Fransen
Environmental Science Center
Syracuse Research Corporation
Syracuse, NY

Julie Klotzbach
Environmental Science Center
Syracuse Research Corporation
Syracuse, NY

David Eastmond
Environmental Toxicology Graduate Program
University of California, Riverside
Riverside, CA

REVIEWERS

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

INTERNAL EPA REVIEWERS

Ted Berner, Office of Research and Development/NCEA
Glinda Cooper, Office of Research and Development/NCEA
Anthony DeAngelo, Office of Research and Development/NHEERL
Karen Hammerstrom, Office of Research and Development/NCEA
Cheryl Scott, Office of Research and Development/NCEA
Joyce Donohue, Office of Water/OST

Genetic Toxicology

Channa Keshava, Office of Research and Development/NCEA
Larry Valcovic, Office of Research and Development/NCEA
YinTak Woo, Office of Prevention, Pesticides and Toxic Substances

Immunotoxicology

Andrew Rooney, Office of Research and Development/NCEA

PBPK

Rob DeWoskin, Office of Research and Development/NCEA
Marina Evans, Office of Research and Development/NHEERL

Mode of Action

Vicki Dellarco, Office of Pollution Prevention
Danielle Devoney, Office of Research and Development/NCEA
Julie Du, Office of Water/OST
Kate Guyton, Office of Research and Development/NCEA
Rita Schoeny, Office of Water/OST
John Whalan, Office of Research and Development/NCEA
YinTak Woo, Office of Prevention, Pesticides and Toxic Substances
Jean Zodrow, U.S. EPA Region 10

EXTERNAL PEER REVIEWERS

Janusz Z. Byczkowski, DABT, D.Sc., Ph.D.
Consultant

Gary Ginsberg, Ph.D.
Connecticut Department of Public Health

Dale Hattis, Ph.D.
Clark University and George Perkins Marsh Institute

Lisa Kamendulis, Ph.D.
Department of Pharmacology and Toxicology, Indiana University

Lawrence Lash, Ph.D.
Department of Pharmacology, Wayne State University School of Medicine

Madhusudan Soni, Ph.D., FACN
Soni and Associates

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

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ 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). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *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, 1998), *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 February 2009.

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 in Table 2-1.

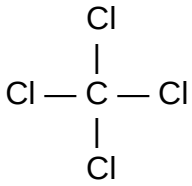


Figure 2-1. Carbon tetrachloride.

Table 2-1. Physical properties and chemical identity of carbon tetrachloride

Parameter	Value	Reference
Chemical Abstract Service Registry Number (CASRN)	56-23-5	
Molecular weight	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 at 25°C	1.15×10^2 mm Hg	NLM, 2003
Density at 20°C	1.5940 g/mL	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 at 25°C	7.93×10^2 mg/L	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 coefficients	log K_{ow} = 2.83 log Koc = 2.04	NLM, 2003; Hansch et al., 1995 ATSDR, 2005
Flash point	Not flammable	NLM, 2003; U.S. Coast Guard, 1999
Autoignition temperature	>1,000°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 at 24°C	0.922 cp	U.S. Coast Guard, 1999
Surface tension at 20°C	0.027 N/m	U.S. Coast Guard, 1999
Henry's law constant at 25°C	2.76×10^{-2} atm m^3/mol	NLM, 2003; Leighton and Calo, 1981
OH reaction rate constant at 25°C	1.20×10^{-16} cm^3/molecule sec	NLM, 2003; Atkinson, 1989
K_{oc}	71	NLM, 2003
Bioconcentration factor	3.2–7.4	NLM, 2003; CITI, 1992
Conversion factors at 25°C	1 mg/m^3 = 0.16 ppm; 1 ppm = 6.29 mg/m^3	NLM, 2003

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 U.S. 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 United States via Title VI of the Clean Air Act Amendments of 1990 (ATSDR, 2005; Doherty, 2000; Holbrook, 1993). The ban on production and import of carbon tetrachloride in developed countries, including the United States, 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 (U.S. EPA, 2007a).

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 the Agency for Toxic Substances and Disease Registry (ATSDR), manufacture of carbon tetrachloride in the United States in the early 2000s was limited to one company (Vulcan Materials Company) at two plants with a combined 130 million pound capacity (ATSDR, 2005); however, these capacities were considered flexible because other chlorinated solvents were 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, 2005). 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). A fraction of carbon tetrachloride in soil may adsorb to the soil organic matter; the sorption of carbon tetrachloride will be affected by the composition of the soil organic matter and water content of the soil (ATSDR, 2005). Bioconcentration factor 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 <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 (GI) tract in humans and animals. There is evidence of GI 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 bolus doses of approximately 18 or 180 mg/kg carbon tetrachloride by oral gavage, 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 3,200 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 GI 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 (Gillespie et al., 1990; Kim et al., 1990a, b). Relative to the neat compound, the initial rate of GI absorption of 25 mg/kg of carbon tetrachloride was faster with administration as a saturated solution in water or 0.25% aqueous Emulphor[1] 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 GI tract and subsequently lower peak blood concentrations and delayed removal from the blood stream (Kim et al., 1990a).

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

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 (≥9,600 ppm) reported symptoms of toxicity within the first minute of exposure; symptoms appeared after 3 minutes in subjects exposed to 30 mg/L (4,800 ppm) (Lehmann and Schmidt-Kehl, 1936). After male Sprague-Dawley rats were exposed at 100 or 1,000 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 5,000 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 4,000 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 1,150 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 the dermally absorbed fraction would be negligible for whole-body exposures to carbon tetrachloride vapor.

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 3,200 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 4,000 ppm for 6 hours showed the largest concentrations of carbon tetrachloride in the fat (1,674 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 eight- 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 inhalation exposure, 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, GI 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 (1,000 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 at which the maxima occurred (T_{max}). Maximal tissue concentrations were reached quickest by oral 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 (1,000 ppm for 2 hours), oral bolus dosing, or gastric infusion over 2 hours

Tissue	Inhalation			Oral bolus			Gastric infusion		
	AUC (µg × min/ mL)	C_{max} (µg/g)	T_{max} (min)	AUC (µg × min/ mL)	C_{max} (µg/g)	T_{max} (min)	AUC (µg × min/ mL)	C_{max} (µg/g)	T_{max} (min)
Liver	2,823	20	30	1,023	58	1	149	0.5	120
Kidney	3,064	25	30	3,029	14	5	800	4	120
Lung	2,952	24	30	2,908	10	15	2,842	6	180
Brain	3,255	28	30	4,223	15	15	2,683	10	150
Fat	230,699	1,506	240	235,471	246	120	165,983	179	360
Heart	2,571	18	30	2,747	10	5	1,900	8	120
Muscle	3,248	18	30	4,117	7	60	2,164	10	150
Spleen	2,035	13	30	4,096	12	5	1,660	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).

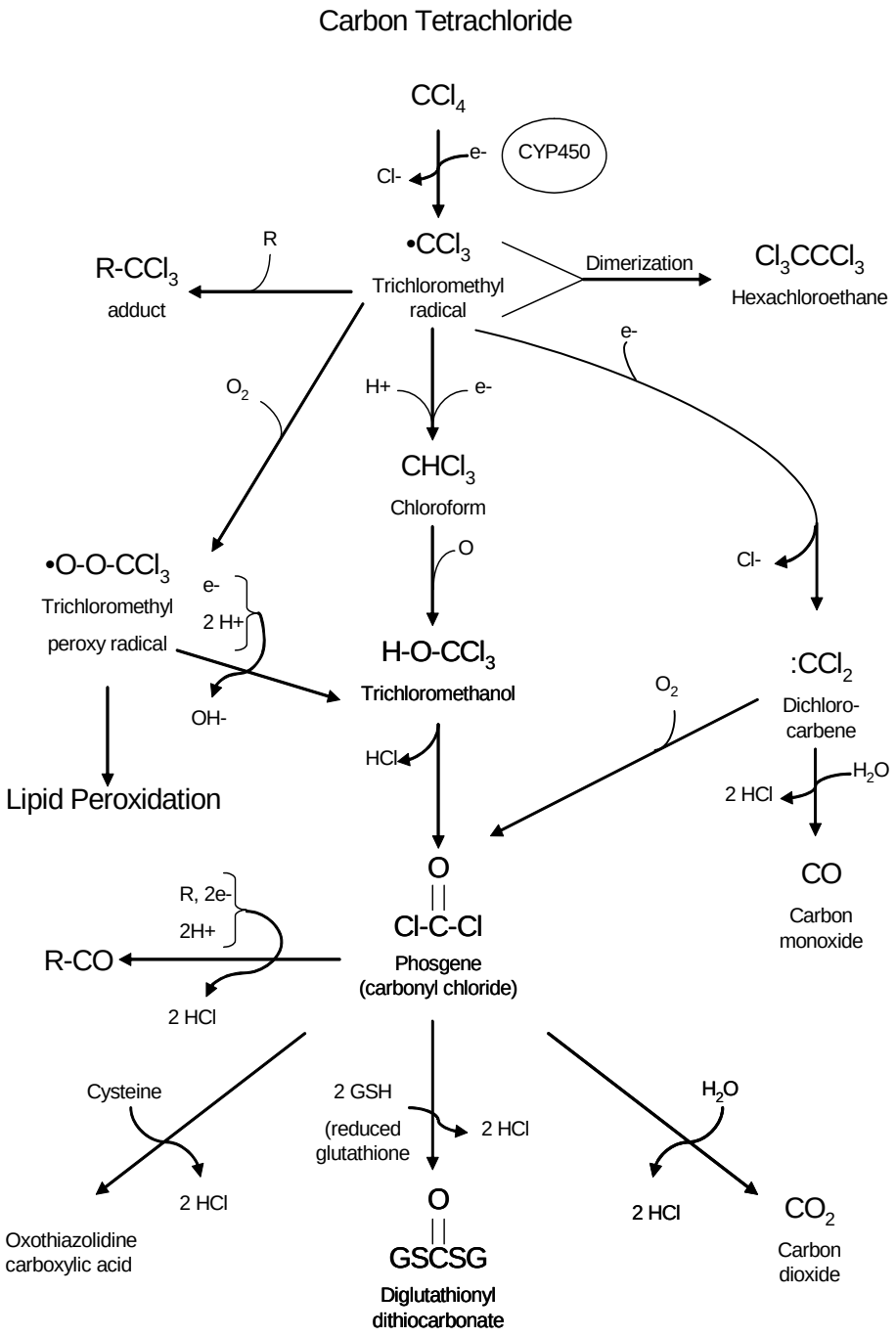
3.2.4. Lactational Transfer

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).



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

Source: ACGIH (2001).

Figure 3-1. Metabolic scheme for carbon tetrachloride.

The initial reaction step is catalyzed by an nicotinamide adenine dinucleotide phosphate (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.

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 rate of conversion of the trichloromethyl radical to the trichloromethyl peroxy radical (and to downstream reaction products with amino acids and lipids) has been estimated to be approximately 10^8–10^9 L/mols (Russell et al., 1990; Slater, 1981; Packer et al., 1978). These rates are sufficiently high to suggest that the rate of production of the trichloromethyl peroxy radical (and, thereby, the rate of elimination of the trichloromethyl radical) may be diffusion limited (10^{10}–10^{12} L/mols; Atkins, 1998). Therefore, limiting factors in the oxidative elimination of the trichloromethyl radical are likely to be reactant concentrations at the site of production of the trichloromethyl radical (e.g., O_2) and/or factors that limit diffusion of the trichloromethyl radical (e.g., diffusion coefficient in cytosol). 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 ($t_{1/2}$) 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). A higher rate of inactivation of CYP450 in the rat compared to humans has potential implications for extrapolating external and internal doses (e.g., rates of metabolism of carbon tetrachloride) across species (see Section 5.2.2.1).

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 (µM)	V_{max} (nmol/min/mg protein)
Human	56.8	2.26
Rat	59.1	3.1
Mouse	29.3	2.86
Hamster	30.2	4.1

K_m= Michaelis-Menten constant; 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 ~2,500 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 ~4,000 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 to 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 $t_{1/2}$ values were about 4 hours (Bruckner et al., 1990).

Reynolds et al. (1984) evaluated elimination parameters during a 24-hour period in rats exposed by oral gavage to [^{14}C]-carbon tetrachloride at doses ranging from 15 to 4,000 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 4,000 mg/kg, peak exhalation rates were 88, 1,550, and 3.4 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% was eliminated 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 1,000 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 <1% of the administered dose.

Sanzgiri et al. (1997) measured the elimination of carbon tetrachloride from tissues in rats exposed to 1,000 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 $t_{1/2}$ of elimination from various tissues are given in Table 3-3. Elimination $t_{1/2}$ values were slowest for fat, which is poorly perfused, but similar for the other tissues.

Table 3-3. Elimination $t_{1/2}$ and apparent clearance of carbon tetrachloride from rat tissues following administration of 179 mg/kg (1,000 ppm, 2 hours) by inhalation, oral bolus dosing, or gastric infusion over 2 hours

	Inhalation		Oral bolus		Gastric infusion	
Tissue	$t_{1/2}$ (min)	Clearance (mL/min/kg)	$t_{1/2}$ (min)	Clearance (mL/min/kg)	$t_{1/2}$ (min)	Clearance (mL/min/kg)
Liver	249	63	323	175	269	1,198
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 <10% of the initial body burden in rats and <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% was eliminated 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, <8% was excreted in urine and <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 $t_{1/2}$ values for the fast and slow phases of 96 and 455 minutes and 89 and 568 minutes, respectively. Similarly, $t_{1/2}$ values for the fast and slow phases of elimination of CO_2 were 305 and 829 minutes on the 8-hour schedule and 455 and 1,824 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 $t_{1/2}$ of elimination in urine and feces for the 2-week exposures were 1,066 and 3,700 minutes for the 8-hour schedule and 944 and 6,700 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 <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 a maximum velocity of enzyme reaction (V_{max}) of 0.92 μmol/hour (0.14 mg/hour) and a Michaelis-Menten constant (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 (L blood/hr)[a]	8	46.4	358
Alveolar ventilation (L air/hr)[a]	8	46.4	358
Tissue volumes (percent of total)			
Liver	4	4	4
Fat	8	10	20[b]
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)[c]	0.35	1.91	12.72
K_m (mg/L)	0.25	0.25[d]	0.25[d]

[a]Allometrically scaled from 15 L/hr × body weight $(BW)^{0.74}$.
[b]Tissue volume for fat in humans is shown in Table 2 of Paustenbach et al. (1988) 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.
[c]Allometrically scaled from 0.65 mg/hour × $BW^{0.7}$.
[d]Assumed to be the same as in rats.

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 to the ¾ power $(BW)^{0.75}$, 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 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 longer $t_{1/2}$ 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% body weight. Model simulations of concentration of carbon tetrachloride in expired air over time were compared with the data of Stewart et al. (1961), who exposed volunteers to 49 ppm carbon tetrachloride for 70 minutes or 10 ppm 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 was still assumed to occur only in the liver and was modeled by a single, saturable pathway that resulted 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 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 (1,800 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; 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 (Thrall et al., 2000; 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 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	2,800
mg protein/g liver[b]	13.8	21.9	17.8	12.8
In vivo V_{max} (mg/hr/kg BW)[c]	0.4	0.79	6.39	1.49
In vivo V_{max} (mg/hr)[d]	0.15	5.97×10^{-2}	1.69	29.15
In vivo V_{max} (mg/hr/mg protein)	1.1×10^{-3}	2.7×10^{-3}	0.016[e]	8.1×10^{-4}
In vivo K_m (mg/L)[c]	0.25	0.46	1.14	0.25[f]
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/hr/mg protein)[h]	0.186	0.1712	0.246	0.135
In vitro K_m (µmol/L)[h]	59.1	29.3	30.2	56.8
In vitro V_{max}/K_m (L/hr/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 Vmax (mg/hr/kg BW) using $BW^{0.7}$ (personal communication; email dated September 5, 2006, from Dr. Karla Thrall, Pacific Northwest National Laboratory, to Susan Rieth, U.S. EPA); humans: calculated (see text).
[e]Corrected from value of 0.14 in Table 5 of Thrall et al. (2000) (personal communication; email dated September 5, 2006, from Dr. Karla Thrall, Pacific Northwest National Laboratory, to Susan Rieth, U.S. EPA).
[f]Assumed to be equal to the rat based on in vitro Km comparisons.
[g]Corrected from value of 0.16 in Table 5 of Thrall et al. (2000) (personal communication; email dated September 5, 2006, from Dr. Karla Thrall, Pacific Northwest National Laboratory, to Susan Rieth, U.S. EPA).
[h]Data from Zangar et al. (2000).
[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 GI compartment to the inhalation model of Paustenbach et al. (1988) to describe uptake of carbon tetrachloride administered by a single oral 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 GI 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 GI 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 body weight. 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) were 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 1,000 ppm carbon tetrachloride for 6 hours. The resulting V_{maxC} and K_m values were 0.37 mg/hour/kg and 1.3 mg/L, respectively. The predicted decreases in chamber carbon tetrachloride concentrations were 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 1,000-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 1,000 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/hour/kg for the 24-hour methanol pretreatment group resulted in 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/hour/kg, which was close to the carbon tetrachloride-only value of 0.11 mg/hour/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

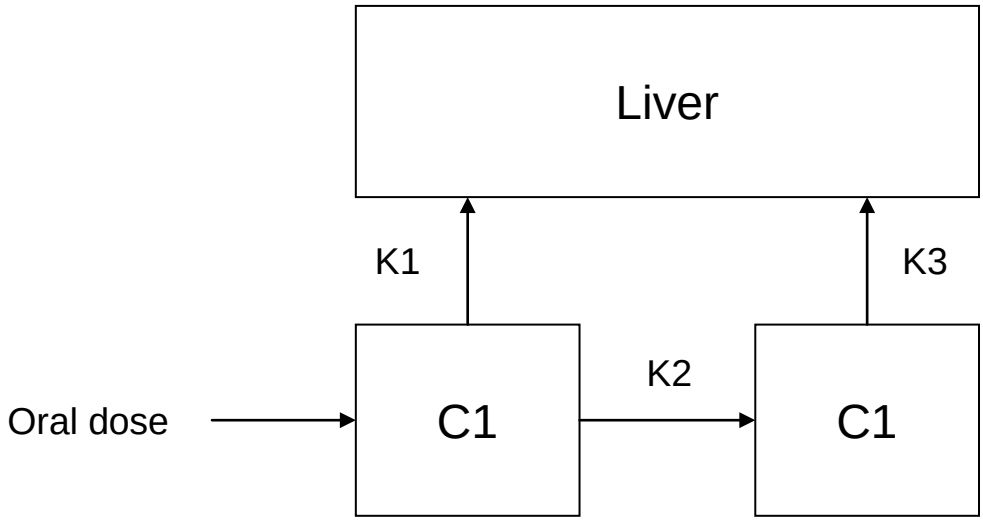
Yoshida et al. (1999) used a classical compartment pharmacokinetic model to derive rates of absorption of carbon tetrachloride in rats exposed at low concentrations in a closed chamber system. Experimentally, rats were exposed at initial concentrations between 10 and 1,000 ppb,

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 four-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 GI tract was simulated as a two-compartment, three-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.



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 hrs^{-1} for 50 and 100 mg/kg doses). Values for K2 and K3 were 2 and 0.05 hr^{-1}, respectively.

Source: Fisher et al. (2004).

Figure 3-2. Two-compartment model for simulating GI absorption of carbon tetrachloride administered to mice as a single oral gavage dose in Emulphor.

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 1,250 ppm (Fisher et al., 2004) in male $B6C3F_1$ mice were used to optimize V_{maxC} and K_m, resulting in values of 1 mg/hour/$kg^{0.75}$ and 0.3 mg/L, respectively. For tetrachloroethylene, gas uptake-derived V_{maxC} and K_m values of 6 mg/hour/$kg^{0.75}$ 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 eight-compartment, flow-limited PBPK model, including compartments for lung, liver, brain, kidney, fat, rapidly and slowly perfused tissues, and the GI tract. Physiological parameter values were taken from the literature (U.S. EPA, 2000e; Brown et al., 1997; Delp et al., 1991). 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 $V_{max, \text{ in vitro tissue}}$/ $V_{max, \text{ in vitro liver}}$. Simulations of open-chamber inhalation exposures (ATSDR, 2005) 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).

Of the PBPK models developed for carbon tetrachloride, the model by Yoon et al. (2007) is the only one that addressed extrahepatic carbon tetrachloride metabolism. Regarding extrahepatic metabolism, it is noted that, although rat kidney cortex and proximal tubules express reasonable levels of CYP2E1 protein and activity for the oxidative metabolism of another CYP2E1 substrate, trichloroethylene (Cummings et al., 2001, 2000b, 1999), the human kidney has been reported by multiple laboratories to not express any detectable CYP2E1 protein (Cummings and Lash, 2000; Cummings et al., 2000a; Amet et al., 1997) and to exhibit little if any oxidative metabolism of trichloroethylene (Cummings and Lash, 2000; Cummings et al., 2000a).

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 GI 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 body weight 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, 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 > the detection limit 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 10th 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 higher levels and were more prevalent in the drinking water supply (i.e., had a 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. ORs 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 GI 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 (≥3,200 ppm). No effect was observed following exposure at 20 mg/L for 5 minutes. Exposure to 30 mg/L (4,800 ppm) for 2.5 minutes resulted in slight drowsiness after 5 minutes. Exposure to 40 mg/L (6,400 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 GI 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 (TBA), 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 23/40 in the low-exposure group, 35/54 in the medium-exposure group, and 2/61 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, 1–5, 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 χ^2 comparison of four levels of alcohol use between exposed and nonexposed). However, there was a slightly higher proportion of very high drinkers (5–7 units every day or >8 units at least 3–4 times/week) in the exposed group (27%) than in controls (20%) ($p = 0.20$ for χ^2 comparison of high alcohol use between exposed and nonexposed). 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) were 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)
TBA (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 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 means of 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 is used in the diagnosis of liver disease. The measurement of serum GGT levels can be used to ascertain whether observed elevations of ALP are due to skeletal disease or 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 (CV), 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).[2] 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 <1 ppm, a no-observed-adverse-effect level (NOAEL) could not be determined.

Seidler et al., 1999

Seidler et al. (1999) evaluated the association between maternal occupational exposure to chemicals and the risk of infants small for gestational age in singleton births in a prospective cohort study of 3,946 pregnant women in West Germany from 1987 to 1988. The final group of

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

[a]Estimated exposures were assumed to be the midpoint of the exposure category.
[b]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.

1,865 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 1,865 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 United States 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; Dumas et al., 2000; Dosemeci et al., 1999; Kernan et al., 1999), occupation/industry information obtained from questionnaires, interviews, or death certificates in combination with a job exposure matrix were 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, $p < 0.001$) (Checkoway et al., 1984; Wilcosky 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 included in the study. Increased mortality was found 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 were 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 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 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 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 oral 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 (2, 4, 6, 8, 10, and 12 weeks, and 2 weeks posttreatment; 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 oral 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[3]. Rats 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. In the low-dose group, histopathological examination of the liver revealed cirrhosis in 2/6 rats and vacuolar degeneration and hepatocellular necrosis in 6/6 rats; in the high-dose group, histopathological examination revealed cirrhosis (as well as degeneration and necrosis) in 6/6 rats. 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 oral 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 to 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

[3]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.

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 2-fold 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 not in other treatment 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 oral 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 oral 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) received daily oral gavage doses of 0, 12, 120, 540, or 1,200 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 were generally 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 had significantly elevated serum LDH and ALT, altered organ weights, and increased incidence of liver lesions (e.g., necrosis in 5/19 in vehicle controls versus 0/20 in untreated controls and 20/20 in the 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 (NCI) studies*

Edwards, 1941

Researchers at 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 oral gavage 2 or 3 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 oral gavage doses of 0.1 mL of olive oil (2 or 3/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 was treated with 0.1 mL of 5% carbon tetrachloride in olive oil (0.005 mL or 8 mg of carbon tetrachloride) 3 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 1–3 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 oral 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 body weight at 4-day intervals or 120 doses of 0.005 mL/g body weight 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 1 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 hepatomas were 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 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 one or two 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 oral 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 (5 males and 5 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 oral 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 (NTP) database search application (NTP, 2007).[4] 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 $B6C3F_1$ mice received oral gavage doses of 1,250 or 2,500 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

[4]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.

other nonneoplastic lesion in mice that was 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 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, 1,260, or 2,520 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 the study authors state that "appropriate controls [were] reserved." 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 exposure levels, and cirrhosis developed at ≥50 ppm. In rats, fatty changes were seen at ≥50 ppm and cirrhosis was noted 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 FEL 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 exposure levels to liver cirrhosis at higher exposure levels. 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 must 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, 1,260, or 2,520 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 were 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 was noted 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, 3 New Zealand rabbits, 2 beagle dogs, and 3 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 an exposure-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, 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, 1,700, or 5,094 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 [CPK], 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-glutathione S-transferase placental (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, exposure-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 exposure-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 CPK were statistically increased in females at ≥30 ppm, 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 an exposure -related fashion in male rats (≥10 ppm) and female rats (≥30 ppm). Significant, exposure-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. 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. Exposure-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:BDF_1 mice (10/sex/group) were exposed (whole body) to 0, 10, 30, 90, 270, or 810 ppm (0, 63, 189, 566, 1,700, or 5,094 mg/m^3) 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 was noted 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. 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 exposure-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, $B6C3F_1$ mice, and Syrian hamsters (10 males/species) were exposed by inhalation to carbon tetrachloride vapor at concentrations of 0, 5, 20, or 100 ppm (0, 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 deoxyribonucleic acid (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 to 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 exposure levels 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*

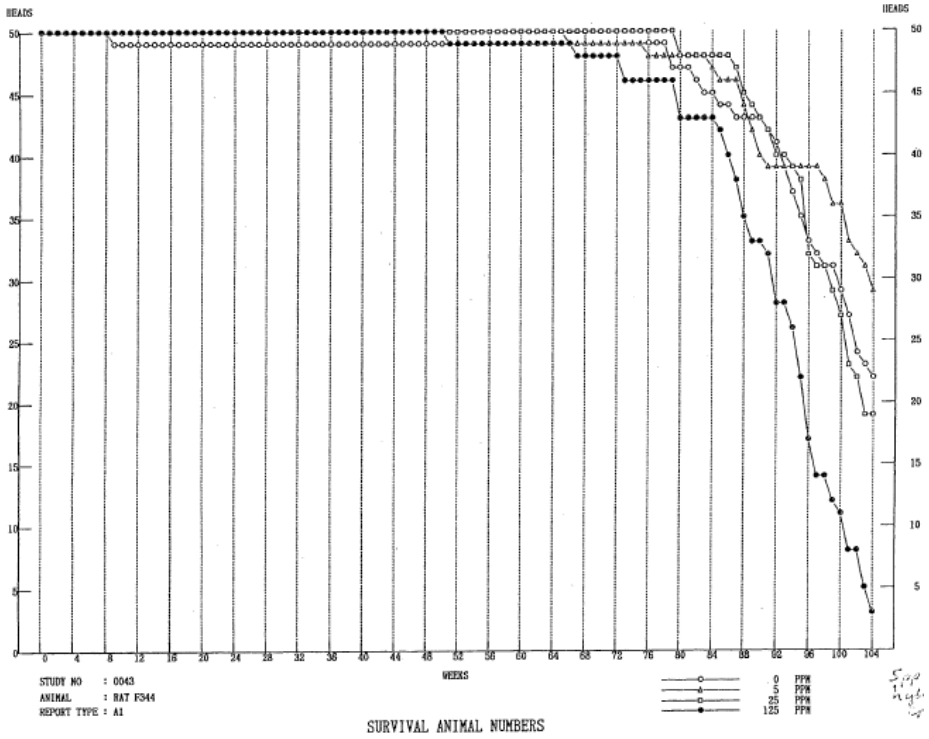
Nagano et al., 2007b; JBRC, 1998

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

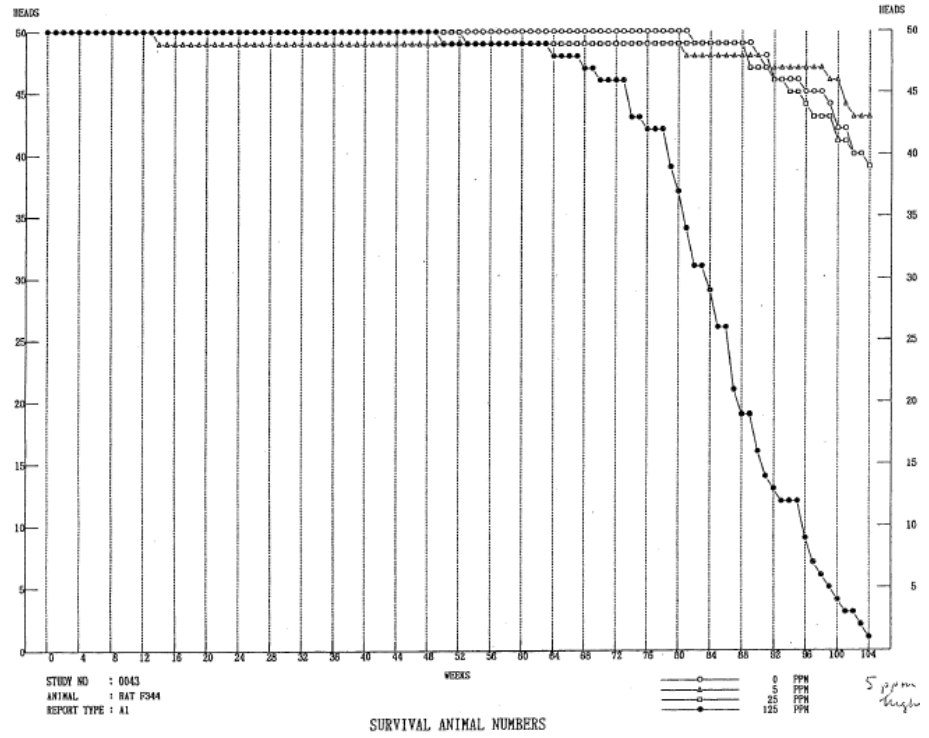
available as an unpublished study by the JBRC [1998].) Animals were observed daily for clinical signs, behavioral changes, and mortality. Body weights were measured once a week for the first 14 weeks and every 2 weeks thereafter. Urinalysis, hematology, and clinical chemistry tests were conducted at 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 for male and female rats 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 (CPN) 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



Source: JBRC (1998)

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

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 (25–63% 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 of 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)[a]	Protein content of urine[b]			
	+	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]The exposure concentrations adjusted to continuous exposure (i.e., multiplied by 5/7 × 6/24) = 0.9, 4.5, and 22.3 ppm.
[b]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+: 1,000 mg/dL (letter dated March 8, 2004, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA).
[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 an exposure-related increase in the severity of chronic nephropathy (progressive glomerulonephrosis[5]) 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 exposure-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).

[5]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]

	Male				Female			
Lesion	**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 were adjusted to continuous exposure (i.e., multiplied by 5/7 × 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.

Sources: 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 was considered 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 (2/1,797) 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]

	Male				Female			
Tumor	**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]

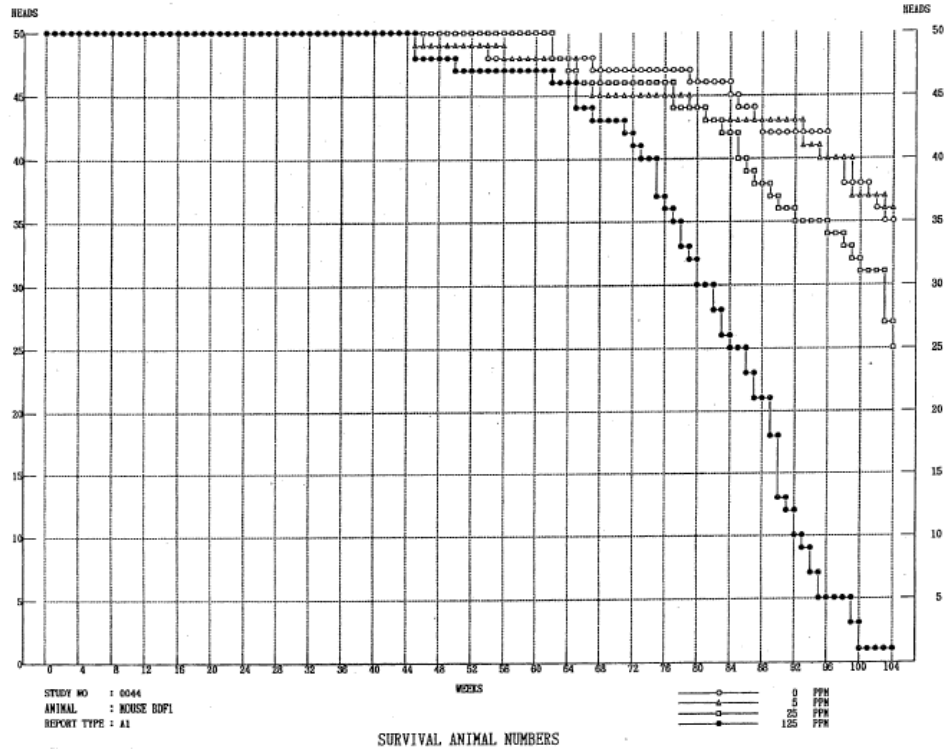
[a]The exposure concentrations adjusted to continuous exposure (i.e., multiplied by 5/7 × 6/24) = 0.9, 4.5, and 22.3 ppm.
[b]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[c]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.01$).
[d]Statistically significant ($p \leq 0.001$ by Fisher's exact test) in comparison to the historical control incidence (2/1,797).

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 to 39 carcinogenicity studies carried out by JBRC; email dated April 5, 2007, from Kasuke Nagano, JBRC, to Susan Rieth, U.S. EPA).

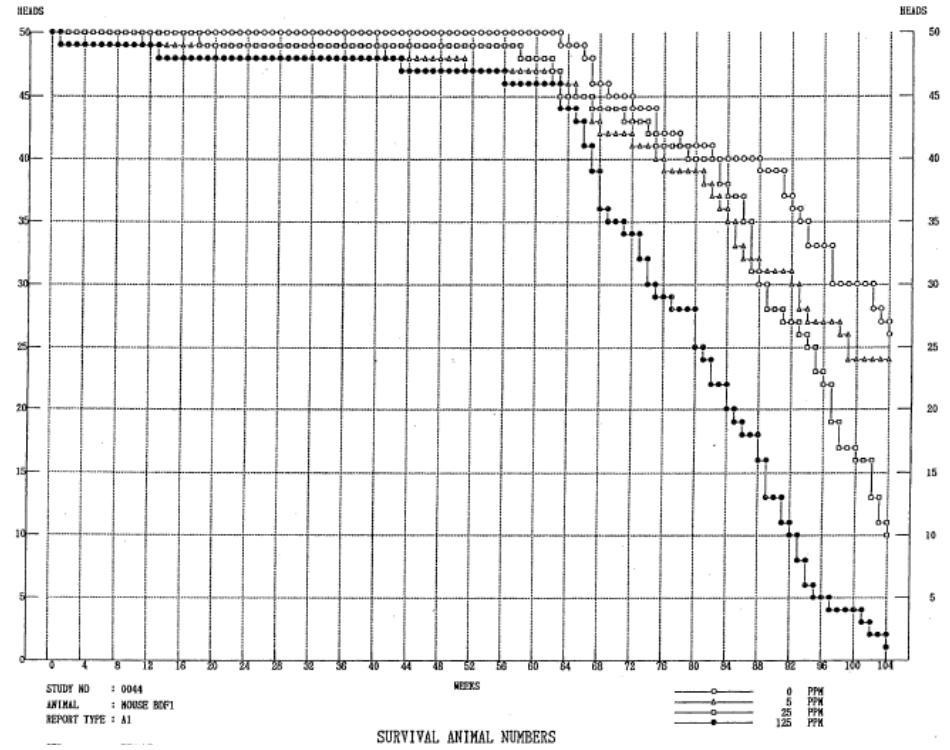
Sources: Nagano et al. (2007b); JBRC (1998).

These researchers also conducted a 2-year study using Crj:BDF_1 mice. Groups of Crj:BDF_1 mice (50/sex/group) were 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 (22–39% reduction at termination).

Male mouse



Female mouse



Source: JBRC (1998)

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

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 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 exposure levels 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:BDF_1 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 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 BDF_1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)[a]

	Male				Female			
Lesion	**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 × 6/24) = 0.9, 4.5, and 22.3 ppm.

Sources: 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:BDF_1 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 or 16%) was statistically significantly elevated compared to the concurrent control group and exceeded the historical control range (2–10%).

Table 4-6. Incidence of liver and adrenal tumors in BDF_1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)[a]

	Male				Female			
Tumor	**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 × 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 was significantly elevated compared with controls by Fisher's exact test ($p \leq 0.01$).
[d]Tumor incidence was significantly elevated compared with controls by Fisher's 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:BDF_1 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:BDF_1 mice in 32 studies at JBRC: 0.3% (range: 0–2%) in both males and females (email dated October 15, 2005, from Kasuke Nagano, JBRC, to Mary Manibusan, U.S. EPA).

Sources: 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:BDF_1 mice in JBRC studies of 0.3% (range: 0–2%) 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 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 oral 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 oral gavage in corn oil on GDs 6–19. 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 were 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 (LH) in the dams (Narotsky et al., 1997a, 1995). In F344 rats administered 150 mg/kg carbon tetrachloride on GD 8, serum LH levels were significantly reduced (by 17–69% at intervals up to 20 hours postdosing) in animals with full-litter resorption; no adverse developmental outcomes were observed in animals that received carbon tetrachloride and human chorionic gonadotropin, which acted as an LH surrogate.

Narotsky et al. (1997b) compared the developmental toxicity of carbon tetrachloride administered to rats by oral 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 PNDs 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 B6D2F_1 mice with 0, 82.6, or 826 mg/kg of carbon tetrachloride by oral gavage in corn oil on GDs 1–5. In this strain, GDs 1–5 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 (1,260 or 2,520 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 (1,260 or 2,520 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 (1,575 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 1,004 ppm (0, 2,101, or 6,316 mg/m^3) for 7 hours/day on GDs 6–15 (Schwetz et al., 1974). Exposures to the two different exposure 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 GDs 6, 13, and 21. 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 1,004-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-exposure group (13%) compared with the concurrent control (2%) but not compared with the low-exposure 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 1,004 ppm. No other increases in individual soft tissue or skeletal anomalies were reported. Maternal toxicity was also observed in both exposure 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 1,004 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 oral gavage, the liver appears to be the primary target organ; damage to the kidney occurs 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 3,188 mg/kg of carbon tetrachloride by oral 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 oral 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 oral 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 2,550 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 7,970 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–

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 oral gavage. After male Sprague-Dawley rats received a single dose of 4,000 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 4,000 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 4,600 ppm, incoordination at 7,300 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 (550 mg/m^3) for 6 hours/day, 5 days/week. The total time-weighted average exposures (concentration × 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 1,000 ppm (630 or 6,300 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 oral 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 1,000 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 1,000 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/hr/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 oral gavage.
[b]100 or 1,000 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 serum glutamic pyruvic transaminase 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 to represent a reasonably comprehensive summary of the available genotoxicity literature. A review of the genotoxicity literature is also provided in Eastmond (2008).

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
S. typhimurium his G46, TA1950	Reverse mutation	Spot test	–	–	4,000 µg/plate	Braun and Schoneich, 1975
S. typhimurium his G46, TA1950	Reverse mutation	Host-mediated assay in male NMRI mice	NA	–	6,400 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	–	–	1,000 µg/plate in DMSO[d]	Brams et al., 1987
S. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation assay	+[d]	+[d]	2,460 µg/plate in methanol	Varma et al., 1988
S. typhimurium TA1535, TA1538	Reverse mutation	Preincubation assay using capped tubes	–	–	1,230 µg/mL	Uehleke et al., 1977
S. typhimurium TA97, TA98, TA100, TA1535, TA1537	Reverse mutation	Preincubation assay using capped tubes	–	–	3,333 µg/plate in DMSO	Zeiger et al., 1988
S. typhimurium TA97, TA98, TA100, TA1535	Reverse mutation	Preincubation assay using capped tubes	–	–	3,333 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 hrs	–	–	ND	Simmon et al., 1977
S. typhimurium TA100, TA1535	Reverse mutation	Gas phase exposure in dessicator for 7–8 hrs	–	–	ND	Simmon and Tardiff, 1978
S. typhimurium TA98, TA100, TA1535	Reverse mutation	Gas phase exposure in closed incubation system for 48 hrs	–	–	2,830 µg/plate	Barber et al., 1981
S. typhimurium TA100, TA1535, TA1537	Reverse mutation	Gas phase exposure in a gas sampling bag for 24 hrs	– (T)	– (T)	50,000 ppm	Araki et al., 2004
S. typhimurium TA98	Reverse mutation	Gas phase exposure in a gas sampling bag for 24 hrs	±	–	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 hrs	±	±	10,000 ppm	Araki et al., 2004
E. coli WP2/pKM101	Reverse mutation	Gas phase exposure in a gas sampling bag for 24 hrs	+	+[e]	5,000 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)	–	–	1,230 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	–	–	5,300 µg/mL	Nakamura et al., 1987
E. coli PQ37	DNA repair	SOS chromotest	–	–	1,540 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

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]		
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

[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 GSH added to the S9 mix. Positive response is based on the magnitude of response as statistical analyses were not performed.

DMSO = dimethyl sulfoxide; SOS = inducible DNA repair system

Table 4-9. Genotoxicity studies of carbon tetrachloride in nonmammalian 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	5,230 µg/mL	Callen et al., 1980
S. cerevisiae D7	Mitotic recombination	Preincubation assay in capped tubes	+ (T)	ND	5,230 µg/mL	Callen et al., 1980
S. cerevisiae D7	Reverse mutation	Preincubation assay in capped tubes	+ (T)	ND	5,230 µg/mL	Callen et al., 1980
S. cerevisiae RS112	Intrachromosomal recombination	Preincubation assay	+ (T)	ND	2,000 µg/mL	Brennan and Schiestl, 1998
S. cerevisiae RS112	Intrachromosomal recombination	Preincubation assay	+ (T)	+ (T)	4,000 µg/mL	Schiestl et al., 1989; Galli and Schiestl, 1998
S. cerevisiae RS112	Interchromosomal recombination	Preincubation assay	+ (T)	+ (T)	4,000 µg/mL	Galli and Schiestl, 1998
S. cerevisiae RS112 (arrested in S phase)	Intrachromosomal recombination	Preincubation assay	–	ND	8,000 µg/mL	Galli and Schiestl, 1998
S. cerevisiae RS112 (arrested in S phase)	Interchromosomal recombination	Preincubation assay	–	ND	8,000 µg/mL	Galli and Schiestl, 1998
S. cerevisiae RS112 (arrested in G1 phase)	Intrachromosomal recombination	Preincubation assay	+ (T)	ND	5,000 µg/mL	Galli and Schiestl, 1998, 1996
S. cerevisiae RS112 (arrested in G1 phase)	Interchromosomal recombination	Preincubation assay	+ (T)	ND	5,000 µg/mL	Galli and Schiestl, 1998, 1996
S. cerevisiae AGY3 (arrested in G2 phase or growing normally)	Intrachromosomal recombination	Preincubation assay	+ (T)	ND	8,000 µg/mL	Galli and Schiestl, 1995
S. cerevisiae D61.M	Aneuploidy	Standard 16-hr incubation or cold-interruption regimen	–	ND	6,400 µg/mL	Whittaker et al., 1989
Aspergillus nidulans P1	Somatic segregation due to cross over and aneuploidy	Plate incorporation assay	+ (T)	ND	0.5%	Gualandi, 1984

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

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
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 2,000 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-Min incubation in sealed tubes	– (T)	– (T)	76 µg/mL	Garry et al., 1990
Human peripheral lymphocytes G_0	Sister chromatid exchange	30-Min incubation in sealed tubes	– (T)	– (T)	48 µg/mL	Garry et al., 1990
Human lymphocytes from two donors	Micronucleus formation	Test conducted in capped tubes	– (2–)[d]	± (1–)[d]	1,540 µg/mL	Tafazoli et al., 1998
Human lymphocytes	DNA breaks	Comet assay	–	–	3,080 µg/mL	Tafazoli et al., 1998
Human lymphocytes	Unscheduled DNA synthesis	4-Hr culture, autoradiography	–	–	16,000 µg/mL	Perocco and Prodi, 1981
Lamb peripheral lymphocytes	Chromosomal aberrations	48-Hr incubation	–	ND	16 µg/mL	Sivikova et al., 2001
Lamb peripheral lymphocytes	Micronucleus formation	48-Hr incubation	+	+	8 µg/mL (without activation) 16 µg/mL (with activation)	Sivikova et al., 2001
Lamb peripheral lymphocytes	Sister chromatid exchange	48-Hr 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	1,540 µg/mL	Doherty et al., 1996
Chinese hamster ovary cells	Chromosomal aberrations	Assay conducted in sealed flasks	–	–	3,000 µg/mL in DMSO	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)	–	1,490 µg/mL (w/out activation) 2,930 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	8,000 µg/mL	Coutino, 1979
V79 Chinese hamster lung cell line	Aneuploidy	3-Hr incubation	+	ND	246 µg/mL	Onfelt, 1987
V79 Chinese hamster lung cell line	c-Mitosis (spindle disturbance)	30-Min incubation	± (T)	ND	492 µg/mL	Onfelt, 1987
Syrian hamster embryo cells	Morphological transformation	Clonal assay	±[f]	ND	3 µg/mL	Amacher and Zelljadt, 1983
Mouse lymphoma L5178Y cells	Mutation at tk locus	4-Hr incubation	ND	– (T)	635 µg/mL	Wangenheim and Bolcsfoldi, 1988
Mouse lymphoma L5178Y cells	DNA strand breaks	Alkaline elution	ND	+(T)	1,007 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 four human donors	Unscheduled DNA synthesis	21.5–24-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

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 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

[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]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 6–48 hrs after dosing	– (T)	NA	8,000 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	1,600 mg/mL by oral gavage	Rossi et al., 1988
Mouse (BDF_1, male)	Micronucleus formation in bone marrow	Analyzed polychromatic erythrocytes from specimens prepared 24 hrs after dosing	– (T)	NA	2,000 mg/kg by oral gavage (2×)	Morita et al., 1997; Suzuki et al., 1997
Mouse (BDF_1, male)	Micronucleus formation in bone marrow	Analyzed polychromatic erythrocytes from specimens prepared 24 hrs after dosing	– (T)	NA	2,000 mg/kg by oral gavage	Morita et al., 1997; Suzuki et al., 1997
Mouse (BDF_1, male)	Micronucleus formation in peripheral blood	Analyzed reticulocytes from specimens prepared 24–72 hrs after dosing	–	NA	3,000 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 hrs after dosing	–[d]	NA	2,000 mg/kg by oral 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 hrs after dosing	– (T)	NA	3,000 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 hrs after dosing	–	NA	2,000 mg/kg by oral 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
Mouse (NMRI, male and female)	DNA strand breaks in liver	Alkaline elution of sample collected 4 hrs after dosing	–	NA	4,000 mg/kg by oral gavage	Schwarz et al., 1979

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, female, partially hepatectomized)	DNA damage in liver	Caffeine elution 4 or 24 hrs after dosing	–	NA	800 mg/kg by oral gavage in corn oil	Stewart, 1981
Rat (F344, male)	DNA strand breaks in liver	Alkaline elution on primary hepatocytes isolated from rats sacrificed 2–48 hrs after dosing	–	NA	400 mg/kg by oral gavage in corn oil	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 hrs after dosing	– (T)	NA	4,000 mg/kg by i.p. injection	Barbin et al., 1983
Rat (Sprague-Dawley, male)	DNA damage in liver	Viscometric assay on rats sacrificed 2 hrs 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 oral gavage in corn oil	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 hrs before sacrifice	–	NA	1,050 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 nonparenchymal cells identified by in situ nick translation 12–96 hrs after dosing	± (T)[e]	NA	1,600 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 nonparenchymal cells identified by in situ nick translation after dosing 2 times/wk until wk 12 with sacrifices at 3, 6, 9, 12, 15, and 18 wks	± (T)[e]	NA	2,000 mg/kg (24 times)	Nakamura and Hotchi, 1992
Mouse (CD-1, male)	DNA damage in liver	Comet assay on liver obtained 0, 3, or 24 hrs after dosing	+ (T)	NA	1,000 mg/kg by oral gavage	Sasaki et al., 1998
Rat (Wistar, male)	DNA fragmentation in liver	TUNEL[f] assay on rats sacrificed 1 d after the second dose	+ (T)	NA	800 mg/kg by ip (2 times)	Cabre et al., 1999
Rat (Wistar, male)	DNA fragmentation in liver	TUNEL[f] assay on rats sacrificed at 10, 15, 20, 25, and 30 hrs after dosing	+ (T)	NA	240 mg/kg in corn oil by i.p.	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 hrs after dosing	–	NA	4,000 mg/kg by oral 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 hrs after dosing	+ (T)	NA	4,000 mg/kg by oral gavage in liquid paraffin	Craddock and Henderson, 1978
Rat (F344, male)	Unscheduled DNA synthesis in liver	Rats sacrificed 2 hrs after dosing; primary hepatocytes isolated by liver perfusion and cultured with [^{3}H]-thymidine	–	NA	100 mg/kg by oral gavage in corn oil	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 (F344, male)	Unscheduled DNA synthesis in liver	Rats sacrificed 2–48 hrs after dosing; primary hepatocytes isolated by liver perfusion and cultured with [^{3}H]-thymidine	– (T)	NA	400 mg/kg by oral gavage in corn oil	Mirsalis et al., 1982
Mouse (B6C3F$_1$, male)	Unscheduled DNA synthesis in liver	Rats sacrificed 12 hrs after dosing; primary hepatocytes isolated by liver perfusion and cultured with [^{3}H]-thymidine	– (T)	NA	100 mg/kg by oral gavage	Madle et al., 1994; Mirsalis, 1987
Mouse (B6C3F$_1$, female)	Unscheduled DNA synthesis in liver	Rats sacrificed 12 hrs 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 hrs after dosing; liver cells isolated and analyzed by autoradiography	– (T)	NA	100 mg/kg by oral gavage in corn oil	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 hrs after dosing	–	NA	8,000 mg/kg	Curtis and Tilley, 1968
Rat (F344, male)	Chromosomal aberrations in liver	Analyzed primary hepatocytes cultured for 48 hrs from rats sacrificed 0–72 hrs after dosing	–	NA	1,600 mg/kg by oral gavage in corn oil	Sawada et al., 1991
Rat (F344, male)	Sister chromatid exchange in liver	Analyzed primary hepatocytes cultured for 48 hrs from rats sacrificed 0–72 hrs after dosing	–	NA	1,600 mg/kg by oral gavage in corn oil	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 (F344, male)	Micronucleus formation in liver	Analyzed primary hepatocytes cultured for 48 hrs from rats sacrificed 0–72 hrs after dosing	–	NA	1,600 mg/kg by oral gavage in corn oil	Sawada et al., 1991
Rat (Wistar, male)	Micronucleus formation in liver	Analyzed primary hepatocytes harvested 72 hrs after dosing, an optimal time to detect micronuclei.	± (T)	NA	3,200 mg/kg by oral gavage in corn oil	Van Goethem et al., 1993
Rat (Wistar, male)	Micronucleus formation in liver	Analyzed primary hepatocytes harvested 72 hrs after dosing, an optimal time to detect micronuclei.	+ (T) [g]	NA	3,200 mg/kg by oral 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 d after dosing and compared with a partially hepatectomized control.	–	NA	15-Min inhalation at 0.05–0.1 mL/5 L	Uryvaeva and Delone, 1995
Mouse ($B6C3F_1$, *lacI* transgenic; Big Blue™, male)	Mutations in *lacI* transgene in liver	The target *lacI* gene is recovered from genomic DNA after five daily doses and the animals sacrificed 7 d after the first dose	– (T)	NA	35 mg/kg-day (5 times)	Mirsalis et al., 1994
Mouse ($CD2F_1$ *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 d later	– (T)	NA	80 mg/kg by oral gavage in corn oil	Lambert et al., 2005; Tombolan et al., 1999
Mouse ($CD2F_1$ *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 d later	– (T)	NA	1,400 mg/kg by oral gavage	Lambert et al., 2005; Hachiya and Motohashi, 2000

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 hrs 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 hrs 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 hrs 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 hrs 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 hrs after dosing	+ (T)	NA	3,200 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 hrs 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 hrs after dosing	±	NA	1,200 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 hrs after dosing	±	NA	1,200 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 hrs after dosing	±	NA	1,200 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 hrs after dosing	+ (T)	NA	1,600 mg/kg by oral 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 d after dosing	+ (T)	NA	0.1 mg/kg by oral gavage in corn oil	Chaudhary et al., 1994
Rat (strain and sex not specified)	DNA adducts in liver	Deoxyguanosine-malondialdehyde adducts measured 48 hrs 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 hrs after treatment	± (T)	NA	160 mg/kg by oral gavage in corn oil	Wang and Liehr, 1995
Rat (F344, male)	DNA adducts in liver	4-HNE-dG adducts formed secondary to lipid peroxidation	+ (T)	NA	3,200 mg/kg i.p. in olive oil	Chung et al., 2000
Rat (F344, female)	DNA adducts in liver, kidney, lung, colon, and forestomach	4-HNE-dG adducts formed secondary to lipid peroxidation. Samples collected 4, 8, 16, or 24 hrs after final dose	+ (T)	NA	500 mg/kg i.p. (1 or 4 times)	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 hrs to 7 d	+ (T)	NA	3,200 mg/kg by oral gavage in olive oil	Takahashi et al., 1998

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 liver	8-OHdG adducts measured at the end of wk 1 after dosage on d 1 and 4	± (T)	NA	400 mg/kg by s.c. injection (2 times)	Iwai et al., 2002
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- and 14-mo animals dosed for 3 d and sacrificed on d 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 hrs after a single injection	+ (T)	NA	1,200 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 wks after two injections given a wk part.	– (T)	NA	1,200 mg/kg by i.p. in corn oil	Nath et al., 1990

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 methylation in liver	Hydrolyzed DNA was analyzed for aberrant methylation as increases in 7-methylguanine and O^6-methylguanine, 12 hrs after dosing	+ (T)	NA	1,000 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 2 times per wk for 3 wks	Varela-Moreiras et al., 1995

[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; i.m. = intramuscular, s.c. = subcutaneous.
[d]The small statistically significant increase detected was considered biologically insignificant by the authors (and other reviewers).
[e]At this dose, a roughly threefold 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.

4-HNE-dG = deoxyguanosine adducts of 4-HNE; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; 8-oxo-7,8-dihydro-2'-deoxyguanosine = 8-oxodG

Note: The data in the paper by Sarkar et al (1999) were judged to be insufficiently reliable to be included in the table.

4.4.2.1. *Genotoxicity Studies: Prokaryotic Organisms*

As shown in Table 4-8, 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. 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 inducible DNA repair system (SOS) induction assay using strain TA1535/pSK1002 (Nakamura et al., 1987). More varied results were seen in experiments using *Escherichia coli*. Carbon tetrachloride was negative in a SOS chromotest assay (Brams et al., 1987), 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 *E. coli* 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 preincubation 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 were unusually low and the induced response was within the control values reported by others (Damment et al., 2005; Martinez et al.,

2000). Additionally, a 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.

Carbon tetrachloride was also positive in the repair-proficient WP2/pKM101 strain of *E. coli*. A doubling in mutant frequency was observed at the 5,000 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 GST. 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 (Martinez et al., 2000; Gatehouse et al., 1994). 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 10-fold increase in mutants in the WP2/pKM101 experiments without S9. However, approximately half of the observed increase was 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 CYP450 monooxygenase-mediated formation of the trichloromethyl radical (Weber et al., 2003; Halliwell and Gutteridge, 1999). 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, dimethyl sulfoxide (DMSO), and ethanol are also metabolized by the CYP450 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).

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 CYP450 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

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.

4.4.2.2. *Genotoxicity Studies: Nonmammalian 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 >20-fold increases in gene conversion and mitotic conversion and a 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 8,000 µg/mL, induced a 25-fold increase in intrachromosomal recombinants with no increase in interchromosomal recombination. Toxicity was >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, 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 *S. cerevisiae*, the assays using *A. 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 and an approximately 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 10-fold increase in chromosome segregation at the highest (0.08%) concentration tested. Toxicity at this concentration was 72%. More modest effects (approximately threefold) 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 (0.01–0.03%; 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 CYP450 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 nontarget 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 (5–20 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 (>1,500 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 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 micronucleus and SCE experiments, the addition of vitamin E and selenium to the cultures protected against the increases in micronucleus and SCE, implicating a role for free radicals in the observed genotoxic effects. In spite of the protective effects of the antioxidants, these studies observed 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, and 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 five- and ninefold increases 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 nontarget 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 1,640 media with horse serum and DMEM with fetal bovine serum. It was negative in the RPMI medium with 0 transformants among 2,665 colonies. In DMEM, one transformed colony was seen in 2,003 colonies scored. Although this was considered a positive result by the authors, the increase is not statistically significant, does not meet criteria for a positive result (Kerckaert et

al., 1996), and falls within the normal control frequencies of 0–0.8% 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 (MDA) 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 2-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 nonspecific mechanisms. The binding studies using radiolabeled carbon tetrachloride (for discussion, see the following sections) provide limited evidence that bioactivated carbon tetrachloride can bind directly to DNA. As seen in nonmammalian 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 nontarget 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 nontarget 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 BDF_1 mice (Morita et al., 1997; Suzuki 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 2,000 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 studies, and positive results were seen in four studies. When positive or equivocal responses were seen, they consistently occurred at cytotoxic doses. A brief overview of each of the positive studies is provided below.

Nakamura and Hotchi (1992) observed an increase in DNA breakage in their studies of DNA breakage in nonparenchymal 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/or gene expression. 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 24–36 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.

UDS 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 4,000 mg/kg carbon tetrachloride increased the synthesis of DNA in nonreplicating 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 2-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).

In six other studies conducted using the currently recommended autographic detection method, 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.

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 (8,000 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 3,200 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 multicentromeric rat probe, the authors attributed the increase in micronucleus 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.

Sarkar et al. (1999) reported that the administration of carbon tetrachloride to mice over a 5-week period resulted in increases in structural chromosome aberrations in liver cells. However, because of the numerous and significant methodological issues with these experiments, 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. The transgenic mouse models used to evaluate carbon tetrachloride (lac I, $B6C3F_1$; *lacz* $CD2F_1$ Muta™Mice) represent normal immunocompetent rodent strains with the addition of reporter genes for identification of mutational events. Negative results were seen in each of the three studies. As reported by Mirsalis and coworkers, transgenic $B6C3F_1$ lacI mice were treated with five daily doses of carbon tetrachloride at 35 mg/kg-day and the animals were sacrificed 7 days after the first dose (Mirsalis, 1995; Mirsalis et al., 1994). 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 1,000-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 was administered at 80 mg/kg by i.p. injection to *lacz* transgenic $CD2F_1$ mice (Muta™Mice) and the animals were sacrificed 14 days later (Lambert et al., 2005; Tombolan et al., 1999). 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 nontransgenic $CD2F_1$ mice receiving an intragastric dose of carbon tetrachloride, significant increases in absolute and relative liver weights were seen beginning 2 days after treatment. The percent of hepatocytes labeling with BrdU during the last 2 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 $CD2F_1$ *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 1,400 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}–100.4×10^{-6}). The mutant frequencies for the other three mice exceeded the upper end of the control range by 3–49%. 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 (Lambert et al., 2005; Heddle et al., 2000). 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 nonpretreated 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 (3,200 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 disintegrations per minute (dpm). 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 1,200 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 [$\times 10^6$]). 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 nonnecrotizing 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 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 4-hydroxynonenal (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-MDA 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 4 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 MDA 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-MDA 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 MDA formed. They noted that under the same conditions, previous studies have shown that large concentrations of MDA adducts with lysine, but not deoxyguanosine-MDA, 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 4 hours after treatment with 160 mg/kg and 1,600 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 × 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 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 1,200 mg/kg. The

observed decrease is also similar to the decrease in the deoxyguanosine-MDA 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 4-HNE-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 4-HNE-dG (deoxyguanosine adducts of 4-HNE) adduct were seen in the livers of F344 rats treated with a single 3,200 mg/kg dose of carbon tetrachloride. Twenty-four hours after treatment, the levels of the 4-HNE-dG adducts were increased 37-fold as compared to those of control animals (104 nmol/mol guanine versus 2.8 nmol/mol guanine). The adducts were persistent as significant levels of the 4-HNE-dG adducts (88 nmol/mod guanine) were present 72 hours after dosing.

The formation of 1,N2-propanodeoxyguanosine adducts of 4-HNE (4-HNE-dGp) 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 postinjection, or by four injections at 24-hour intervals with the sacrifice occurring 8 hours after the final dose. In the single injection studies, increases in 4-HNE-dGp adducts were seen in the lung and colon at various times and in the forestomach at all three time points. 4-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 multidose studies, significant increases were seen in the liver (2.2-fold) and the forestomach (1.7-fold). The levels of 4-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 MDA in the liver after treatment with carbon tetrachloride (3.8 per 10^7 normal nucleotides; Chaudhary et al., 1994) and 4-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 3,200 mg/kg by oral gavage and sacrificed at 6 and 12 hours, and 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 messenger RNA (mRNA) expression were also increased by 5.1- and 1.7-fold, respectively. MDA plus 4-HNE 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 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 months old) and older (14 months old) female CD-1 mice. Twenty-four hours posttreatment, 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 1,200 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 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]-postlabeling 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 1,200 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 (1,200 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 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 (1981), increases in 7-methylguanine and O^6-methylguanine were seen in liver DNA 12 hours after rats were administered a single 1,000 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 nonspecific 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, GSH, 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*

U.S. 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 nontarget tissues. This prioritization scheme is also consistent with EPA's cancer 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 MOA. 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 the 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. Nonrandom 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. 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 noncoding 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 limited evidence for the formation of DNA adducts derived directly from carbon tetrachloride.

UDS. 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. Initiation-promotion Studies

Tsujimura et al. (2008) examined the potential of carbon tetrachloride to induce preneoplastic lesions in rat liver. Male rats (15/group) were exposed to carbon tetrachloride

vapor using nose-only inhalation exposure at concentrations of 0, 1, 5, 25, or 125 ppm for 6 hours/day, 6 days/week for 6 weeks. The numbers and area of GST-P positive foci were determined. Investigators also evaluated liver tissue for histopathological changes and measured serum chemistry parameters and carbon tetrachloride concentrations in blood.

Absolute and relative liver weights were statistically significantly increased at concentrations ≥25 ppm. The areas (mm^2/cm^2) and numbers (number per cm^2) of GST-P positive foci were statistically significantly increased in the carbon tetrachloride-exposed rats at 25 and 125 ppm, but not at concentrations of 1 and 5 ppm. Histopathological examination of the liver revealed centrilobular ballooning of hepatocytes, interlobular fibrosis, increased mitoses of hepatocytes, and eosinophilic foci in all 125-ppm exposed rats. At 25 ppm, centrilobular ballooning of hepatocytes was reported. Investigators observed microgranuloma in 14/15 rats exposed to 25 ppm, but not in any rats exposed to 5 ppm or 125 ppm. Exposure-related changes in liver enzymes were reported. ALP was increased at ≥5 ppm, and AST and ALT were increased at ≥25 ppm. At 125 ppm, gamma-glutamyl transpeptidase activity and total cholesterol were increased.

Bull et al. (2004) used an initiation-promotion study design to examine how dichloroacetate, trichloroacetate, and carbon tetrachloride, three liver carcinogens that appear to induce liver tumors by different MOAs, might interact when given as mixed exposures. Only the carbon tetrachloride results are summarized here. $B6C3F_1$ mice were initiated by the tumor initiator, vinyl carbamate (3 mg/kg at 2 weeks of age), and then promoted by carbon tetrachloride for 18, 24, 30, and 36 weeks beginning at weaning (21 days of age). Initial carbon tetrachloride doses (50, 100, and 500 mg/kg-day by oral gavage) were too high for study purposes and were reduced to 5, 20, and 50 mg/kg-day. Dose-related increases in mean tumor volume were observed with 20 and 50 mg/kg-day carbon tetrachloride, but each produced equal numbers of tumors at 36 weeks. At doses ≥100 mg/kg-day, substantial increases in the number of tumors per animal were observed, but the mean tumor size decreased. The investigators concluded that this finding suggests that initiation occurs at carbon tetrachloride doses of ≥100 mg/kg-day, perhaps as a result of a high-dose inflammatory response that is known to occur with high doses of carbon tetrachloride. The investigators observed that trichloroacetate substantially increased the numbers of tumors observed at early time points when combined with carbon tetrachloride and suggested that the interaction between carbon tetrachloride and trichloroacetate may be explained through stimulation of the growth of cells with differing phenotypes.

4.4.4. 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 (≥4,800 ppm). In an acute inhalation study in rats, signs of central nervous system depression occurred at ≥4,600 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 1,370 ppm (one-tailed 90% CI: 465 ppm) for mice.

4.4.5. 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 were, 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 B6C3F$_1$ mice at doses of 0, 500, 1,000, or 1,500 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 ≥1,000 mg/kg-day, and total protein at 1,500 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 1,500 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 B6C3F$_1$ mice given carbon tetrachloride by i.p. injection or oral gavage in corn oil. Oral or i.p. administration of 500–5,000 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 1,000 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 5–1,000 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 1,000 mg/kg-day by oral gavage in corn oil to B6C3F$_1$ 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 1,000 mg/kg-day) and a decrease in glucose (by 20% at 1,000 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 1,000 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 (1,590 mg/kg) carbon tetrachloride in olive oil twice weekly by oral 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 weights 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%). NK 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 oral 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 oral 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 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 (0, 630, 1,260, or 1,890 mg/m^3) of carbon tetrachloride vapor (Ban et al., 2003). Exposure duration was not reported; however, the maximum exposure period was most likely <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 MOA 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). Discussion of the roles of metabolism, lipid peroxidation, and disruption of calcium homeostasis in carbon tetrachloride toxicity is presented 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).

Carbon tetrachloride administered in vivo to guinea pigs decreased microsomal CYP450 concentrations in the adrenal gland, providing evidence that the adrenal cortex is an active site of carbon tetrachloride metabolism (Colby et al., 1981). In the adrenal gland, necrosis caused by carbon tetrachloride is localized to the innermost region of the cortex, the zona reticularis, where there is far greater activation of carbon tetrachloride by microsomal enzymes than other regions of the adrenal cortex (Colby et al., 1994). The profile of CYP450 isozymes in the adrenal is directed principally toward steroid metabolism and bears little resemblance to that in the liver. Using an in vitro model with isolated tissue from guinea pig adrenal zona reticularis, Colby et al. (1994) reported that carbon tetrachloride is specifically activated by a 52 kDa CY450 enzyme associated with xenobiotic metabolism.

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).

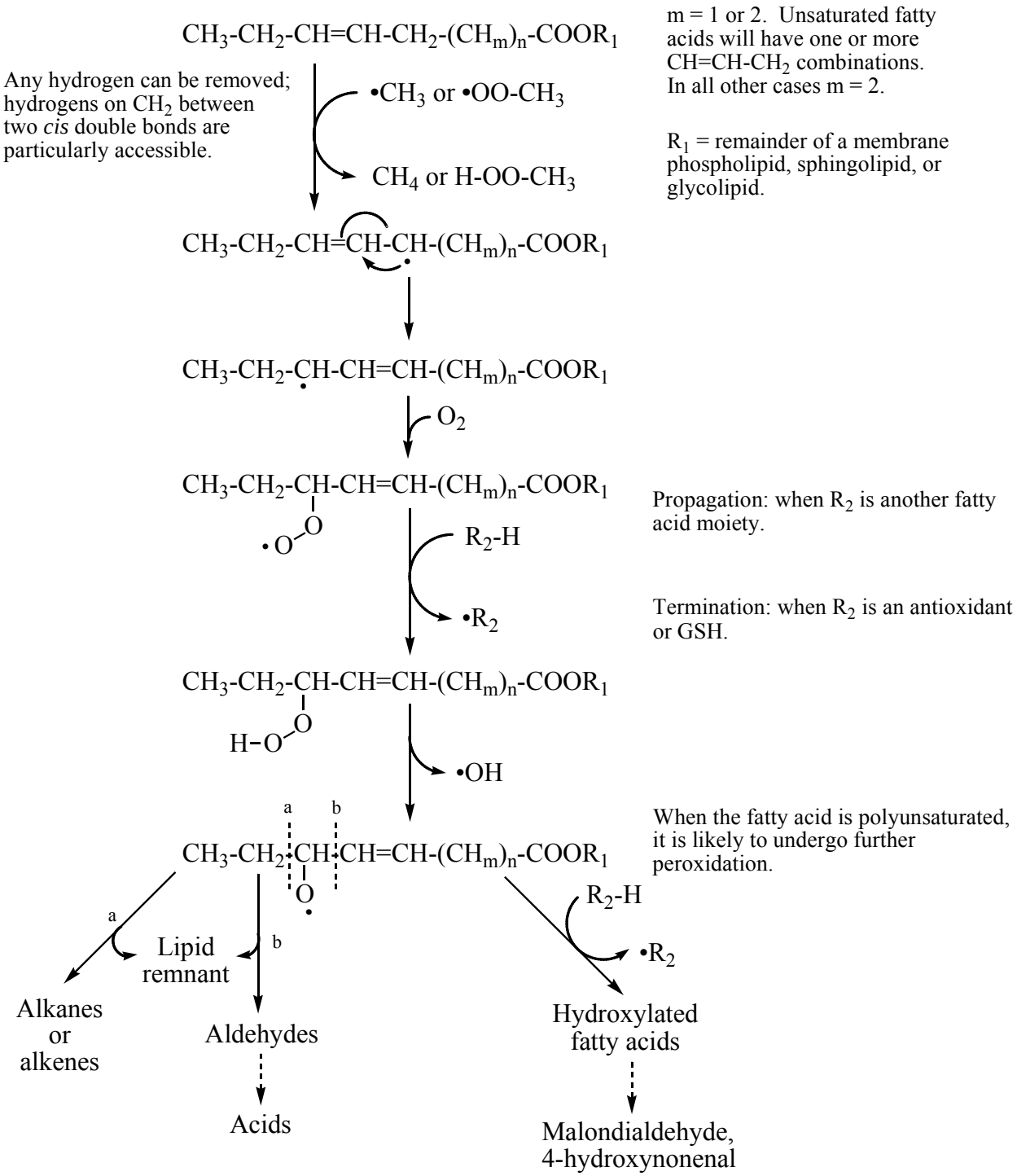


Figure 4-3. Lipid peroxidation.

Although the trichloromethyl radical can also initiate lipid peroxidation, it does so at a 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.

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-HNE and MDA.

In vitro studies have shown that 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 4-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 4-HNE-dGp (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 4-HNE-dG adducts in the liver were elevated 1.5- to 2-fold compared with those in controls. The authors concluded that these promutagenic adducts are evidence of radical-initiated lipid peroxidation, which can lead to cancer if not

repaired effectively. Other studies have also indicated that lipid peroxidation byproducts 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 4-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 et al., 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 oral 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).

Ciccoli et al. (1978) reported that binding of carbon tetrachloride radicals to cellular lipids occurred in rat extrahepatic tissues, although to a lesser extent than the liver. Almost half of the radioactivity from [^{14}C]-labeled carbon tetrachloride incorporated into phospholipids was found in the liver (47%); in other tissues, incorporation into phospholipids was found in intestinal mucosa (24%), kidney (9%), adrenal gland (8%), and lung (5%), while spleen, testis, brain, heart, and skeletal muscle lipids showed minor levels of radioactivity. Fatty acid methyl esters prepared from the phospholipids of intestinal mucosa and kidney exhibited an electron capture detector response similar to the liver (indicating free radical reaction); however, other tissues with low-level [^{14}C] incorporation showed no electron capture detector response. In an in vitro model, Colby (1981) and Colby et al. (1994) provided evidence that carbon tetrachloride can stimulate lipid peroxidation in adrenal micosomes. Incubation of carbon tetrachloride plus NADPH produced a decrease in guinea pig adrenal microsomal CYP450 content and stimulated lipid peroxidation in adrenal zona reticularis microsomes (as indicated by rate of MDA production). In the absence of NADPH, carbon tetrachloride did not affect lipid peroxidation and little covalent binding was demonstrable.

Lipid peroxidation byproducts 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 (>twofold) at 4 mM; lower concentrations were not studied. The formation of MDA-DNA adducts correlated 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

GSH 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 GSH 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 and water. Cellular levels of GSH are restored through the activity of GSH reductase using NADPH + H^+ as the hydrogen donor (Klaassen, 1996).

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 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 GSH). 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 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 that generates 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 calcium ions from the cytosol to the endoplasmic reticulum hydrolyzing 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 <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 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 were, 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 (PH) (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 $B6C3F_1$ mice treated with 250 or 500 mg/kg carbon tetrachloride in corn oil by oral 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 1,000 mg/kg carbon tetrachloride in corn oil) was abolished upon addition of TGF-β-specific antibodies to the assay. Jeon et al. (1997) reported elevations of TGF-β1 mRNA in the liver of $B6C3F_1$ 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 (TNF-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 1,275 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 4,000 mg/kg of carbon tetrachloride by oral gavage in corn oil, with or without i.p. injection of 2,000 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 3,190 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-κβ 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 PH. 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 PH), 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 PH. 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, 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 2/3 PH 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-κβ and AP-1; steady-state levels of TNF-α 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-α mRNA, activation of NF-κβ and AP-1, and induction of iNOS. Lead nitrate induced NF-κβ, TNF-α, 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-α mRNA, activation of NF-κβ and AP-1, or induction of iNOS, although the number of hepatocytes entering S phase 18–24 hours after nafenopin 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

3 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 1,594 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

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-d)	LOAEL (mg/kg-d)	Effects at the LOAEL	Reference
Subchronic studies					
Dog (6/sex)	28 d in gelatin capsule: 797 mg/kg-d	Not determined	797	Increased ALT, OCT; fatty vacuolization with single cell necrosis in liver	Litchfield and Gartland, 1974
Dog (3 F)	8 wks in gelatin capsule: 32 mg/kg-d	32	Not determined	No increases in serum enzymes; no liver histopathology	Litchfield and Gartland, 1974
Rat (15–16 M/ group)	5 d/wk for 12 wks by oral gavage in corn oil: 0, 1, 10, or 33 mg/kg-d	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 d/wk for 12 wks by oral gavage in corn oil: 0, 20, or 40 mg/kg-d; sacrificed at intervals from 1 to 15 d postexposure	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 d/wk for 13 wks by oral gavage in corn oil: 0, 25, or 100 mg/kg-d	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 d/wk for 13 wks by oral gavage in 1% Emulphor: 0, 25, or 100 mg/kg-d	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 d/wk for 12 wks by oral gavage in corn oil: 0, 1.2, 12, or 120 mg/kg-d	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 d/wk for 12 wks by oral gavage in 1% Tween-60: 0, 1.2, 12, or 120 mg/kg-d	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
Mouse (20/sex/ group)	7 d/wk for 13 wks by oral gavage in corn oil: 0, 12, 120, 540, or 1,200 mg/kg-d	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 d by oral gavage in corn oil: 0, 40, 80, or 160 mg/kg-d	40	80	Necrosis in liver	Eschenbrenner and Miller, 1946

Table 4-13. Oral toxicity studies for carbon tetrachloride

Species	Dose/duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the LOAEL	Reference
Chronic studies					
Rat (50/sex/ group)	5 d/wk for 78 wks by oral gavage in corn oil: 0, 47, or 94 mg/kg-d for males; 0, 80, or 159 mg/kg-d for females	Not determined	47	Increased mortality; cirrhosis in liver	NCI, 1977, 1976a, b
Mouse (50/sex/ group)	5 days/wk for 78 wks by oral gavage in corn oil: 0, 1,250, or 2,500 mg/kg-d	Not determined	1,250 (FEL)	Markedly increased mortality; cirrhosis and other toxic lesions in liver; adrenal pheochromocytoma	NCI, 1977, 1976a, b
Gestational exposure studies					
Rat (29 gravid F)	2 d on GDs 7–11 by oral gavage in corn oil: 478 mg/kg-d	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 oral gavage in corn oil: 0, 112.5, or 150 mg/kg-d	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 oral gavage in corn oil: 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 oral gavage in 10% Emulphor: 0, 25, 50, or 75 mg/kg-d	25	50	Piloerection; slightly increased full-litter resorption	Narotsky et al., 1997b
Mouse (≥8 gravid F/group)	GDs 1–5 by oral 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-d × [5 d/wk ÷ 7 d/wk] = 0.71 mg/kg-d).

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 GDs 6–15 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., 1997a, 1995). 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 did not show these effects (Hamlin et al., 1993).

Adrenal adenoma and pheochromocytomas were observed in mice exposed to carbon tetrachloride by oral gavage in an NCI bioassay in which carbon tetrachloride was used as a positive control for liver tumors (Weisburger, 1977). 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 1,250 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 oral 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., 1997b; 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. (1997b) found that the

influence of vehicle could be dose-dependent. In their study of developmental toxicity, Narotsky et al. (1997b) 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 volatile organic compounds 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., 1990b).

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

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 GI 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 1–4 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 those causing 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).

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 hrs/d, 5 d/wk for 10.5 mo: 0, 50, 100, 200, or 400 ppm	Not determined	50 [12][a]	Fatty change in liver	Smyth et al., 1936
Guinea pig (24 mixed sex/group)	8 hrs/d, 5 d/wk for 10.5 mo: 0, 25, 50, 100, 200, or 400 ppm	Not determined	25 [6][a] (FEL)	Increased mortality; reduced body weight gain; fatty change in liver	Smyth et al., 1936
Monkey (4/group)	8 hrs/d, 5 d/wk for 10.5 mo: 0, 50, or 200 ppm	Not determined	50 [12][a]	Mild fatty change and degeneration in liver	Smyth et al., 1936
Rat (15–25/sex/ group)	7 hrs/d, 5 d/wk for 6 mo: 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 hrs/d, 5 d/wk for 6 mo: 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 hrs/d, 5 d/wk for 6 mo: 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 hrs/d, 5 d/wk for 6 mo: 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 hrs/d, 7 d/wk for 13 wks: 0, 1 (in n-octane), or 10 ppm	1	10	Reduced body weight gain; enlarged liver with fatty change	Prendergast et al., 1967
Guinea pig (15/group)	24 hrs/d, 7 d/wk for 13 wks: 0, 1 (in n-octane), or 10 ppm	1	10	Reduced body weight gain; enlarged liver with fatty change; three died, though mortality also reported in control group	Prendergast et al., 1967
Rabbit (3/group)	24 hrs/d, 7 d/wk for 13 wks: 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 hrs/d, 7 d/wk for 13 wks: 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 hrs/d, 7 d/wk for 13 wks: 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 hrs/d, 5 d/wk for 13 wks: 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 hrs/d, 5 d/wk for 13 wks: 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

Table 4-14. Inhalation toxicity studies for carbon tetrachloride

Species	Duration/ concentration	NOAEL (ppm)	LOAEL (ppm)	Effects at the LOAEL	Reference
Rat (10 M/ group)	6 hrs/d, 5 d/wk for 12 wks: 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 hrs/d, 5 d/wk for 12 wks: 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 hrs/d, 5 d/wk for 12 wks: 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 hrs/d, 5 d/wk for 104 wks: 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
Mouse (50/sex/ Group)	6 hrs/d, 5 d/wk for 104 wks: 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 pheochro-mocytoma (males)	Nagano et al., 2007b; JBRC, 1998
Gestational exposure study					
Rat (22–23 gravid F/group)	7 hrs/d on GDs 6–15: 0, 334, or 1,004 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 × [6 hours/day ÷ 24 hours/day × 5 days/week ÷ 7 days/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 are 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 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 unknown. 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 2 years of exposure to carbon tetrachloride, proteinuria in 5-ppm rats did not 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 than 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 is not 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 MOA 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 MOA (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.

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, the exact sequence or contribution of cellular mechanisms leading from the key event of metabolism to carbon tetrachloride-induced hepatotoxicity (cell death) is uncertain. A description of how carbon tetrachloride-induced noncancer effects may coincide with a hypothesized mode of carcinogenic action for carbon tetrachloride can be found in Figure 4-4.

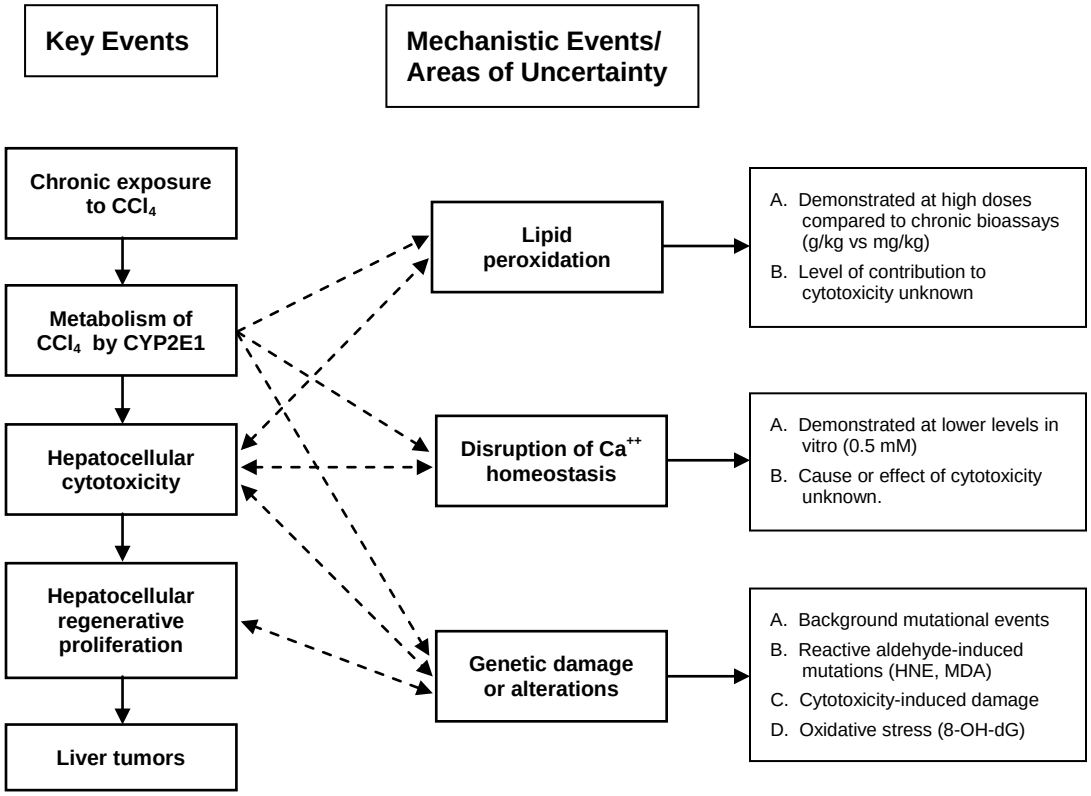


Figure 4-4. Hypothesized carcinogenic MOA.

Although most mechanistic studies for carbon tetrachloride have concentrated on hepatic effects, some studies provide evidence for a similar MOA 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 MOA 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 is "likely to be carcinogenic to humans" based on: (1) inadequate evidence of carcinogenicity in humans and (2) sufficient evidence in animals by oral and inhalation exposure, i.e., hepatic tumors in multiple species (rat, mouse, and hamster) and pheochromocytomas (adrenal gland tumors) in mice.

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the cancer descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of carbon tetrachloride demonstrates that tumors occur in tissues remote from the site of absorption. 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, and to induce pheochromocytomas in mice by oral and inhalation exposure. Information on the carcinogenic effects of carbon tetrachloride via the dermal route in humans and animals is limited or absent. Data on the absorption of carbon tetrachloride reveal that the chemical is readily absorbed via all routes of exposure, including oral, inhalation, and dermal. Therefore, based on the observance of

systemic tumors following oral and inhalation exposure and absorption by all routes of exposure, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, carbon tetrachloride is considered "likely to be carcinogenic to humans" by all routes of 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 [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 was statistically significantly increased compared to concurrent and historical controls.

A hypothesized carcinogenic MOA for carbon tetrachloride-induced liver tumors 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. This MOA appears to play a significant role at relatively high exposures, driving the steep increase in liver tumors in this exposure range. Data to characterize key events at low-exposure levels, however, are limited. This is of particular concern for liver tumor MOA considerations in light of (1) the finding that liver tumors in female mice occurred at noncytotoxic exposure levels (Nagano et al., 2007b; JBRC, 1998); (2) the potential for genotoxicity at low doses; and (3) the fundamental reactivity of direct and indirect products of carbon tetrachloride metabolism. Therefore, the MOA for carbon tetrachloride carcinogenicity across all exposure levels is unknown at this time. Hypothesized MOAs are discussed further in Sections 4.7.3 and 4.7.4 below.

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 oral 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 (oral 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.

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 (1,250 or 2,500 mg/kg, 5 day/week for 78 weeks) that produced close to 100% incidence of hepatocellular carcinoma. The incidence of adrenal adenomas and pheochromocytomas 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 induced tumors in an 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 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, 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. Significant increases were also observed in the incidence of benign adrenal pheochromocytomas in male mice at 25 or 125 ppm and female mice at 125 ppm.

Specifically, pheochromocytomas were identified in 32/50 high-exposure 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-exposure female mice. 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 dose of 86 mg/kg-day induced hepatomas in C3H mice (Kiplinger and Kensler, 1963).

Carbon tetrachloride has been extensively studied for its genotoxic and mutagenic effects. Overall, results are largely negative. 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 have been described in Section 4.4.2.1 and 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.

4.7.3. Mode of Action Information for Liver Tumors

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

Carbon tetrachloride produced liver tumors in rats, mice, and hamsters in studies using various experimental designs by oral and inhalation exposure (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). A hypothesized MOA for carbon tetrachloride-induced liver tumors is described graphically in Figure 4-4.

Hypothesized key events. Hypothesized 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.

Metabolism of carbon tetrachloride is identified as a key event based on the following: (1) reactive metabolites are present in the liver (Stoyanovsky and Cederbaum, 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 CYP450s result in potentiating effects to carbon tetrachloride-induced toxicity (Section 4.8.6).

The resulting hepatocellular toxicity 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. Tumorigenesis through this hypothesized MOA resulting from carbon tetrachloride-induced toxicity is believed to require persistent hepatocellular cytotoxicity and regenerative cellular proliferation for tumor formation.

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

Strain, species	Exposure	Hepatic necrosis/ degeneration	Hyperplasia/ regeneration	Reference
Sprague-Dawley rat (male)	Oral, 12 wks 24 mg/kg-d (adjusted)	Necrosis	Bile duct hyperplasia	Bruckner et al., 1986
F344 rat (male)	Oral, 12 wks 14 mg/kg-d (adjusted)	Necrosis		Allis et al., 1990
Sprague-Dawley rat (male)	Oral, 13 wks 71 mg/kg-d (adjusted)	Necrosis	Nodular hepatic, bile duct, and oval cell hyperplasia	Koporec et al., 1995
CD-1 mouse	Oral, 13 wks 12 mg/kg-d	Necrosis	Bile duct hyperplasia	Hayes et al., 1986
CD-1 mouse	Oral, 12 wks 8.6 mg/kg-d (adjusted)	Necrosis		Condie et al., 1986
Strain A mouse	Oral, 120 d (30 doses) 80 mg/kg-d[a]	Necrosis		Eschenbrenner and Miller, 1946
$B6C3F_1$ mouse	Oral, 78 wks, 892 mg/kg-d[a] (adjusted)		Bile duct proliferation	NCI, 1977, 1976a, b
F344 rat (male)	Inhalation, 12 wks 18 ppm (adjusted)[b]	Necrosis	BrdU-negative hepatocytes	Benson and Springer, 1999
$B6C3F_1$ mouse (male)	Inhalation, 12 wks 4 ppm (adjusted)[b]	Necrosis	BrdU-positive hepatocytes	Benson and Springer, 1999
Syrian hamster (male)	Inhalation, 12 wks 18 ppm (adjusted)[b]	Necrosis	BrdU-positive hepatocytes	Benson and Springer, 1999
Wistar rat	Inhalation, 6 mo 42 ppm	Necrosis		Adams et al., 1952
Hartley guinea pig	Inhalation, 13 wks 10 ppm (continuous)	Hepatocellular degeneration	Hepatocellular regeneration	Prendergast et al., 1967
Hartley guinea pig; Sprague-Dawley or Long-Evans rat	Inhalation, 6 wks 20 ppm (adjusted)[b]	Necrosis, hepatocellular degeneration	Hepatocellular regeneration, bile duct proliferation	Prendergast et al., 1967
F344 rat	Inhalation, 13 wks 2 ppm (adjusted)[b]		Mitosis, bile duct proliferation, foci	Nagano et al., 2007a; JBRC, 1998
BDF_1 mouse	Inhalation, 13 wks 5–48 ppm (adjusted)[b]		Bile duct proliferation: 5 ppm, female; 16 ppm, male; mitosis: 16 ppm, male; 48 ppm, female; foci: 48 ppm both sexes	Nagano et al., 2007a; JBRC, 1998

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

Strain, species	Exposure	Hepatic necrosis/ degeneration	Hyperplasia/ regeneration	Reference
F344 rat	Inhalation, 104 wks 5–22 ppm (adjusted)[b]		Foci: 5 ppm, female; 22 ppm, male[a]	Nagano et al., 2007b; JBRC, 1998
BDF_1 mouse	Inhalation, 104 wks 5 ppm[b] (adjusted)[b]	Degeneration in males; necrosis in females		Nagano et al., 2007b; JBRC, 1998

[a]Hepatic tumors detected at this level.
[b]This concentration was adjusted to continuous exposure (e.g., a factor of 6/24 × 5/7 applied used for an inhalation exposure administered 6 hrs/d, 5 d/wk).

Other mechanistic events hypothesized to contribute to liver tumor induction. Other biological events, including lipid peroxidation, disturbances in calcium homeostasis, and genetic damage, are possibly involved in the induction of liver tumors by carbon tetrachloride; however, the contribution of these events has not been established. Therefore, whether these mechanistic events represent key events in carbon tetrachloride's carcinogenicity is unknown.

In general, mechanistic studies of carbon tetrachloride-induced 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. These studies do not adequately characterize cellular responses that may occur at exposures below those that induced 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 other effects such as cytotoxicity or genotoxicity 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 $t_{1/2}$ 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), the doses at which this effect has been demonstrated do not allow for a 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 or other cellular events hypothesized to contribute to tumorigenicity. Some studies present evidence that disturbances in calcium homeostasis may not be a necessary event for cell death (Albano et al., 1989; Clawson, 1989). Similarly, 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).

The role of genetic damage or alteration to DNA in the cancer MOA(s) for carbon tetrachloride has not been 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. The reactivity of these radicals, however, 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, 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). Methodological problems with these studies, however, confound interpretation of 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 positive genotoxic results are generally found at high exposure levels and 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. Development of mutations by lipid peroxidation-induced DNA damage 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., 2006). Thus, 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.

Multiple 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, measurement of genetic damage to DNA has not 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). Assays that measure genetic damaging events at and below exposure levels of carbon tetrachloride that induce 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.

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 an increased incidence of liver tumors, independent of species, gender, or route of administration (Nagano et al., 2007b; 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). 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. 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).

In the 2-year inhalation studies in rats and mice by JBRC (Nagano et al., 2007b; JBRC, 1998), which are the best documented of the available chronic studies, livers of male and female rats and male mice with adenomas or carcinomas also expressed nonneoplastic changes, including degenerative changes, fatty liver, fibrosis, cirrhosis, and bile duct proliferation. This association was not observed, however, in low-exposure (5-ppm or 0.9-ppm duration adjusted) female mice, where an increased incidence of liver adenomas occurred in the absence of evidence of hepatocellular cytotoxicity.

Eschenbrenner and Miller (1946) reported the 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 1 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.

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 and Dalton, 1942; Edwards et al., 1942; Edwards, 1941; 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 almost 100% incidence in male and female mice of both dose groups (i.e., liver tumors were observed in 179 of 183 exposed male and female 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. The JBRC inhalation studies in rats and mice (Nagano et al., 2007b; JBRC, 1998), which used exposure concentrations of 5, 25, or 125 ppm, showed an increase in the incidence of liver tumors (hepatocellular adenomas or carcinomas) in rats and mice of both sexes with increasing exposure concentration (see Tables 4-4 and 4-5).

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 in rats were observed at an exposure level associated with hepatotoxicity following subchronic and chronic exposure; 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, but less consistent, dose-response relationship for cytotoxicity and tumor formation was observed for mice (Nagano et al., 2007b; JBRC, 1998). In mice exposed for 13 weeks, exposure-dependent histopathological findings indicative of cytotoxicity, damage, proliferation, and preneoplastic changes were observed. In male mice, histopathological findings indicative of fatty change were observed in male mice exposed to ≥10 ppm and in female mice exposed to ≥30 ppm carbon tetrachloride. In male and female mice exposed to ≥30 ppm, a significantly increased incidence of liver collapse was observed. Liver collapse was characterized by shrunken parenchymal tissue over the centilobular area, presumably resulting from the necrotic loss of hepatocytes, and accompanied by proliferation of the bile ducts and oval cells. In male and female mice exposed to ≥270 ppm, the incidences of nuclear enlargement of hepatocytes with atypia and altered cell foci were significantly increased. The incidence of liver adenomas and carcinomas in male mice in the 104-week study was increased compared to concurrent controls at ≥25 ppm, an exposure level that also produced cytotoxicity and similar to an exposure level (30 ppm) that produced a proliferative response in the 13-week study. In female mice, however, the incidence of hepatocellular adenomas was statistically elevated at 5 ppm (0.9-ppm adjusted) compared to concurrent controls, although hepatocellular damage was not observed.

Thus, the dose-response relationships between cytotoxicity and liver tumors demonstrated by the JBRC bioassay in male and female rats and male mice generally support a prominent role for cytotoxicity, regeneration, and proliferation in the MOA for carbon tetrachloride-induced carcinogenesis at higher exposure levels; however, data for the female mouse suggest a lack of dose-response concordance for the proposed key events.

As summarized in Table 4-15, several subchronic inhalation and oral studies demonstrate 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 to continuous exposure) 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 et al., 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 (1,590 mg/kg)[a]	Sprague-Dawley rats; three strains of mice (A/J, BALB/cJ, and C57B1/6J	Increased conjugated dienes in 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 treated animals; iron binding eliminated lipid peroxidation (ethane) in 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	Twofold induction of 4-HNE-dG adducts	Wacker et al., 2001
Lipid peroxidation	3,200 mg/kg	Female F344 rats	37-Fold induction of 4-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	1,590 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. (3,980 or 1,590 mg/kg)[a]	Male Sprague-Dawley rats	Conjugated dienes or incorporation of [^{14}C] labeled carbon tetrachloride was not significantly prevented by several antioxidants	de Ferreyra et al., 1975
Lipid peroxidation	1,590 mg/kg	Rat	2.5-Fold increase TBARS over controls	Hartley et al., 1999
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

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

End point	Dose of carbon tetrachloride	Test system	Result	Reference
GSH modulation	0.5 ml (2.38 mL/kg) injected i.p. (800 mg/kg)[a]	Wistar rats	GSH decreased at 5 wks	Cabre et al., 2000
GSH modulation	Pretreated with 2 g/kg GSH 30 min prior to 1,590 mg/kg i.p. carbon tetrachloride	Male Sprague-Dawley rats	GSH pretreatment partially prevented hepatic necrosis	Gorla et al., 1983
GSH modulation	1,600 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 (46–1,540 mg/L)	In vitro hepatocytes	Increased activity of phosphorylase a and decreased activity of endoplasmic reticulum Ca++ pump; effects only observed at concentrations >1 mM	Long and Moore, 1986
Altered Ca++ homeostasis	1 mL/kg injected i.p. (1,590 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 (3,980 mg/kg)[a]	Male Sprague-Dawley rats	85% reduction in ATP-dependent Ca++ uptake and endoplasmic reticulum capacity	Moore et al., 1976
Altered Ca++ homeostasis	0.03 mL/100 g to 0.125 mL/100 g body weight (1.25 mL/kg by feeding tube) (0.48–1,990 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. (4,770 mg/kg)[a]	Male Sprague-Dawley rats	Co-treated with CBZ (calpain inhibitor), decreased mortality 50% from lethal dose of carbon tetrachloride	Limaye et al., 2003

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

End point	Dose of carbon tetrachloride	Test system	Result	Reference
Phospholipase activity	1 mL/kg injected i.p. (1,590 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 1.594 g/mL at 20°C.

An additional area of 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, a low-dose mutagenic effect cannot be excluded**.**

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 MOA. Acute toxicity studies on rodents treated orally with carbon tetrachloride show 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). As reviewed in Sections 4.2.1.1 and 4.2.2.1, 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 hypothesized MOA 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 and formation of •O-O-CCl_3 (immediate and ongoing)	13 wks		104 wks		Liver tumors (104 wks)
		Hepato-toxicity[b]	Regeneration and proliferation[c]	Hepato-toxicity[b]	Regeneration and proliferation[c]	
5 (0.9)	+[d]			—	—	—
10 (1.8)	+[d]	+/—[e]	—			
25 (4.5)	+[d]			+	—	+/—[f]
30 (5.4)	+[d]	+	+			
90 (16.1)	+[d]	+	+			
125 (22.3)	+[d]			+	+	+
270 (48.2)	+[d]	+	+			
810 (145)	+[d]	+	+			

[a]The exposure concentration in parentheses is the concentration adjusted to continuous exposure (i.e., multiplied by 5/7 × 6/24)
[b]As indicated based on histopathological findings, including fatty change, fibrosis, cirrhosis, and/or necrosis.
[c]As indicated based on histopathological findings, including proliferation and hyperplasia (and in the 13-wk study, mitosis).
[d]+ = Studies demonstrating key event were not conducted as part of the JBRC 13- and 104-wk 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.
[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%), an increase that was statistically significant compared to the historical control.

Note: Different exposure concentrations were used in the 13- and 104-wk JBRC bioassays. Blank cells indicate exposure concentrations not tested in either the 13- or 104-wk study.

+ = evidence demonstrating key event; — = no evidence demonstrating key event; +/— = equivocal

Sources: 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 for carcinogenicity.

4.7.3.3. *Other Possible Modes of Action*

Genotoxicity. The available genotoxicity studies for carbon tetrachloride are summarized in Section 4.4.2. 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. Overall, the database is comprised of largely negative results. Many of the positive assays were conducted at doses/concentrations that also produced cytotoxicity; however, positive results were also reported in the absence of accompanying cytotoxicity.

Many of the positive genotoxicity findings, including the following, are consistent with compounds that induce oxidative and/or peroxidative damage: (1) two positive mutation/DNA damage studies in *E. coli* WP2 strains particularly sensitive to oxidative damage; (2) intrachromosomal recombination induced by carbon tetrachloride in *S. cerevisiae* consistent with DNA breakage originating from various reactive species produced subsequent to formation of the trichloromethyl radical (e.g., trichloromethyl peroxy radical, reactive aldehydes, and other lipid peroxidation products (see Figure 4-3)); (3) evidence from in vitro and in vivo assays of DNA breakage and fragmentation; 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

An area of significant uncertainty is the possibility of genetically damaging events occurring at or below doses that induced tumors in laboratory rodents. The possibility exists that reactive aldehydes generated at low levels could contribute to the genotoxicity of carbon tetrachloride. Because genotoxicity and mechanistic data in this portion of the dose-response curve are limited, a low-dose mutagenic effect cannot be excluded.

Epigenetic effects and changes in gene expression. As summarized in Section 4.4.2.4, a number of studies have reported alterations in liver DNA methylation. For instance, Varela-Moreiras et al. (1995) investigated the effect of short-term administration of carbon tetrachloride on hepatic DNA methylation and on SAM and 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 or GSH, 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.

Changes in the expression of specific genes in response to carbon tetrachloride exposure have been investigated in the liver of rodents and in cultured human hepatoma cell line (see Section 4.5.7) (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. 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-κβ 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) 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 PH. 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 PH), 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 PH. 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, 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-κβ and AP-1; steady-state levels of TNF-α mRNA; and induction of the iNOS. Liver regeneration after treatment with carbon tetrachloride was associated with an increase in steady-state levels of TNF-α mRNA, activation of NF-κβ and AP-1, and induction of iNOS. Lead nitrate induced NF-κβ, TNF-α, 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-α mRNA, activation of NF-κβ and AP-1, or induction of iNOS, although the number of hepatocytes entering S phase 18–24 hours after nafenopin was similar to that seen after PH. The findings from these two studies suggest 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.

4.7.3.4. *Conclusions About the Hypothesized Mode of Action*

The carcinogenic MOA for liver tumors induced by carbon tetrachloride is unknown. However, biological support exists for several of the hypothesized mechanistic events for induction of liver tumors by carbon tetrachloride. Metabolism of carbon tetrachloride by CYP2E1 to the trichloromethyl radical and other subsequently generated reactive species, including products of lipid peroxidation, resulting in sustained cytotoxicity and regenerative cell proliferation has been postulated as a MOA. This potential MOA has been extensively investigated, and appears to be a major factor driving the steep nonlinear increase in liver tumor dose-response at relatively high carbon tetrachloride exposures. Genotoxicity has also been extensively investigated; however, various confounding factors and other challenges in evaluating the genotoxicity database for carbon tetrachloride limit the ability to establish whether mutagenicity as a key event is operative across all exposure levels. Thus, at high exposures the hypothesized cytotoxicity-regenerative proliferation-based MOA as well as other (e.g., mutagenic) MOA(s) may be operative, but it is not possible to delineate the contribution of these possible MOA(s) to carbon tetrachloride tumor response. Inconsistencies in the database supporting a potential role for the cytotoxicity-regenerative proliferation-based MOA at the low end of the experimental exposure range, especially the formation of liver adenomas in female mice at a noncytotoxic exposure level, suggest that MOA(s) that are independent of cytotoxicity and regenerative cell proliferation may play a role in carbon tetrachloride liver tumor induction in this range.

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. Thus, the fundamental reactivity of direct and indirect products of carbon tetrachloride metabolism can reasonably be expected to play a role in carbon tetrachloride carcinogenicity at all levels of exposure to carbon tetrachloride.

Although the extensive genotoxicity database for carbon tetrachloride suggests that the chemical is not likely a direct acting mutagen, the database is complex and raises various issues (see Table 4-12) that make it difficult to ascertain the potential genotoxicity of carbon tetrachloride at exposures below which there is overt cytotoxicity. Positive genotoxicity findings have generally been observed at exposures that induce cytotoxicity and regenerative cell proliferation. Because of the difficulties in detecting genotoxic effects following treatment with carbon tetrachloride, the many studies conducted at relatively high doses lack information regarding dose-response and fail to characterize the role of genotoxicity at low carbon tetrachloride exposure levels.

In summary, biological support exists for a hypothetical MOA involving metabolism of carbon tetrachloride by CYP2E1, sustained cytotoxicity, and regenerative cell proliferation as key events in the cancer mode of action at high exposures. Linear processes would likely dominate the dose-response relationship at low exposures (i.e., exposures that are not cytotoxic).

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

Although data are inadequate to determine the operative MOA for rodent liver tumors at low exposures, none of the available data suggest that the hypothesized MOAs are biologically precluded in 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 hypothesized MOA 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 (NTP, 2007; NCI, 1977, 1976a, b; Weisburger, 1977) and inhalation exposure (Nagano et al., 2007b; JBRC, 1998), but not in rats by either route of exposure. The MOA by which carbon tetrachloride induces pheochromocytomas in mice is not known.

Research on the mechanism(s) by which carbon tetrachloride induces toxicity in the adrenal gland is largely limited to short-term studies of carbon tetrachloride enzyme activation in the adrenal tissue. Colby et al. (1994, 1981) reported that carbon tetrachloride has induced adrenocortical necrosis in humans, although reports of effects of carbon tetrachloride on the human adrenal gland were not independently identified. In experimental animals, acute exposure to carbon tetrachloride has produced adrenal necrosis, with effects localized to the zona reticularis, the innermost region of the cortex (Brogan et al., 1984). This localization of toxicity appears to be the result of greater activation of carbon tetrachloride by microsomal enzymes in the zona reticularis (Colby et al., 1994; Brogan and Colby, 1983). In vitro studies showed that preincubation of adrenal microsomes with 1-aminobenzotriazole (ABT) a CYP450 suicide inhibitor, prevented the effects of carbon tetrachloride on lipid peroxidation and covalent binding (Colby et al., 1994). It would appear that carbon tetrachloride metabolism plays a role in the induction of toxicity in the adrenal gland as it does in the liver.

Malendowicz and Colby (1982) administered 0.2 mL (~1,750 mg/kg) carbon tetrachloride to Wistar rats by oral gavage once a week for 20 weeks or 0.1 mL (~880 mg/kg) per day for 7 or 14 days. The investigators suggested that carbon tetrachloride may influence plasma corticosteroid levels through effects on adrenal steroid production as well as hepatic reductive steroid metabolism, resulting in overall decreases in circulating corticosteroid concentrations. No evidence is available, however, that suggests any association between effects on corticosteroid balance and induction of pheochromocytomas.

In general, few chemicals have been reported to cause pheochromocytomas in mice. Of 514 technical reports published by NTP, exposure to only seven chemicals has been associated with pheochromocytomas in mice (Tischler et al., 2004; Hill et al., 2003). Greim et al. (2009) identified bioassays (many of these being the same NTP bioassays identified by Hill et al., 2003) for nine chemicals that showed an increased incidence of mouse pheochromocytomas. Tischler et al. (2004) found no apparent common denominator among the chemicals that induced mouse pheochromocytomas. Greim et al. (2009) hypothesized MOAs for the induction of mouse pheochromocytomas that included endocrine disturbance, impairment of mitochondrial function, uncoupling of oxidative phosphorylation, hepatoxicity, and nephrotoxicity leading to impaired calcium homeostasis, but provided no support for any of these hypothesized MOAs.

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. These factors are described further below.

Hypothesized events that may be involved in carbon tetrachloride-induced liver toxicity and carcinogenicity (e.g., metabolism to trichloromethyl radical by CYP2E1 and subsequent formation of trichloromethyl peroxy radical; cytotoxicity; sustained regenerative and

proliferative changes in the liver in response to hepatotoxicity; epigenetic alterations; gene expression changes; and DNA damage and fixation leading to mutagenic activity) involve metabolic and cellular processes common to cells at all life stages. Because metabolism is a hypothesized key event in carbon tetrachloride toxicity, heterogeneity in the human population in microsomal enzymes responsible for carbon tetrachloride metabolism could also influence susceptibility to carbon tetrachloride. Quantitative information on variation in human hepatic levels of CYP2E1 and other CYP450 enzymes demonstrates considerable intrahuman variability. For example, Lipscomb et al. (1997) reported a sevenfold range in activity of CYP2E1 among hepatic microsomal samples from 23 subjects. Snawder and Lipscomb (2000) demonstrated 36-, 13-, 11-, 2-, 12-, and 22-fold differences in CYP1A, CYP2B, CYP2C6, CYP2C11, CYP2E1, and CYP3A protein content, respectively, between the highest and lowest samples from 40 samples of microsomes from adult human liver organ donors.

4.8.1. Possible Childhood Susceptibility

Limited data on CYP450 enzymes 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 MOA 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 $t_{1/2}$ results for several therapeutic drugs metabolized by the CYP3A family (Ginsberg et al., 2002), enzyme levels were 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 that 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.

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 (7–17 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. For inhalation exposures, the arterial blood flow does not perfuse the liver before reaching the fetus; therefore, 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 MOA 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 years (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 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, 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 γ-glutamylcysteine ligase (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 was 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 7 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 that 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 nonlethal 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 g ethanol/day and were the only individuals to develop severe hepatotoxicity and nephrotoxicity 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 <50 g ethanol/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 were 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 are thus 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 oral 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 administered carbon tetrachloride 5 days/week by oral gavage in corn oil (0.71 and 7.1 mg/kg-day, respectively, adjusted to daily exposure). Condie et al. (1986) identified a NOAEL of 1.2 mg/kg and a LOAEL of 12 mg/kg in similarly treated mice (0.86 and 8.6 mg/kg-day, respectively, adjusted to daily exposure). 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 (including serum enzyme changes and liver histopathology) 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.

Serum chemistry data. Condie et al. (1986) reported liver enzyme changes in carbon tetrachloride-exposed mice; however, the median of 8–12 determinations was reported without a standard error (SE) or SD (only the minimum and maximum of the range were reported). Without a mean and SE or SD, BMD analysis cannot be performed. Therefore, the NOAEL of 1.2 mg/kg-day, 5 days/week, was identified as a possible POD for this data set.

Serum chemistry data in male rats from Bruckner et al. (1986) are presented in Table 5-1. Of the enzymes monitored, only SDH showed a clear statistically and biologically significant increase in the 10 mg/kg dose group. The data for the 10- and 12-week blood draws were similar. Therefore, both 10- and 12-week data were used for dose-response modeling using BMD methods.

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 a 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) 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 was 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 are 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 (see the section on liver histopathologic changes below). Thus, carbon tetrachloride-induced elevations in SDH and ALT are valid markers of histopathological changes to the liver in the Bruckner et al. (1986) study.

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

Daily dose (mg/kg-d)	SDH (IU/mL)[a]		OCT (nmol CO_2/mL)[a]		ALT (IU/mL)[a]	
	10 wks	12 wks	10 wks	12 wks	10 wks	12 wks
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 ± SE for groups of five rats at 10 wks and seven to nine rats at 12 wks.
[b]$p < 0.05$.

Source: Bruckner et al. (1986).

All of the models for continuous data in U.S. EPA's benchmark dose software (BMDS) (version 1.4.1) (U.S. EPA, 2007b) were fit to the 10- and 12-week SDH data. An increase in SDH activity 2 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 for the 10- and 12-week SDH data are presented in Appendix B. None of the models for continuous data in BMDS provided an adequate fit of the 12-week SDH data. 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 Akaike's Information Criterion [AIC] value) and was therefore selected as the basis for a candidate POD; this model estimated a BMD_{2X} of 7.32 mg/kg-day and the 95% lower confidence limit on the BMD ($BMDL_{2X}$) of 5.46 mg/kg-day.

BMD modeling was also performed using the 10- and 12-week OCT and ALT data from Bruckner et al. (1986) (see Appendix B for a more detailed summary of model results). 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, yielding a BMD_{2X} and $BMDL_{2X}$ of 14.7 and 13.21 mg/kg-day, respectively; however, as shown in Table 5-1, the standard error of the mean (SEM) 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, relatively high uncertainty is associated with this quantitative analysis using the 10-week ALT data set. The polynomial and power models provided adequate fits of the 12-week ALT data (based on a goodness-of-fit *p*-value ≥0.1). The polynomial model, which provided a better fit (based on lower AIC values) of the data using both n = 7 and 9, estimated a BMD_{2X} and $BMDL_{2X}$ of 13.0 and 11.8 mg/kg-day, respectively. The values of the BMD and BMDL were not sensitive to the value of n.

Overall, a dose-response analysis of 10- and 12-week liver enzyme data from Bruckner et al. (1986) reveals that the 10-week SDH data provide the most sensitive estimates of the BMD_{2X} and $BMDL_{2X}$, or 7.32 and 5.46 mg/kg-day, respectively. For purposes of comparison across chemicals, the BMD and BMDL corresponding to a change in the mean response equal to one control SD from the control mean were also calculated for the 10-week SDH data, consistent with BMD guidance (U.S. EPA, 2000c). The BMD_{1SD} and $BMDL_{1SD}$ were 5.5 and 3.8 mg/kg-day, respectively.

Liver histopathologic changes. 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 exhibit inductions of hepatic lesions due to carbon tetrachloride at 10–12 mg/kg-day. 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 Bruckner et al. (1986) implied that incidence was 100% for lipid vacuolation in the 10 mg/kg-day dose group.

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

Daily dose (mg/kg-d)	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-day 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-day to 100% at 10 mg/kg-day.

In the Condie et al. (1986) study, exposure to carbon tetrachloride by oral 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-day. However, only necrosis (minimal to mild) in males and hepatocytomegaly (severity unranked) in males and females treated using a 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. For all three of these histopathologic lesions, incidence increased from 0% in the 1.2 mg/kg-day group to 60–90% in the 12 mg/kg-day group.

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-d	1.2 mg/kg-d	12 mg/kg-d	120 mg/kg-d
Male	Corn oil	Necrosis	0/10	0/9	9/10[a]	9/10[a]
Male	Corn oil	Hepatocytomegaly	0/10	0/9	8/10[a]	10/10[a]
Female	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).

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-day in male rats and 1.2 and 12 mg/kg-day 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 LOAELs (approximately 10–12 mg/kg-day) in these studies, the response rate was 60–100%, whereas the response at the dose below the LOAEL was 0%. The incidence data do, however, support the BMD_{2X} of 7.32 mg/kg and $BMDL_{2X}$ of 5.46 mg/kg-day estimated from the increase in 10-week serum SDH observed in the Bruckner et al. (1986) study.

The NOAEL of 1.2 mg/kg-day, 5 days/week for liver enzyme changes from Condie et al. (1986) was considered as a POD for the carbon tetrachloride RfD; however, the data provided in the study report were insufficient to allow BMD modeling of these data. Because the NOAELs and LOAELs in the Bruckner et al. (1986) study were similar to those in Condie et al. (1986), and because the data reported in Bruckner et al. (1986) supported BMD modeling and thus provided better resolution of the dose-response relationship in the low-dose region, the $BMDL_{2X}$ based on Bruckner et al. (1986) was selected as the POD for the carbon tetrachloride RfD.

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 multicompartment model in which first-order absorption from 6 to 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 command in Advanced Continuous Simulation Language (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/hour) 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 two-compartment, three-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 two-compartment, three-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 nonlinear function of carbon tetrachloride delivery to the liver (i.e., from absorption and from arterial blood). 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. Therefore, EPA does not consider currently available PBPK models to be adequate for interspecies dosimetry extrapolations of carbon tetrachloride administered to animals by oral gavage (e.g., in corn oil) to continuous exposures in humans. As described in Section 5.4.3.4, however, the human PBPK model has been used to extrapolate dosimetry in the human across routes (i.e., inhalation to oral).

As noted above, 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 selected as the POD for

derivation of the RfD. The $BMDL_{2X}$ of 5.46 mg/kg-day was derived from a study 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:

$$BMDL_{2X\text{-}ADJ} = BMDL_{2X} \times 5 \text{ days}/7 \text{ days} \quad \text{Eq. (5-1)}$$
$$= 5.46 \text{ mg/kg-day} \times 5 \text{ days}/7 \text{ days}$$
$$= 3.9 \text{ mg/kg-day}$$

5.1.3. Reference Dose Derivation—Including Application of Uncertainty Factors (UFs)

An RfD of 0.004 mg/kg-day for carbon tetrachloride is derived by applying a composite UF of 1,000 to the $BMDL_{2X\text{-}ADJ}$ of 3.9 mg/kg-day, as follows:

$$RfD = BMDL_{2X\text{-}ADJ}/UF \quad \text{Eq. (5-2)}$$
$$= 3.9 \text{ mg/kg-day}/1{,}000$$
$$= 0.0039 \text{ mg/kg-day or}$$
$$0.004 \text{ mg/kg-day (rounded to one significant figure)}$$

The composite UF of 1,000 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 database deficiencies.

- A default 10-fold UF for intraspecies differences (UF_H) 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. Intrahuman variability in CYP450 levels that are responsible for metabolism of carbon tetrachloride to reactive metabolites has been documented (see Section 4.8). This variation in CYP450, which is likely influenced by 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 (UF_A) 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 or a suitable PBPK model, a UF of 10 is included.

- A UF of 3 ($10^{0.5}$) for subchronic to chronic extrapolation (UF_S) 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 0.9–4 ppm (see Table 4-14), which is similar to or within fourfold of the NOAEL from the JBRC chronic inhalation bioassay.

  (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 4 days of exposure in an 11-day oral toxicity study, and increases in liver enzymes at week 2 in a 12-week oral toxicity study.

  Thus, the data suggest that an increase in the duration of the exposure may not increase the incidence and/or severity of the liver toxicity.

- A UF to account for extrapolation from a LOAEL to a NOAEL (UF_L) 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 2 times the control mean was selected under an assumption that it represents a minimal biologically significant change.

- A UF to account for deficiencies in the database (UF_D) 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_D of 3 was applied to account for the lack of a multigeneration reproductive toxicity study.

5.1.4. Reference Dose Comparison Information

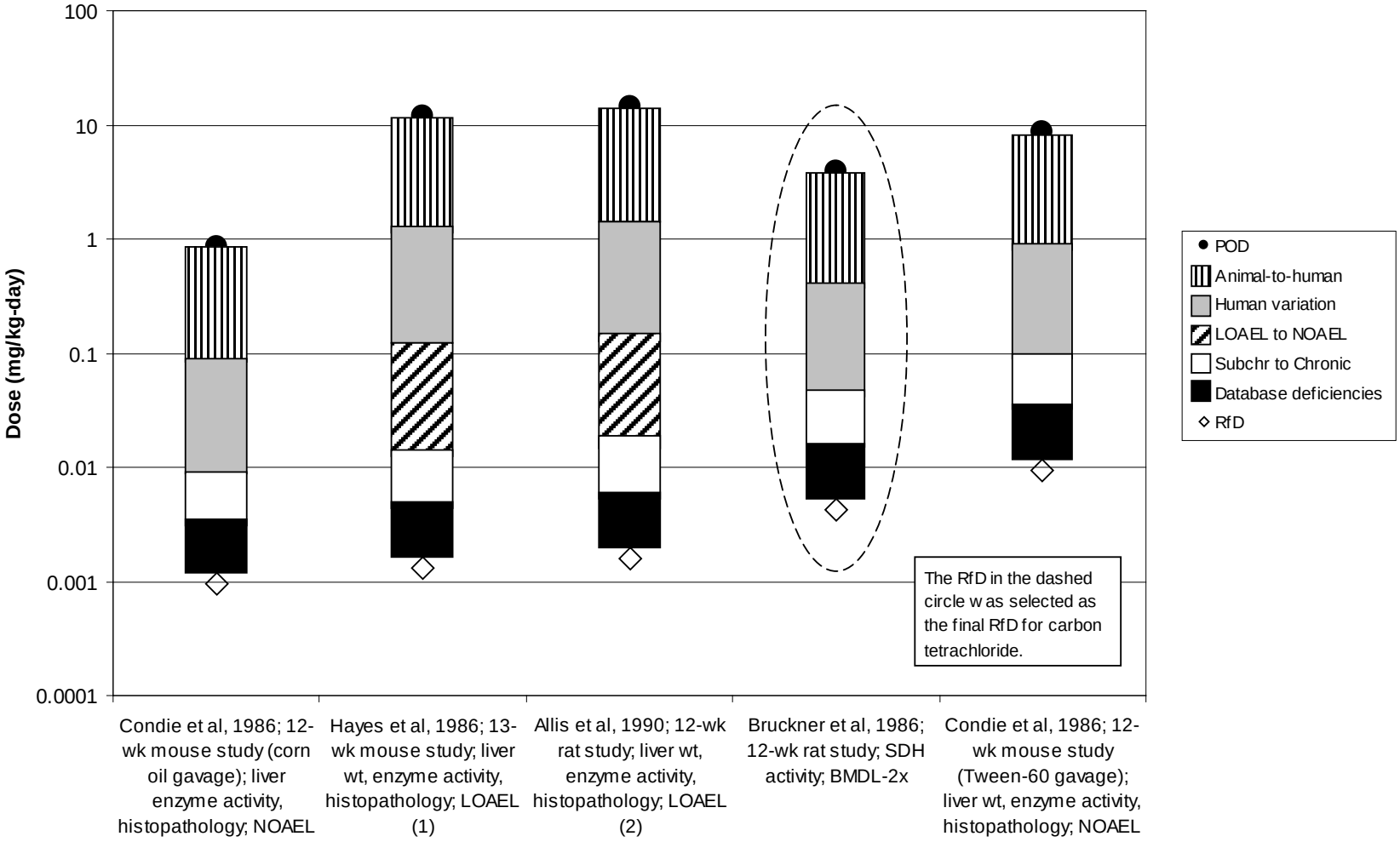
PODs and potential oral RfDs based on selected studies included in Table 4-13 are arrayed in Figures 5-1 to 5-3, and provide perspective on the RfD supported by Bruckner et al. (1986). These figures should be interpreted with caution because the PODs across studies are not necessarily comparable, nor 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 potential 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.

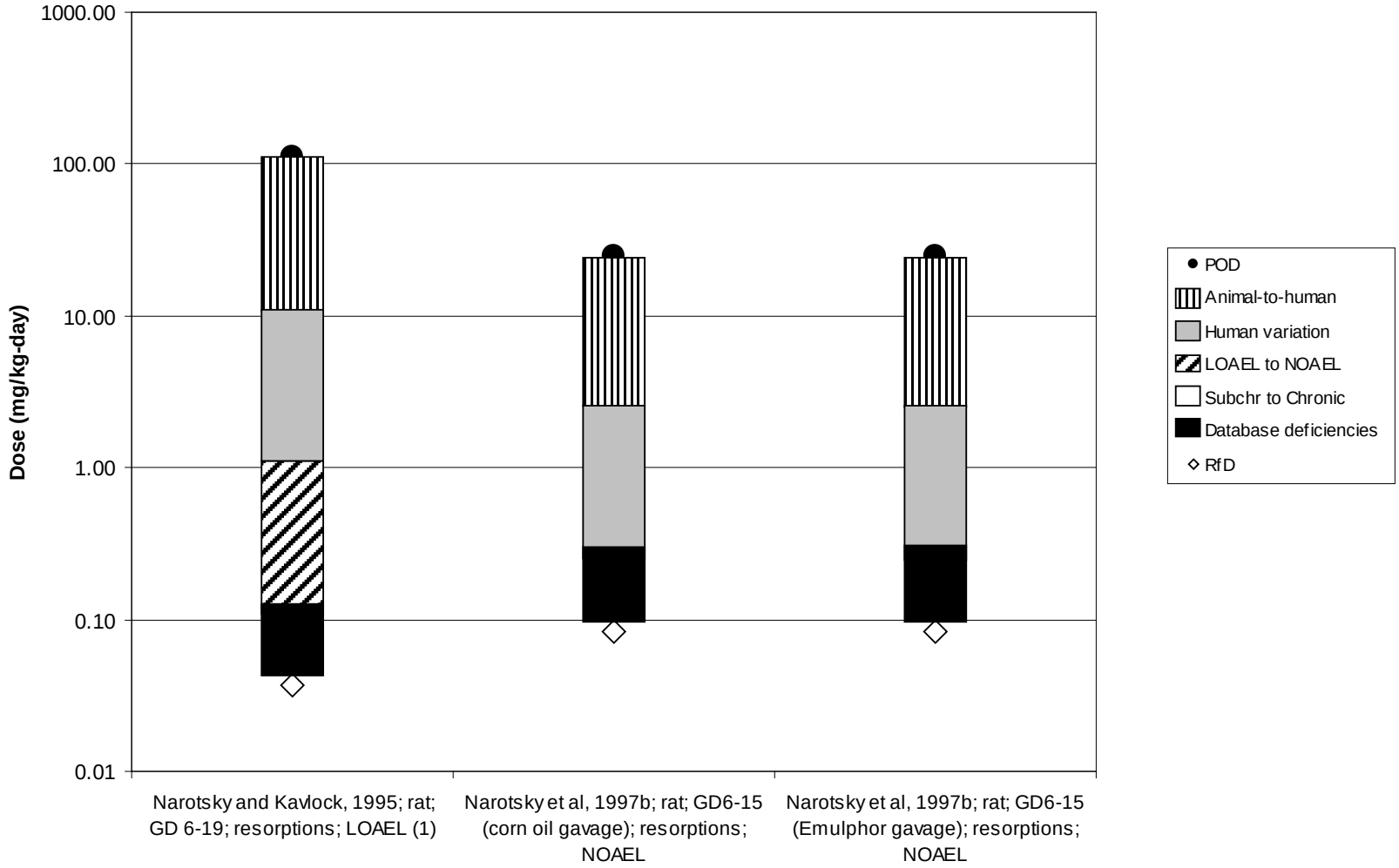
Studies in experimental animals have also found that relatively high doses of carbon tetrachloride during gestation can produce prenatal loss; these doses also produced overt toxic effects in the dams. A graphical display of dose-response information from three developmental studies is provided in Figure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



(1) Magnitude of effect at the LOAEL: liver weight (↑ 15–19%); enzyme activity (↑ ≤ sixfold); 100% necrosis.
(2) Magnitude of effect at the LOAEL: liver weight (↑ 30%); enzyme activity (↑ 3–5 times); 100% necrosis.

Figure 5-1. PODs (mg/kg-day) with corresponding potential oral reference values that would result if liver toxicity was used as the critical effect.



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

Figure 5-2. PODs (mg/kg-day) with corresponding potential oral reference values that would result if developmental toxicity was used as the critical effect.

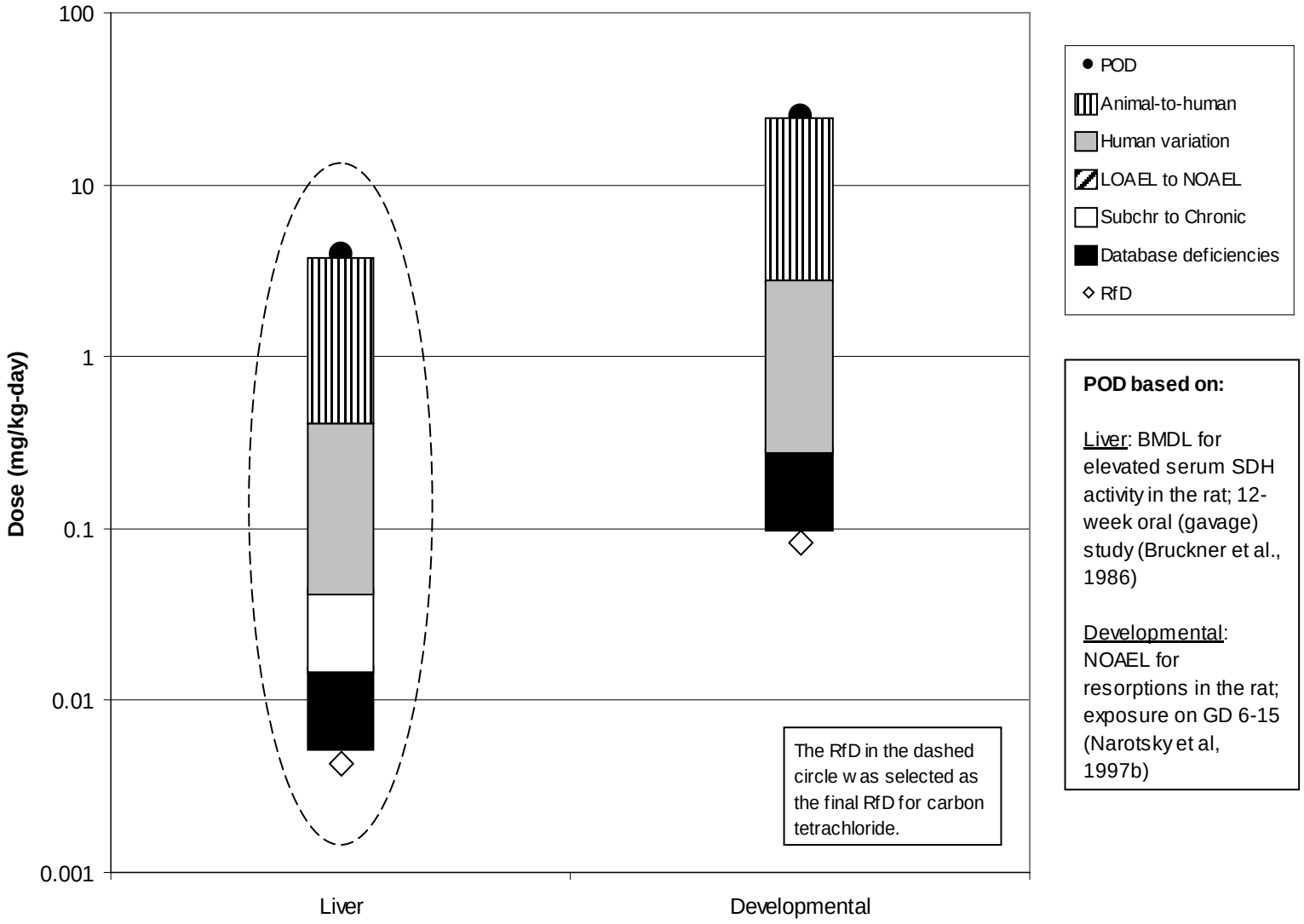


Figure 5-3. PODs (mg/kg-day) with corresponding potential oral reference values that would result if alternative endpoints were used as the critical effect.

5.1.5. Previous Reference Dose Assessment

The previous oral RfD for carbon tetrachloride (verified on May 20, 1985 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 oral 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 1,000 (3 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.

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 suggests an effect of occupational carbon tetrachloride exposure on the liver at exposures in the range of >1–3.9 ppm (6.3–24.5 mg/m^3); this exposure range is considered a LOAEL. The low exposure category in this study (≤1 ppm or ≤6.3 mg/m^3) is 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 the 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)[6] 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 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 an exposure-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 exposure groups, including the control, and an exposure-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. Epidemiological literature, in particular a cross-sectional study of hepatic function in carbon tetrachloride-exposed workers (Tomenson et al., 1995), reported some evidence of carbon tetrachloride-associated effects on hepatic serum enzymes. Subchronic studies in a number of experimental species (Benson and Springer, 1999; Prendergast et al., 1967; Adams et al., 1952) identified a NOAEL for liver effects in the range of 0.9–4 ppm (adjusted to continuous exposure). These subchronic studies used exposure durations of 12–26 weeks (versus 104 weeks in the JBRC bioassay) and 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. Renal effects were observed in the JBRC chronic

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

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 supports liver toxicity as the endpoint of concern.

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. 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, that could represent a potential noncancer health hazard, 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 considered less sensitive than liver endpoints and therefore were not selected 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 exposure-response analysis because this histopathologic lesion is indicative of cellular damage and appears to be a more sensitive endpoint than other histopathologic changes (i.e., fibrosis and cirrhosis) that were also present in 25-ppm rats in the JBRC study. General information on liver toxicants reveals that a sufficient intracellular concentration of fatty acids can lead to injury of cell membranes, thereby

contributing to necrosis, inflammation, and progression to fibrosis and cirrhosis (Lieber, 2004; Brunt and Tiniakos, 2002). 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 40–90%). Further, reliable liver enzyme data were not available for 125-ppm rats or mice because of the high mortality at this exposure concentration (1–3 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, compared with fatty change, for exposure-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 potential 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.

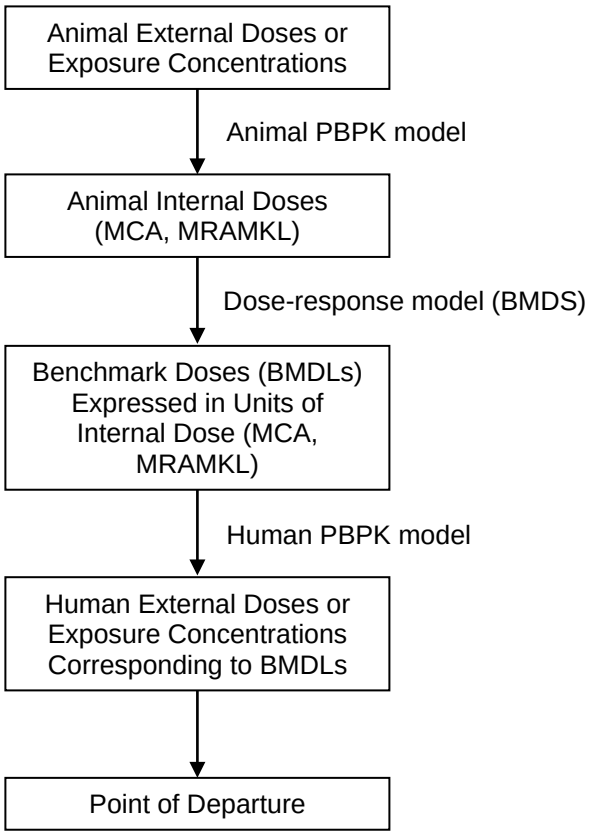
Table 5-4. Nonneoplastic lesions (fatty change) in F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

Species	Sex	Lesion type	Lesion severity[a]	Number of rats with lesions			
				Exposure concentration			
				0 ppm	5 ppm	25 ppm	125 ppm
Rat	Male[b]	Fatty change	1+ and 2+	4	7	39	49
Rat	Female[c]	Fatty change	1+, 2+, and 3+	6	7	49	46

[a]Severity rating: 1+, 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.

Sources: Nagano et al. (2007b); JBRC (1998).

The general procedure for analysis of the animal bioassay data for PBPK analysis 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.



MCA, mean arterial concentration, time-averaged arterial blood concentration of carbon tetrachloride (μmol/L); MRAMKL, mean rate of metabolism in the liver, time-averaged rate of metabolism of carbon tetrachloride (μmol/hr/kg liver); PBPK, physiologically-based pharmacokinetics model

Figure 5-4. Process for analyzing animal bioassay data for deriving noncancer toxicity values and cancer IURs and SFs using PBPK modeling.

5.2.2.1. *Physiologically Based Pharmacokinetic 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 Section 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, 2006c) that addresses the application and evaluation of PBPK models. The PBPK model was used to simulate internal dose metrics corresponding to intermittent exposure (6 hours/day, 5 days/week) to concentrations of 5, 25, and 125 ppm, as studied in the 2-year bioassay (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 MOA 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 (mean arterial concentration [MCA], μmol/L); and (2) time-averaged rate of metabolism of carbon tetrachloride (mean rate of metabolism in the liver [MRAMKL], μmol/hour/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 MOA in producing liver toxicity (described in Section 4.5). The primary reactive metabolites that are thought to participate in carbon tetrachloride toxicity are the trichloromethyl radical ($\cdot CCl_3$) and the trichloromethyl peroxy radical ($O\text{-}OCCl_3$), although other reactive species may also contribute to a lesser extent (e.g., dichlorocarbene, $:CCl_2$). The role of these species in oxidative injury is discussed further in Sections 4.5.2 and 4.5.3. The trichloromethyl radical is a product of carbon tetrachloride metabolism by CYP450. It rapidly reacts with oxygen to produce the corresponding peroxy radical, which is more highly reactive than the trichloromethyl radical (Russell et al., 1990; Slater, 1981; Packer et al., 1978). The trichloromethyl peroxy radical is thought to be the dominant intermediate in the initiation of lipid peroxidation associated with carbon tetrachloride hepatotoxicity (Slater, 1981). Elimination of trichloromethyl radical, by reaction with oxygen to form the trichloromethyl peroxy radical and downstream reaction products with amino acids, protein, and lipid, is extremely rapid (e.g., near the limits of diffusion) relative to the production of the trichloromethyl radical by CYP450. (See Section 3.3 for a discussion of the rates of conversion of carbon tetrachloride to the trichloromethyl radical and the trichloromethyl radical to the trichloromethyl peroxy radical.) The large difference in rates of production and elimination of the trichloromethyl radical (i.e., 10^7–10^8 fold difference) has several implications. (1) Limiting factors in the elimination of the trichloromethyl radical are likely to be reactant concentrations at the site of production of the trichloromethyl radical (e.g., O_2,) and/or factors that limit diffusion of the trichloromethyl radical (e.g., diffusion coefficient in cytosol). (2) Similarly, limiting factors in the elimination of the trichloromethyl peroxy radical are likely to be reactant concentrations (e.g., intracellular amino acids, lipid, protein, nonprotein sulfhydryls) and/or diffusion, all of which are expected to be similar in rodents and humans. (3) These elimination reactions are likely to occur within a relatively short diffusion distance from the site of production of the trichloromethyl radical (Slater, 1981), resulting in most (if not all) of the production of reaction products of the trichloromethyl and trichloromethyl peroxy radicals occurring within the tissue where trichloromethyl radical is produced (e.g., within liver and other tissues having appreciable CYP450 activity). Suicide inhibition and destruction of CYP450 by carbon tetrachloride is consistent with the reactivity of these species on a very local histological scale. It follows from the above considerations that equal rates of hepatic metabolism of carbon tetrachloride by CYP450 in rodents and humans would be expected to yield equal rates of elimination of trichloromethyl and trichloromethyl peroxy radicals.

Finally, carbon tetrachloride metabolism is known to lead to lipid peroxidation. Once initiated, lipid peroxidation is a self-perpetuating process that continues as a chain reaction (MacNee and Rahman, 2004). As such, the generation of lipid peroxides is not expected to be enzymatically driven. Accordingly, the rate of hepatic metabolism of carbon tetrachloride should be a reasonable internal dose surrogate for these radical species in liver.

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 and induction); (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 5-3 and 5-4):

$$MCA = \frac{AUC_{CA}}{t} \qquad \text{Eq. (5-3)}$$

$$MRAMKL = \frac{AUC_{RAMKL}}{t} = \frac{AMKL}{t} \qquad \text{Eq. (5-4)}$$

where:
MCA = time-averaged arterial blood concentration of carbon tetrachloride (µmol/L)
AUC_{CA} = area under the arterial concentration (CA) – time profile (µmol·hour/L)
MRAMKL = time-averaged rate of metabolism of carbon tetrachloride (µmol/hour/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/hour/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/hour/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/hour/kg $BW^{0.70}$ resulted in lower values for the MCA dose metric and higher

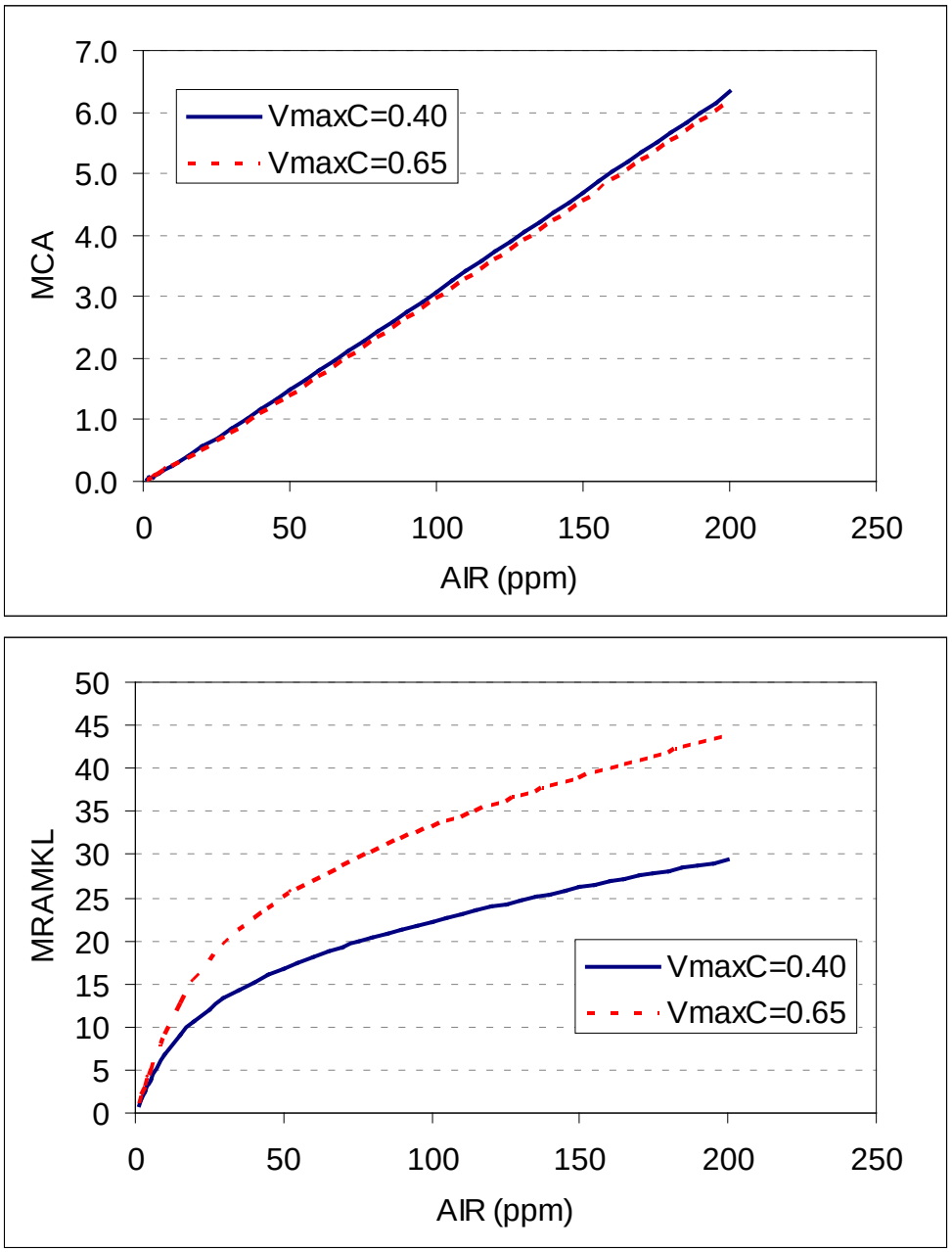
values for the MRAMKL dose metric (Table 5-5). Comparisons of internal doses predicted for various exposure concentrations are shown in Figure 5-5. The effect of varying V_{maxC} on MRAMKL becomes more pronounced as exposure concentration increases. This pattern reflects the increasing influence of V_{max} 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[a]

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

[a]Values are for 0.452 kg rat.

Sources: Thrall et al. (2000); Paustenbach et al. (1988); Gargas et al. (1986).



Dose metrics shown are MCA (µmol/L, upper panel), and MRAMKL (µmol/hr/kg liver, lower panel). The dose metrics are plotted against exposure concentration (6 hrs/d, 5 days/wk, 2 yrs) for a 0.452 kg rat.

Sources: Thrall et al. (2000); Paustenbach et al. (1988).

Figure 5-5. Internal dose metrics predicted by the PBPK rat model.

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 (i.e., 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, 2007b) were fit to the incidence data for rats.

Internal doses associated with a 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 in U.S. EPA (2000c), "[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[a]

		BMD modeling[b]			HEC (mg/m^3)	
BMR	**Metric**	**$V_{maxC(R)}$ = 0.4**	**$V_{maxC(R)}$ = 0.65**	**$V_{maxC(H)}$**	**$V_{maxC(R)}$ = 0.4**	**$V_{maxC(R)}$ = 0.65**
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)
0.1	MCA (μmol/L)	BMD_{10}: 0.14 (34.26) $BMDL_{10}$: 0.079 (19.68)	BMD_{10}: 0.12 (32.36) $BMDL_{10}$: 0.071 (19.42)	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 (μmol/hr/kg liver)	BMD_{10}: 3.26 (26.27) $BMDL_{10}$: 2.59 (20.38)	BMD_{10}: 4.60 (28.72) $BMDL_{10}$: 3.65 (22.42)	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

[a]Rats were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, 25, 125 ppm. V_{maxC}, maximum rate of metabolism in humans (H) or rat (R), $mg/hr/kg\ BW^{0.70}$
[b]MCA, log-logistic model provided the best fit; MRAMKL, logistic model provided the best fit. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]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)[a]

		BMD modeling[a]			HEC (mg/m^3)	
BMR	**Metric**	**$V_{maxC(R)}$ = 0.4**	**$V_{maxC(R)}$ = 0.65**	**$V_{maxC(H)}$**	**$V_{maxC(R)}$ = 0.4**	**$V_{maxC(R)}$ = 0.65**
(1)[b]	(2)	(3)	(4)	(5)	(6)	(7)
0.1	MCA (μmol/L)	BMD_{10}: 0.12 (29.53) $BMDL_{10}$: 0.085 (21.13)	BMD_{10}: 0.11 (29.75) $BMDL_{10}$: 0.078 (21.29)	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 (μmol/hr/ kg liver)	BMD_{10}: 3.77 (31.97) $BMDL_{10}$: 2.82 (22.37)	BMD_{10}: 5.42 (34.35) $BMDL_{10}$: 3.75 (23.08)	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

[a]Rats were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, 25 ppm (125 ppm dose dropped).
[b]MCA, multistage (2); MRAMKL, multistage (3). Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]Number in parentheses indicates the column number.

5.2.2.3. *Physiologically Based Pharmacokinetic 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 Thrall et al. (2000), Benson and Springer (1999), and Paustenbach et al. (1988). The human PBPK model was used to estimate continuous 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 (0.63–629 mg/m^3); for MRAMKL, the human PBPK model was run at intervals from 1 to 300 ppm (6.3–1,887 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); and
- 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)

(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/hour/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/hour/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/hour/kg $BW^{0.70}$ for hamsters, based on the results of closed chamber gas uptake studies. The value of 1.49 mg/hour/kg $BW^{0.70}$ for humans (Thrall et al., 2000; Benson

and Springer, 1999), the value of 1.70 mg/hour/kg $BW^{0.70}$ for the hamster (Thrall et al., 2000), and the two values estimated for the rat (0.4, 0.65 mg/hour/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 MOA 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/hour/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/hour/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 was selected as the POD for RfC derivation.

5.2.3. Reference Concentration 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:

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

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 database deficiencies.

- A default 10-fold UF for intraspecies differences (UF_H) 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. Intrahuman variability in CYP450 levels that are responsible for metabolism of carbon tetrachloride to reactive metabolites has been documented (see Section 4.8). This variation in CYP450, which is likely influenced by 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 applied for individual variability.

- A UF of 3 ($10^{0.5}$) was selected for interspecies extrapolation (UF_A) 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. A pharmacokinetic model was used to adjust for pharmacokinetic differences across species; therefore, an additional UF was not included for pharmacokinetic differences between species. In the absence of data to quantify specific interspecies differences for cellular protective mechanisms, a UF of 3 is applied to account for species differences in pharmacodynamics.

- A UF to account for extrapolation from a LOAEL to a NOAEL (UF_L) 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.

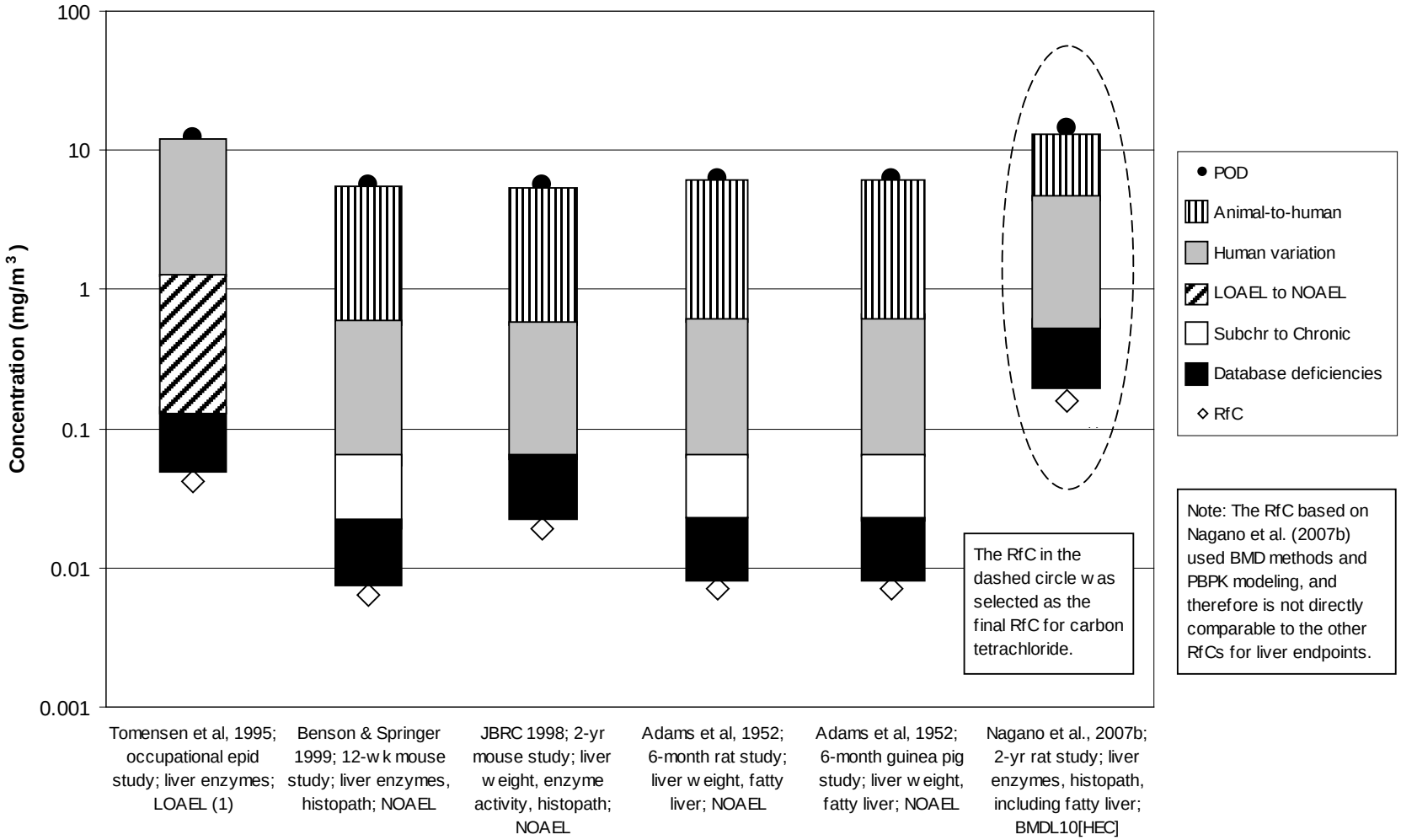
- A UF to extrapolate from a subchronic to a chronic exposure duration (UF_S) was not necessary because the RfC was derived from a study using a chronic exposure protocol.

- A UF to account for database deficiencies (UF_D) 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 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 do not achieve adult levels in humans until sometime between 1 and 10 years. Thus, lifestage 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.

The available information suggests that further developmental toxicity testing would not likely result in a POD lower than that based on liver toxicity. The database lacks an adequate multigeneration study of reproductive function by any route of exposure; therefore, a UF of 3 was applied.

5.2.4. Reference Concentration Comparison Information

PODs and potential 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 a UF of 10) was used for other animal data sets. To some extent, the confidence associated with the resulting potential 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.



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

Figure 5-6. PODs (mg/m^3) with corresponding potential inhalation reference values that would result if liver toxicity was used as the critical effect.

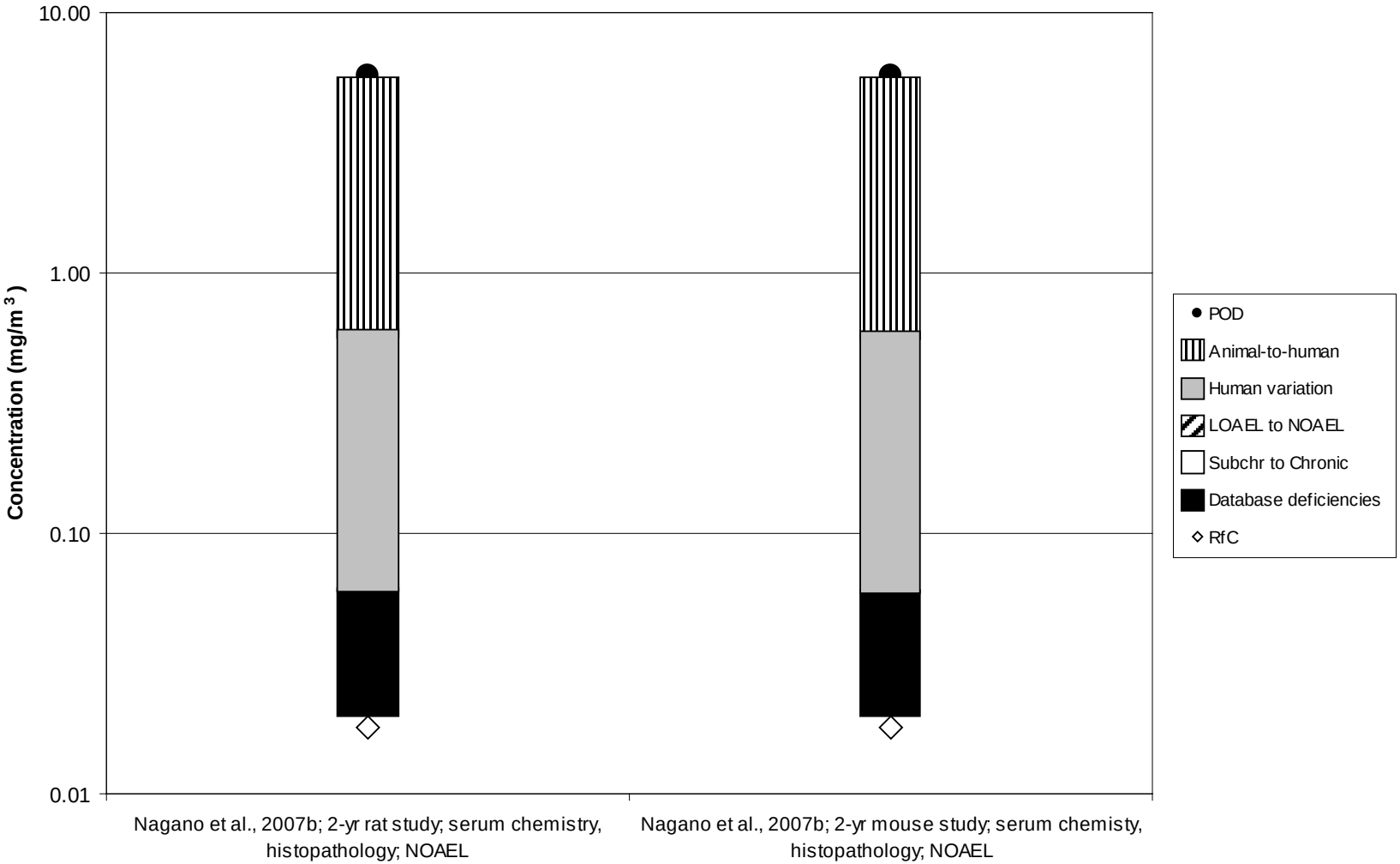


Figure 5-7. PODs (mg/m^3) with corresponding potential inhalation reference values that would result if kidney toxicity was used as the critical effect.

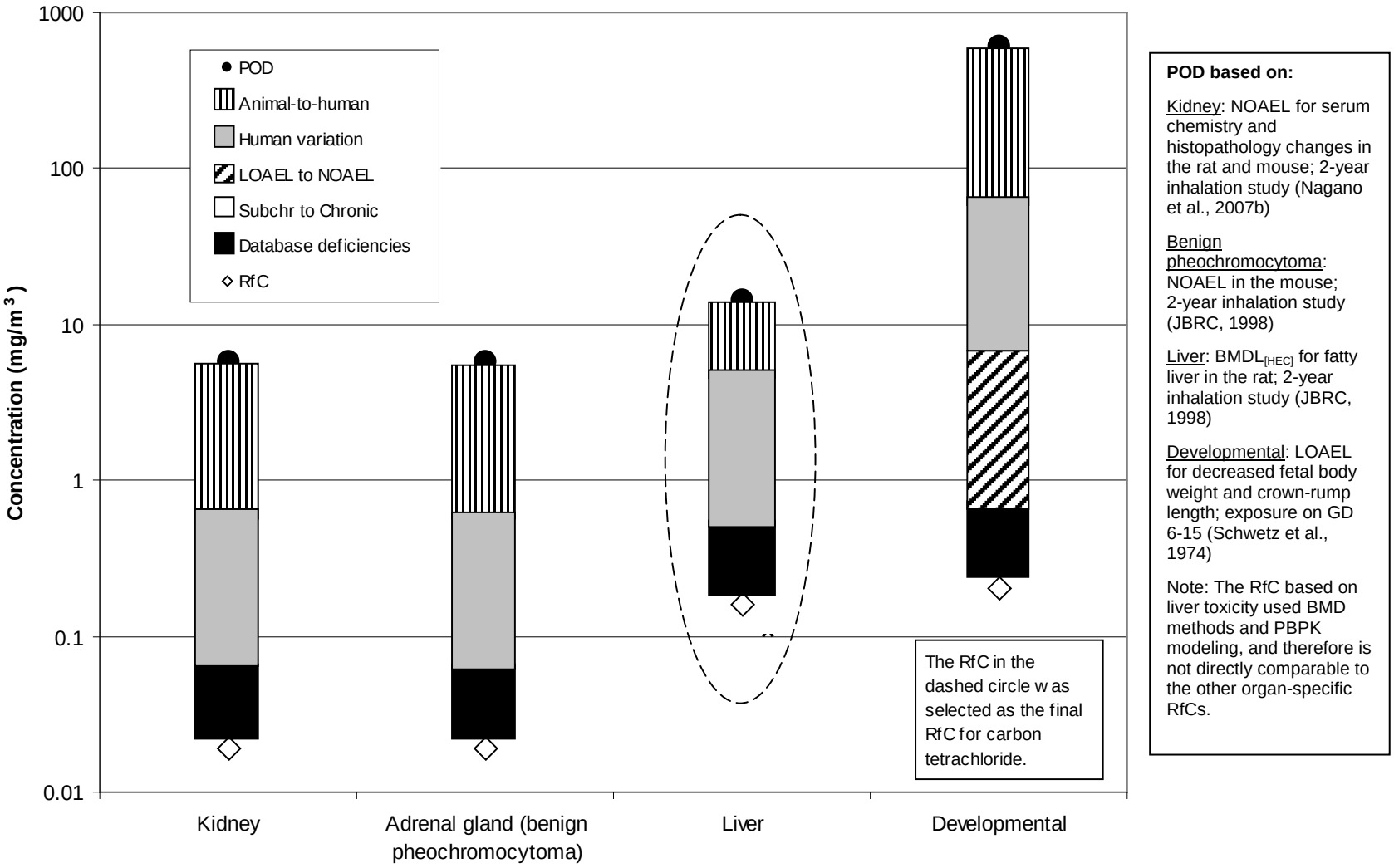


Figure 5-8. PODs (mg/m^3) with corresponding potential inhalation reference values that would result if alternative endpoints were used as the critical effect.

As discussed in Section 4.6.2, the liver and kidney are the predominant targets of carbon tetrachloride toxicity in laboratory animals in subchronic and chronic inhalation studies (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 (adjusted to continuous exposure).[7] 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 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. Potential 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 UF to the POD, (i.e., smaller degree of uncertainty than with other data sets to which the default UF of 10 for interspecies extrapolation was applied). The RfC derived using data from the JBRC rat study is consistent

[7]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) × (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.

with the potential RfC derived from the Tomensen et al. (1995) study. Tomensen et al. (1995) 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 potential RfC is estimated 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 potential RfC estimated from Tomensen et al. (1995) serum enzyme data would be 0.1 mg/m^3. Thus, the potential RfCs of 0.04–0.1 mg/m^3 estimated from Tomensen et al. (1995) are consistent with the RfC of 0.1 mg/m^3 derived from the JBRC rat bioassay (Nagano et al., 2007b; JBRC, 1998), and supports this 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 potential RfC for developmental toxicity. While this relatively large UF drives down the value of the potential RfC for developmental toxicity, the RfC based on liver effects should be adequately protective.

5.2.5. Previous Reference Concentration 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 (see Sections 5.1.2 and 5.1.3 for the RfD and Sections 5.2.2 and 5.2.3 for the RfC), the UF 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 2-year bioassay. This kidney finding occurred at an exposure level fivefold 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.

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 differences 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 related to model structure and parameters (e.g., inclusion of appropriate parameters and interrelationships between parameters), and to values assigned to parameters. A sensitivity analysis was performed for the human PBPK model (see Section C.4 in Appendix C). The maximum rate of metabolism (V_{maxC}) was a sensitive parameter for both dose metrics utilized. Other sensitive chemical-specific parameters included the blood:air partition coefficient and Michaelis-Menten coefficient for metabolism (K_m) 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.

In general, relatively high confidence is assumed for values of physiological parameters (e.g., tissue volumes and blood flows), since these are amenable to direct (and corroborated) observation in animals and humans. Similarly, relatively high confidence is also assumed for values of partition coefficients that have been directly measured in rodent and human tissues, especially if independent estimates yield values within expected intra- and inter-laboratory variability. Although different values for the blood:air partition coefficient were used in the human (2.64; Paustenbach et al.,1988) and rat model (4.52; Gargas et al., 1986), these differences are within a range of expected variability for these parameter values, within and across species. Studies in which identical methods have been applied to estimation of blood:air partition coefficients have obtained variation in values across species. For example, estimates of partition coefficients for carbon tetrachloride in humans (H) and rats (R) from a single laboratory were 2.73 ± 0.23 (SE) and 4.52 ± 0.35, respectively (H/R = 0.60; Gargas et al., 1989). The above value for humans is similar to the value reported by Paustenbach et al. (1988), based on similar methods, 2.64 ± 0.07 (SE), and that was used in PBPK modeling in the current assessment. Estimates for 59 chemicals in human and rat blood yielded H/R ratios that ranged from 0.33 to 1.08 (mean = 0.64; Gargas et al., 1989). Estimates for seven chemicals in human and mouse (M) blood yielded H/M ratios of 0.32–1.54 (mean = 0.68; Gargas et al., 1989).

Independent estimates of partition coefficients for carbon tetrachloride in the same species (i.e., estimates from different laboratories) have also shown variability to different degrees (e.g., for mouse: 7.83 ± 2.18 [SD, Thrall et al., 2000] and 3.8 [SD not reported, Fisher et al., 2004]). Independent estimates for rats are: 4.52 ± 0.35 (SE, Gargas et al., 1986), 5.49 ± 0.95 (SD, Evans et al., 1994), and 4.11 ± 0.25 (Uemitsu, 1986). Mechanisms for apparent differences across species may include interspecies differences in blood composition, including lipid, protein, and water (Béliveau et al., 2005). Inter-laboratory variability in methods may also contribute to variability in values reported from different laboratories (e.g., preservatives and metabolic inhibitors added to blood). Given the above observations and the absence of conclusive evidence to argue that the interspecies differences in reported values for the blood:air partition coefficient are not real differences, EPA has used the reported measured values for blood:air partition coefficients in each species in PBPK modeling to support derivation of toxicity values. These are the values used in the reported calibration of each model (e.g., human, mouse, rat) and would be most consistent with other estimated parameters (e.g., V_{max}, K_m) that also relied on the measured values for partition coefficients (see below).

Metabolism parameters in PBPK models used in this analysis were fit to gas uptake data in rodents. In this procedure, elimination kinetics from the chamber atmosphere (after accounting for leaks and adsorption) are attributed to metabolism, and metabolism parameter values (V_{max}, K_m) are adjusted to achieve the best fit to observations. A range of chamber concentrations is studied, presumed to span $<K_m$ and $>K_m$, in order to provide sensitivity of the data fitting procedure to values of K_m. Although this procedure yields a measurement of whole-body metabolism kinetics, parameters other than V_{max} and K_m may influence chamber elimination kinetics (e.g., parameters that influence carbon tetrachloride concentration in the liver such as tissue:blood partition coefficients). Uncertainties in estimates for these parameters will contribute to uncertainties in the estimates of V_{max} and K_m. For these reasons, greater uncertainty is assumed for estimates of metabolism parameters than for physiological parameters and directly measured tissue partition coefficients. Additional uncertainty enters the dosimetry calculations from extrapolation of values for V_{max} and K_m from rodents to humans, when, as in this case, no validating estimates of these parameters in humans are available. For all of the above reasons, various values for V_{maxC} were considered in modeling rodents and humans in order to capture reported uncertainty in carbon tetrachloride metabolism kinetics in the estimates of rodent internal dose metrics and corresponding HECs.

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 two- to sevenfold 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/hour/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/hour/kg $BW^{0.70}$ for hamsters, based on the results of closed chamber gas uptake studies. The value of 1.49 mg/hour/kg $BW^{0.70}$ for humans (Thrall et al., 2000; Benson and Springer, 1999), the value of 1.70 mg/hour/kg $BW^{0.70}$ for the hamster (Thrall et al., 2000), and the two values estimated for the rat (0.4 and 0.65 mg/hour/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/hour/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 twofold higher.

Suicide inhibition of CYP450 was not explicitly simulated in PBPK models used to predict internal doses of carbon tetrachloride or to extrapolate external doses across species. However, since estimates for V_{max} and K_m were based on in vivo gas uptake studies, the parameter estimates reflect time-averaged estimates of these parameters during the 6-hour duration (1–1,000 ppm) gas uptake measurements (Thrall et al., 2000; Gargas et al., 1986), during which suicide inhibition of CYP450 probably occurred (Uemitsu, 1986). Therefore, average rates of metabolism simulated in the animal PBPK models for exposure concentrations (e.g., 5–125 ppm) and exposure durations (6 hours) would be expected to reflect the average Michaelis-Menten parameter values estimated in gas uptake studies for similar exposure concentrations and durations. What may not be accurately captured in the simulations are the effects of repeated daily exposures on CYP450 activity and metabolism rates (i.e., cumulative

effects of suicide inhibition and induction). The rat PBPK model was able to simulate fat [^{14}C] levels observed during repeated exposures to [^{14}C]-labeled carbon tetrachloride (8 hours/day, 5 days/7 days over 14 days) and excretion rates of [^{14}C] following cessation of repeated exposures to [^{14}C]-labeled carbon tetrachloride (Paustenbach et al., 1988), suggesting that metabolism rates over this duration of exposure were not substantially over- or under-predicted by the model.

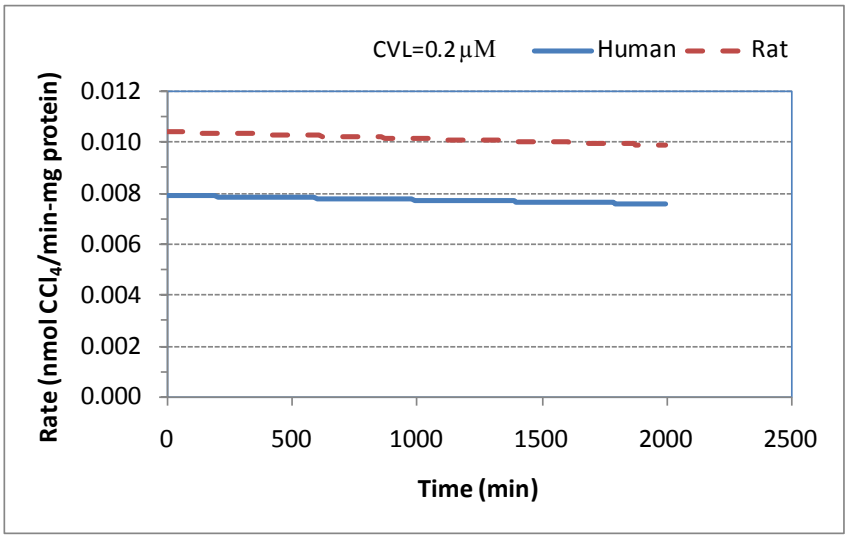
Nevertheless, suicide inhibition, in the absence of induction of CYP450, would be expected to decrease the rate of metabolism of carbon tetrachloride; therefore, models that do not simulate suicide inhibition may over-predict the dose metric MRAMKL (rate of metabolism of carbon tetrachloride per kg liver). The dose metric MCA (concentration of carbon tetrachloride in blood) is relatively insensitive to V_{max} (as a result of the competing respiratory elimination pathways for carbon tetrachloride) and, therefore, quasi-steady-state values for the MCA metric would not be expected to be appreciably affected by suicide inhibition of CYP450.

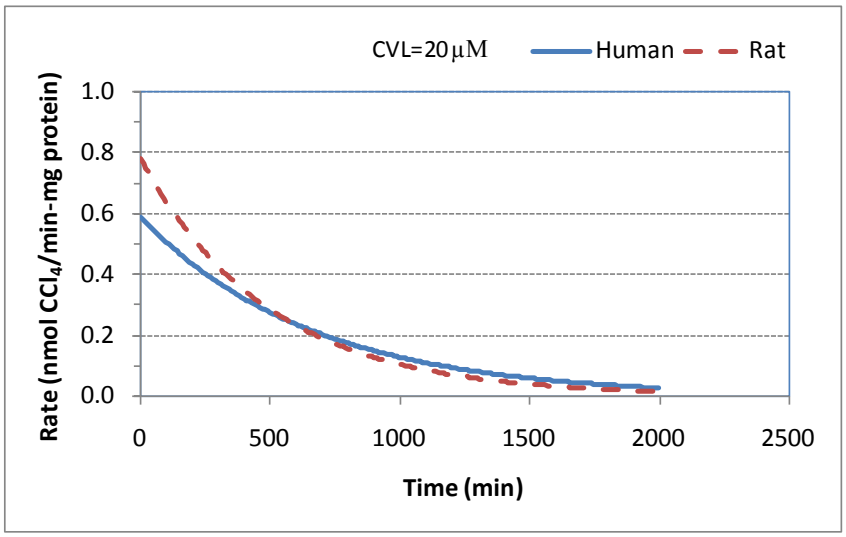
Over-prediction of MRAMKL could have several potential effects on the interspecies extrapolation of carbon tetrachloride dosimetry. Over-prediction of MRAMKL in the animal models may result in over- or under-prediction of BMD and BMDL values, depending on the dose range and form of the dose-response model (e.g., linear versus nonlinear). Over-prediction of the BMDL selected as the POD may result in over-prediction of the corresponding HEC. On the other hand, over-prediction of MRAMKL in the human PBPK model would result in under-prediction of the corresponding HEC.

The direction and magnitude of the effect of suicide inhibition of CYP450 on the RfC (which was based on the MRAMKL metric) cannot be determined with any certainty without validated animal and human PBPK models that simulate CYP450 suicide inhibition and induction. However, it is possible to approximate the relative magnitudes of effect that interspecies differences in suicide inhibition rates might have on CYP450 metabolism rates, by making some simplifying assumptions. The magnitude of the effect of the inactivation of CYP450 on the rate of carbon tetrachloride metabolism will depend on the inactivation coefficient (i.e., mole CYP450 inactivated/mole carbon tetrachloride metabolized), levels of CYP450 in liver (nmol CYP450/g liver), V_{max} (nmol/minute/g liver), K_m (μM in liver), and carbon tetrachloride concentration in liver relative to V_{max} (e.g., $<<K_m$ versus $\geq K_m$). Given similar values for V_{max} and K_m in rat and human liver (Zanger et al., 2000), the dominant variables affecting interspecies differences in suicide inhibition would be the inactivation coefficient, which is substantially greater in the rat (≈0.04) compared to humans (≈0.005; Manno et al., 1992, 1988), and higher basal liver CYP450 levels in rats (1.5 nmol CYP450/mg microsomal protein) compared to humans (0.2 nmol CYP450 mg protein; Manno et al., 1992).

Figures 5-9 and 5-10 compare predicted effects of suicide inhibition on CYP450 activity in rat and human liver microsomes, assuming (1) a constant substrate (carbon tetrachloride) concentration; (2) effects of suicide inhibition are restricted to removal of CYP450 activity and,

thereby, reduction of V_{max} (i.e., no effects on K_m); and (3) the removal rate of CYP450 is given by the suicide inhibition coefficient (moles CYP450 inactivated/mole carbon tetrachloride metabolized). As shown in Figures 5-9 and 5-10, at carbon tetrachloride concentrations in liver (PBPK model parameter *CVL*) similar to those predicted by the PBPK models (0.1–0.2 μM) for exposures corresponding to the POD for the derivation of the RfC (14.3 mg/m^3, 2.27 ppm), inhibition has a relatively minor effect on metabolism rate, and rates of decline of metabolism are similar in rat and human microsomes. At higher concentrations (approaching the K_m), inhibition is more pronounced. Although this simple model of suicide inhibition in isolated liver microsome preparations cannot accurately reflect all events that occur in vivo (e.g., effects of CYP450 inhibition and carbon tetrachloride concentrations in liver, CYP450 induction), the model supports the conclusion that suicide inhibition would have relatively minor effects on the extrapolation of carbon tetrachloride external doses across species, in the low-dose range relevant to the derivation of the RfC.

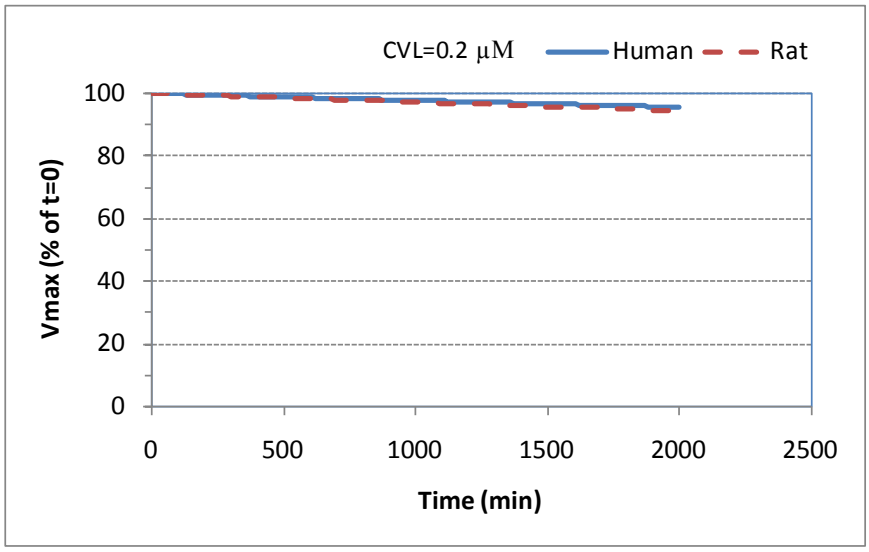


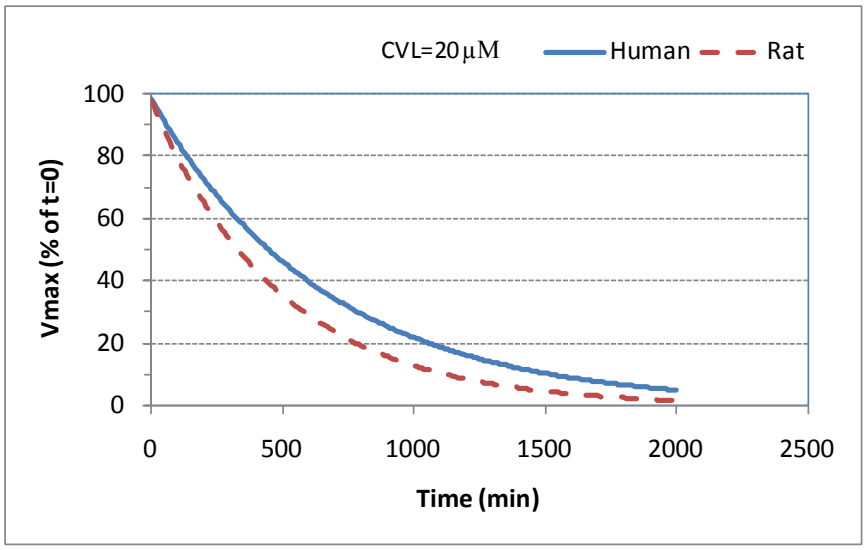


Rate is expressed in units of nmol carbon tetrachloride metabolized/min/mg protein. Rate is expressed in units of nmol carbon tetrachloride metabolized/min/mg protein. Time is in units of minutes of reaction time and assumes instantaneous inactivation, whereas observed kinetics of inactivation appears to be first-order with multiple phases (half-lives of ~3–4 and 29 min).

Sources: Manno et al. (1998, 1992).

Figure 5-9. Comparison of suicide inhibition profiles for liver CYP450 in microsomes prepared from rat and human liver at substrate (carbon tetrachloride) concentrations (*CVL*) similar to those predicted by the PBPK models (0.2 µM) for exposures corresponding to the POD for the derivation of the RfC (14.3 mg/m^3, 2.27 ppm), and at 10-fold higher concentrations (20 µM).





The change in V_{max} (percent of value at time = 0) is shown in the vertical axis. Time is in units of minutes of reaction time and assumes instantaneous inactivation, whereas observed kinetics of inactivation appears to be first-order with multiple phases (half-lives of ~3–4 and 29 min).

Sources: Manno et al. (1998, 1992).

Figure 5-10. Comparison of suicide inhibition profiles for liver CYP450 in microsomes prepared from rat and human liver at substrate (carbon tetrachloride) concentrations (*CVL*) similar to those predicted by the PBPK models (0.2 µM) for exposures corresponding to the POD for the derivation of the RfC (14.3 mg/m^3, 2.27 ppm), and at 10-fold higher concentrations (20 µM).

An adequate PBPK model for the oral pathway was not available; 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, an UF of 10 was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfD.

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 (Lipscomb and Kedderis, 2002). Carbon tetrachloride-specific data on human variation is not available. Quantitative information on variation in human hepatic levels of CYP2E1 and other CYP450 enzymes is available, however, and demonstrates considerable intrahuman variability (see Section 4.8 for additional information). 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 are 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. Considering the database for carbon tetrachloride, 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 the toxicity assays. Investigators have variably reported that (compared to an aqueous vehicle) corn oil either enhanced carbon tetrachloride toxicity (Narotsky et al., 1997b; 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 (Narotsky et al., 1997b; Raymond and Plaa, 1997). The

polyethoxylated vegetable oil Emulphor has been shown to not 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 the exposures were estimated qualitatively based on historical information. These studies, therefore, can provide only limited 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 adenomas and 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 (NTP, 2007; Weisburger, 1977) and inhalation (Nagano et al., 2007b; JBRC, 1998) exposure. No increase in pheochromocytomas was observed in rats.

Selection of a low-dose extrapolation approach to assess cancer risk for carbon tetrachloride was guided by U.S. EPA's (2005a) *Guidelines for Carcinogen Risk Assessment*. According to these guidelines, 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.

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. 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.

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."

As discussed in Section 4.7.3.4, the mechanisms underlying the induction of liver toxicity by carbon tetrachloride have been extensively investigated. Biological support exists for a hypothetical MOA involving metabolism of carbon tetrachloride by CYP2E1, sustained cytotoxicity, and regenerative cell proliferation as key events driving the steep nonlinear increase in liver tumor dose-response at relatively high carbon tetrachloride exposures. However, several pieces of evidence suggest that carbon tetrachloride carcinogenicity is not explained by a cytotoxic-proliferative MOA.

At lower exposure levels, the correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors is inconsistent. 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 MOA. An increased incidence of hepatocellular adenomas occurred in the low-exposure (0.9-ppm adjusted) female mouse in the absence of nonneoplastic liver toxicity, raising the possibility of another MOA operating in addition to or in conjunction with the cytotoxic-proliferative MOA. Other considerations suggest that the carbon tetrachloride database is insufficient to rule out other MOAs 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 species that also can damage DNA and other macromolecules. The potential exists for biologically-active carbon tetrachloride metabolites to react with macromolecules at low exposures (i.e., exposure levels below doses that are cytotoxic); however, data to characterize this low-exposure activity are limited. Thus, as described above and in Section 4.7.3.4, the carcinogenic MOA for carbon tetrachloride is not known. Therefore, consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), linear low-dose extrapolation as a default approach is applied to data for liver tumors and pheochromocytomas.

However, some of the external peer review panel members noted that the available data for carbon tetrachloride provide scientific support for a MOA for liver tumors involving metabolism to reactive intermediates, hepatocellular toxicity, and sustained regenerative and proliferative changes that is consistent with a nonlinear extrapolation approach. Thus, these

panel members did not support the application of a default linear extrapolation approach. (See Appendix A: Summary of External Peer Review and Public Comments and Disposition.)

As noted by several peer review panel members, there is empirical evidence that supports the hypothesis that liver carcinogenicity occurs at carbon tetrachloride exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response, and that exposures that do not cause sustained cytotoxicity and regenerative cell proliferation would be protective of liver tumors if this is the primary MOA. Therefore, an alternative nonlinear extrapolation approach for carbon tetrachloride liver tumors is presented in Section 5.4.5. As discussed above and in Section 4.7.3.4, although there is biological support for a hypothesized cytotoxicity-regenerative MOA as a major MOA driving the steep nonlinear increase in liver tumor dose-response at relatively high carbon tetrachloride exposures, evidence suggests that carbon tetrachloride-induced liver tumors are not explained only by this MOA.

At high exposures, both the cytotoxicity-regenerative proliferation-based MOA and a mutagenicity-based MOA may be operative, but it is not possible to delineate the contribution of these potential MOA(s) to carbon tetrachloride tumor response. Additionally, inconsistencies at the low end of the experimental exposure range (including evidence from the JBRC bioassay of liver adenomas in female mice at a noncytotoxic exposure level 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) MOAs independent of cytotoxicity and regenerative cell proliferation may be operative in this range. Furthermore, the fundamental reactivity of direct and indirect products of carbon tetrachloride metabolism can reasonably be expected to play a role in carbon tetrachloride carcinogenicity at all exposure levels. Linear processes associated with this fundamental reactivity would likely dominate the dose-response relationship at low exposures (i.e., exposure levels below those that are cytotoxic).

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 (including NRC, 2009) have provided broad and long-standing scientific arguments in favor of low-dose linear approaches to cancer risk assessment 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 Lutz et al., 2005; Zeise et al., 1987) 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, for example, genetic variability, disease states, nutrition, age).

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

In summary, while an alternative nonlinear approach for carbon tetrachloride-induced liver cancer is presented in Section 5.4.5, EPA recommends the application of linear extrapolation in the absence of a carcinogenic MOA for carbon tetrachloride and broader science considerations. For carbon tetrachloride-induced pheochromocytomas, a linear extrapolation approach is also recommended in the absence of MOA information for this tumor.

5.4.1. Choice of Study/Data—with Rationale and Justification

5.4.1.1. *Inhalation Data*

As noted previously, epidemiological studies of populations exposed to carbon tetrachloride provide only limited 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 BDF_1 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.

5.4.1.2. *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 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 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. Dose-Response Data

5.4.2.1. *Inhalation Data*

Dose-response modeling was performed for five tumor responses from the JBRC bioassay: adenoma or carcinoma of the liver in female rats, adenoma or 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 BDF_1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

	Male				Female			
Tumor	**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's exact test ($p \leq 0.01$).

Sources: Nagano et al. (2007b); JBRC (1998).

Table 5-9. Incidence of adrenal tumors (pheochromocytomas) in BDF_1 mice exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)

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

[a]All pheochromocytomas in the mouse were benign with the exception of one malignant pheochromocytoma in the 125-ppm male mouse group.
[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's exact test ($p \leq 0.01$).

Sources: Nagano et al. (2007b); JBRC (1998).

The male rat incidence data for liver adenomas or carcinomas were not modeled because this data set lacked the resolution desired for dose-response modeling. Tumor frequency increased from control levels to close to maximal response without any intervening exposure levels having submaximal responses. In the female rat, lower but biologically significant levels of response were seen at intermediate exposure 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 χ^2 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 high exposure-level 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. The decision to develop dose-response models for pheochromocytomas was based on guidance provided in the *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 presence of one observed malignant tumor in the mouse study also suggests the 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 NTP, 2007 and Weisburger, 1977); the status as benign or malignant was

not specified. Finally, Salmenkivi et al. (2004) observed that while most pheochromocytomas are benign, differentiating a benign tumor from a malignant tumor only by histological criteria is difficult. Thus, it was considered appropriate to conduct dose-response modeling for pheochromocytomas.

Some of the external peer review panel members noted the human relevance of carbon tetrachloride-induced mouse pheochromocytomas was questionable or uncertain and consequently not appropriate for a quantitative assessment of human cancer risk (see Appendix A: Summary of External Peer Review and Public Comments and Disposition). The panel members who disagreed with using pheochromocytoma data as the basis for cancer risk values did so largely for the following reasons: (1) an excess incidence of pheochromocytomas has not been observed in humans exposed to carbon tetrachloride, (2) pheochromocytomas were induced by carbon tetrachloride exposure in mice only (there was no increase in exposed rats), and (3) the tumors are almost always benign.

As observed by the peer review panelists, an association between carbon tetrachloride exposure and increased risk of pheochromocytomas in human populations has not been demonstrated. However, epidemiological studies have not been conducted to date to investigate whether such an association exists. U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that site concordance between animals and humans is not always assumed. Therefore, the lack of evidence for carbon tetrachloride-induced pheochromocytomas in humans does not preclude that tumors in rodents are generally indicative of a potential for carcinogenicity in humans.

The relevance of rodent pheochromocytomas as a model for human cancer risk has been the subject of discussion in the scientific literature (e.g., Greim et al., 2009; Powers et al., 2008). The spontaneous occurrence of pheochromocytomas, as well as metastases, in the mouse is relatively rare. The lifetime incidence in wild-type laboratory mice has been reported as ≤3% by Tischler et al. (2004, 1996). However, 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 catecholamine-producing neuroendocrine tumors (Eisenhofer et al., 2004; Salmenkivi et al., 2004; Tischler et al., 1996). 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), the enzyme that produces epinephrine from norepinephrine; human pheochromocytomas are also usually PNMT-positive (Tischler et al., 1996).

In humans, pheochromocytomas 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). Rates of malignant transformation of 10% (Salmenkivi et al., 2004; Sweeney, 2009) to approximately 36% (Ohta et al., 2006) have been reported. In light of the parallels between mouse and human

pheochromocytomas, the mouse model has been considered as a potentially appropriate model for human adrenal medullary tumors (Tischler et al., 1996).

Therefore, EPA considers mouse pheochromocytomas to be relevant to humans. Consequently, the incidence data for this tumor type are appropriate for consideration in the cancer dose-response analysis for carbon tetrachloride.

5.4.2.2. *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 IUR were considered appropriate for estimation of an oral SF. The route-to-route extrapolation method is described further below.

5.4.3. Dose Adjustments and Extrapolation Methods

5.4.3.1. *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 U.S. 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 HECs and human equivalent doses (HEDs) by applying the human PBPK model.

5.4.3.2. Physiologically Based Pharmacokinetic *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) (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, 2006c), 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 MOA 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/hour/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 MOA in producing liver toxicity (described in Section 4.5). Further support for rate of hepatic metabolism as a dose metric is provided in Section 5.2.2.1. 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. Based on the model and estimates of Michaelis-Menten parameters for carbon tetrachloride metabolism in lung and kidney, metabolism in each of these tissues was estimated to be <1% of that in the liver, and 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.

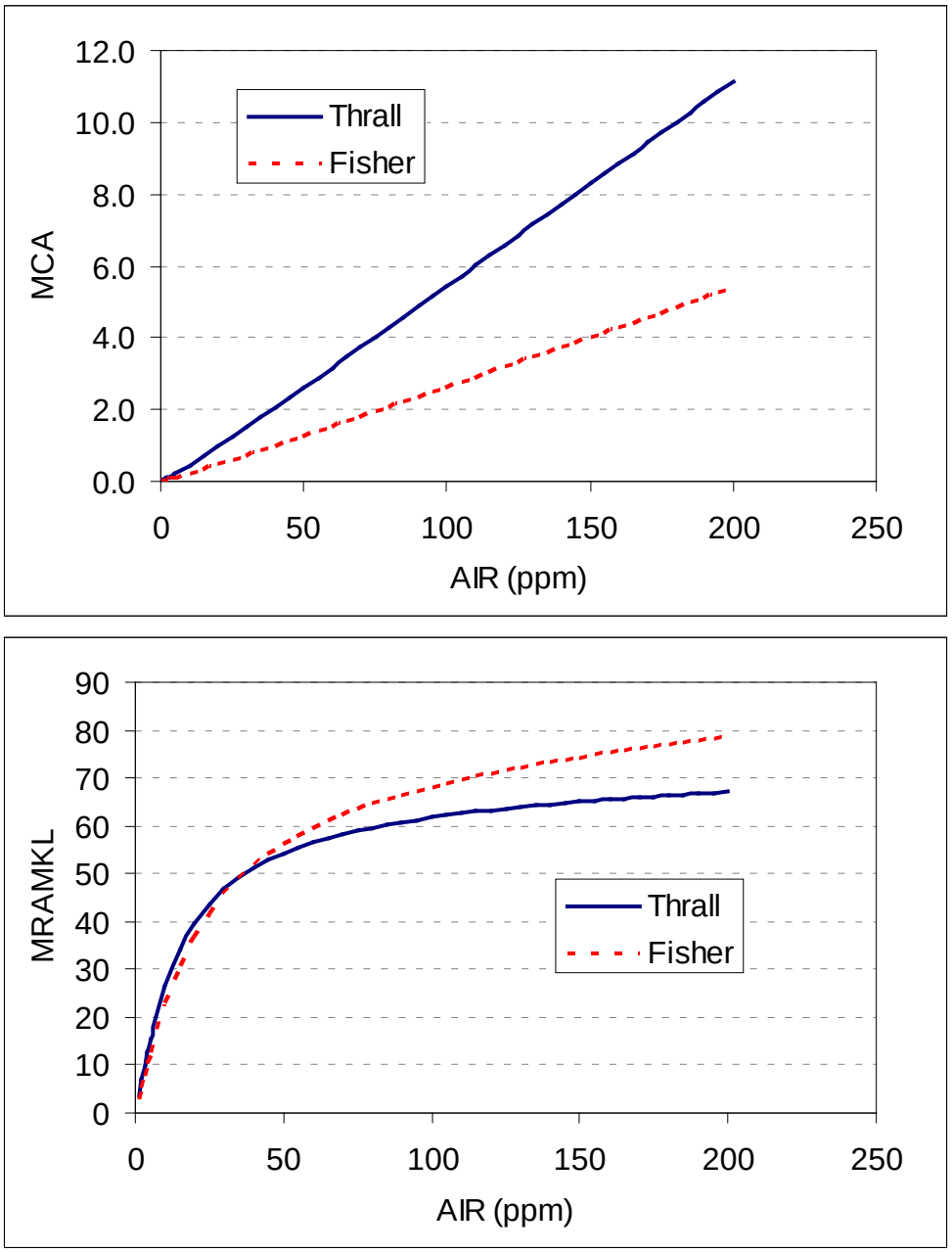
The two dose metrics, MCA and MRAMKL, were simulated in the rat and mouse PBPK models as time-averaged values, with the averaging time being the chronic exposure period (e.g., 2 years). See Equations 5-1 and 5-2 (Section 5.2.2.1) for the calculation of the time-averaged dose metrics.

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 5-11.

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

Exposure (ppm)	MCA (µmol/L)		MRAMKL (µmol/hr/kg liver)	
	Fisher et al.	Thrall et al.	Fisher et al.	Thrall et al.
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

[a]Values are for 0.036 kg mouse..



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 0.036 kg mouse.

Figure 5-11. Internal dose metrics predicted from the Fisher et al. (2004) and Thrall et al. (2000) PBPK mouse models.

5.4.3.3. *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 tumor incidence reflects that of benign or malignant tumors combined (i.e., hepatocellular adenomas or carcinomas; benign or malignant pheochromocytomas). 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). The multistage model in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007b) 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 or 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% (a 10% BMR is commonly used in BMD modeling as a means of facilitating comparison across assessments and endpoints) 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 the hypothesized cytotoxic-proliferative MOA.

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 or carcinoma) in female F344 rats and corresponding HEC and HED values[a]

Metric	BMD modeling[b]		$V_{maxC(H)}$	HEC (mg/m^3)		HED (mg/kg-d)	
	$V_{maxC(R)}$ = 0.4	$V_{maxC(R)}$ = 0.65		$V_{maxC(R)}$ = 0.4	$V_{maxC(R)}$ = 0.65	$V_{maxC(R)}$ = 0.4	$V_{maxC(R)}$ = 0.65
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA (µmol/L)	BMD_5: 0.61 (136.8) $BMDL_5$: 0.39 (90.29)	BMD_5: 0.59 (143.2) $BMDL_5$: 0.35 (88.94)	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 (µmol/hr/ kg liver)	BMD_5: 9.82 (109.0) $BMDL_5$: 8.40 (85.71)	BMD_5: 14.6 (116.4) $BMDL_5$: 12.3 (91.37)	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

[a]Rats were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Internal doses modeled correspond to exposure concentrations: 0, 5, 25, or 125 ppm.
[b]MCA, multistage (2-stage); MRAMKL, multistage (4-stage). BMR = 5%. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]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 2/1,797 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 (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 or carcinoma) in female F344 rats (high dose dropped) and corresponding HEC and HED values[a]

Metric	BMD modeling[b]		$V_{maxC(H)}$	HEC (mg/m^3)		HED (mg/kg-day)	
	$V_{maxC(R)}$ = 0.4	$V_{maxC(R)}$ = 0.65		$V_{maxC(R)}$ = 0.4	$V_{maxC(R)}$ = 0.65	$V_{maxC(R)}$ = 0.4	$V_{maxC(R)}$ = 0.65
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA (µmol/L)	BMD_5: 0.65 (145.1) $BMDL_5$: 0.35 (81.62)	BMD_5: 0.60 (145.4) $BMDL_5$: 0.32 (81.88)	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 (µmol/hr/kg liver)	BMD_5: 11.6 (145.1) $BMDL_5$: 6.92 (65.27)	BMD_5: 16.7 (143.3) $BMDL_5$: 9.76 (67.82)	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

[a]Rats were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Internal doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure was dropped).
[b]MCA, multistage (2-stage); MRAMKL, multistage (2-stage). BMR = 5%. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]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 or carcinomas. 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 MOA. 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 5.4.2.1, the multistage model fits below were conducted without use of the highest exposure concentration data, an approach commonly used in BMD modeling when 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 or carcinoma) in female BDF_1 mice (high dose dropped) and corresponding HEC and HED values[a]

Metric	BMD modeling[b]		$V_{maxC(H)}$	HEC (mg/m^3)		HED (mg/kg-day)	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA (µmol/L)	BMD_{10}: 0.10 (28.41) $BMDL_{10}$: 0.047 (13.54)	BMD_{10}: 0.19 (28.20) $BMDL_{10}$: 0.088 (13.42)	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 (µmol/hr/ kg liver)	BMD_{10}: 9.71 (23.45) $BMDL_{10}$: 6.32 (14.82)	BMD_{10}: 10.4 (19.98) $BMDL_{10}$: 7.59 (14.16)	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

[a]Mice were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped). Fisher, Fisher et al. (2004) model; Thrall, Thrall et al. (2000) model; [b]MCA, multistage (2-stage); MRAMKL, multistage (2-stage). BMR (benchmark response) = 10%. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]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). As discussed further in Section 5.4.4.2, the analysis based on the control and 5-ppm exposure groups provides a less informative characterization of the dose-response curve than does the analysis based on the control, 5-ppm and 25-ppm exposure groups. The latter analysis does, however, provide some information on the dose-response relationship for liver tumors in the female mouse at concentrations below levels documented to cause cell replication.

Table 5-14. BMD values for incidence data for liver tumors (adenoma or carcinoma) in female BDF_1 mice (two highest doses dropped) and corresponding HEC and HED values[a]

Metric	BMD modeling[b]		$V_{maxC(H)}$	HEC (mg/m^3)		HED (mg/kg-day)	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA (µmol/L)	BMD_{10}: 0.10 (28.41) $BMDL_{10}$: 0.044 (12.68)	BMD_{10}: 0.20 (29.61) $BMDL_{10}$: 0.085 (12.97)	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 (µmol/hr/ kg liver)	BMD_{10}: 11.6 (28.51) $BMDL_{10}$: 5.05 (11.72)	BMD_{10}: 14.2 (28.49) $BMDL_{10}$: 6.16 (11.33)	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

[a]Mice were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0 or 5 ppm (25 and 125 ppm exposures were dropped). Fisher, Fisher et al. (2004) model; Thrall, Thrall et al. (2000) model
[b]MCA, multistage (2-stage); MRAMKL, multistage (2-stage). BMR = 10%. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]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 or 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 or 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 exposure 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 or carcinoma) in male BDF_1 mice (high dose dropped) and corresponding HEC and HED values[a]

Metric	BMD modeling[b]		$V_{maxC(H}$	HEC (mg/m^3)		HED (mg/kg-day)	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA (µmol/L)	BMD_{10}: 0.19 (52.89) $BMDL_{10}$: 0.064 (18.35)	BMD_{10}: 0.39 (55.37) $BMDL_{10}$: 0.12 (18.14)	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 (µmol/hr/ kg liver)	BMD_{10}: 13.4 (33.52) $BMDL_{10}$: 7.31 (17.29)	BMD_{10}: 14.2 (28.49) $BMDL_{10}$: 8.82 (16.66)	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

[a]Mice were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped). Fisher, Fisher et al. (2004) model; Thrall, Thrall et al. (2000) model.
[b]MCA, multistage (3-stage); MRAMKL, multistage (3-stage). BMR = 10%. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]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 BDF_1 mice and corresponding HEC and HED values[a]

Metric	BMD modeling[b]		$V_{maxC(H)}$	HEC (mg/m^3)		HED (mg/kg-day)	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA (µmol/L)	BMD_{10}: 1.43 (3529) $BMDL_{10}$: 1.14 (285.2)	BMD_{10}: 2.95 (353.3) $BMDL_{10}$: 2.34 (284.8)	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

[a]Mice were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, 25, or 125 ppm. Fisher, Fisher et al. (2004) model; Thrall, Thrall et al. (2000) model.
[b]Multistage (2-stage) model. BMR = 10%. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c] Number in parentheses indicates the column number.

Table 5-17. BMD values for incidence data for pheochromocytomas in male BDF_1 mice and corresponding HEC and HED values[a]

Metric	BMD modeling[b]		$V_{maxC(H)}$	HEC (mg/m^3)		HED (mg/kg-day)	
	Fisher	Thrall		Fisher	Thrall	Fisher	Thrall
(1)[c]	(2)	(3)	(4)	(5)	(6)	(7)	(8)
MCA (µmol/L)	BMD_{10}: 0.26 (71.36) $BMDL_{10}$: 0.15 (42.13)	BMD_{10}: 0.53 (73.43) $BMDL_{10}$: 0.30 (43.38)	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

[a]Mice were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, 25, or 125 ppm. Fisher, Fisher et al. (2004) model; Thrall, Thrall et al. (2000) model.
[b]log-probit model. BMR = 10%. Values in parentheses are animal exposure concentrations (mg/m^3) corresponding to BMD and BMDL values.
[c]Number in parentheses indicates the column number.

See Appendix E for the BMDS model outputs and graphs of the modeled data.

5.4.3.4. *Physiologically Based Pharmacokinetic 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 HECs (in mg/m^3) or 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 (0.63–629 mg/m^3); for MRAMKL, the human PBPK model was run at intervals from 1 to 300 ppm (6.3–1,887 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 GI tract to liver (RGIL, mg/kg-day) that yielded the same internal doses. Values for uptake (RGIL) were used as estimates of HEDs (mg/kg-day). This simple method of approximating the HED from the RGIL assumes that a given ingestion dose of carbon tetrachloride (mg/kg-day) would result in the same dose delivered from the GI tract to the liver and that the liver dose would be delivered at a constant rate during the day (i.e., conceptually equivalent to, and simulated in the PBPK model as, a constant rate of infusion of carbon tetrachloride into the liver). HED values derived from RGIL values are approximations because they do not account for the possibility that bioavailability of ingested carbon tetrachloride may be <100% or that the rate of absorption of ingested carbon tetrachloride may not be constant throughout the day (i.e., bolus effects). Thus, this approximation method assumes complete absorption from the GI tract. Information on bioavailability and absorption kinetics of carbon tetrachloride in humans is not available. However, as discussed in Section 5.1.2, under certain dosing conditions (e.g., oral gavage in corn oil), absorption of carbon tetrachloride from the GI tract may actually exhibit pulsatile behavior (Fisher et al., 2004; Semino et al., 1997; Gallo et al., 1993).

(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 GI 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 GI 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/hour/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/hour/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 5.4.3.2, 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/hour/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 5.4.4 below).

5.4.4. Inhalation Unit Risk and Oral Slope Factor

5.4.4.1. *Inhalation Unit Risk*

IUR estimates based on the five tumor data sets analyzed in Section 5.4.3.3 were calculated as follows:

$$IUR = BMR / HEC \qquad \text{Eq. (5-6)}$$

The IURs are provided in Table 5-18. The highest IUR was associated with pheochromocytomas in the male mouse (5.6×10^{-6} $[\mu g/m^3]^{-1}$, or rounded to one significant figure, 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 5.4.3.3, 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 IUR estimates using linear low-dose extrapolation approach

Tumor	Exposure groups modeled	Model parameters	HEC (mg/m^3)	Average HEC (mg/m^3)[a]	IUR estimate[b] $(\mu g/m^3)^{-1}$
Female rat hepatocellular adenoma or carcinoma	0, 5, 25, 125 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	39.63	49.48	1.0×10^{-6}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	59.32		
	0, 5, 25 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	32.33	39.37	1.3×10^{-6}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	46.41		
Female mouse hepatocellular adenoma or carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	29.46	32.55	3.1×10^{-6}
		MRAMKL; Thrall model BMR = 10%	35.64		
	0, 5 ppm	MRAMKL; Fisher model BMR = 10%	23.37	26.03	3.8×10^{-6}
		MRAMKL; Thrall model BMR = 10%	28.68		
Male mouse hepatocellular adenoma or carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	34.25	37.98	2.6×10^{-6}
		MRAMKL; Thrall model BMR = 10%	41.70		
Female mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	88.17	131.4	7.6×10^{-7}
		MCA; Thrall model BMR = 10%	174.69		
Male mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	12.00	17.78	5.6×10^{-6}
		MCA; Thrall model BMR = 10%	23.56		

[a]For the rat, the average represents an arithmetic mean of the two HEC values based on V_{maxR} values of 0.65 and 0.4 mg/hr/kg $BW^{0.70}$; for the mouse, the average represents an arithmetic mean of the two HEC values based the Fisher and Thall models.
[b]The IUR was calculated as the BMR ÷ HEC.

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 the

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.

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. The slope of the linear extrapolation from the central estimate based on pheochromocytomas in the male mouse is calculated as $0.1 \div (3.13 \times 10^4$ $\mu g/m^3) = 3.2 \times 10^{-6}$ $(\mu g/m^3)^{-1}$, or rounded to one significant figure, 3×10^{-6} $(\mu g/m^3)^{-1}$.[8]

5.4.4.2. *Oral Slope Factor*

Oral SF estimates based on the five inhalation tumor data sets analyzed in Section 5.4.3.3 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 (6.5×10^{-2} $[mg/kg\text{-}day]^{-1}$, or rounded to one significant figure, 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 appropriate. 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} versus 8×10^{-2} $[mg/kg\text{-}day]^{-1}$). A similar analysis of female rat liver tumor data

[8]The slope of the linear extrapolation from the central estimate POD was calculated based on incidence data for pheochromocytomas in male BDF1 mice. The central estimate POD (expressed as HEC) is:

	BMC_{10}[a]			HEC (central estimate)[b] (mg/m^3)		
Metric	**Fisher**	**Thrall**	$V_{maxC(H)}$	**Fisher**	**Thrall**	**Average**[c]
MCA	0.26	0.53	1.49	20.7	41.9	31.3

Mice were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, 25, or 125 ppm. Fisher, Fisher et al. (2004) model; Thrall, Thrall et al. (2000) model.
[a]Log-probit model. BMR = 10%.
[b]The HEC values corresponding to BMC_{10} values (as central estimates) were calculated using the values in Table C-6 in Appendix C.
[c]The average represents an arithmetic mean of the two HEC values based the Fisher and Thrall models.

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} versus 3×10^{-2} [mg/kg-day]$^{-1}$; see Table 5-19).

Table 5-19. Summary of oral SF estimates using linear low-dose extrapolation approach and route-to-route extrapolation

Tumor	Dose groups modeled	Model parameters	HED (mg/kg-d)	Average HED (mg/kg-d)[a]	Oral SF estimate (mg/kg-d)$^{-1}$
Female rat hepatocellular adenoma or carcinoma	0, 5, 25, 125 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	1.88	2.34	2.1×10^{-2}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	2.81		
	0, 5, 25 ppm	MRAMKL; V_{maxR} = 0.4 BMR = 5%	1.53	1.86	2.7×10^{-2}
		MRAMKL; V_{maxR} = 0.65 BMR = 5%	2.20		
Female mouse hepatocellular adenoma or carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	1.40	1.54	6.5×10^{-2}
		MRAMKL; Thrall model BMR = 10%	1.69		
	0, 5 ppm	MRAMKL; Fisher model BMR = 10%	1.11	1.24	8.1×10^{-2}
		MRAMKL; Thrall model BMR = 10%	1.36		
Male mouse hepatocellular adenoma or carcinoma	0, 5, 25 ppm	MRAMKL; Fisher model BMR = 10%	1.62	1.8	5.6×10^{-2}
		MRAMKL; Thrall model BMR = 10%	1.98		
Female mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	14.2	19.27	5.2×10^{-3}
		MCA; Thrall model BMR = 10%	24.34		
Male mouse pheochromocytoma	0, 5, 25, 125 ppm	MCA; Fisher model BMR = 10%	3.04	4.12	2.4×10^{-2}
		MCA; Thrall model BMR = 10%	5.21		

[a]For the rat, the average represents an arithmetic mean of the two HEC values based on V_{maxR} values of 0.65 and 0.4 mg/hr/kg $BW^{0.70}$; for the mouse, the average represents an arithmetic mean of the two HEC values based the Fisher and Thall models.
[b]The oral SF was calculated as the BMR ÷ HED.

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.

The oral SF for carbon tetrachloride is estimated as 7×10^{-2} (mg/kg-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. The slope of the linear extrapolation from the central estimate based on female mouse liver tumors is calculated as $0.1 \div 2.27$ mg/kg-day $= 4.4 \times 10^{-2}$ (mg/kg-day)$^{-1}$, or rounded to one significant figure, 4×10^{-2} (mg/kg-day)$^{-1}$.[9]

Whereas the male mouse pheochromocytoma data set yielded the highest estimate of the IUR for carbon tetrachloride based on analysis of tumor data from the JBRC bioassay (Nagano et al., 2007b) and application of PBPK modeling for interspecies extrapolation, female mouse liver tumor data yielded the highest estimate of the oral SF based on the data from the same bioassay and route-to-route extrapolation using PBPK modeling. While it may appear counterintuitive that the use of data from a single inhalation bioassay (Nagano et al., 2007b) could result in the use of different data sets for estimating cancer potency by the oral and inhalation routes, the situation arises because of the use in PBPK modeling of different dose metrics for the liver and adrenal gland that could result in different relationships between environmental exposure and internal dose within a species (i.e., rat in the current bioassay) and across species (i.e., rats and humans).

[9]The slope of the linear extrapolation from the central estimate POD was calculated based on incidence data for liver tumors in female BDF1 mice. The central estimate POD (expressed as HED) is:

Metric	BMD_{10}[a]		$V_{maxC(H)}$	HED (central estimate)[b] (mg/kg-d)		
	Fisher	Thrall		Fisher	Thrall	Average[c]
MRAMKL (μmol/hr/kg liver)	9.71	10.4	1.49	2.19	2.35	2.27

Mice were exposed to carbon tetrachloride vapor for 104 wks (6 hrs/d, 5 d/wk). Doses modeled correspond to exposure concentrations: 0, 5, or 25 ppm (125 ppm exposure dropped). Fisher, Fisher et al. (2004) model; Thrall, Thrall et al. (2000) model.
[a]Multistage model. BMR = 10%.
[b]The HED values corresponding to BMD_{10} values (as central estimates) were calculated using the values in Table C-10 in Appendix C.
[c]The average represents an arithmetic mean of the two HED values based the Fisher and Thall models.

5.4.5. Nonlinear Extrapolation Approach

As noted above, empirical evidence for carbon tetrachloride, particularly from studies using relatively high exposure levels, provides support for a MOA for liver tumors that includes the following hypothesized 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 postulated 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 MOA for carbon tetrachloride liver carcinogenicity, a nonlinear approach to low-dose extrapolation may be 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, respectively. Doses (or concentrations) of carbon tetrachloride below the RfD (or RfC) that do not cause sustained hepatocellular cytotoxicity and regenerative cell proliferation would be expected to be protective of liver tumors if this is the primary MOA for liver tumors. This harmonized approach between noncancer and cancer endpoints utilizes a key event (cytotoxicity or hepatotoxicity) in the hypothesized nonlinear MOA to derive the RfD and RfC. Based on a MOA 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 MOA information specific to that tumor type and therefore does not apply to pheochromocytomas for which the MOA is unknown. 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.

As noted above, a nonlinear approach for assessing the risk of liver tumors assumes that a cytotoxic-proliferative MOA is the primary MOA for liver tumors. In light of evidence that suggests that carbon tetrachloride-induced liver tumors are not explained only by this MOA, EPA recommends a linear low-dose extrapolation approach be applied for carbon tetrachloride rather than a nonlinear approach.

5.4.6. 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. As noted in U.S. EPA's *Guidelines for Carcinogen Risk Assessment*, "… agents observed to produce tumors in both humans and animals have produced tumors either at the same site (e.g., vinyl chloride) or different sites (e.g., benzene) (NRC, 1994). Hence, site concordance is not always assumed between animals and humans." Thus, it is not clear whether the tumors observed in rodent bioassays would be predictive of human tumors of the same or different sites.

The MOA for liver tumor induction has not been established, but the hypothesized MOAs that have been investigated are assumed to be relevant to humans (Section 4.7.3.5). There is no available 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 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). The relevance of mouse pheochromocytomas to humans is considered in Section 5.4.2. 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 adrenal medullary tumors (Hill et al., 2003), and the MOA for this tumor in mice is unknown. However, parallels between this tumor in the mouse and human led investigators to conclude 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, the available experimental evidence supports a conclusion that mouse pheochromocytomas are relevant to humans.

Choice of low-dose extrapolation approach. The MOA is a key determinant of which approach to apply for estimating low-dose cancer risk. The MOA of carbon tetrachloride liver carcinogenicity has been investigated extensively; however, much of this research has been conducted at relatively high exposure levels. The MOA(s) at low exposure levels is not known.

For liver tumors, a nonlinear extrapolation approach was explored in Section 5.4.5 as an alternative to the linear low-dose extrapolation approach for cancer risk estimation. Such an approach would be supported by a conclusion that the hypothesized cytotoxicity-proliferative MOA is operative at all doses; however, evidence inconsistent with the nonlinear approach includes the finding of female mouse hepatocarcinogenicity at noncytotoxic doses, potential for genotoxicity at low doses, the fundamental reactivity of the chemical, and the absence of MOA information regarding the observed pheochromocytomas in mice.

The linear extrapolation approach assumes that some cancer risk exists at all nonzero exposures, and that this risk increases linearly with exposure. While consistent with the recognized biological reactivity of carbon tetrachloride, uncertainties in this low-dose extrapolation approach are associated with the lack of MOA information at low exposures. Additional MOA 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 estimating the magnitude of liver tumor risk.

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 provides 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 IUR for liver tumors. The impact on the IUR derived from pheochromocytoma data is unknown.

Under the linear low-dose extrapolation approach, 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 MOA for this tumor. MOA information to inform the approach to low-dose extrapolation for 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 than 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 GI tract to the liver and that absorption of carbon tetrachloride from the GI tract is essentially complete. 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. To the extent that GI absorption is less than 100%, the current estimation method for route-to-route extrapolation would tend to overestimate the SF.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed through CIs. 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 POD 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 IUR and, using route-to-route extrapolation, the oral SF. 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, using two species (rats and mice), four exposure groups, including an appropriate untreated control, and 50 animals/sex/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 adequate for developing an IUR or oral SF were unavailable.

Choice of species/gender. For liver tumors, modeling was performed using the 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 increased from control levels to close to maximal responses without any intervening exposure levels having submaximal responses. In the female mice and rats, lower but biologically significant levels of tumor response were seen at intermediate exposure levels. Also, notably, increased levels of hepatocellular proliferation were not reported for rodents at these intermediate levels, so that dose-response modeling based on these data may be more applicable to an evaluation of cancer risk at noncytotoxic exposures. There is no indication that male rodents are more sensitive to carbon tetrachloride liver tumor induction or that use of female data 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), genetic polymorphisms in drug metabolism enzymes, transporters, and receptors (all of which can markedly affect susceptibility to a toxic chemical), 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	As noted in U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), "…site concordance is not always assumed between animals and humans." Thus, it is not clear whether the tumors observed in rodent bioassays would be predictive of human tumors of the same or different sites. Liver: The experimental animal literature shows carbon tetrachloride to consistently induce liver tumors across species and routes of exposure. Although there is no evidence in humans for hepatic cancer associated with carbon tetrachloride exposure, the hypothesized MOAs are considered relevant to humans. Experimental animals and humans are similar 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 a potenially appropriate model for human adrenal medullary tumors.
Low-dose extrapolation approach	Departure from U.S. 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 MOA that is consistent with a nonlinear extrapolation approach; however, other evidence suggests that hepatocarcinogenicity may not be explained only in terms of this hypothesized MOA. Where data are not strong enough to ascertain the MOA, U.S. EPA's *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 U.S. EPA's *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.

Table 5-20. Summary of uncertainty in the carbon tetrachloride cancer risk assessment

Consideration/ approach	Impact on cancer risk estimate	Decision	Justification
Route-to-route extrapolation using PBPK model	The magnitude of uncertainty cannot be quantified; however, assumption of complete GI absorption may overestimate the SF.	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 GI tract to the liver.
Statistical uncertainty at POD	↓ IUR and SF by 1.5–1.8-fold if BMD used as the POD rather than lower bound on POD	BMDL (preferred approach for calculating reasonable upper bound SF)	Size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure.
Bioassay	Alternative bioassay, if available, could ↑ or ↓ SF 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, genetic polymorphisms, 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.

5.4.7. 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 SF 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 (NCI, 1977, 1976a, b; Edwards et al., 1942), 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} $[mg/kg\text{-}day]^{-1}$) is approximately twofold smaller than the previous SF.

An IUR of 1.5×10^{-5} $(\mu g/m^3)^{-1}$ was derived previously from the oral SF 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} $[\mu g/m^3]^{-1}$) was derived using a chronic inhalation bioassay (Nagano et al., 2007b) 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 <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.

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, reactive 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.

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.

Examination of rodent bioassay data at relatively high doses reveals a general correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors. At lower exposure levels, however, the correspondence between hepatocellular cytotoxicity and regenerative hyperplasia and the induction of liver tumors is

inconsistent with this MOA hypothesis. In particular, an increased incidence of hepatocellular adenomas in the low-exposure (5-ppm) female mouse in the absence of nonneoplastic liver toxicity (Nagano et al., 2007b; JBRC, 1998) suggests that mouse hepatocarcinogenicity cannot simply be explained in terms of the hypothesized cytotoxic-proliferative MOA.

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 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. 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 products of carbon tetrachloride metabolism, uncertainties about genotoxic activity at low exposures, and empirical data from rodent bioassays that suggest that mouse hepatocarcinogenicity cannot be explained in terms of a cytotoxic-proliferative MOA alone, the MOA(s) for carbon tetrachloride-induced liver tumors at low exposure levels is/are unknown. The MOA for pheochromocytomas induced by carbon tetrachloride is unknown.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), carbon tetrachloride is "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 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 2 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, 2007b) were fit to the 10- and 12-week SDH data. None of the models for continuous data in BMDS provided an adequate fit of the 12-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 allow for 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 1,000 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 1,000 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 database deficiencies. 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 a lack of 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. 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 UFs, and potential RfDs for comparison to the RfD 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 oral gavage study is a well-conducted, peer-reviewed study that used three dose groups plus a control and collected interim data at 2-week intervals. The study is limited by relatively small group sizes (five to nine rats/group) and investigation of only two target organs (liver and kidney). Confidence in the oral database is medium. The chronic bioassay by NCI provided complete nonneoplastic and neoplastic incidence data; however, because of the marked hepatotoxicity in dosed rats even 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 contains information on developmental toxicity, but lacks an adequate multigeneration study of reproductive function. 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 (Thrall et al., 2000; Benson and Springer, 1999; Paustenbach et al., 1988); 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 $BMDL_x$. 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, 2007b) 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 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 (or $10^{0.5}$) to extrapolate from rats to humans, and a factor of 3 (or $10^{0.5}$) 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 a lack of a multigeneration reproductive toxicity.

To provide perspective on the RfC derived using data from the JBRC inhalation bioassay in the rat, PODs and potential inhalation RfCs based on other selected studies of carbon tetrachloride inhalation toxicity are arrayed in Figures 5-6 to 5-8. 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 UFs, and potential RfDs for comparison to the RfD derived from JBRC liver data. Benign pheochromocytomas from the adrenal gland medulla, which 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 (rats and mice), three exposure groups, and 50 animals/sex/group. 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 2-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

The MOA of carbon tetrachloride-induced liver tumors and pheochromocytomas has not been established. Therefore, consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a low-dose linear extrapolation approach has been applied to the quantitative evaluation of 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 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-day]$^{-1}$).

An alternative nonlinear approach was also presented for quantitative dose-response analysis of liver tumor data consistent with the evidence that supports a hypothesized MOA 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. Biological support exists for these hypothesized mechanistic events. Under this hypothesized MOA, liver carcinogenicity occurs at carbon tetrachloride exposures that also induce hepatocellular toxicity and a sustained regenerative and proliferative response; exposures that do not cause hepatotoxicity are not expected to result in liver cancer. The RfD and RfC were quantitatively derived based upon hepatotoxicity (cytotoxicity), a key event for the hypothesized nonlinear MOA. Therefore, under an assumption of nonlinearity, doses (or concentrations) of carbon tetrachloride below the RfD of 0.004 mg/kg-day or RfC of 0.1 mg/m^3 that do not cause sustained cytotoxicity and regenerative cell proliferation would be expected to be protective of liver tumors if this is the primary MOA for liver tumors. The application of a nonlinear approach for liver tumors is based on MOA information specific to that tumor type and does not apply to the occurrence of pheochromocytomas for which the MOA is unknown. Therefore, 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. As noted in Section 5.4, EPA recommends the application of a linear extrapolation approach for both carbon tetrachloride-induced liver tumors and pheochromocytomas.

Uncertainties in the cancer dose-response assessment. Major uncertainties in the cancer assessment are described below:

Relevance to humans. As noted in EPA's *Guidelines for Carcinogen Risk Assessment*, "… agents observed to produce tumors in both humans and animals have produced tumors either at the same site (e.g., vinyl chloride) or different sites (e.g., benzene) (NRC, 1994). Hence, site concordance is not always assumed between animals and humans." Thus, it is not clear whether the tumors observed in rodent bioassays would be predictive of human tumors of the same or different sites.

The MOA for liver tumor induction has not been established, but the hypothesized MOAs that have been investigated are assumed to be relevant to humans (Section 4.7.3.5). There is no available 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 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). The relevance of mouse pheochromocytomas to humans is considered in Section 5.4.2. 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 adrenal medullary tumors (Hill et al., 2003), and the MOA for this tumor in mice is unknown. However, parallels between this tumor in the mouse and human led investigators to conclude 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, the available experimental evidence supports a conclusion that mouse pheochromocytomas are relevant to humans.

Choice of low-dose extrapolation approach. The MOA is a key determinant of which approach to apply for estimating low-dose cancer risk. The MOA of carbon tetrachloride liver carcinogenicity has been investigated extensively; however, much of this research has been conducted at relatively high exposure levels. The MOA(s) at low exposure levels is not known.

For liver tumors, a nonlinear extrapolation approach was explored in Section 5.4.5 as an alternative to the linear low-dose extrapolation approach for cancer risk estimation. Such an approach would be supported by a conclusion that the hypothesized cytotoxicity-proliferative MOA is operative at all doses; however, evidence inconsistent with the nonlinear approach includes the finding of female mouse hepatocarcinogenicity at non-cytotoxic doses, potential for genotoxicity at low doses, fundamental reactivity of the chemical, and the absence of MOA information regarding the observed pheochromocytomas in mice.

The linear extrapolation approach assumes that some cancer risk exists at all nonzero exposures, and that this risk increases linearly with exposure. While consistent with the recognized biological reactivity of carbon tetrachloride, uncertainties in this low-dose extrapolation approach are associated with the lack of MOA information at low exposures. Additional MOA 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 estimating the magnitude of liver tumor risk.

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 provides 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 IUR for liver tumors. The impact on the IUR derived from pheochromocytoma data is unknown.

Under the linear low-dose extrapolation approach, 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 MOA for this tumor. MOA information to inform the approach to low-dose extrapolation for 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 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 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 than 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 GI tract to the liver and that absorption of carbon tetrachloride from the GI tract is essentially complete. 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. To the extent that GI absorption is less than 100%, the current estimation method for route-to-route extrapolation would tend to overestimate the SF.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed through CIs. 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 POD 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 IUR and, using route-to-route extrapolation, the oral SF. 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, using two species (rats and mice), four exposure groups, including an appropriate untreated control, and 50 animals/sex/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 adequate for developing an IUR or oral SF were unavailable.

Choice of species/gender. For liver tumors, modeling was performed using the 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 increased from control levels to close to maximal responses without any intervening exposure levels having submaximal responses. In the female mice and rats, lower but biologically significant levels of tumor response were seen at intermediate exposure levels. Also, notably, increased levels of hepatocellular proliferation were not reported

for rodents at these intermediate levels, so that dose-response modeling based on these data may be more applicable to an evaluation of cancer risk at noncytotoxic exposures. There is no indication that male rodents are more sensitive to carbon tetrachloride liver tumor induction or that use of female data 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), genetic polymorphisms in drug metabolism enzymes, transporters, and receptors (all of which can markedly affect susceptibility to a toxic chemical), 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.

Adelman, R; Saul, RL; Ames, BN. (1988) Oxidative damage to DNA: relation to species metabolic rate and life span. Proc Natl Acad Sci USA 85:2706–2708.

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.

Albano, E; Carini, R; Parola, M; et al. (1989). Effects of carbon tetrachloride on calcium homeostasis. A critical reconsideration. Biochem Pharmacol 38:2719–2225.

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.

Amet, Y; Berthou, F; Fournier, G; et al. (1997) Cytochrome P450 4A and 2E1 expression in human kidney microsomes. Biochem Pharmacol 53:765–771.

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.

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.

Anderson, MW; Reynolds, SH; You, M; et al. (1992) Role of proto-oncogene activation in carcinogenesis. Environ Health Perspect 98:13–24.

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, TN: Oak Ridge National Laboratory. EPA/600/6-88/004. PB88-196019.

Atkins, P. (1998) Diffusion controlled reactions. In: Physical chemistry (6th ed.), New York, NY: Freeman, pp. 825–828.

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). (2005) Toxicological profile for carbon tetrachloride. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. PB2006-100002. Available online at http://www.atsdr.cdc.gov/toxprofiles (accessed June 30, 2009).

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):310–322.

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; 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.

Badger, DA; Kuester, RK; Sauer, JM; et al. (1997) Gadolinium chloride reduces cytochrome P450: Relevance to chemical-induced hepatotoxicity. Toxicology 121:143–153.

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.

Béliveau, M; Lipscomb, J; Tardif, R; et al. (2005) Quantiative structure-property relationships for interspecies extrapolation of the inhalation pharmacokinetics of organic chemicals. Chem Res Toxicol 18:475–485.

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. (1983) Application and results of whole-body autoradiography in distribution studies of organic solvents. Crit Rev Toxicol 12:59–118.

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.

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.

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.

Brogan, WC; Colby, HD. (1983) Carbon tetrachloride (CCl_4) toxicity in the guinea pig adrenal cortex. West VA Med J 79(12):274.

Brogan, WC; Eacho, PI; Hinton, DE; et al. (1984) Effects of carbon tetrachloride on adrenocortical structure and function in guinea pigs. Toxicol Appl Pharmacol 75:118–127.

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. 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.

Brunt, EM; Tiniakos, DG. (2002) Pathology of steatohepatitis. Best Practices Res Clin Gastroenterol 16(5):691–707.

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.

Bull, RJ; Sasser, LB; Lei, XC. (2004) Interactions in the tumor-promoting activity of carbon tetrachloride, trichloroacetate, and dichloroacetate in the liver of male B6C3F1 mice. Toxicology 199:169–183.

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; Loury, EL; et al. (1989) Use of primary cultures of human hepatocytes in toxicology studies. Cancer Res 49:1075–1084.

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.

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.

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. Am Stat 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, 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.

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.

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. Am Stat 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.

Ciccoli, L; Casini, AF; Beneditti, A. (1978) Free radical damage produced by carbon tetrachloride in the lipids of various rat tissues. Agents Actions 8(3):303–310.

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.

Colby, HD. (1981) Chemical suppression of steriodogenesis. Environ Health Perspect 38:119–127.

Colby, HD; Brogan, WC; Miles, PR. (1981) Carbon tetrachloride-induced changes in adrenal microsomal mixed-function oxidases and lipid peroxidation. Toxicol Appl Pharmacol 60:492–499.

Colby, HD; Purcell, H; Kominami, S; et al. (1994) Adrenal activation of carbon tetrachloride: role of microsomal P450 isozymes. Toxicology 94:31–40.

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 14:857–863.

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 261:4542–4548.

Correa, P. (1996) Morphology and natural history of cancer precursors. In: Schottenfield, D; Fraumeni, JF; eds. Cancer epidemiology and prevention. New York, NY: 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 (QSAR) 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; et al. (1976) Fundamental carcinogenic processes and their implications for low dose4 risk assessment. Cancer Res 36:2973–2979.

Cummings, BS; Lash, LH. (2000) Metabolism and toxicity of trichloroethylene and S-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells. Toxicol Sci 53:458–466.

Cummings, BS; Zangar, RC; Novak, RF; et al. (1999) Cellular distribution of cytochromes P-450 in the rat kidney. Drug Metab Dispos 27:542–548.

Cummings, BS; Lasker, JM; Lash, LH. (2000a) Expression of glutathione-dependent enzymes and cytochrome P450s in freshly isolated and primary cultures of proximal tubular cells from human kidney. J Pharmacol Exp Ther 293:677–685.

Cummings, BS; Parker, JC; Lash, LH. (2000b) Role of cytochrome P450 and glutathione S-transferase in metabolism and cytotoxicity of trichloroethylene in rat kidney. Biochem Pharmacol 59:531–543.

Cummings, BS; Parker, JC; Lash, LH. (2001) Cytochrome P-450-dependent metabolism of trichloroethylene in rat kidney. Toxicol Sci 60:11–19.

Curren, RD; Yang, LL; Conklin, PM; et al. (1988) Mutagenesis of xeroderma pigmentosum fibroblasts by acrolein. Mutat Res 209:17–22.

Curtis, HJ; 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; 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.

Davies, B; Morris, T (1993) Physiological parameters in laboratory animals and humans. Pharm Res 10:1093–1095.

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.

Dedrick, RL; Bishoff, KB. (1980) Species similarities in pharmacokinetics. Fed Proc 39:54–59.

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.

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.

Delp, MD; Manning, RO; Bruckner, JV; et al. (1991) Distribution of cardiac output during diurnal changes of activity in rats. Am J Physiol 261:H1487–H1493.

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.

Dianzani, MU. (1984) Lipid peroxidation and haloalkylation: two distinct mechanisms for CCl4-induced liver damage. In: Calandra, S; Carulli, N; Salvioli, G.. eds. Liver and lipid metabolism, Amsterdam: Elsevier Exerpta Medica, pp. 39–50

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.

Diaz Gomez, MI; Castro, JA. (1981) Reaction of trichloromethyl free radicals with deoxyribonucleic acid bases. Res Commun Chem Pathol Pharmacol 32:147–153.

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.

Doherty, RE. (2000) A history of the production and use of carbon tetrachloride, tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane in the United States: Part 1—historical background; carbon tetrachloride and tetrachloroethylene. J Environ Forensics 1:69–81.

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.

Eastmond, DA. (2008) Evaluating genotoxicity data to identify a mode of action and its application in estimating cancer risk at low doses: a case study involving carbon tetrachloride. Environ Mol Mut 49:132–141.

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.

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.

Elkins, HB. (1942) Maximal allowable concentrations. II. Carbon tetrachloride. J Ind Hyg Toxicol 24:233–235.

El Masri, HA; Thomas, RS; Sabados, GR; et al. (1996) Physiologically based pharmacokinetic/pharmacodynamic modeling of the toxicologic interaction between carbon tetrachloride and kepone. Arch Toxicol 70:704–713.

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-LW; 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. EMEA/CHMP/SWP/150115/2006. Available online at http://www.emea.europa.eu/index/indexh1.htm (accessed June 23, 2009).

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; et al. (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). U.S. Food and Drug Administration. Available online at http://www.fda.gov/cder/livertox/preclinical.pdf (accessed June 24, 2009).

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 370:209–221.

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; et al. (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, NY: 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.

Greim, H; Hartwig, A; Reuter, U; et al. (2009) Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. Crit Rev Toxicol 39(8):695-718.

Grube, K; Bürkle, A. (1992) PolycADP-ribose polymerase activity in mononuclear leukocytes of 12 mammailian species correlates with species-specific life span. Proc Natl Acad Sci USA 89:11759–11763.

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. Mol 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. New York, NY: Oxford University Press.

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 Pathol 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.

Hart, RN; Setlow, RB. (1974) Correlation between deoxyribonucleic acid excision-repair and lifespan in a number of mammalian species. Proc Natl Acad Sci USA 71:2169–2173.

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.

Hattis, D; Rahmioglu, N; Verma, P; et al. (2009) Preliminary operational classification system for non-mutagenic modes of action for carcinogenesis. Crit Rev Toxicol 39(2):97–98.

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. Fed 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):51–56.

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.

ILSI/EPA (International Life Sciences Institute/U.S. Environmental Protection Agency). 1994. Physiological Parameter Values for PBPK Models. International Life Sciences Institute Risk Science Institute, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency.

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.

Jegga, A; Inga, A, Menendaez, D; et al. (2008) Functional evolution of the p53 regulatory network through its target response elements. Proc Natl Acad Sci USA 105:944–949.

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.

Kaporec, 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.

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):158–169.

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.

King-Herbert, A; Thayer, K. (2006) NTP workshop: Animal models for the NTP rodent cancer bioassay: Stocks and strains—Should we switch? Toxicol Pathol 34:802–805.

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: Snyder, R; Parke, DV; Kocsis, JJ; et al.; eds. Biological reactive intermediates-II: chemical mechanisms and biological effects. New York, NY: Plenum Press, pp. 769–777.

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. Regul Toxicol Pharmacol 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. Radiat Res 164:810–819.

Kurtz, AJ; 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; 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.

Lieber, CS. (2004) Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 34:9–19.

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.

Lindstedt, SL; Calder, WA. (1981) Body size, physiological time, and longevity of homeothermic animals. Quart Rev Biol 56:1–16.

Lipscomb, JC; Kedderis, GL. (2002) Incorporating human interindividual biotransformation variance in health risk assessment. Sci Total Environ 288:13–21.

Lipscomb, JC; Garrett, CM; Snawder, JE. (1997) Cytochrome P450-dependent metabolism of trichloroethylene: interindividual differences in humans. Toxicol Appl Pharmacol 142:311–318.

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, WK. (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. Toxicol Appl Pharmacol 207:S565–S569.

MacNee, W; Rahman, I. (2004) Oxidative stress in chronic obstructive pulmonary disease. In: Oxygen/nitrogen radicals: lung injury and disease. Vallyathan, V; Shi, X; Castranova, V; eds. New York, NY: Marcel Dekker, Inc.

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):263–285.

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.

Malendowicz, LK; Colby, HD. (1982) Effects of carbon tetrachloride on adrenocortical function in rats. Toxicol Appl Pharmacol 65:32–37.

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, 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.

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.

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-κB and AP-1 or with expression of tumor necrosis factor α. Hepatology 25:585–592.

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. (1995) Transgenic models for detection of mutations in tumors and normal tissues of rodents. Toxicol Lett 82–83:131–134.

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; Monforte, JA; 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.

Morley, AA; Turner, DR. (1999) The contribution of exogenous and endogenous mutagens to in vivo mutations. Mutat Res 428(1–2):11–15.

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; Sofuni, T. (2000) Appropriate levels of cytotoxicity for genotoxicity tests using mammalian cells in vitro. Environ Mol Mutagen 35:202–205.

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.

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. Inhal Toxicol 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.

Natarajan, SK; Basivireddy, J; Ramachandran, A; et al. (2006) Renal damage in experimentally-induced cirrhosis in rats: role of oxygen free radicals. Hepatology 43:1248–1456.

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.

NCI (National Cancer Institute). (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 (National Cancer Institute). (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 online at http://toxnet.nlm.nih.gov (accessed June 24, 2009).

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.

NRC (National Research Council). (1994) Science and judgment in risk assessment. Washington, DC: National Academy Press.

NRC (National Research Council). (2009) Science and decisions: advancing risk assessment. 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 online at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm (accessed June 24, 2009).

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.

Ohta, S; Lai, EW; Taniguchi, S; et al. (2006) Animal models of pheochromocytoma including NIH initial experience. Ann NY Acad Sci 1073:300-305.

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 (CCl_3O_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.

Powers, JF; Picard, KL; Nyska, A, et al. (2008) Adrenergic differentiation and Ret expression in rat pheochromocytomas. Endocr Pathol 19:9-16.

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.

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.

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.

Rikans, LE; Hornbrook, KR; Cai, Y. (1994) Carbon tetrachloride hepatotoxicity as a function of age in female Fischer 344 rats. Mech Ageing Dev 76:89–99.

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.

Russell, JJ; Seetula, JA; Gutman, D; et al. (1990) Kinetics and thermochemistry of the equilibrium $CCl_3 + O_2 \approx CCl_3O_2$. J Phys Chem 94:3277–3283.

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):288–297.

Sanzgiri, UY; Bruckner, JV. (1997) Effect of Emulphor, an emulsifier, on the pharmacokinetic and hepatotoxicity of oral carbon tetrachloride in the rat. Fundam 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. Am Stat 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.

Snawder, JE; Lipscomb, JC. (2000) Interindividual variance of cytochrome P450 forms in human hepatic microsomes: correlation of individual forms with xenobiotic metabolism and implications in risk assessment. Regul Toxicol Pharmacol 32:200–209.

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. Available online at http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/manual14.pdf (accessed June 24, 2009).

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.

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, AI. (1996) Thiol oxidation and cytochrome P450-dependent metabolism of CCl4 triggers Ca2+ release from liver microsomes. Biochemistry 35:15839–15845.

Stoyanovsky, DA; Cederbaum, AL. (1999) Metabolism of carbon tetrachloride to trichloromethyl radical: An ESR and HPLC-EC study. Chem Res Toxicol 12(8):730–736.

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.

Sweeney, AT. (2009) Pheochromocytoma. eMedicine. Available online at http://emedicine.medscape.com/article/124059-overview. Updated July 31, 2009.

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) Fundamentals of clinical chemistry. Philadelphia, PA: 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.

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.

Tischler, AS; Sheldon, W; Gray, R. (1996) Immunohistochemical and morphological characterization of spontaneously occurring pheochromocytomas in the aging mouse. Vet Pathol 33:512–520.

Tischler, AS; Powers, JF; Alroy, J. (2004) Animal models of pheochromocytoma. Histol Histopathol 19:883–895.

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.

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; Bruckner, JV. (1976) The participation of 2-butanone in 2-butanol-induced potentiation of carbon tetrachloride hepatotoxicity. J Pharmacol Exp Therap 196:493–500.

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.

Travis, CC. (1990) Tissue dosimetry for reactive metabolites. Risk Anal 10:317–321.

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.

Tsuda, H; Matsumoto, K; Ogino, H, et al. (1993) Demonstration of initiation potential of carcinogens by induction of preneoplastic glutathione S-transferase P-form-positive liver cell foci: possible in vivo assay system for environmental carcinogens. Jpn J Cancer Res 84:230–236.

Tsujimura, K; Ichinose, F; Hara, T; et al. (2008) The inhalation exposure of carbon tetrachloride promote rat liver carcinogenesis in a medium-term liver bioassay. Toxicol Letters 176:207–214.

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.

Uemitsu, N. (1986) Inhalation pharmacokinetics of carbon tetrachloride in rats based on arterial blood:inhaled air concentration ratios. Toxicol Appl Pharmacol 83:20–29.

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 online at http://www.chrismanual.com/findform.htm (accessed June 23, 2009).

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014–34025. Available online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006–34012. Available online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (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 online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798–63826. Available online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799 (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (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 online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R-94/007. Available online at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=42601 (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1996a) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274–56322. Available online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1996b) Symposium on natural attenuation of chlorinated organics in groundwater. Office of Research and Development, Washington, DC; EPA-540/R-96/509. National Technical Information Service, Springfield, VA; AD-A319114/5.

U.S. EPA (Environmental Protection Agency). (1998) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926–26954.

U.S. EPA (Environmental Protection Agency). (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 online at http://www.epa.gov/iris/backgrd.html (accessed June 23, 2009).

U.S. EPA (Environmental Protection Agency). (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 online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2000c) Benchmark dose technical guidance document. External review draft. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (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 online at http://www.epa.gov/iris (accessed June 24, 2009).

U.S. EPA (Environmental Protection Agency). (2001a) Toxicological review of chloroform. Integrated Risk Information System (IRIS), National Center for Environmental Assessment, Washington, DC; EPA/635/R-01/001. Available online at http://www.epa.gov/iris (accessed June 24, 2009).

U.S. EPA (Environmental Protection Agency). (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 (Environmental Protection Agency). (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available online at http://www.epa.gov/iris/backgrd.html (accessed June 24, 2009).

U.S. EPA (Environmental Protection Agency). (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001b. Available online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/P-03/003F. Available online at http://www.epa.gov/iris/backgrd.html (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2006a) Science policy council handbook: Peer review. 3rd edition. Science Policy Council, Washington, DC. Available online at http://www.epa.gov/ncea/iris/Peer_Review_Handbook_2006_3rd_edition.pdf (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (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 online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363 (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (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 (Environmental Protection Agency). (2007a) Protection of stratospheric ozone: extension of global laboratory and analytical use exemption for essential class I ozone-depleting substances. Federal Register 72(177):52332–52337. Available online at http://frwebgate4.access.gpo.gov/cgi-bin/PDFgate.cgi?WAISdocID=467979422640+16+2+0&WAISaction=retrieve (accessed July 1, 2009.)

U.S. EPA (Environmental Protection Agency). (2007b) Benchmark dose software (BMDS) version 1.4.1. last modified February 2007. Available online at http://www.epa.gov/ncea/bmds.htm (accessed June 23, 2009).

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 Goethem, F; de Stoppelaar, J; Hoebee, B; Kirsch-Volders, M. (1995) Identification of clastogenic and/or aneugenic events during the preneoplastic stages of experimental rat hepatocarcinogenicity by fluorescence in site hybridization. Carcinogenesis 16(8):1825–1834.

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.

Vijg, J; Mullaart, E; van der Schans, GP; et al. (1984) Kinetics of ultraviolet induced DNA excision repair in rat and human fibroblasts. Mutat Res 132:129–138.

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.

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.

West, GB; Woodruff, WH; Brown, JH. (2002) Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. Proc Natl Acad Sci USA 99:2473–2478

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; WiIson, R; Crouch, EAC. (1987) Dose-response relationships for carcinogens: a review. Environ Health Perspect 73:259–308.

APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The *Toxicological Review of Carbon Tetrachloride* (dated May 2008) has undergone a formal external peer review performed by scientists in accordance with the EPA guidance on peer review (U.S. EPA, 2006a, 2000a). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. An external peer review workshop was held October 14, 2008. EPA received no scientific comments on this assessment from the public.

EXTERNAL PEER REVIEW PANEL COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

I. General Comments

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazards?

Comments: All six peer reviewers agreed or generally agreed that the Toxicological Review was logical and clear. One of these reviewers noted that certain aspects of the pharmacokinetic modeling were not sufficiently described. One reviewer considered the Toxicological Review to be concise, whereas three reviewers did not. Two of these reviewers pointed to redundancy in the document and suggested that text be synthesized in a tabular format, or that discussions of the MOA be shortened with reference to the initial text location. One reviewer suggested changes for improved accuracy or clarity throughout the Toxicological Review and identified some relevant references that were not cited. This reviewer considered these errors or lack of analysis to be relatively minor and ones that might not significantly influence the overall evaluation of noncancer and cancer hazards (although may modestly influence the specific UFs used).

Response: A more detailed discussion of the PBPK modeling was provided in the Toxicological Review (see response to RfC Charge Question #5 for more detailed response). Sections of the

Toxicological Review, and in particular those sections dealing with MOA, were revised to reduce redundancy. Errors or omissions of references identified by the peer reviewers were addressed.

Comments: One reviewer identified two main problems with the utilization of the available evidence: (1) the use of the rate of metabolism per unit liver tissue dose metric for PBPK modeling with no additional pharmacokinetic correction between species, and (2) the selection of a doubling of a particular enzyme level as the BMR, to be identified as the functional replacement for a NOAEL. Another reviewer questioned the large range in the value of the blood/air partition coefficient in humans and rats.

Response: These comments are addressed in response to comments on RfD Charge Question #3 and RfC Charge Question #5.

Comment: One reviewer noted that CYP enzyme inactivation is more severe in the rat (1 molecule of enzyme lost for every 26 molecules of substrate metabolized in the rat versus 1 molecule of enzyme lost for every 196 molecules of substrate metabolized in the human) and that a 7.5-fold difference in metabolism-dependent inactivation would be expected to have a large influence on the extent of carbon tetrachloride bioactivation and potential for increased risk in humans as compared to rats. This reviewer further observed that a 27% lower V_{max} in humans versus rats (based on Table 3-5, in vitro and in vivo metabolism data for four species) may mitigate some of the effect of omitting consideration of interspecies differences in rates of CYP inactivation.

Response: Suicide inhibition is identified in the Toxicological Review as a contributor to uncertainty in the application of PBPK models to interspecies extrapolation of carbon tetrachloride toxicokinetics (Section 5.2.2.1); however, the uncertainty was not addressed quantitatively because information and models to support such an assessment are not currently available. A discussion of major issues associated with the fact that suicide inhibition of CYP450 was not explicitly simulated in PBPK models (to predict internal doses of carbon tetrachloride or to extrapolate external doses across species) was added to Section 3.3, Metabolism, and Section 5.3, Uncertainties in the Oral RfD and Inhalation RfC, under the subheading "Animal to human extrapolation." Based on the analyses presented in Section 5.3, the model supports the conclusion that suicide inhibition would have relatively minor effects on the extrapolation of carbon tetrachloride external exposures across species in the low-dose range relevant to the derivation of the RfC.

Comment: One reviewer pointed to the discussion of Yoon et al. (2007) regarding the extrahepatic metabolism of carbon tetrachloride. This reviewer noted that while rat kidney cortex and proximal tubules express reasonable levels of CYP2E1 protein and activity for the oxidative metabolism of the CYP2E1 substrate trichloroethylene, human kidney has been reported by multiple laboratories to not express any detectable CYP2E1 protein and to exhibit little if any oxidative metabolism of trichloroethylene. This reviewer acknowledged that because extrahepatic metabolism is calculated to contribute only a minor proportion to total metabolism (<1%), this interspecies difference has no significant influence on the conclusions. For the sake of correctness, however, this reviewer recommended that these interspecies (rodent versus human) and interorgan (kidney versus liver) differences in CYP2E1 expression and activity be properly noted. Six references (Cummings et al., 2001, 2000a, b, 1999; Cummings and Lash, 2000; Amet et al., 1997) were provided by this reviewer for consideration.

Response: Pertinent findings from the literature cited by the reviewer were incorporated in Sections 3.5 and 5.4.3.2.

Comments: One reviewer offered several comments regarding the interpretation of genotoxicity studies and the strength of the conclusions that were synthesized from those studies. Although the reviewer did not necessarily disagree with the qualitative conclusions provided in Section 4.4.2, the reviewer suggested that stronger statements may be achievable in this section related to the results of the genotoxicity studies for carbon tetrachloride. Lastly, this reviewer suggested that brief descriptions of in vivo mouse strains be added to Section 4.4.2.4.

Response: Section 4.4.2.5 is intended to provide a summary of the genotoxicity literature for carbon tetrachloride and observations about interpretation of positive and negative findings in particular bioassays. Conclusions related to carbon tetrachloride's genotoxic potential as it relates to MOA are presented in Section 4.7.3.4. A brief description of the transgenic mouse strains was added to Section 4.4.2.4 (Mutations in transgenic mice).

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of carbon tetrachloride.

Comments: Peer reviewers identified the following additional studies for consideration:

Colby, HD; Purcell, H; Kominami, S; et al. (1994) Adrenal activation of carbon tetrachloride: role of microsomal P450 isozymes. Toxicology 94:31–40.

Eastmond, DA. (2008) Evaluating genotoxicity data to identify a mode of action and its application in estimating cancer risk at low doses: a case study involving carbon tetrachloride. Environ Mol Mutagen 49:132–141.

Two reviewers identified additional initiation-promotion studies (see below). One of these reviewers noted that these types of studies should be part of the evidence that carbon tetrachloride is a well-known promoter at high dose, and suggested that this literature be examined to see if data are available for the evaluation of initiating potential of carbon tetrachloride in such two-stage designs.

Tsuda, H; Matsumoto, K; Ogino, H; et al. (1993) Demonstration of initiation potential of carcinogens by induction of preneoplastic glutathione S-transferase P-form-positive liver cell foci: possible in vivo assay system for environmental carcinogens. Jpn J Cancer Res 84:230–236.

Tsujimura, K; Ichinose, F; Hara, T; et al. (2008) The inhalation exposure of carbon tetrachloride promote rat liver carcinogenesis in a medium-term liver bioassay. Toxicol Lett 176(3):207–214.

Bull, RJ; Sasser, LB; Lei, XC. (2004) Interactions in the tumor-promoting activity of carbon tetrachloride, trichloroacetate, and dichloroacetate in the liver of male B6C3F1 mice. Toxicology 199(2–3):169–183.

One reviewer stated that the following references on renal vs. hepatic CYP2E1 in rats versus humans and on the human interindividual variability in CYP expression should be considered:

Amet, Y; Berthou, F; Fournier, G; et al. (1997) Cytochrome P450 4A and 2E1 expression in human kidney microsomes. Biochem Pharmacol 53:765–771.

Cummings, BS; Lash, LH. (2000) Metabolism and toxicity of trichloroethylene and S-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells. Toxicol Sci 53:458–466.

Cummings, BS; Lasker, JM; Lash, LH. (2000a) Expression of glutathione-dependent enzymes and cytochrome P450s in freshly isolated and primary cultures of proximal tubular cells from human kidney. J Pharmacol Exp Ther 293:677–685.

Cummings, BS; Parker, JC; Lash, LH. (2000b) Role of cytochrome P450 and glutathione S-transferase α in metabolism and cytotoxicity of trichloroethylene in rat kidney. Biochem Pharmacol 59:531–543.

Cummings, BS; Parker, JC; Lash, LH. (2001) Cytochrome P-450-dependent metabolism of trichloroethylene in rat kidney. Toxicol Sci 60:11–19.

Cummings, BS; Zangar, RC; Novak, RF; et al. (1999) Cellular distribution of cytochromes P-450 in the rat kidney. Drug Metab Dispos 27:542–548.

Two reviewers were not aware of any additional studies that should be included in the assessment.

Response: A discussion of the promotion study by Tsujimura et al. (2008) and initiation-promotion study by Bull et al. (2004) was added to the Toxicological Review (Section 4.4.3). A summary of Tsuda et al. (1993) was not added. In this study, carbon tetrachloride was used as a promoter, but the lack of an appropriate control limited the utility of this study for evaluating carbon tetrachloride promotion properties.

A summary of the findings of Colby et al. (1994) on carbon tetrachloride-induction of effects on the adrenal gland was added to Sections 4.5 (mechanistic data) and 4.7.4 (MOA for pheochromocytomas). Citation to the paper by Eastmond (2008) was added.

A discussion of renal versus hepatic CYP2E1 in rats and humans and human interindividual variability in CYP expression based on Amet et al. (1997), Cummings and Lash (2000), and Cummings et al. (2001, 2000a, b, 1999) was added to Section 3.5.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of carbon tetrachloride.

Comments: The peer reviewers identified the following areas of research to increase confidence in the database.

Carcinogenicity/chronic toxicity:

- Studies that characterize carcinogenic activity at lower dose levels (i.e., a bioassay with lower doses, and/or studies evaluating preneoplastic lesion development at lower dose levels).

- A new oral cancer bioassay with administration of a wide range of doses, including those below which hepatotoxicity occurs, to eliminate the need for route-to-route extrapolation as well as to provide better data for RfD estimation.

- Studies on cancer endpoints in CYP2E1 knockout mice (either cancer bioassay or studies of preneoplastic lesions).

- Repeat of studies where control animals exhibit higher rates of liver cancer than historical controls.

- Classical initiation-promotion liver studies in which low doses of carbon tetrachloride are given in conjunction with promoters (PH, phorbol esters, etc.) to determine whether carbon tetrachloride has initiating potential in rodent liver. One reviewer suggested such studies using a system described in Tsujimura et al. (2008), where various amounts/durations of carbon tetrachloride are administered either before known promoters of liver tumors or after known initiators to improve our information on dose response for different kinds of cancer-enhancing activities for carbon tetrachloride.

- Studies of the mechanism of adrenal tumor induction to understand if parent compound or a metabolite is the key dose metrics for pharmacokinetic modeling and whether there is a potential nonlinearity in the dose response. Colby et al. (1994) was identified as a possible useful start.

Genetic toxicology:

- Assessments of genotoxicity and mutagenicity at lower (noncytolethal) dose levels to establish whether DNA damage can really occur at doses relevant to environmental or occupational exposures.

Kinetic information:

- Measurements in comparable rat and human liver metabolism systems of the rates of destruction of the reactive metabolites of carbon tetrachloride or steady-state concentrations of those metabolites as indexed by rates of formation of metabolite-specific adducts. If metabolite elimination rates are in fact slower in people than in rodents, then steady state concentrations of metabolites should be greater in humans than in the rodent systems for a given rate of metabolite formation. To be fully credible, such comparisons should be done with fresh liver systems (e.g., slices, isolated hepatocytes) that preserve as much of the in vivo concentrations of enzymes and cofactors as possible.

- More complete human metabolism data in both liver and extrahepatic tissues.

- More complete analysis of human variation, including genetic polymorphisms, in enzymes that metabolize carbon tetrachloride, including CYP2E1 and CYP3A4.

Noncancer toxicity:

- Studies of developmental toxicity and reproductive toxicity (including a multigenerational toxicity study).

- Studies to explore the potential for carbon tetrachloride to be endocrine disruptive (hormonal mimic or impairment of hormonal systems).

- Studies to elucidate dose-response relationships for more sensitive tests of liver effects, including cell replication, lipid peroxidation, and SAM depletion.

- In general, enhanced assessment of toxicity at lower dose levels.

Epidemiology:

- Epidemiology studies of exposed workers to follow up on the suggestive evidence of lymphocytic cancer and further explore the potential for adrenal, liver, and other tumors. The epidemiology studies may be enhanced by phenotyping individuals for CYP2E1 level and by genotyping individuals for GSH transferase polymorphisms and for other factors that may modify anti-oxidant and cellular defense status.

Response: No response needed.

4. Please comment on the identification and characterization of sources of uncertainty in Sections 5 and 6 of the Toxicological Review. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Comments: Three peer reviewers believed that the key sources of uncertainty were adequately discussed. Two reviewers offered specific comments on individual UFs; these comments are summarized and addressed in response to RfD Charge Question #4.

One reviewer stated that the characterization of uncertainty in Sections 5 and 6 could be more complete and more descriptive, and suggested that thought be given to weighting these uncertainties in terms of how much they affect the confidence in the overall assessment (low, medium, or high importance). This reviewer identified the following uncertainties not specifically elaborated in text or tables: (1) dose metric for adrenal tumors; (2) interaction with other chemicals that may induce or inhibit CYP2E1 or detoxification pathways; (3) disease processes (specifically diabetes as a condition that could elevate CYP2E1 levels, leading to additional uncertainty over population variability) and genetic polymorphisms; and (4) uncertainty regarding effect of time-weight averaging exposure in the Japanese inhalation bioassay and whether hepatotoxicity may be occurring and then repaired at the 5-ppm exposure level so that the net result is no evidence of toxicity at this exposure level. This reviewer stated that an attempt at expressing this on page 238 needed to be made more coherent and further developed in the uncertainty section.

One reviewer recommended that the implicit assumption of passive destruction of the reactive metabolites at identical rates in humans and rodents be articulated and raised questions as to whether the appropriate causal dose metric (gross metabolism rate versus AUC of the active metabolites) was used and interspecies projections correctly performed.

Response: Discussion of uncertainties associated with the dose metrics (both for effects on the liver and adrenal gland) was expanded in Section 5.3, Uncertainties in the Oral RfD and Inhalation RfC, under the subheading "Animal to human extrapolation." U.S. EPA judged that the discussions of interactions of carbon tetrachloride with other chemicals and susceptibility associated with disease processes and genetic polymorphisms are most appropriately addressed in Section 4.8, Susceptible Populations and Life Stages, and were not expanded on in Sections 5 or 6. The exposure regimen used in the JBRC inhalation bioassay (Nagano et al., 2007b) and the adjustment of the intermittent exposure (6 hours/day) to continuous 24-hour exposure was taken into account in the PBPK modeling of the experimental exposure. U.S. EPA does not consider this a significant source of uncertainty to be included in Sections 5 or 6. The comment regarding differences in rates of passive destruction of the reactive metabolites across species and

implications for selection of dose metric is addressed in response to comments on RfC Charge Question #5.

Chemical-Specific Charge Questions:

(A) Oral reference dose (RfD) for carbon tetrachloride

1. A 12-week oral gavage study in the rat by Bruckner et al. (1986) was selected as the basis for the RfD. Please comment on whether the selection of this study as the principal study is scientifically justified. Has this study been transparently and objectively described in the Toxicological Review? Are the criteria and rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: Four reviewers considered the selection of the Bruckner et al. (1986) study to be scientifically justified and the rationale for its selection clearly explained. One of these reviewers suggested that the discussion be consolidated to make reasoning for this selection even more transparent. A fifth reviewer stated that "The choice of the Bruckner et al. (1986) … study is not clearly incorrect," but would have considered preferable some integrative calculation across different data sets rather than the determination of the RfD based on a single study and single data set within that study. The sixth reviewer stated that the choice of Bruckner et al. (1986) may in fact be the best choice for RfD derivation, although this reviewer observed that leakage of SDH may not be the most sensitive indicator of hepatotoxicity and suggested that consideration be given to low-dose biochemical perturbations (as an additional source of database uncertainty in RfD derivation). This reviewer noted that evidence suggests that other carbon tetrachloride effects may be detectable at lower doses, although this is not known because careful dose-response studies for these effects have not been reported down to low doses.

One reviewer's comment concerning the use of 10-week versus 12-week data from the Bruckner et al. (1986) study is summarized in response to RfD Charge Question #2.

Response: In response to the reviewer who suggested that some integrative calculation across different data sets would have been preferable, U.S. EPA notes that BMD analysis was performed using data for SDH, OCT, and ALT. As detailed in Appendix B, none of the models in BMDS provided an adequate fit to the OCT data. ALT data provided higher BMD and BMDL values than did SDH data. In light of the analysis by Travlos et al. (1996) of serum liver enzymes as predictors of hepatotoxicity that showed SDH to be a more sensitive predictor of histopathological changes than ALT, U.S. EPA considers the BMDL based on SDH data alone to be a sensitive and appropriate basis for the carbon tetrachloride RfD. U.S. EPA performed an

integrative analysis by considering serum enzyme changes in the context of levels that also induced histopathological changes (see Section 5.1.2). Indicators of hepatotoxicity possibly more sensitive than increases in SDH could not be considered because no such experimental data have been collected for carbon tetrachloride.

2. An increase in serum sorbitol dehydrogenase (SDH) activity was selected as the most appropriate critical effect for the RfD because it is considered by EPA to be an indicator of hepatocellular injury and a biomarker of an adverse effect. Please comment on whether the rationale for the selection of this critical effect is scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the Toxicological Review? Please provide a detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comments: Five reviewers agreed that the selection of SDH activity as the critical effect was scientifically justified, and four of these reviewers specifically considered the selection of the critical effect to have been adequately explained in the Toxicological Review. A sixth reviewer stated that within the limits of the testing done and parameters measured, SDH activity may be the most useful and reasonably sensitive endpoint to date, but noted the uncertainty due to data gaps in reproductive testing, the potential for low-dose biochemical effects that are part of the hepatotoxic MOA, and the lack of a long-term oral study with adequate sensitivity and histopathology to test whether liver histopathology could be more sensitive than liver enzyme leakage for POD selection.

One of the reviewers who concurred with selection of SDH activity as the critical effect questioned why SDH rather than some of the more commonly measured parameters, such as AST or ALT, was chosen, and suggested that the Toxicological Review include a statement as to how typically SDH is used as a metric of hepatic function.

Two reviewers questioned the exclusion of the data for the 12-week time point (on the basis that group sizes for the 12-week data were provided as a range of seven to nine rats whereas the group size for the 10-week data was five rats). One of these reviewers noted that a range of group sizes does not make the data unusable and suggested that the BMD analysis be conducted by assuming eight animals/group at all doses. A statistical analysis performed by the second reviewer revealed that the uncertainty in the SDs estimated for the 12-week-exposure groups was less than the uncertainty in the estimates of SD for the 10-week exposures that were selected for BMD analysis (a CV of 27.5% for the 12-week-exposure groups versus a CV of 36.7% for the 10-week exposure groups, assuming the same mean and SD). Therefore, this reviewer recommended that the 12-week results be used in preference to the 10-week results, or preferably that the BMD calculations be performed for both periods of exposure and that the results be combined in some reasonable way.

Response: U.S. EPA recognizes the possibility that if studies were conducted that involved longer exposure durations or examined other endpoints of toxicity (e.g., reproductive toxicity endpoints or low-dose biochemical effects), a more sensitive endpoint for carbon tetrachloride could be identified. This uncertainty is addressed by the application of UFs for database deficiencies (UF = 3) and subchronic to chronic extrapolation (UF = 3).

In response to the question of how typically SDH is used as a metric of hepatic function, U.S. EPA notes that Travlos et al. (1996) reviewed serum enzyme data for 61 l3-week toxicity studies in male and female F344 rats conducted for the NTP by eight contract laboratories following a standard protocol established by the NTP. Of these 61 studies, SDH was measured in male rats in 58 of the studies and in females in 57 studies. ALT was measured in male rats in 61 studies and female rats in 60 studies. This review, while limited to NTP protocols, suggests that SDH is a commonly measured metric of hepatic function. SDH was selected as the critical effect for the carbon tetrachloride RfD because it was the most sensitive of the three serum enzymes (SDH, OCT, and ALT) measured by Bruckner et al. (1986).

An analysis of serum enzyme data collected after 12-weeks of exposure in the Bruckner et al. (1986) study was added to Appendix B and integrated in Section 5.1.2. As discussed in Section 5.1.2, the BMDL based on 10-week SDH data provided the lowest POD and was retained as the basis for the RfD.

3. Benchmark dose (BMD) modeling methods were applied to SDH data to derive the point of departure (POD) for the RfD. Please comment on whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., an increase in SDH activity two times the control mean) scientifically justified? Has it been transparently and objectively described? Please identify and provide rationales for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Comments: All peer reviewers considered BMD modeling for use in deriving the POD to be appropriate.

Three peer reviewers considered the selection of an increase in SDH activity 2 times the control mean as the BMR to be scientifically justified. One reviewer considered a twofold SDH increase to be too large a change to be considered the functional equivalent of a NOAEL. This reviewer recommended a shift in the mean of 1 SD (based on observations in the control group) as the BMR. This reviewer observed that in the current assessment the SD for the control group is $0.4 \times 5^{0.5} = 0.9$; 1 SD above the mean would be about 4.4 IU/mL rather than the doubling to

7 IU/mL that was used for the BMR. This reviewer further observed that a doubling of the group mean enzyme level represents a movement for the average animal of about 3.5/0.9 = 3.9 SDs. Two reviewers did not provide comments on the selection of the BMR.

One reviewer's comment concerning the use of 10-week versus 12-week data from the Bruckner et al. (1986) study is summarized in response to RfD Charge Question #2.

Response: U.S. EPA notes that a BMD in not equivalent to a NOAEL. Rather, a BMD (or BMC) is defined as "a dose or concentration that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background" (IRIS glossary at http://www.epa.gov/ncea/iris/help_gloss.htm#b). The BMR of a twofold SDH increase was selected consistent with U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), that states that "[i]f there is a minimal level of change in the endpoint that is generally considered to be biologically significant (for example, a change in average adult body weight of 10%, or the doubling of average level for some liver enzyme), then that amount of change can be used to define the BMR." As discussed in Section 5.1.2, the scientific literature supports a twofold increase in liver enzyme levels as a minimally biologically significant change. U.S. EPA's BMD guidance further suggests that "a change in the mean equal to one control standard deviation from the control mean" be used "in the absence of any other idea of what level of response to consider adverse." For purposes of comparison across chemicals, the BMD and BMDL corresponding to a change in the mean response equal to one control SD from the control mean were also calculated for the 10-week SDH data and are presented in Section 5.1.2 and Appendix B.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factors:

- **An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the RfD because the available quantitative information on the variability in human response to carbon tetrachloride is considered insufficient to move away from the default uncertainty factor of 10.**
- **A subchronic to chronic uncertainty factor of 3, rather than a default of 10, was used in light of limited chronic oral study data and more extensive inhalation study data that informed the progression of toxicity from subchronic to chronic exposure durations.**

- **A database uncertainty factor of 3 was used to account for lack of adequate reproductive toxicity data for carbon tetrachloride, and in particular absence of a multigeneration reproductive toxicity study.**

Are the criteria and rationale for the selection of these uncertainty factors transparently and objectively described in the document? Please comment on whether the application of these uncertainty factors has been scientifically justified?

Comments: Five peer reviewers considered the UF for intraspecies extrapolation of 10 and for subchronic to chronic extrapolation of 3 to be scientifically justified; four reviewers considered the database UF of 3 to be scientifically justified. Although the charge did not include a specific question about the interspecies (animal to human) UF, two reviewers agreed that the UF of 10 was appropriate. Four reviewers considered the criteria and rationale for the selection of the UFs to be transparent and objective.

Of the reviewers who considered the intra- and interspecies UFs to be appropriate, one reviewer observed that the lack of a PBPK model for refining the RfD derivation requires further explanation given the use of this technique to extrapolate kinetics across species for inhalation exposure. A second reviewer observed (response to General Charge Question #4) that the clarity of the text could be improved by listing the applied UFs in a separate table with the abbreviations UF_A, UF_H, UF_L, UF_S, and UF_D.

One reviewer did not consider the rationale for the intraspecies (human variability) UF to be scientifically justified. This reviewer observed that the Toxicological Review did not seem to account for much of the known information on variation and genetic polymorphisms in CYP2E1 and CYP3A4 or for the stated differences in rates of enzyme inactivation in rat and human liver microsomes.

One reviewer considered the justification for the subchronic to chronic UF of 3 provided in the Toxicological Review (i.e., that inhalation studies failed to show a difference between subchronic and chronic dose response) to be weak. This reviewer observed that inhalation exposure may not be as sensitive as oral exposure to the buildup of toxicity from carbon tetrachloride dosing (related to first-pass delivery from oral but not inhalation exposure and to oral gavage dosing that delivers a higher acute dose compared to inhalation—factors that can combine to cause the peak exposure at the target site to be greater after oral exposure).

Two reviewers raised questions about the database UF, but did not offer an alternative value for this factor. One of these reviewers noted that it could be argued that the database UF of 3 is too low in light of possible upstream effects in the form of lipid peroxidation, GSH depletion, macromolecular binding, and derangement in calcium homeostasis. This reviewer further acknowledged because low-dose mechanistic studies are unavailable and because the point at which any perturbations might be considered adverse would be difficult to establish, a database UF of 3 can be acceptable under the current circumstances. The second reviewer

pointed to the following language in the discussion of data gaps—"the absence of these types of studies (i.e., an adequate multigeneration study of reproductive toxicity) introduces uncertainty… the magnitude of this uncertainty cannot be quantified"—and asked, if the magnitude of uncertainty due to missing data is unknown, why would not the default UF of 10 be used rather than an UF of 3.

Response: Further discussion of the rationale for not applying a PBPK model for RfD derivation was added to Section 5.1.2.

The abbreviations UF_A, UF_H, UF_L, UF_S, and UF_D were added to the Toxicological Review in the discussion of the UFs.

Additional discussion of CYP450 variation in the human population was added to Section 4.8, Susceptible Populations and Life Stages. Reference to this section was added to the justification for the intraspecies UF for both the RfD and RfC.

With regard to the comment on the subchronic to chronic UF of 3, U.S. EPA notes that inhalation study information that revealed no difference between subchronic and chronic dose response was only one of the factors that contributed to U.S. EPA's determination that a full 10-fold UF for subchronic to chronic extrapolation was not warranted. Other considerations included available chronic oral toxicity data and the observation of early onset of toxicity following oral exposure.

Consistent with input from several peer reviewers, a database UF of 3 was retained. A database UF of 3 to account for the lack of a multigeneration reproductive toxicity study in the presence of developmental toxicity information is consistent with U.S. EPA practice (U.S. EPA, 2002).

(B) Inhalation reference concentration (RfC) for carbon tetrachloride

1. The JBRC et al. (1998) 2-year inhalation bioassay in the rat was selected as the basis for the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Has the rationale for this selection been transparently and objectively described in the Toxicological Review? Are the criteria and rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: All six peer reviewers considered the selection of JBRC et al. (1998) as the principal study to be appropriate.

Response: No response needed.

2. Fatty changes in the liver was selected as the critical effect for the RfC because it is considered by EPA to be an adverse effect. Please comment on whether the selection of this critical effect is scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the Toxicological Review? Please comment on whether EPA's rationale about the adverse of the critical effect has been adequately and transparently described and is supported by the available data. Please provide a detailed explanation. Please identify and provide the rationales for any other endpoints that should be considered in the selection of the critical effect.

Comments: Five peer reviewers considered the selection of fatty changes in the liver as the critical effect to be scientifically justified.

A sixth reviewer considered liver effects to be the most sensitive endpoint for deriving an RfC, and the criteria and rationale for the selection of fatty changes as the critical effect to be transparently and objectively described in the Toxicological Review. This reviewer suggested that additional discussion and literature citations be included to firm the association between fatty liver (seen in this study) and assumed cell damage. This reviewer observed that fibrotic changes in the liver may be more representative of sustained cellular damage and therefore the more biologically relevant endpoint, but further that since the NOAEL and LOAEL for fatty liver changes and fibrosis were the same, selecting fibrosis as the critical effect would not change the NOAEL and LOAEL values used to derive the RfC.

Response: Fatty liver was retained as the critical effect for the RfC and discussion of the association between fatty liver and subsequent liver fibrosis and cirrhosis was added to Section 5.2.1.

3. An increase in the severity (but not incidence) of proteinuria in low-dose male and female rats was reported in the 2-year JBRC (1998) bioassay. Because the biological significance of this finding in F344/DuCrj rats was considered unclear (see Section 4.6.2 of the Toxicological Review), proteinuria was not used as the critical effect for the RfC. Please comment on whether the decision not to use proteinuria as the critical effect is scientifically sound and has been transparently and objectively described in the Toxicological Review.

Comments: Five peer reviewers agreed that the decision not to use proteinuria as the critical effect was scientifically sound and transparently and objectively described. One of these reviewers further proposed the inclusion of an analysis of the implications for the RfC of using proteinuria as the basis for calculating an RfC, and recommended the inclusion of alternative

RfC calculations using this endpoint to make the consequence of the choice of liver effects as the primary focus for the RfC more transparent.

One reviewer considered this decision to be "a questionable call by U.S. EPA." This reviewer stated that arguments against using the chronic proteinuria data are not compelling because relying upon the subchronic study to dictate the dose response for chronic nephrotoxicity may underestimate the potential for the kidney to accumulate damage related to carbon tetrachloride. This reviewer considered proteinuria to be a logical early signal of renal pathology, with the high frequency in aged animals making interpretation more complex. This reviewer suggested that the description in Section 4.6.2 show incidence and severity data for this endpoint and related renal toxicity endpoints to better document the relevance (or lack thereof) of proteinuria to carbon tetrachloride risk. This reviewer observed that the proteinuria data add to the uncertainty regarding proper selection of the key endpoint.

Response: The implications of not using proteinuria as the basis for the RfC are discussed in Section 5.3, Uncertainties in the Oral RfD and Inhalation RfC, Selection of the critical effect for reference value determination. The text was revised to indicate that proteinuria occurred at an exposure level fivefold lower than the concentration associated with fatty liver. Because the dose-response analysis of data for incidence of fatty liver incorporated BMD and PBPK modeling, the consequence of an alternative analysis using proteinuria data (without the application of BMD and PBPK modeling) cannot be directly established. Given the uncertainties in the proteinuria findings in the rat, U.S. EPA determined that an analysis of kidney data using BMD and PBPK modeling is not warranted. U.S. EPA notes that a statement is included in Section 5.3, acknowledging that use of proteinuria data as the critical effect would have yielded a lower POD than the liver data.

In response to the reviewer who did not find compelling U.S. EPA's argument for not using proteinuria as the basis for the RfD because of subchronic study data considerations, U.S. EPA notes that conclusions about the biological significance of proteinuria were based on a number of considerations in addition to analysis of the 13-week study findings, including: (1) 100% incidence of proteinuria in all rats, including the control, (2) ≥90% incidence of 3+ or 4+ proteinuria in all rats, including the control, (3) lack of progression of proteinuria in the 5-ppm rats after 2 years of exposures, i.e., lack of treatment-related increases in incidence or severity of other renal changes, (4) the occurrence of proteinuria in an animal model (F344 rat) known for its high incidence of spontaneous, age-related CPN that complicates interpretation of kidney findings, and (5) the body of carbon tetrachloride literature that suggests that the liver is a more sensitive target organ than the kidney. U.S. EPA notes that Table 4-2 in Section 4.2.2.2 presents all available information on proteinuria from the JBRC study. Thus, documentation of incidence and severity data for proteinuria was not repeated in the synthesis section (Section

4.6.2), as recommended by the reviewer; however, reference to Table 4-2 is provided in this section.

4. BMD methods were applied to incidence data for fatty changes in the liver to derive the POD for the RfC. Please provide comments on whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the BMR selected for use in deriving the POD (i.e., 10% extra risk of fatty liver) been scientifically justified? Has it been transparently and objectively described? Please identify and provide rationales for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Comments: All six peer reviewers considered BMD modeling and the choice of the BMR for use in deriving the POD to be appropriate.

Response: No response needed.

5. PBPK modeling was used to extrapolate the POD from rats to humans and from inhalation to oral dose estimates. Please comment on whether the PBPK modeling for interspecies and route-to-route extrapolation is scientifically justified. Has the modeling been transparently and objectively described in the Toxicological Review? Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported? Has the sensitivity analysis been clearly presented, and appropriately characterized and considered? Has the uncertainty been accurately captured and considered?

Comments: Three reviewers considered the application of PBPK models for interspecies extrapolation to be scientifically appropriate and transparently described.

Response: No response needed.

Comment: One reviewer observed that the description of PBPK modeling applied to extrapolate animal to human carbon tetrachloride dosimetry (Appendix C) is a potential source of confusion because of the overwhelming amount of detailed (and sometime redundant) information and inconsistencies in Section 3.5 (e.g., different values of QCC and QPC).

Response: Inconsistencies in the body of the Toxicological Review and Appendix C were corrected. The objective of Section 3.5 is to describe models that have been reported in the literature, whereas Appendix C describes the models and parameter values used in the implementation of these models in deriving toxicity values. Exact concordance between parameter values in the two sections is not expected, since some parameter values were selected based on consideration of multiple factors (e.g., multiple independent estimates of the values), and units of parameters reported in the literature were not always the same as the units of parameters implemented in the models described in Appendix C. For example, the reviewer noted a discrepancy between the values for QPC and QCC reported in Section 3.5 (Table 3-4) based on Paustenbach et al. (1988) and those reported in Table C-2 of Appendix C. Values in Table 3-4 are for QC and QP (L/hour) for a 0.42-kg rat and 70-kg human (as reported in Paustenbach et al., 1988), whereas in Table C-2, these values were converted to values for QCC and QPC (L/hour/kg $BW^{0.74}$), as described in Paustenbach et al. (1988). The same applies to values for V_{max} reported in Table 3-4; corresponding values for V_{maxC} (mg/hour/kg $BW^{0.7}$) are reported in Table C-2. The data presentation in Sections 3.5 and Appendix C was made clearer by including the unscaled values for these parameters in the revised footnote to Table 3-4 (for comparison to Table C-2).

Data presented in Table C-1 were incorrectly cited as a personal communication on page C-2 (and in Section 3.5); these data were reported in Thrall et al. (2000). The text was revised accordingly.

Comment: One reviewer pointed to inconsistencies in reporting PBPK model parameterization between the text (Table 3-4) and Appendix C (Table C-2), in particular with respect to human cardiac output (QC) and alveolar ventilation (QP).

Response: Values of 256 L/hr for QC and 254 L/hour for QP were derived from Table 2 of Paustenbach et al. (1988); however, in the text on page 196 of the same publication, the values are given for QC and QP as 348 L/hr for a 70-kg human, as derived from QCC (or QPC) = 15 L/hour × $BW^{0.74}$. The reason for the discrepancy between the text and Table 2 of Paustenbach et al. (1988) is not apparent. To improve clarity and comparability between Table 3-4 and Table C-2, Table 3-4 was revised to present the scaled values from the allometric scaling functions for cardiac output and alveolar ventilation (i.e., 15 L/hour × $BW^{0.74}$) and V_{max} (i.e., 0.65 mg/hour × $BW^{0.7}$) reported by Paustenbach et al. (1988).

Comment: One reviewer stated that, in light of nonlinear pharmacokinetics and toxicodynamics, it would be more appropriate to apply the UFs relevant to animal and human variability to internal dosimetrics rather than to the predicted human external exposure concentration of carbon tetrachloride.

Response: The reviewer is suggesting that the UF used to account for possible interspecies differences in pharmacokinetics and/or pharmacodynamics (e.g., $10^{0.5}$ used in derivation of the RfC) be applied to the animal internal dosimetry (e.g., MCA, MRAMKL) and not to the HEC. This approach might yield a lower reference value than if the same UF is applied to the human external dose, if the unadjusted human external dose was in the nonlinear range of the external dose-internal dose relationship. However, as a general principle, the U.S. EPA applies UFs to estimates of HECs for the following reasons. The HEC is intended to be an estimate of the most likely value for the HEC equivalent to the POD from the internal dose-response relationship. UFs are then applied to the most likely estimate to account for various categories of uncertainty that might result in an overestimation of the HEC. Applying all UFs to the HEC achieves greater transparency in the quantitative treatment of uncertainty than distributing UFs across different points in the derivation of the HEC.

Comment: One reviewer noted that the two rates (MCA and MRAMKL) selected as internal dosimetry for derivation of the RfC and cancer SF are both time-averaged values. The reviewer further claimed that the dynamics of the PBPK model prediction were lost because the animal exposure dosage was also adjusted from 6/24 hours and 5/7 days to the average continuous exposure of 24 hours/day, 7 days/week.

Response: Inhalation exposures to animals were simulated in PBPK models as 6 hours/day, 5 days/week exposures. Simulations of equivalent human exposures assumed continuous (24 hours/day, 7 days/week) exposures. The text was revised to increase transparency (Section 5.2.2.1).

Comment: One reviewer suggested that the PBPK modeling be made more transparent by listing the inhalation concentration in the rodent corresponding to the BMD and BMDL.

Response: Tables that present HECs (Tables 5-6, 5-7, and 5-11 though 5-17) were revised to include external exposure concentrations that correspond to reported BMD and BMDL values.

Comment: One reviewer recommended that the text explain the major rodent-human differences that yield greater dosimetry in rodents and the confidence one has that these physiologic and metabolic differences are accurate (e.g., the percentage of body fat and metabolic rate appears to be backfits).

Response: Values for tissue volumes (i.e., fraction of body weight) and metabolism parameters were taken from the documentation on the models (i.e., Thrall et al., 2000; Paustenbach et al.,

1988). The metabolism parameters were derived in the above studies from fitting data on closed chamber elimination kinetics. Paustenbach et al. (1988) also adjusted values for the fat fraction (VFC) and blood flow (QFC) of rats to improve fit to the gas uptake data. The values used in the Paustenbach et al. (1988) model and in the PBPK models used to derive toxicity values in the Toxicological Review (VFC, 8%; QFC, 4%) are within the range of reported observations for these parameters: VFC, 7–15% for adult rats weighing 250–500 g; QFC, 4–7% (ILSI, 1994; Davies and Morris, 1983).

Results of sensitivity analyses of the two internal dose metrics used in deriving toxicity values (MCA, MRAMKL) are provided in Appendix C.4. The relative volume of fat (i.e., fat volume as a fraction of body weight, VFC) was not a sensitive parameter for either dose metric (sensitivity coefficient <0.01). The metabolism parameter V_{maxC} was a sensitive parameter for both dose metrics (sensitivity coefficient ≥0.1). Increasing V_{maxC} decreases MCA and increases MRAMKL.

Section 5.3 (animal to human extrapolation) was revised to discuss the relative confidence in PBPK model parameter values, including physiological parameters, partition coefficients, and metabolism parameters.

Comment: One reviewer observed that the blood:air partition coefficients was measured as being lower in humans than rodents and suggested that the confidence in these data be described as it is pivotal in creating cross species dosimetry differences. A second reviewer considered it unusual that there should be a large range of values for the blood:air partition coefficient (2.73–4.20 in humans, Fisher et al., 1997 and Gargas et al., 1989; 4.52 for rats, Gargas et al., 1986).

Response: Confidence in parameter values that are measured in the species being simulated (e.g., blood:air partition coefficient) are, in general, considered to be more certain than those that are extrapolated across species by applying generic allometric scaling factors (e.g., V_{max}, K_m). The importance of uncertainty in the estimate of the blood:air partition coefficient depends on the internal dose metric used in the internal dosimetry modeling. The MRAMKL metric (used as the basis for the RfC and oral cancer SF) is relatively insensitive to uncertainties in the blood:air partition coefficient, whereas the MCA metric (used as the basis for the cancer IUR) is highly sensitive to this partition coefficient. The sensitivity analysis for this and other model parameters is presented in Appendix C.4, Figures C-14 and C-15.

Although different values for the blood:air partition coefficient were used in the human and rat models for carbon tetrachloride, these differences were within a range of expected variability for these parameter values, within and across species. Section 5.3 was revised to provide additional discussion of literature values for the blood:air partition coefficient.

Comment: One reviewer disagreed with the choice of dose metric in the PBPK model and its interspecies projection. This reviewer noted that the implicit conclusion made in the assessment, that there will be equal toxic and carcinogenic effects across species for an equal rate of production of reactive metabolites per unit liver tissue, would be correct if the rates of destruction of the reactive metabolites across species are the same. The reviewer further noted, however, that evidence to support this assumption was not provided. This reviewer recommended that the implicit assumption of passive destruction of the reactive metabolites at identical rates in humans versus rodents be articulated, together with the mechanistic reasoning and prior experience with other chemicals that could lead to different assumptions as to the appropriate causal dose metric (gross metabolism rate versus AUC of the active metabolites) and interspecies projection rules for causally-relevant delivered dose.

This reviewer further stated that unless both the production and loss of the reactive metabolites can be included in pharmacokinetic models based on reasonable empirical data, U.S. EPA should apply the $BW^{-0.25}$ correction to account for likely slower elimination of the active metabolites in humans relative to rats.

Response: This comment applies to the carbon tetrachloride RfC and IUR, both of which were derived using a PBPK model for interspecies extrapolation and rate of metabolism of carbon tetrachloride in the liver as the dose metric. U.S. EPA acknowledges uncertainty in the assumption of equal toxic and carcinogenic effects in liver across species for an equal rate of production of metabolites per unit liver. Species differences could arise from various mechanisms, including quantitative differences in clearance of reactive metabolites of carbon tetrachloride and quantitative differences in mechanisms that participate in quenching lipid peroxide cascades and/or repairing lipid peroxides (e.g., glutathione peroxidase), that scavenge or reduce oxygen radicals (e.g., superoxide dismutase, GSH), or that repair DNA damage (e.g., glycolases, ligases, polymerases).

Nevertheless, empirical data specific to carbon tetrachloride metabolism indicate that equal rates of metabolism of carbon tetrachloride by CYP450 in rodents and humans would be expected to yield equal rates of elimination of trichloromethyl and trichloromethyl peroxy radicals. Sections 3.3 and 5.2.2.1 were revised to include a discussion of the generation and elimination of reactive metabolites of carbon tetrachloride. Based on the considerations presented in Section 5.2.2.1, U.S. EPA determined that a reasonable modeling approximation was to simulate the elimination of the trichloromethyl radical, in both rodents and humans, as occurring with the same, high rate relative to the much slower production of the radical. This is analogous to a flow-limited system, in which the amounts of reaction products of the trichloromethyl radical produced over time (i.e., AUC) are limited by the rate of production of the trichloromethyl radical (i.e., via CYP450) and the availability of reactants for the trichloromethyl and trichloromethyl peroxy radicals (e.g., intracellular amino acids, lipid, protein

in the liver). In carbon tetrachloride PBPK models applied in the current assessment, interspecies scaling of the production of the trichloromethyl radical is modeled with species-specific values for Michaelis-Menten rate coefficients for CYP450-mediated metabolism of carbon tetrachloride (i.e., V_{max} scaled to $BW^{0.7}$). Tissue concentrations of reactants for the trichloromethyl and trichloromethyl peroxy radicals (e.g., amino acids, lipid, protein) are assumed to be the same in rodent and human liver. Therefore, the AUC for the concentration of trichloromethyl and trichloromethyl peroxy radicals in liver would be expected to scale with the rates of production of metabolism of carbon tetrachloride to the trichloromethyl radical in liver, which are simulated in both species, and with liver volumes, which scale directly with body weight (i.e., liver volume is assumed to be 0.04 of body weight). Given the highly reactive nature of carbon tetrachloride and the available rate constant information for carbon tetrachloride metabolites, the additional scaling factor of the elimination rate proposed by the reviewer (i.e., $BW^{-0.25}$) is not necessary. Scaling dosimetry of reactive metabolites that are eliminated by spontaneous processes (i.e., not metabolism) directly with body weight (i.e., BW^{1}) has been discussed elsewhere (e.g., Travis, 1990). It is emphasized that the determination not to apply an additional scaling factor of $BW^{-0.25}$ was based on the strength of the available carbon tetrachloride data and information on the biochemical reaction mechanism and should not be construed as precedent for other compounds where such data and information are not available.

Comment: One reviewer observed that the HECs obtained from V_{maxC} values of 0.4 and 0.65 were averaged. This reviewer noted that using the lower value might be considered more conservative, but that use of either the average of the two HECs or the lower value yielded the same RfC when rounded to one significant figure.

Response: The rationale for averaging (i.e., that there is no empirical basis for selecting either end of the range as the more likely estimate for the RfC) is provided in Section 5.2.2.3.

Comment: One reviewer pointed to the absence of explicit source codes of PBPK model(s) used in POD extrapolations for derivation of the RfC (both, CSL and CMD files).

Response: ACSL csl file, and m files for the human, rat, and mouse models were added as a new Appendix F.

6. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfC. If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factors:

- **An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the RfC because the available quantitative information on the variability in human response to carbon tetrachloride is considered insufficient to move away from the default uncertainty factor of 10.**
- **An interspecies uncertainty factor of 3 was used to address pharmacodynamic uncertainty only, because PBPK modeling was used to address pharmacokinetic extrapolation from rodents to humans. This contrasts with using the full default interspecies uncertainty factor of 10 for the RfD where an oral PBPK model to support interspecies extrapolation is not available.**
- **A database uncertainty factor of 3 was used to account for lack of adequate reproductive toxicity data for carbon tetrachloride, and in particular absence of a multigeneration reproductive toxicity study.**

Are the criteria and rationale for the selection of these uncertainty factors transparently and objectively described in the document? Please comment on whether the application of these uncertainty factors has been scientifically justified?

Comments: All six peer reviewers agreed with the application of an intraspecies UF of 10. One of these reviewers noted while information is available regarding CYP expression that was not considered and that could mitigate some of the variability, the choice of the default UF of 10 is probably reasonable based on the desire to err on the side of conservatism.

Five reviewers agreed with the application of an interspecies UF of 3. One of these reviewers suggested that a discussion of whether the use of an UF of 3 was adequate for the interspecies extrapolation from rat to hamster be included to provide support for the use of an interspecies UF. A sixth reviewer reiterated that a $BW^{-0.25}$ correction should be added to account for likely slower elimination of the active metabolites in humans relative to rats, which would lower the RfC by a factor of about fourfold $([70/0.25]^{-0.25})$.

Five reviewers agreed with the application of a database UF of 3. A sixth reviewer observed that there may be sufficient uncertainty with regards to proteinuria being the driving endpoint instead of fatty liver to increase the database UF to 10. In lieu of this, this reviewer noted that U.S. EPA could model the proteinuria data to study the implications of this apparent lowest LOAEL and either use that determination directly for RfC derivation, or use it to further inform the magnitude of the database UF.

Two reviewers specifically offered the opinion that the criteria and rationale for the selection of the UFs were transparent and objective. A third offered the same opinion, with the exception of the failure in the document to correct for the likely difference in detoxification rate of the active metabolites in humans versus rodents.

One reviewer reiterated the suggestions on improvement of the clarity of presentation of UFs (see RfD Charge Question #5) and further recommended that UFs be applied to the internal

dosimetric rather than to the predicted human external exposure concentration of carbon tetrachloride.

Response: With respect to the interspecies UF, U.S. EPA does not consider that a discussion of interspecies extrapolation from rat to hamster would provide relevant support for the interspecies UF applied to account for uncertainty in the extrapolation from data in rats to humans. The comments related to application of body weight scaling for interspecies extrapolation and application of UFs to the internal dosimetric are addressed in response to comments on RfC Charge Question #5. The comment related to uncertainty associated with data for proteinuria is addressed in response to comments on RfC Charge Question #3. The comment related to correction for likely differences in detoxification rate of the active metabolites in humans versus rodents is addressed in response to comments on General Charge Question #1. The comment regarding the clarity of the UF presentation and application is addressed in response to comments on RfD Charge Question #5. The abbreviations UF_A, UF_H, UF_L, UF_S, and UF_D were added in the discussion of the UFs applied in deriving the RfC.

(C) Carcinogenicity of carbon tetrachloride

1. Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), the Agency concluded that carbon tetrachloride is likely to be carcinogenic to humans by all routes of exposure. Please comment on the cancer weight of evidence characterization. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described? Do the available data for both liver tumors in rats and mice and pheochromocytomas in mice support the conclusion that carbon tetrachloride is a likely human carcinogen? Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described?

Comments: Four peer reviewers agreed with the conclusion that carbon tetrachloride is "likely to be carcinogenic to humans" by all routes of exposure.

One reviewer expressed concerns about the overall conclusion that carbon tetrachloride should be considered "likely to be carcinogenic to humans" by all routes of exposure, as it was not clear how this related to the previous assessment from 1991 that assigned a weight-of-evidence descriptor of "probably a human carcinogen." This reviewer stated that the previous conclusion was based on sufficient evidence in animals whereas the newly proposed designation of "likely to be carcinogenic to humans" would be based on sufficient evidence in animals and humans. This reviewer further observed that considering that liver effects are considered to be primary, it was unclear how the absence of liver tumors in humans could be reconciled with the designation of "likely to be carcinogenic to humans."

Another reviewer did not specifically offer a comment on the cancer weight-of-evidence descriptor. Rather, this reviewer offered the opinion that it is logical to postulate that hepatocarcinogenicity could be mechanistically relevant to humans, but believed there is no such a parallelism with mouse pheochromocytomas, and that the conclusion that "…experimental evidence for pheochromocytomas is potentially relevant to humans…" bears a great degree of uncertainty. This reviewer suggested that the uncertainty regarding pheochromocytoma in humans should be better emphasized in Section 6.2.3, Relevance to humans.

Response: As noted in U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), evidence consistent with the descriptor of "likely to be carcinogenic to humans" covers a broad range of data combinations, including, for example, "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" (U.S. EPA, 2005a, p. 2–55). The cancer findings for carbon tetrachloride, which include tumors in three species (rat, mouse, and hamster), two sites (liver and adrenal gland), and two routes of exposure (oral and inhalation), are consistent with this cancer weight-of-evidence descriptor.

In response to the reviewer who suggested that the uncertainty regarding pheochromocytomas in humans be better emphasized in Section 6.2.3, Relevance to humans, U.S. EPA notes that the fact that pheochromocytomas were observed only in mice does not necessarily lead to the conclusion of uncertain relevance to humans. As noted in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), "agents observed to produce tumors in both humans and animals have produced tumors either at the same site... or different sites," and therefore "site concordance is not always assumed between animals and humans," particularly where MOA information does not lead to an anticipation of site concordance. Text discussing the human relevance of mouse pheochromocytomas was added to Sections 4.7.4 and 5.4.2. U.S. EPA considers the discussion of uncertainties related to the relevance of mouse pheochromocytomas to humans to be appropriately captured in Section 6.2.3, Relevance to humans, where it is acknowledged that the relevance is unknown, but that the mouse has been identified as a potentially appropriate model for human adrenal medullary tumors.

2. In the Toxicological Review, EPA discussed a MOA for liver cancer involving metabolism, cytotoxicity, and regenerative proliferation leading to tumor induction as key events occurring at relatively high exposure levels. EPA also discussed that carbon tetrachloride carcinogenicity may not be explained by a cytotoxic-proliferative MOA only and that a MOA involving genetic damage may also be operative at high exposure levels and may predominate at noncytotoxic (low) exposures. Please provide detailed comments on whether this analysis regarding carbon tetrachloride's MOA(s) is scientifically justified. In particular, please provide comments on EPA's evaluation of the carbon tetrachloride

genotoxicity database and EPA's judgments about potential low-dose genotoxicity given the limited information at low doses. Has the MOA for liver cancer been transparently and objectively described in the document? Considerations should include the scientific support regarding the plausibility for each of the hypothesized MOAs, and the characterization of uncertainty regarding these MOAs.

Comments: The peer reviewers offered a range of opinions on U.S. EPA's presentation of hypothesized cancer MOAs for carbon tetrachloride. Three reviewers generally agreed that the inclusion of hypothesized MOAs at high and low doses is appropriate. One of these reviewers observed that the various MOA discussions in the document tended to emphasize the high-dose phenomena, with the low-dose MOA discussion brought in secondarily mostly to explain one data point rather than as a primary mechanism with sufficient footing to drive low-dose extrapolation. A second reviewer emphasized that reactive metabolites are expected to be formed at low and high doses. A third reviewer noted that available evidence supporting the cancer MOA involving hepatic cytotoxicity, necrosis, and cellular regeneration is well presented and more convincing than a MOA involving genetic damage, but both MOAs appear to contribute.

Three reviewers questioned whether a second MOA involving low-dose genetic damage was adequately scientifically supported. One of these reviewers considered that the discussion of MOAs involved in hepatocarcinogenicity of carbon tetrachloride considered only the two extreme alternatives in a somewhat simplistic manner (e.g., either cytotoxicity/regeneration or genetic damage) and avoided discussing the epigenetic mechanisms that this reviewer believed were most probably involved in both cancer and noncancer effects caused by environmentally-relevant, low concentrations of carbon tetrachloride, a pro-oxidant chemical. This reviewer believed that an increased proliferation rate appears earlier and at significantly lower concentrations than those needed for noticeable cytotoxicity, and further that the biomarkers of cellular proliferation relate to the dose of pro-oxidant nonlinearly. This reviewer concluded that it is likely that the epigenetic mechanisms (e.g., oncogene depression or activation) rather than genotoxicity or necrosis/regeneration may be responsible for carcinogenicity observed in carbon tetrachloride-treated animals and that, therefore, genotoxicity at low exposures is not a plausible MOA for carbon tetrachloride.

The second of these three reviewers concluded that the preponderance of data for carbon tetrachloride supports a MOA for liver tumors that includes the following key events: (1) metabolism to reactive intermediates, (2) radical-induced mechanisms leading to hepatocellular toxicity, and (3) sustained regenerative and proliferative changes, and that these key events are consistent with a hypothesis that exposures that do not cause hepatotoxicity are not expected to result in liver cancer. This reviewer stated that the scientific basis for this MOA

and the characterization of uncertainties for this MOA were adequately addressed and described in the Toxicological Review.

The third reviewer believed that there are ample data to support a MOA involving cytotoxicity-proliferation. With the exception of the unexplained hepatocellular adenomas in female mice at low doses, this reviewer knew of no data that support any other mechanism of action. While appropriate to suggest an additional mechanism to be consistent with unexplained data, this reviewer was not sure that the mouse data provide a strong rationale for an alternate MOA.

Response: Section 4.7 was rewritten to more clearly articulate the hypothesized liver tumor MOA at high and low exposure levels. One characteristic of the mechanistic database that was evaluated in the MOA analysis is that the majority of available studies were conducted at relatively high doses (Table 4-16). Evidence for an epigenetic component to the cancer MOA was added to Section 4.7.3.3.

Support for MOAs other than a cytotoxicity/regenerative proliferation MOA is not limited to the incidence of female mouse liver tumors (Nagano et al., 2007b). Other considerations that suggest that the carbon tetrachloride database is insufficient to rule out other MOAs at low exposure levels include: (1) carbon tetrachloride's general reactivity (i.e., carbon tetrachloride is metabolized to the reactive species trichloromethyl and trichloromethyl peroxy radical that can react with cellular constituents and lead to formation of reactive oxygen species that also can damage DNA and other macromolecules) and (2) insufficient data to ascertain whether or not carbon tetrachloride is genotoxic at low exposures. EPA retained the recommendation to apply a linear extrapolation approach for carbon tetrachloride; however, an expanded discussion of the nonlinear and linear extrapolation approaches was added to Section 5.4.

Comments: Two of the six reviewers provided specific opinions related to the weight of evidence for genotoxicity. One of these reviewers stated that the genetic toxicology database is overall not supportive of mutagenesis as being a primary mechanism; however, given important uncertainties in the genotoxicity studies, the fact that carbon tetrachloride has showed a genotoxic effect in the absence of S-9 mix in a few studies, and the likely formation of radicals at sub-toxic doses, this reviewer thought some consideration should be given to genotoxicity as the explanation for the tumor response in the JBRC study at relatively low, nontoxic levels. The second reviewer believed that using a weight-of-evidence approach, the scientific data show that carbon tetrachloride is not genotoxic or mutagenic and therefore did not agree with the conclusions drawn in the Toxicological Review concerning potential for low-dose genotoxicity of carbon tetrachloride. This reviewer also suggested that carbon tetrachloride could induce a hormetic response in that moderate "priming doses" of liver toxicants such as carbon

tetrachloride can induce detoxifying and/or DNA repair enzymes and reduce or prevent cellular damage caused by carbon tetrachloride.

Response: U.S. EPA maintains that, given the highly reactive biological activity of carbon tetrachloride and demonstration of a genotoxic response at high-exposure levels, the contribution of genotoxicity to the cancer MOA for carbon tetrachloride cannot be excluded. Significant literature that suggests that carbon tetrachloride induces a hormetic response is unavailable. The three citations on hormesis provided by the peer reviewer are papers on chloroform or a general review of hormesis and are not specific to carbon tetrachloride.

3. Regarding liver cancer, two approaches to dose-response assessment for the inhalation exposure route are presented in the Toxicological Review—a nonlinear low-dose approach and a linear low-dose extrapolation approach. Do you agree with EPA regarding the support for a nonlinear extrapolation approach consistent with a MOA involving hepatocellular cytotoxicity and regenerative hyperplasia? Do you agree with EPA regarding the support for applying the default linear extrapolation approach due to uncertainty in understanding the cancer MOA at low doses? Please provide detailed comments on whether the inclusion of both approaches to dose-response assessment is scientifically sound and transparently and objectively described in the document.

Comments: Three reviewers generally agreed with the presentation of both linear and nonlinear approaches in the assessment. One of these reviewers concluded that given the suggestive evidence of low-dose carcinogenesis below toxicity thresholds and uncertainties with respect to genotoxicity, the recommendation in the Toxicological Review of a linear low-dose modeling approach is a prudent way to deal with the uncertainties in a reasonably health protective manner. This reviewer suggested that the Toxicological Review attempt to bring these different approaches together into a unified synthesis and provide perspective on the difference between the approaches (i.e., that if one chose the nonlinear approach, one would be out of bounds for protecting public health if, in fact, the low-dose linear model is correct). The second of these reviewers thought it appropriate to present the low-dose linear approach, but considered it to be a default approach with little scientific support, whereas the nonlinear extrapolation approach has a good deal of scientific support from the literature. This reviewer believed that the document fell short in not making some judgment as to the relative strength of the two proposed approaches. The third reviewer believed that both nonlinear and linear approaches were well described in the document and that a well-balanced explanation of the support and deficiencies for both methods was clearly presented. This reviewer further noted that while the nonlinear extrapolation approach appears more consistent with the MOA involving hepatocellular cytotoxicity and

regenerative hyperplasia, the default linear approach may also be considered given the uncertainty in understanding the cancer MOA at low doses.

One reviewer did not agree with the application of the default linear extrapolation approach due to uncertainty in understanding the cancer MOA at low doses. Rather, this reviewer believed that the available data supported key events involving hepatocellular cytotoxicity and regenerative hyperplasia consistent with a nonlinear MOA. Further, this reviewer questioned the biological significance of female mouse liver tumors (18%) at 5 ppm in the JBRC study that was statistically significantly elevated relative to historical but not study controls, and that was lower than the incidence produced by 25 ppm (88%) and 125 ppm (98%) carbon tetrachloride in male mice or by 125 ppm in either male or female rats (80–88%). This reviewer also noted that epidemiological studies have not identified an association between human exposures to carbon tetrachloride and increased liver cancer incidence.

In contrast, another reviewer disagreed with a presentation of the nonlinear threshold-implying calculations. This reviewer suggested that where linear and upward-turning nonlinear MOAs are present in the same system, the dose response in the low-dose region will tend toward linearity. In this case, U.S. EPA should therefore do the best it can to estimate the low-dose slope. The reviewer appended an extended excerpt from a 2007 white paper prepared for U.S. EPA discussing relevant issues. In addition, this reviewer proposed the depletion of SAM as one of the likely components of the carbon tetrachloride MOA mentioned in the Toxicological Review. This reviewer noted as relevant the discussion of the dose-response relationship for dichloroacetate (DCA), which is also thought to act via this process (and cited: Hattis, D; Rahmioglu, N; Verma, P; et al. (2009) A preliminary operational classification system for nonmutagenic MOAs for carcinogenesis. Crit Rev Toxicol 39(2):97–98). According to this reviewer, the proposed MOA for dichloroacetate is decreased methylation of the promoter regions of the proto-oncogenes c-jun and c-myc and increased expression of the corresponding m-RNAs. Data from a 2-year carcinogenesis dose-response study for dichloroacetate did not indicate appreciable nonlinearity over the fairly wide dose range studied. A sixth reviewer did not provide a specific opinion as to whether the inclusion of both approaches to dose-response assessment is supported. This reviewer raised questions as to the support for a linear approach, noting that under the assumption that the short-lived free radical metabolites of carbon tetrachloride and the peroxidative products are responsible for hepatocarcinogenicity, it may be unrealistic to expect a linear proliferative response versus time-averaged integrated carbon tetrachloride dosimetrics. Given this and consistent with both epigenetic cancer and noncancer MOA (e.g., represented by the RfD/RfC), this reviewer considered a nonlinear approach to dose-response to be more appropriate and more relevant to potential hepatocarcinogenesis than the linear extrapolations or even a simplified MOA involving hepatocellular cytotoxicity and regenerative hyperplasia.

Response: Consistent with the overall input received from the peer reviewers, a default linear approach and a nonlinear approach (as an alternative low-dose extrapolation approach) were retained. EPA continues to recommend the linear extrapolation approach. However, text was added to Section 5.4 to expand the discussion of the support of application of the nonlinear and linear extrapolation approaches.

With respect to the recommendation suggested by two reviewers that the assessment provide either a unified synthesis of the linear and nonlinear approaches or judgment as to the relative strength of the two approaches, U.S. EPA notes that providing the risk at an exposure equivalent to the RfD or RfC under the assumption of the linear low-dose extrapolation approach is essentially conducting a risk assessment for an exposure scenario of lifetime exposure at that exposure level, and thus falls outside the scope of an IRIS Toxicological Review. The linear low-dose extrapolation approach was selected because the cancer MOA for carbon tetrachloride is unknown; this basis for selecting an extrapolation approach does not lend itself to reaching judgments as to the relative strength of the linear and nonlinear approaches. Rather, a characterization of uncertainties in the extrapolation approach, as provided in Section 5.4.6, is considered more appropriate. In response to the reviewer who disagreed with the application of the default linear extrapolation on the basis that data support key events consistent with a nonlinear MOA, U.S. EPA notes that the incidence of hepatocellular adenomas in 5-ppm female mice in the JBRC study was, in fact, statistically elevated relative to both historical and study controls and that the combined incidence of hepatocellular adenomas and carcinomas showed a positive trend. As this reviewer observed, epidemiological studies have not identified an association between human exposures to carbon tetrachloride and increased liver cancer incidence; however, no case-control studies were identified that specifically looked for this association. Further, U.S. EPA notes that site concordance is not necessarily assumed between animals and humans (U.S. EPA, 2005a).

In response to the reviewer who provided a white paper entitled, "Uncertainties in Risk Assessment for Carcinogenesis: A Road Map Toward Practical Improvements," U.S. EPA notes that much of the discussion in this white paper extends beyond the scope of the carbon tetrachloride assessment, although some of the concepts that support the application of a low-dose linear extrapolation are presented in Section 5.4, Cancer Assessment.

Additional discussion of the role of SAM depletion in the carbon tetrachloride cancer MOA was added to Section 4.7.3.3.

Additional responses to comments regarding U.S. EPA's decision to recommend the linear low-dose extrapolation approach for assessing cancer risk are provided in response to Carcinogenicity Charge Question #8.

4. Is EPA's characterization of mouse pheochromocytomas, including their relevance to human cancer risk, transparently and objectively described in the Toxicological Review?

EPA applied a linear extrapolation approach to pheochromocytoma data from the JBRC inhalation bioassay in mice in the absence of MOA information. Please comment on the scientific justification for quantification of cancer risk for this tumor type, considering relevance to humans. Has the dose-response modeling been appropriately and objectively conducted? Are the results objectively and transparently described?

Comments: Two peer reviewers agreed with the characterization of pheochromocytomas as relevant to humans, the dose-response assessment, and with the characterization of the uncertainty in dose response. One of these reviewers expressed the opinion that the fact that the tumors are benign does not materially detract from their relevance as indicators of a carbon tetrachloride-induced carcinogenic process. This reviewer cited Colby et al. (1994) as additional evidence for carbon tetrachloride activation in the rodent and suggested that additional literature search and evaluation be conducted regarding the potential MOA for adrenal tumors. This reviewer further suggested that greater emphasis be placed on the uncertainties in PBPK modeling and cancer risk estimation for this endpoint given the lack of MOA information and uncertainty with regards to the key dose metric for estimating internal dose and risk. The second reviewer believed that it would be informative to include an alternative linear low-dose model estimate based on liver tumors only and an addition of a statement about the fraction of animals with pheochromocytomas that also had liver tumors. Finally, this reviewer thought it would be useful to put the result in perspective by showing where the carbon tetrachloride SF fits among the SFs calculated for other small MW chlorinated hydrocarbons (e.g., vinyl chloride, methylene chloride).

Three peer reviewers did not agree with U.S. EPA's approach to pheochromocytomas. Two reviewers observed that pheochromocytomas may represent a species-specific finding and that no increases in pheochromocytomas have been observed in epidemiological studies. For these reasons, one of these two reviewers did not consider a linear extrapolation based on pheochromocytomas in mice to be justified. A third reviewer did not agree that data for pheochromocytomas should override the conclusions based on the use of liver tumors as the primary response because: (1) pheochromocytomas have been observed at higher doses than those that cause liver tumors; (2) the relevance to humans is questionable as this tumor has not been previously observed in carbon tetrachloride-exposed individuals; and (3) these tumors are almost always benign.

One reviewer did not directly address the charge question. This reviewer observed that the issue of mouse pheochromocytomas was adequately described qualitatively and quantitatively and characterized sufficiently in this Toxicological Review; however, their relevance to human cancer risk was considered highly uncertain. In response to Carcinogenicity Charge Question #6, however, this same reviewer stated that data for mouse pheochromocytomas with an uncertain relevance to humans should not be used in derivation of cancer risk values.

Response: A search of the literature for additional information on the effects of carbon tetrachloride on the adrenal gland was performed. Relevant findings, including those of Colby et al. (1994), were added to Sections 4.5 (mechanistic data) and 4.7.4 (MOA for pheochromocytomas).

Uncertainties in the PBPK modeling for pheochromocytomas, and in particular selection of the dose metric, given the lack of MOA information for this tumor were addressed in Section 5.4.3.2.

Estimates of cancer risk using a linear low-dose extrapolation approach are presented for all individual tumor types in Tables 5-18 and 5-19. A statement about the fraction of animals with pheochromocytomas that also had liver tumors has no bearing on the estimate of cancer risk and is not necessary.

The presentation of cancer SFs or unit risks for other small molecular weight chlorinated hydrocarbons is outside the scope of the *Toxicological Review for Carbon Tetrachloride*.

In response to comments regarding the use of pheochromocytoma data for cancer dose-response modeling, EPA considers the pheochromocytomas observed in mice to be relevant to humans. Consequently, incidence data for this tumor type is appropriate for consideration in the cancer dose-response analysis for carbon tetrachloride. Section 5.4.2 was expanded to include a discussion of the evidence that supports the potential human relevance of mouse pheochromocytomas. Uncertainties associated with the quantification of cancer risk based on the different tumor types are discussed in Section 5.4.6.

5. Nonlinear approach: The Toxicological Review finds that the RfD of 0.004 mg/kg-day and the RfC of 0.1 mg/m^3 be used to assess liver cancer risk for carbon tetrachloride under the assumption of a MOA consistent with low-dose nonlinearity. Please provide detailed comments on whether this nonlinear approach is scientifically justified. Has this approach been transparently and objectively described in the document? Are there other nonlinear approaches to evaluating liver cancer risk for carbon tetrachloride that should be presented in the Toxicological Review? Please comment on the utility of including these alternative nonlinear approaches. Please comment on the confidence that EPA should have that there is not a cancer risk for exposures below the level of the RfD/RfC.

Comments: Four peer reviewers generally considered the nonlinear approach to be appropriately presented in the Toxicological Review and to be the preferred and more scientifically supported approach for carbon tetrachloride cancer assessment. One of these reviewers observed that while the linear approach is health-protective, it can result in exaggerated risk estimates in comparison to the alternative approach (i.e., an epigenetic approach discussed by this reviewer in response to Cancer Charge Question #2), which this reviewer considered insufficiently explored in this

Toxicological Review. The other three reviewers did not identify alternative nonlinear approaches that should be applied to characterize liver cancer risk from carbon tetrachloride.

It was the opinion of two reviewers that the choice of the low-dose linear approach was the most clear, prudent and scientifically defensible approach. One of these reviewers noted that while the nonlinear approach is reasonable to consider, the disparity between the nonlinear and linear approaches is so large that they cannot easily be used in tandem when making risk judgments. This reviewer further noted that an advocate of the nonlinear approach might use an additional UF for potential carcinogenicity (e.g., 10x) to get the target dose down in a range that has a better chance of protecting against both cancer and noncancer endpoints—an approach that has been used for "Group C" carcinogens in certain regulatory settings (e.g., U.S. EPA's Office of Drinking Water). The second reviewer did not consider a low-dose nonlinear assumption to be compatible with the expected linear production of DNA reactive metabolites at low doses.

Response: Section 5.4, Cancer Assessment, was restructured to make it clear that U.S. EPA has applied a linear extrapolation approach to the carbon tetrachloride tumor data consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). These guidelines recommend the application of a linear extrapolation approach 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." U.S. EPA considers the current understanding of carbon tetrachloride-induced liver tumors and pheochromocytomas to be consistent with the application of a linear extrapolation approach. Discussion of a nonlinear approach was moved to Section 5.4.5 and is presented as an alternative approach supported by empirical (bioassay) evidence for liver cancer at relatively high exposures of carbon tetrachloride. Text was added to Sections 5.4 and 5.4.2 to expand the discussion of the extrapolation approaches.

Additional consideration to the suggestion that an epigenetic approach be considered is addressed in the response to Cancer Charge Question #2.

6. Linear extrapolation: The Toxicological Review describes the alternative approaches for incorporating low-dose linearity that were applied to four tumor datasets from JBRC (1998) (female rat and mouse liver tumors and male and female mouse pheochromocytomas). These included (1) POD-based straight line risk calculations and (2) similar risk calculations (for liver tumor data sets only) that examined the effect on risk estimates of using only data on carbon tetrachloride cancer response at exposure levels below those for which increased cell replication was reported. In addition, a Bayesian approach was applied to male mouse pheochromocytoma data to investigate the distribution of the slope parameter in the log-probit model. Please comment on whether

the linear extrapolation approaches are scientifically plausible given potential for a cytotoxic MOA at higher doses and other MOAs at lower doses. Please comment on EPA's choice of using data for pheochromocytomas in the male mouse as the basis for the inhalation unit risk and data for female mouse liver tumors as the basis for the oral slope factor. Has the rationale for including a low-dose linear extrapolation been transparently and objectively described in the document? In the above analyses, a BMR of 5% was used for the female rat liver tumor data set, and a BMR of 10% was used for the other tumor data sets. Please comment on the scientific justification for the selection of these BMRs. Is the rationale transparently and objectively described in the document?

Comments: Two reviewers generally concurred with U.S. EPA's linear low-dose analysis. Two reviewers considered it appropriate to present a linear low-dose extrapolation approach as an alternative approach, but that based on available evidence, the nonlinear method seems more appropriate. One of these two reviewers recommended the addition of an evaluative statement regarding the likelihood that a linear approach is correct as compared with the nonlinear extrapolation approach. A fifth reviewer believed that the linear extrapolation of cancer data to low-dose exposures to carbon tetrachloride is difficult to defend and is not a preferable approach. A sixth reviewer did not agree that a linear assessment is justified for carbon tetrachloride.

Response: U.S. EPA notes that a linear extrapolation approach was selected as a default approach in large part because of the absence of an understanding of carbon tetrachloride tumor induction in the low-dose region. According to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a linear extrapolation approach is selected because it is generally considered to be a health-protective approach. As such, it is not possible to provide a statement of the likelihood that either the linear or nonlinear approach is correct. See also the response to Cancer Charge Question #5 to which similar comments regarding the linear and nonlinear approaches were provided.

Comments: Two reviewers agreed that the Toxicological Review clearly describes the procedures (i.e., assumptions and modeling) for low-dose linear extrapolation and a third generally agreed with the U.S. EPA analyses and choices. A fourth reviewer considered the cancer modeling approaches to be "reasonable explorations of the dose response at low dose." This reviewer further noted, however, that little importance should be given to the run of the inhalation data in which the top doses were discarded as that is a dose response involving only a low dose and a control and that this exercise should just be seen as a screening-level cross check. It was suggested that other techniques to test whether low-dose response is compatible with the remainder of the dose response might be more helpful in determining whether the dose response might be different if more subtoxic doses were available.

Response: Text was added to Section 5.4.3.3, under Female BDF_1 mouse—hepatocellular adenomas or carcinomas, noting the limitations of the dose-response analysis based on control and 5-ppm liver tumor data only. Section 5.4.4.2 describes this analysis as a less informative characterization of the dose-response curve than the analysis based on data for the control, 5-ppm, and 25-ppm exposure groups. While limited, this analysis revealed that the elimination of data points with evidence of cell replication had small impact on the estimate of the IUR (see Table 5-18). U.S. EPA is not aware of other techniques that could be applied to this tumor data set to explore the effects of cytotoxicity on the shape of the dose-response curve.

Comments: One reviewer stated that the use of data for pheochromocytomas in the male mouse as the basis for the IUR appears sound and provides the highest risk estimate. Two reviewers questioned the relevance of pheochromocytomas to humans, and one of these two further indicated that these tumors should not be used in derivation of a cancer risk value.

One reviewer expressed some reservations about the exclusive use of a probit model for the pheochromocytoma Bayesian analysis because it implies an individual threshold-type dose response for which there is no specific justification.

One reviewer questioned the switching of tumor endpoint when going from the IUR to the oral SF given that both the oral and inhalation SFs are based upon the same (inhalation) bioassay. This reviewer further observed that a systemic target site like the adrenal gland would logically be the risk driver for inhalation exposure and the liver would be the driver for oral exposure because oral exposure leads to first pass metabolism in the liver whereas inhalation exposure leads to greater systemic doses of parent compound and more opportunity for extra-hepatic targeting of the tumor response. Given the uncertainties in the MOA and PBPK modeling of the adrenal tumor dose response, this reviewer believed that it may be more appropriate to use female liver tumors as the basis for both the IUR and oral SF. If retained, the reviewer recommended a straightforward explanation of the reasons for switching endpoint with dose route.

Response: Comments regarding the relevance of pheochromocytomas and use of pheochromocytoma data for derivation of cancer risk estimates are addressed in response to Carcinogenicity Charge Question #4.

The log-probit model (without restriction on the slope parameter) was the only model in the BMDS suite of models that provided an adequate fit (a *p*-value for fit ≥ 0.1 as recommended by the BMDS guidance [U.S. EPA, 2000c]) and was therefore the model used to estimate the POD. Bayesian analysis was used to provide more detail on why restricting the slope parameter is inappropriate.

An explanation for the use of different tumor types as the basis for the IUR and oral SF where tumor incidence data from the same study (i.e., Nagano et al., 2007b) were used as the basis for both values was added to Section 5.4.4.2.

Comment: Two reviewers considered the choice of a BMR of 5% for female rat liver tumor data and a BMR of 10% for the other tumor data sets to be scientifically justified. One reviewer stated that the objective of using a BMR of 5% for all tumor sites (i.e., a POD as far removed from the hepatotoxic portion of dose response as possible) made sense, but this reviewer believed that backing off to the 10% BMR for all tumor endpoints other than female rat liver was not well justified. This reviewer suggested a graphic depiction of where on the dose-response curve a 5 and 10% BMR lies and description of potential risk implications (i.e., would you tend to get higher SF with a 5 versus 10% BMR?). Other reviewers did not offer an opinion regarding selection of the BMR.

Response: The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that for each tumor response, a POD from the observed data be estimated to mark the beginning of extrapolation to lower doses and that this POD be an estimated dose near the lower end of the observed range without significant extrapolation to lower doses. Appendix E provides the dose-response curves from BMDS for the models that provided the best fit of the tumor data from the JBRC bioassay (Nagano et al., 2007b) (see list of tumor data sets modeled in Table 5-18). As the plots in Appendix E show, a BMR of 10% for male and female mouse liver tumors and male mouse pheochromocytomas results in a BMDL at the low end of the observed range (i.e., on either side of the lowest exposure concentration). Use of a BMR of 5% would move the BMDL lower on the dose-response curve and would result in more significant extrapolation below the observed range. For these tumor data sets, therefore, a BMR of 5% is not supported.

In the case of female mouse pheochromocytomas, the current BMR of 10% yields a BMDL somewhat above the mid-dose group. Because there were no pheochromocytomas in the control, low-, or mid-dose groups in the female mouse, a BMR below the mid-dose group would, for this data set, be outside the observed range (i.e., the range that produced a tumor response). Therefore, for this pheochromocytoma data set, a BMR of 5% is similarly not supported.

7. The conclusion was reached that studies of carbon tetrachloride carcinogenicity by the oral exposure route are not sufficient to derive a quantitative estimate of cancer risk using oral cancer response data and low-dose linear approaches. Please provide detailed comments on whether this judgment is scientifically justified. Has EPA's judgment been transparently and objectively described in the Toxicological Review? EPA used a PBPK model to extrapolate inhalation data to derive an oral cancer risk estimate. Please comment on EPA's application of a PBPK model for route-to-route extrapolation to derive

an oral cancer risk estimate from the inhalation data. Please provide detailed comments on whether this approach is scientifically justified. Has EPA's judgment been transparently and objectively described in the document?

Comments: Three reviewers supported the conclusion that studies on carbon tetrachloride carcinogenicity by the oral route were insufficient to derive a quantitative estimate of cancer risk. One reviewer considered U.S. EPA's judgment that oral studies provide inadequate data for dose-response assessment to be "basically correct," with the one possible exception of the adrenal tumor response seen in the oral NCI mouse study in which high doses yielded approximately a 50% response in male mice and 20% response in female mice. This reviewer observed that analysis of adrenal tumor data could provide interesting comparison to the inhalation dose response for this endpoint after correction for internal dose differences across dose routes. A fifth reviewer agreed that U.S. EPA had sound reasons for concluding that the available carcinogenesis studies by the oral route are considerably less than ideal, but added that it is not impossible to use these data.

Response: In response to the reviewer who suggested that a comparison be made of inhalation exposure concentrations corresponding to the BMD for 20 and 50% adrenal tumor responses in the mouse inhalation study with equivalent oral doses (i.e., in terms of internal dose metrics, MCA and/or MRAMKL), U.S. EPA noted that the available PBPK models are not considered adequate for simulating internal doses from oral gavage studies (e.g., NCI, 1977). Challenges in simulating absorption kinetics in carbon tetrachloride gavage studies include pulsatile absorption kinetics, which are vehicle-dependent (e.g., corn oil, Emulphor) and may be dose-dependent (Fisher et al., 2004; Semino et al., 1997; Gallo et al., 1993). Available models that have been developed to simulate the relatively complex kinetics of carbon tetrachloride absorption in rodent oral gavage studies have required calibration of the absorption parameters to the specific observations being simulated and have not been successfully validated to extrapolation to other dosing regimens (Fisher et al., 2004; Semino et al., 1997; Gallo et al., 1993). The uncertainties in applying existing PBPK models for this purpose are described in Section 5.1.2.

Comments: Three reviewers considered the use of a PBPK model for the inhalation-to-oral exposure extrapolation to be supported. One of these three reviewers qualified this comment with the observation that the analysis used a low-dose linear extrapolation, an approach with which this reviewer was not in agreement. One reviewer reiterated a previous comment on PBPK model use in derivation of the RfC; i.e., that the use of time-weighted carbon tetrachloride dosimetry has a questionable relevance to mechanisms of carcinogenesis.

Response: With regard to the comments on low-dose linear extrapolation, see response to comments under Cancer Charge Questions 3 and 6. The comment related to the use of time-weighted carbon tetrachloride dosimetry is addressed in response to RfC Charge Question #5 where this reviewer offered the same comment.

Comment: One reviewer did not consider the inhalation to oral extrapolation to be adequately explained, including the basis for the RGIL and the assumptions of whether human oral exposure is relatively constant or bolus in nature.

Response: The RGIL parameter is an estimate of the rate of transfer of carbon tetrachloride from the GI tract to the liver (mg/kg-day) that is equivalent, in terms of internal dose (i.e., MCL or MRAMKL), to a continuous inhalation exposure (HEC, mg/m^3). In extrapolating human inhalation exposures to equivalent human ingestion doses (HED), the RGIL value was used to estimate the HED. Section 5.4.3.4 was revised to clarify that HED values were calculated from the predicted relationship (i.e., from the human PBPK model) between the HEC and RGIL for the purpose of making the route-to-route extrapolation.

Values for RGIL in humans cannot be derived from available studies for two reasons: (1) no ingestion studies have been reported that allow estimates to be made of carbon tetrachloride bioavailability or absorption kinetics in humans; and (2) studies conducted in rodents in which animals received oral gavage doses of carbon tetrachloride have shown that absorption kinetics can be complex (e.g., pulsatile), dependent on vehicle (e.g., corn oil, Emulphor, water), and may also be dose-dependent (Fisher et al., 2004; Semino et al., 1997; Gallo et al., 1993). Based on the absence of data on absorption kinetics and bioavailability in humans, the simplest conceptual model was adopted for the purpose of making the inhalation-to-oral extrapolation in humans, where the primary interest is a continuous exposure scenario (e.g., drinking water); it was assumed that bioavailability was 100% and absorption rate during the day for any given oral dosage (mg/kg-day) was constant. The Toxicological Review (Section 5.4.3.4) was revised to clarify this point.

Note that the RGIL was used for route-to-route extrapolation in humans in deriving the oral cancer SF; the absence of an adequate model for simulating bioavailability and absorption kinetics in oral gavage studies conducted in rodents precluded using PBPK models for animal-to-human extrapolations of internal dosimetry in deriving the RfD (see Section 5.1.2).

Comment: One reviewer found some contradiction in the document because PBPK modeling was not used in connection with the interspecies projection of the RfD but was used to derive an oral cancer SF.

Response: In the derivation of the oral SF, the human PBPK model was used to extrapolate from inhalation exposures to oral dosages that would result in the same values for internal dose metrics. In applying the PBPK model, however, it is acknowledged that this approach would only approximate oral dosimetry because it does not account for oral bioavailability or absorption kinetics, information that is not available for carbon tetrachloride (see Section 5.4.3.4). Had route-to-route extrapolation not been performed, an oral SF could not have been derived because oral cancer bioassay data for carbon tetrachloride are not adequate for dose-response analysis (see Section 5.4.1.2). U.S. EPA considers the uncertainties associated with use of the PBPK model for route-to-route extrapolation to be acceptable and preferable to not having any quantitative estimate of oral cancer potency for carbon tetrachloride on IRIS.

On the other hand, a route-to-route extrapolation was not needed for deriving the RfD, since adequate animal oral studies were available. The determination made in the derivation of the RfD was that PBPK models were not sufficiently developed to extrapolate internal dose estimates across species, in particular for extrapolation of internal doses resulting from oral gavage doses (e.g., in corn oil or other vehicles) to continuous exposures of carbon tetrachloride exposures (e.g., in water or food; see Section 5.1.2).

U.S. EPA does not view these two determinations (i.e., to use a PBPK model for dosimetry extrapolations across routes for continuous exposures in humans, but not use PBPK models for dosimetry extrapolations across species and from oral gavage dosing in one species to continuous dosing in humans) to be in conflict. The Toxicological Review has been revised to include these considerations (see Section 5.1.2).

8. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* provides guidance on choosing an approach for dose-response extrapolation below the observed data. Relevant language related to choosing an extrapolation approach is provided in Section 5.4.3 of the Toxicological Review. In this section of the Toxicological Review, a linear low-dose extrapolation approach is recommended for assessing carbon tetrachloride cancer risk over a nonlinear approach due to uncertainty in understanding the cancer MOA as well as some bioassay evidence inconsistent with a nonlinear MOA at low exposure levels. Please comment on the scientific justification for this recommendation. Has this recommendation been transparently and objectively described in the document?

Comments: Two reviewers generally agreed with the recommendation to use a low-dose linear model.

One reviewer believed it appropriate to present a possible, alternative risk assessment approach; however, this reviewer identified concerns with both the validity of some of the data and their relevance to humans that makes the linear approach much less likely to yield accurate

estimates of risk. This reviewer also observed that the document lacks an evaluation of the likelihood of one approach over the other providing an accurate assessment.

One reviewer concurred that an alternative MOA may be operative in the carbon tetrachloride carcinogenesis at low exposure levels and considered the linear low-dose extrapolation approach to be adequately described, but stated that "the data lacks for support of a linear approach."

One reviewer stated that the recommendation to apply a linear low-dose extrapolation approach was not convincing, and suggested that an alternative nonlinear approach be used. The reviewer also noted that the oral cancer SF at a 10^{-6} risk level would require enforcing a concentration in drinking water below practical quantifiable limits for carbon tetrachloride. Another reviewer reiterated their disagreement with the recommendation based on the following: (1) "the slight increase in tumor response (5 ppm, female mice) was limited to female mice, and not in male mice or male and female rats", and "[t]he tumor response at 5 ppm in female mice (18%) was considerably lower than the incidence produced by 25 (88%) and 125 ppm (98%) carbon tetrachloride in male mice (98%) and by 125 ppm in either male or female rats (80–88%)." Further, epidemiological studies have not identified an association between human exposures to carbon tetrachloride and increased liver cancer incidence; (2) pheochromocytomas in mice were classified as benign, were only observed in mice by two separate routes of administration, and epidemiological studies did not reveal increases in pheochromocytomas in exposed humans; and (3) a weight-of-evidence approach of the scientific data supports that carbon tetrachloride is not genotoxic or mutagenic.

Response: The recommendation to apply a linear extrapolation approach for carbon tetrachloride cancer dose-response assessment is consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) that state that a linear approach should be used with agents whose MOA is considered to be linear in the region below the POD as well as when the available data are insufficient to establish the MOA for a tumor site. U.S. EPA notes that for carbon tetrachloride a linear extrapolation approach was selected as a default approach in large part because of the absence of an understanding of carbon tetrachloride tumor induction in the low-dose region. See also responses to Cancer Charge Questions #3 and 6 that address comments from the peer reviewers similar to those offered in response to the above charge question.

In response to the reviewer who noted that the oral cancer SF would result in drinking water limits below practical quantifiable limits, U.S. EPA notes that drinking water standards (maximum contaminant levels or MCLs) are set as close to the health based level as feasible, but also take into consideration best available technology, treatment techniques, and cost.

APPENDIX B. BENCHMARK DOSE MODELING FOR DERIVING THE REFERENCE DOSE

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-d)	SDH (IU/mL)[a]		OCT (nmol CO_2/mL)[a]		ALT (IU/mL)[a]	
	10 wks	12 wks	10 wks	12 wks	10 wks	12 wks
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 ± SE 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. Benchmark Dose Modeling of Sorbitol Dehydrogenase

SDH data for the 10- and 12-week time points were used for BMD analysis. Because the precise group sizes were not known for the 12-week data (a range of 7–9 rats per group was reported), BMD modeling for 12-week data was run using an n of both 7 and 9 rats/group to bracket the values of the BMD and BMDL.

All of the models for continuous data in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007b) were fit to the 10- and 12-week serum SDH data from Bruckner et al. (1986) (see Table B-1). 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 BMD 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 benchmark response factor (BRMF) entered by the user, or

$$BMR = P0 \pm (BMRF*P0)$$

To achieve a doubling of the control mean, a BMRF of 1 was used. Thus, the BMR was calculated as P0 + (1 × P0) or 2 × P0. It should be noted that BMDS uses the fitted, or estimated,

value for the mean and SD to calculate the BMR and BMD. For example, for the 10-week SDH data, the value estimated by BMDS for the control SDH mean was 2.71 IU/mL (see detailed model run; a box appears around the estimated mean). Thus, for this data set, the BMR using relative deviation (as the BMR type) and a BMRF of 1 was calculated as BMR = 2.71 + (1 × 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, which provided the better fit of the data (based on the lower AIC value), gave an estimated BMD_{2X} of 7.32 mg/kg-day and $BMDL_{2X}$ of 5.46 mg/kg-day (see the detailed model run at the end of this appendix). None of the models for continuous data in BMDS provided an adequate fit of the 12-week SDH data (i.e., the linear, polynomial, and power models yielded a *p*-value <0.0001, and there were insufficient degrees of freedom to run the Hill model).

Table B-2. Model predictions for changes in serum SDH levels (IU/mL) in male rats exposed to carbon tetrachloride for 10 and 12 weeks

Model	*p*-value[a]	AIC for fitted model	BMD_{2X} (mg/kg-d)	$BMDL_{2X}$ (mg/kg-d)
10-Wk data				
Linear[b]	<0.0001	—	—	—
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
12-Wk data: all continuous models provided a significant lack of fit.				

[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.

For purposes of comparison across chemicals, the BMD and BMDL corresponding to a change in the mean response equal to one control SD from the control mean were also calculated for the 10-week SDH data (using the power model, which provided the best fit of the data as described above), consistent with BMD guidance (U.S. EPA, 2000c):

BMD_{1SD}: 5.5 mg/kg-day
$BMDL_{1SD}$: 3.8 mg/kg-day

B.2. Benchmark Dose Modeling of Ornithine Carbamoyl Transferase and Alanine Aminotransferase

BMD modeling was also conducted for OCT and ALT. Available continuous variable models in U.S. EPA's BMDS (linear, polynomial, power, and Hill models; BMDS version 1.4.1; U.S. EPA, 2007b) were fit to the 10- and 12-week data shown in Table B-1 for changes in serum OCT and ALT in male rats exposed to carbon tetrachloride (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 for OCT data are summarized in Table B-3. None of the models for continuous data provided an adequate fit to the 10- or 12-week OCT data (based on a goodness-of-fit p-value ≥ 0.1).

Table B-3. Model predictions for changes in serum OCT levels (nmol CO_2/mL) in male rats exposed to carbon tetrachloride for 10 and 12 weeks

Model	*p* value[a]	AIC for fitted model	BMD_{2X} (mg/kg-day)	$BMDL_{2X}$ (mg/kg-day)
10-Wk data				
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[e]	158.60	10.12	6.52
12-Wk data				
Linear[b]				
n = 7	0.04507	239.05	9.08	5.78
n = 9	0.03479	313.74	9.00	5.79
Polynomial[b,c]				
n = 7 (2nd degree)	0.0499	238.70	14.8	7.62
n = 9 (2nd degree)	0.02905	313.79	14.1	7.14
Power[d]				
n = 7	0.04347	238.93	15.4	7.37
n = 9	0.02376	314.14	14.8	6.80
Hill[d]				
n = 7	NA[e]	241.04	11.7	10.0
n = 9	NA[e]	316.31	11.7	6.60

[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]NA = not available; insufficient degrees of freedom.

Source: Bruckner et al. (1986).

Modeling results for ALT data are summarized in Table B-4. The power model provided an adequate fit of the 10-week ALT data; however, as shown in Table B-1, the SEM 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, relatively high uncertainty is associated with quantitative analysis using the 10-week ALT data set. The polynomial and power models provided adequate fits of the 12-week ALT data (based on a goodness-of-fit *p*-value ≥0.1). The polynomial model, which provided a better fit of the data using both an n = 7 and 9 (based on lower AIC values), gave an estimated BMD_{2X} of 13.0 mg/kg-day and a $BMDL_{2X}$ of 11.8 mg/kg-day. The values of the

BMD and BMDL were not sensitive to the value of n. Model outputs for the ALT data sets are provided at the end of this appendix.

Table B-4. Model predictions for changes in serum ALT levels (IU/mL) in male rats exposed to carbon tetrachloride for 10 and 12 weeks

Model	*p* value[a]	AIC for fitted model	BMD $_{2X}$ (mg/kg-d)	$BMDL_{2X}$ (mg/kg-d)
10-Wk data				
Linear[b]	<0.0001	291.27	33.05	0.0071
Polynomial (3rd degree)[b,c]	0.01022	123.31	13.66	12.7
Power[d]	0.1145	118.70	14.66	13.2
Hill[d]	NA[e]	120.70	NA[f]	NA[f]
12-Wk data				
Linear[b]				
n = 7	<0.0001	353.58	Failed	0.66
n = 9	<0.0001	454.34	Failed	0.53
Polynomial[b,c]				
n = 7 (3rd degree)	**0.5311**	**159.75**	**13.0**	**11.8**
n = 9 (3rd degree)	**0.631**	**212.94**	**13.0**	**11.8**
Power[d]				
n = 7	0.6561	160.72	12.9	11.9
n = 9	0.8388	214.13	12.9	11.8
Hill[d]				
n = 7	NA	162.72	10.88	Failed
n = 9	NA	216.13	11.8	Failed

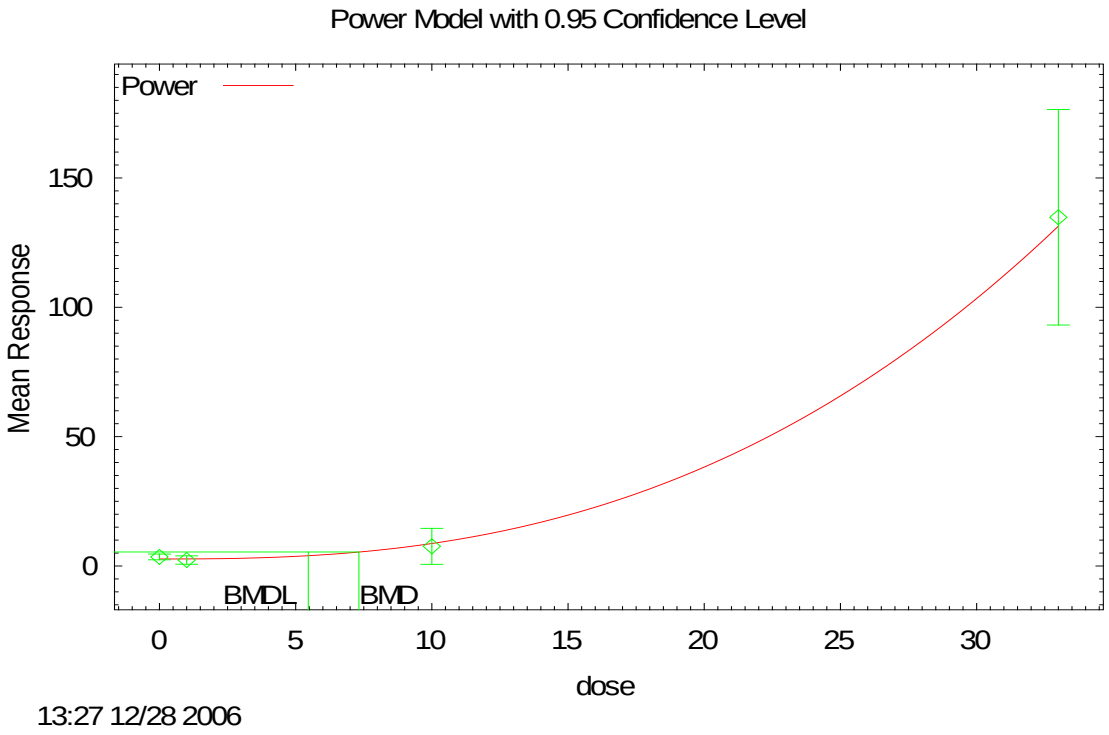
[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Betas restricted to ≥0.
[c]Insufficient degrees of freedom to fit higher degree polynomials.
[d]Power restricted to ≥1.
[e,f]NA = not available; insufficient degrees of freedom (BMD software could not generate a model output).

Source: Bruckner et al. (1986).

BMDS model output for SDH levels (10 weeks)

```
 ======================================================================
     Power Model. (Version: 2.6;  Date: 12/06/2005)
     Input Data File: G:\CARBON TET\BMD\RFD\SDH.(d)
     Gnuplot Plotting File:  G:\CARBON TET\BMD\RFD\SDH.plt
 ======================================================================

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
```


Power Model with 0.95 Confidence Level
Power
150
100
50
0
Mean Response
BMDL
BMD
0
5
10
15
20
25
30
dose
13:27 12/28 2006

BMDS model output for ALT levels (10 weeks)

```
 ================================================================
     Power Model. (Version: 2.6;  Date: 12/06/2005)
     Input Data File: G:\CARBON TET\BMD\RFD\ALT.(d)
     Gnuplot Plotting File:  G:\CARBON TET\BMD\RFD\ALT.plt

 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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 =       139449
                          rho =            0
                      control =           18
                        slope =      3.57427
                        power =      1.46475


          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha          rho      control        slope        power

    alpha            1        -0.97        -0.11        -0.51         0.64

      rho        -0.97            1        0.068         0.56         -0.7

   control        -0.11        0.068            1        -0.34         0.27

     slope        -0.51         0.56        -0.34            1        -0.98

     power         0.64         -0.7         0.27        -0.98            1


                                Parameter Estimates

                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha      0.000240402      0.000362454        -0.000469995           0.0009508
           rho          3.29767         0.461545             2.39305             4.20228
       control          19.0745         0.631297             17.8372             20.3119
         slope      0.000186379       0.00024718        -0.000298084         0.000670842
         power          4.29657         0.473086             3.36933              5.2238


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0     5         18         19.1          2.2            2           -1.2
    1     5         20         19.1          2.2            2           1.03
```

10	5	23	22.8	2.2	2.68	0.197
33	5	617	643	747	661	-0.0863

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	-126.223088	5	262.446176
A2	-52.674727	8	121.349453
A3	-53.102306	6	118.204612
fitted	-54.347748	5	118.695497
R	-131.425960	2	266.851919

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	157.502	6	<.0001
Test 2	147.097	3	<.0001
Test 3	0.855159	2	0.6521
Test 4	2.49088	1	0.1145

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 greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

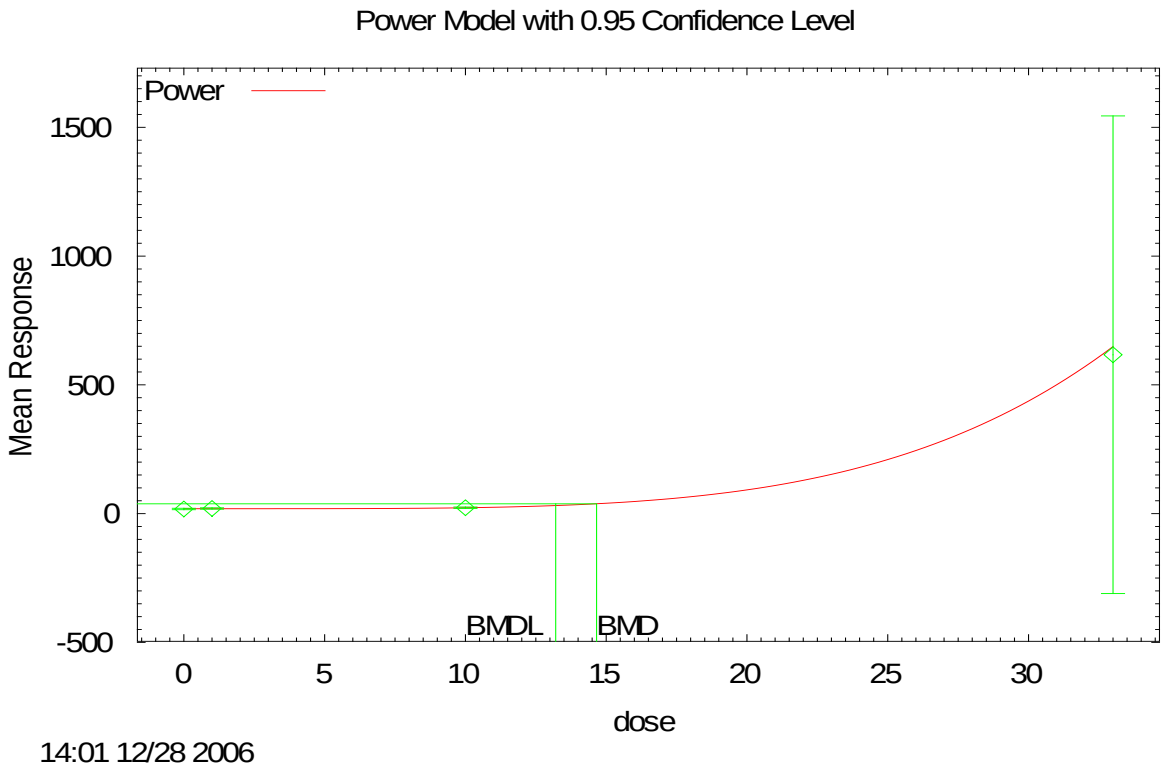
Specified effect = 1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 14.6575

BMDL = 13.205


Power Model with 0.95 Confidence Level
Power
1500
1000
500
0
-500
Mean Response
BMDL
BMD
0
5
10
15
20
25
30
dose
14:01 12/28 2006

BMDS model output for ALT levels (12 weeks)

```
 ======================================================================
     Polynomial Model. (Version: 2.7;  Date: 12/06/2005)
     Input Data File: G:\CARBON TET\BMD\RFD\BRUCKNER 12-WK DATA\ALT-12N7.(d)
     Gnuplot Plotting File:  G:\CARBON TET\BMD\RFD\BRUCKNER 12-WK DATA\ALT-12N7.plt

 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose(mg/kg-d)
   The polynomial coefficients are restricted to be positive
   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 =            1
                           rho =            0
                        beta_0 =           20
                        beta_1 =            0
                        beta_2 =            0
                        beta_3 =            0


                                  Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          alpha      8.5932e-005     8.29597e-005       -7.66661e-005          0.00024853
            rho          3.65721         0.244569             3.17787             4.13656
         beta_0          19.3388         0.558043             18.2451             20.4325
         beta_1                0               NA
         beta_2                0               NA
         beta_3       0.00876918      0.00160884          0.00561592           0.0119224

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


           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 )

                  alpha          rho       beta_0       beta_3

     alpha            1            0            0            0

       rho            0            1            0            0

    beta_0            0            0            1            0

    beta_3            0            0            0            1
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	7	20	19.3	0.8	2.09	0.838
1	7	19	19.3	2.6	2.09	-0.44
10	7	27	28.1	5.3	4.13	-0.709
33	7	502	334	357	383	1.16

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	-157.028066	5	324.056132
A2	-69.790014	8	155.580028
A3	-75.241703	6	162.483407
fitted	-75.874569	4	159.749137
R	-170.807125	2	345.614250

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	202.034	6	<.0001
Test 2	174.476	3	<.0001
Test 3	10.9034	2	0.004289
Test 4	1.26573	2	0.5311

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

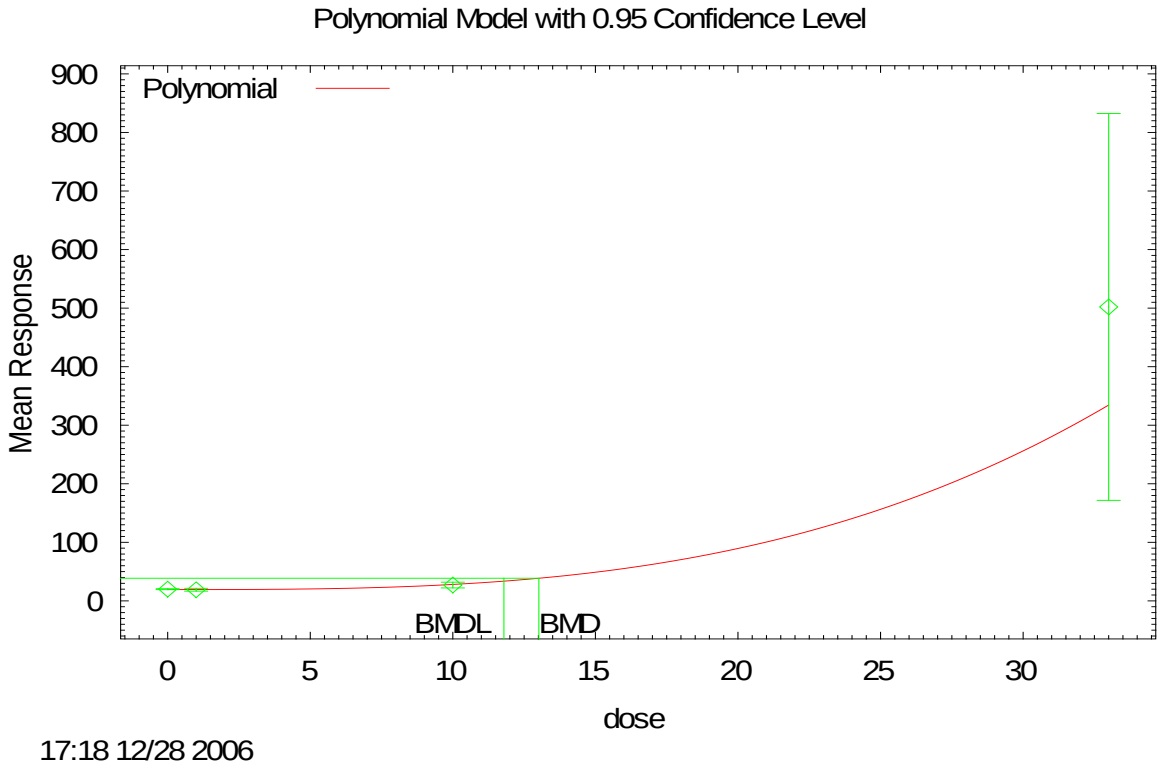
The p-value for Test 3 is less than .1. You may want to consider a different variance model

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

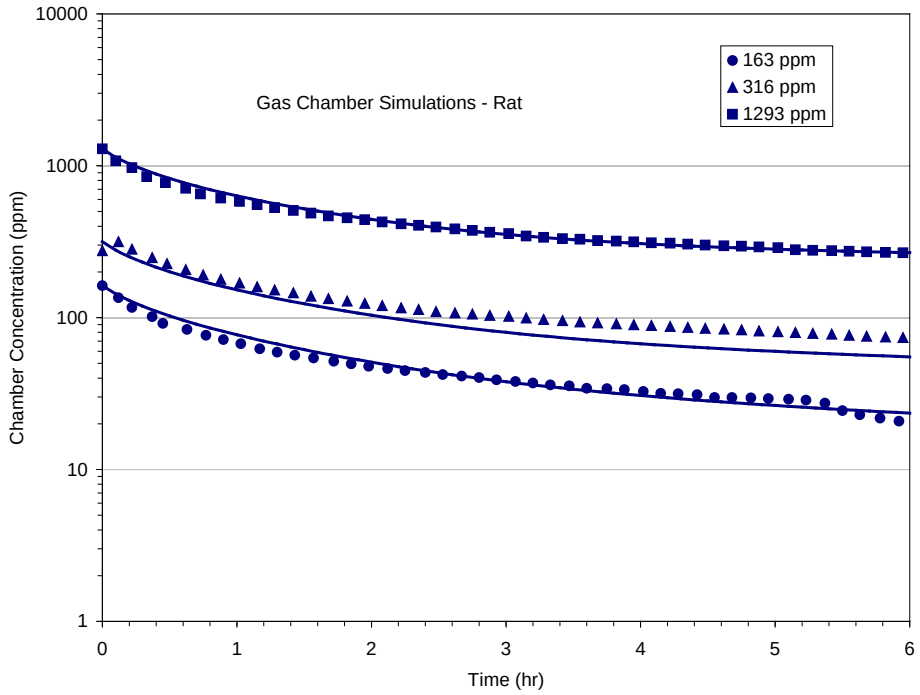
```
Risk Type        =     Relative risk
Confidence level =          0.95
             BMD =       13.0164

            BMDL =        11.791
```



APPENDIX C. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING

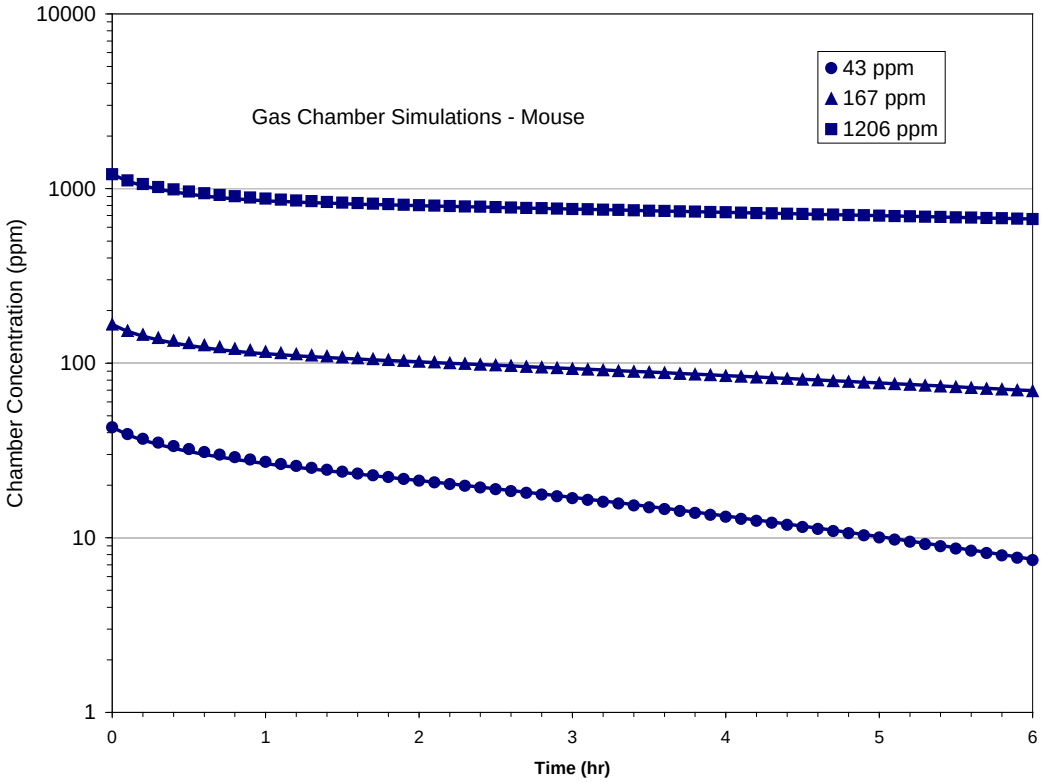
C.1. Thrall et al. (2000) and Paustenbach et al. (1988) PBPK Models (rat, mouse, human)

Detailed summaries of the Thrall et al. (2000) and Paustenbach et al. (1988) PBPK models appear in Section 3.5. Source code for the rat, mouse, and hamster models (reported in Thrall et al., 2000) in ACSL was provided to Syracuse Research Corporation 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 carbon tetrachloride concentrations as a function of exposure times match those reported in Figure 2 of Thrall et al. (2000).



Data points are observations (provided by Thrall) for exposures for three rats per chamber (BW, 0.24 kg); lines are simulations. The nonspecific 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-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 seven mice per chamber (BW, 0.024 kg); lines are simulations. The nonspecific 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.

Figure C-2. Comparison of observed and predicted chamber carbon tetrachloride concentrations in closed chamber studies conducted in mice.

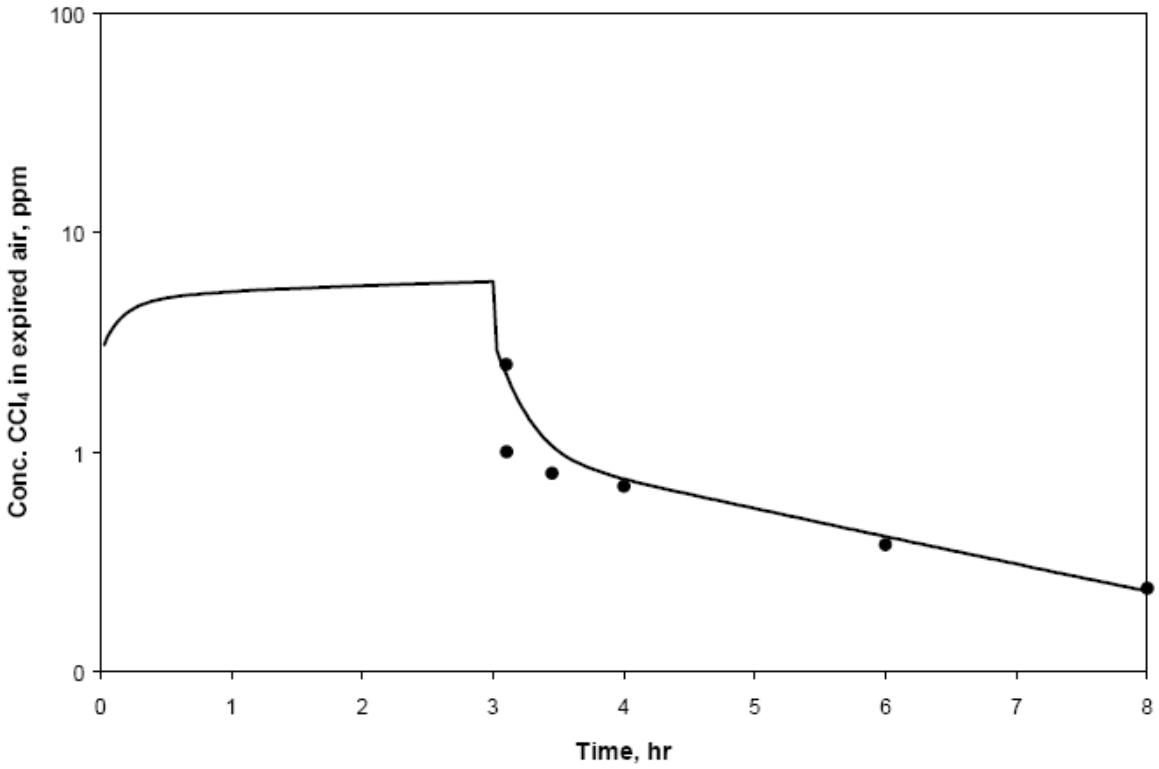
As noted above, Thrall et al. (2000) compared model predictions for the rat and mouse with experimental data collected over a 48-hour period following a 4-hour nose-only inhalation exposure to 20 ppm of [^{14}C]-carbon tetrachloride. This comparison of PBPK model-predicted and experimentally-observed 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 ± 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).

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



Sources: 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 min (data from Stewart et al., 1961).

Parameter values for the rat and human models used in the Thrall et al. (2000) and Paustenbach et al. (1988) 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[e]
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 $BW^{0.74}$)	15[c,d]	15[c]
QPC	Alveolar ventilation rate (L/hour-kg $BW^{0.74}$)	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[f]	2.64[c]
PL	Liver:blood partition coefficient	3.14[f]	3.14[f]
PF	Fat:blood partition coefficient	79.42[f]	79.42[f]
PS	Slowly-perfused partition coefficient	1[f]	1[f]
PR	Readily-perfused partition coefficient	3.14[g]	3.14[g]
V_{maxC}	Maximum rate of metabolism (mg/hour-kg $BW^{0.7}$)	0.4[f],0.65[c]	0.4[f], 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 Thrall et al. (2000) and Paustenbach et al. (1988) 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 hrs/d, 5 d/wk (based on Nagano et al., 2007b; JBRC, 1998).
[c]Paustenbach et al. (1988).
[d]Thrall et al. (2000).
[e]Adjusted from reported value of 0.1 in Paustenbach et al. (1988).
[f]Gargas et al. (1986).
[g]Partition coefficient for readily-perfused is assumed to be equal to that of liver.

Table C-3. Parameter values for mouse models[a]

Parameter	Definition	Thrall et al. (2000)	Fisher et al. (2004)
BW	BW (kg)	0.036[b]	—
VLC	Liver volume (fraction of body)	0.04[c]	0.04[d]
VFC	Fat volume (fraction of body)	0.04[c]	0.04[d]
VSC	Slowly-perfused tissue volume (fraction of body)	0.78[c]	0.69[d]
VRC	Richly-perfused tissue volume (fraction of body)	0.05[c]	0.14[d]
QCC	Cardiac output (L/hr-kg BW^{SF})[e,f]	28[c,e]	30[d,f]
QPC	Alveolar ventilation rate (L/hr-kg BW^{SF})[e,f]	28[c,e]	30[d,f]
QLC	Liver blood flow (fraction of cardiac output)	0.24[c]	0.24[d]
QFC	Fat blood flow (fraction of cardiac output)	0.05[c]	0.05[d]
QSC	Slowly-perfused blood flow (fraction of cardiac output)	0.19[c]	0.17[d]
QRC	Richly-perfused blood flow (fraction of cardiac output)	0.52[c]	0.54[d]
PB	Blood:air partition coefficient	7.83[g]	3.8[h]
PL	Liver:blood partition coefficient	2.08[g]	4.8[h]
PF	Fat:blood partition coefficient	23.0[g]	91.4[h]
PS	Slowly-perfused partition coefficient	0.61[g]	2.5[h]
PR	Richly-perfused partition coefficient	2.08[g]	4.8[h]
V_{maxC}	Maximum rate of metabolism (mg/hr-kg BW^{SF})[i,j]	0.79[g,i]	1[h,j]
K_{mX}	Michaelis-Menten coefficient for metabolism (mg/L)	0.46[g]	0.3[h]
K1	GI absorption rate coefficient C1-liver (hr^{-1})	—	0.4, 10[k]
K2	GI absorption rate coefficient C1-C2 (hr^{-1})	—	2[k]
K2	GI absorption rate coefficient C2-liver (hr^{-1})	—	0.05[l]

[a]See Thrall et al. (2000) and Paustenbach et al. (1988) 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]Brown et al., 1997
[e]SF, scaling factor; QC (L/hr)=QCC*$BW^{0.74}$; QP (L/hr)=QPC*$BW^{0.74}$
[f]Scaling factor; QC (L/hr)=QCC*$BW^{0.75}$; QP (L/hr)=QPC*$BW^{0.75}$
[g]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.
[h]Fisher et al. (2004) vial equilibrium measurements
[i]SF, scaling factor; $V_{max}=V_{BmaxC}*BW^{0.70}$
[j]$V_{max} = V_{BmaxC}*BW^{0.75}$
[k]Fisher et al. (2004) fit to closed chamber data.
[l]Fisher et al. (2004) fit to 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 Physiologically Based Pharmacokinetic 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. 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/hour/kg$^{0.75}$ (Fisher et al., 2004) compared to 0.46 mg/L, 0.79 mg/hour/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/hour (Thrall et al., 2000) and 0.082 mg/hour (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.

C.3. Physiologically Based Pharmacokinetic 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 Thrall et al. (2000), Paustenbach et al. (1988), 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 RGIL (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 5.4.3.4.

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/hour/kg BW$^{0.70}$) are provided in Tables C-4 to C-11. Figures C-4 to C-11 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-12 and C-13).

Table C-4. Interspecies conversion factors based on MCA dose metric (V_{maxC} = 0.40)

EC (ppm)	EC (mg/m^3)	MCA (µmol/L)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg-d/ 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

Table C-5. Interspecies conversion factors based on MCA dose metric (V_{maxC} = 0.65)

EC (ppm)	EC (mg/m^3)	MCA (µmol/L)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg-d/ 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

Table C-6. Interspecies conversion factors based on MCA dose metric (V_{maxC} = 1.49)

EC (ppm)	EC (mg/m^3)	MCA (µmol/L)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg-d/ 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

Table C-7. Interspecies conversion factors based on MCA dose metric (V_{maxC} = 1.70)

EC (ppm)	EC mg/m^3)	MCA (µmol/L)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MCA (mg/m^3/ µmol/L)	RGIL/MCA (mg/kg-d/ 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

Table C-8. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 0.40)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-day)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/ kg liver)	RGIL/MRAMKL(mg/kg-d/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	1,006	16.53	47.63	0.04732	60.873	2.880
170	1,069	16.63	50.60	0.04732	64.318	3.043
180	1,132	16.71	53.57	0.04731	67.765	3.206
190	1,195	16.78	56.54	0.04731	71.213	3.369
200	1,258	16.85	59.52	0.04731	74.662	3.532
210	1,321	16.91	62.49	0.04730	78.112	3.695
220	1,384	16.97	65.46	0.04730	81.563	3.858
230	1,447	17.02	68.43	0.04730	85.015	4.021
240	1,510	17.06	71.40	0.04730	88.468	4.184
250	1,573	17.11	74.37	0.04729	91.921	4.347
260	1,636	17.15	77.34	0.04729	95.375	4.510
270	1,698	17.19	80.31	0.04728	98.830	4.673
280	1,761	17.22	83.28	0.04728	102.284	4.836

Table C-8. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 0.40)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-day)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/ kg liver)	RGIL/MRAMKL(mg/kg-d/µmol/hr/kg liver)
290	1,824	17.25	86.24	0.04728	105.740	4.999
300	1,887	17.28	89.21	0.04728	109.195	5.162

Table C-9. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 0.65)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/ kg liver)	RGIL/MRAMKL (mg/kg-d/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	1,006	26.78	47.63	0.04732	37.578	1.778
170	1,069	26.94	50.60	0.04732	39.691	1.878
180	1,132	27.08	53.57	0.04731	41.805	1.978
190	1,195	27.21	56.55	0.04731	43.922	2.078
200	1,258	27.33	59.52	0.04731	46.039	2.178
210	1,321	27.43	62.49	0.04730	48.158	2.278
220	1,384	27.52	65.46	0.04730	50.278	2.378
230	1,447	27.61	68.44	0.04730	52.398	2.479
240	1,510	27.69	71.40	0.04730	54.519	2.579
250	1,573	27.76	74.38	0.04730	56.641	2.679
260	1,636	27.83	77.34	0.04729	58.764	2.779
270	1,698	27.89	80.32	0.04729	60.887	2.879
280	1,761	27.95	83.28	0.04728	63.010	2.979

Table C-9. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 0.65)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/ kg liver)	RGIL/MRAMKL (mg/kg-d/µmol/hr/ kg liver)
290	1,824	28.01	86.25	0.04728	65.134	3.080
300	1,887	28.06	89.22	0.04728	67.259	3.180

Table C-10. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 1.49)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/ kg liver)	RGIL/MRAMKL (mg/kg-d/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	1,006	60.67	47.64	0.04733	16.590	0.785
170	1,069	61.11	50.61	0.04733	17.499	0.828
180	1,132	61.50	53.59	0.04733	18.410	0.871
190	1,195	61.85	56.56	0.04732	19.323	0.914
200	1,258	62.17	59.53	0.04732	20.238	0.958
210	1,321	62.45	62.51	0.04732	21.154	1.001
220	1,384	62.70	65.47	0.04731	22.071	1.044
230	1,447	62.93	68.45	0.04731	22.989	1.088
240	1,510	63.14	71.42	0.04730	23.909	1.131
250	1,573	63.34	74.39	0.04730	24.829	1.175
260	1,636	63.52	77.36	0.04730	25.750	1.218
270	1,698	63.68	80.33	0.04730	26.671	1.261
280	1,761	63.83	83.30	0.04729	27.593	1.305

Table C-10. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 1.49)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/ kg liver)	RGIL/MRAMKL (mg/kg-d/µmol/hr/ kg liver)
290	1,824	63.97	86.27	0.04729	28.516	1.349
300	1,887	64.10	89.24	0.04729	29.439	1.392

Table C-11. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 1.70)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/kg liver)	RGIL/MRAMKL (mg/kg-d/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	1,006	68.98	47.64	0.04733	14.591	0.691
170	1,069	69.51	50.61	0.04733	15.383	0.728
180	1,132	69.99	53.59	0.04733	16.179	0.766
190	1,195	70.40	56.56	0.04733	16.976	0.803
200	1,258	70.78	59.53	0.04732	17.775	0.841
210	1,321	71.11	62.51	0.04732	18.576	0.879
220	1,384	71.42	65.48	0.04731	19.378	0.917
230	1,447	71.69	68.45	0.04731	20.181	0.955
240	1,510	71.94	71.42	0.04731	20.985	0.993
250	1,573	72.17	74.39	0.04731	21.790	1.031
260	1,636	72.38	77.36	0.04730	22.596	1.069
270	1,698	72.57	80.34	0.04730	23.403	1.107
280	1,761	72.75	83.30	0.04730	24.210	1.145

Table C-11. Interspecies conversion factors based on MRAMKL dose metric (V_{maxC} = 1.70)

EC (ppm)	EC (mg/m^3)	MRAMKL (µmol/hr/kg liver)	RGIL (mg/kg-d)	RGIL/EC (mg/kg-d/ mg/m^3)	EC/MRAMKL (mg/m^3/µmol/hr/kg liver)	RGIL/MRAMKL (mg/kg-d/µmol/hr/ kg liver)
290	1,824	72.92	86.28	0.04730	25.017	1.183
300	1,887	73.07	89.24	0.04729	25.825	1.221

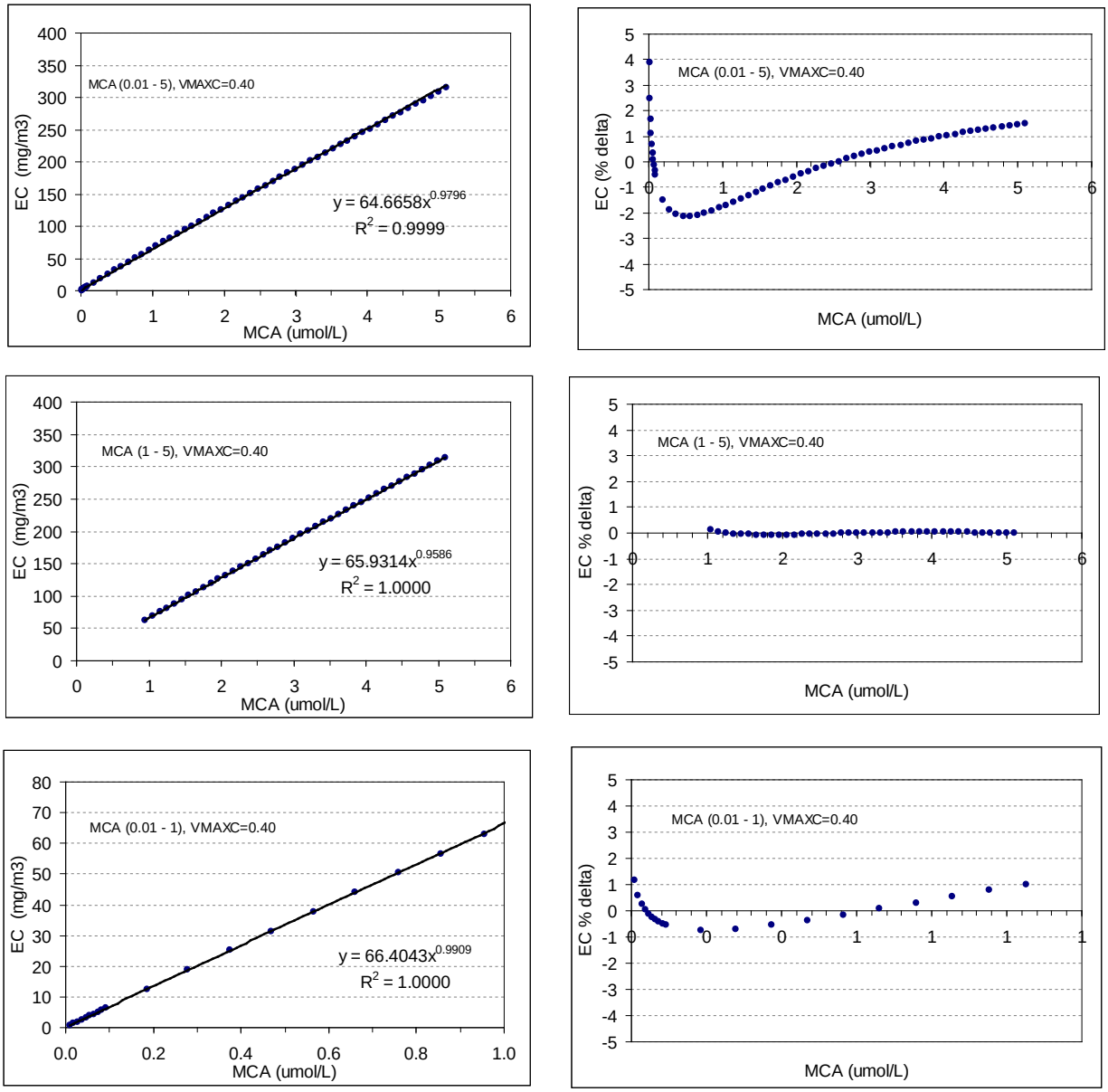


Figure C-4. Relationship between internal dose metric MCA and equivalent EC (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 0.40 mg/hour/kg $BW^{0.70}$.

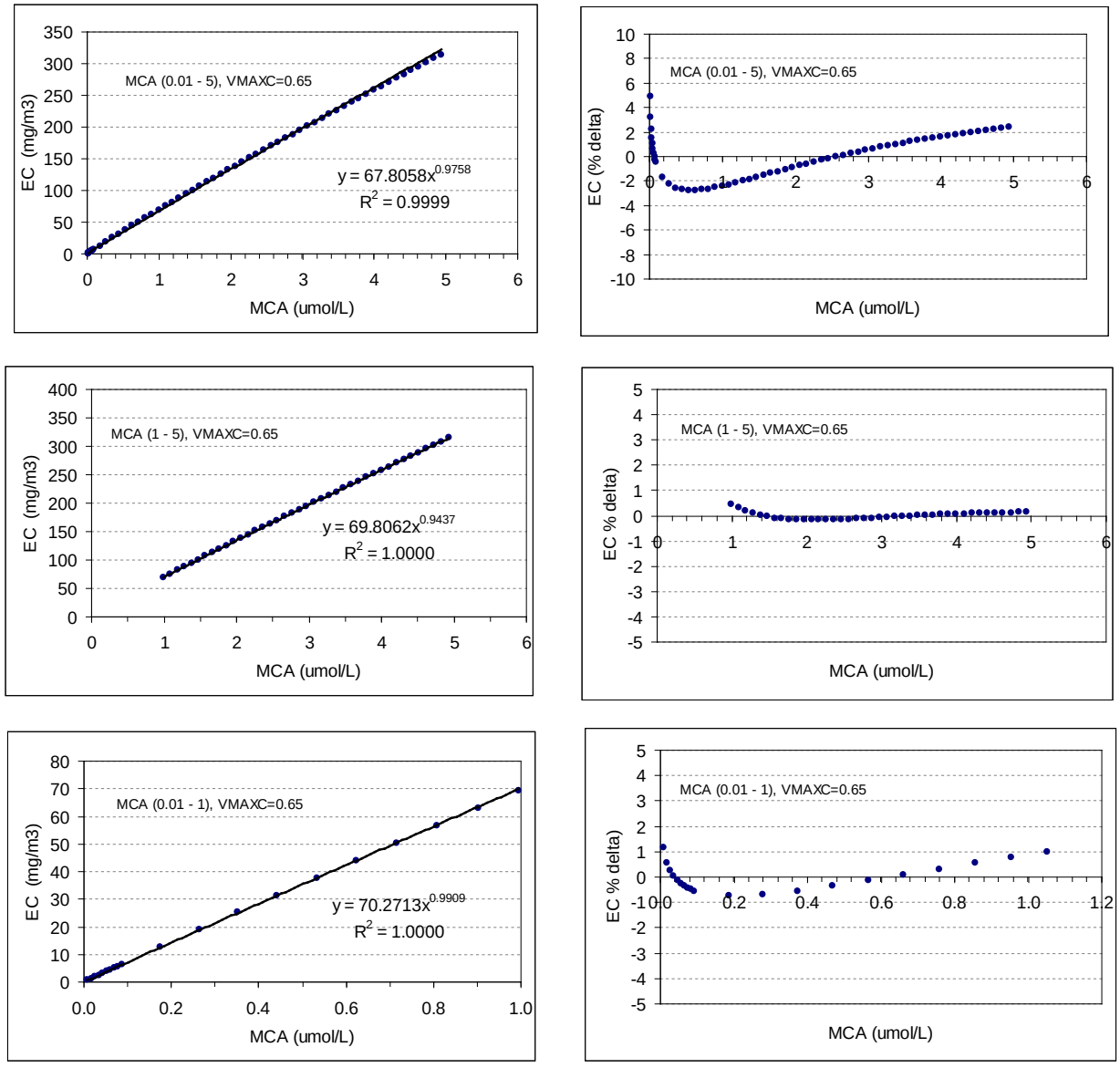


Figure C-5. Relationship between internal dose metric MCA and equivalent EC (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 0.65 mg/hour/kg $BW^{0.70}$.

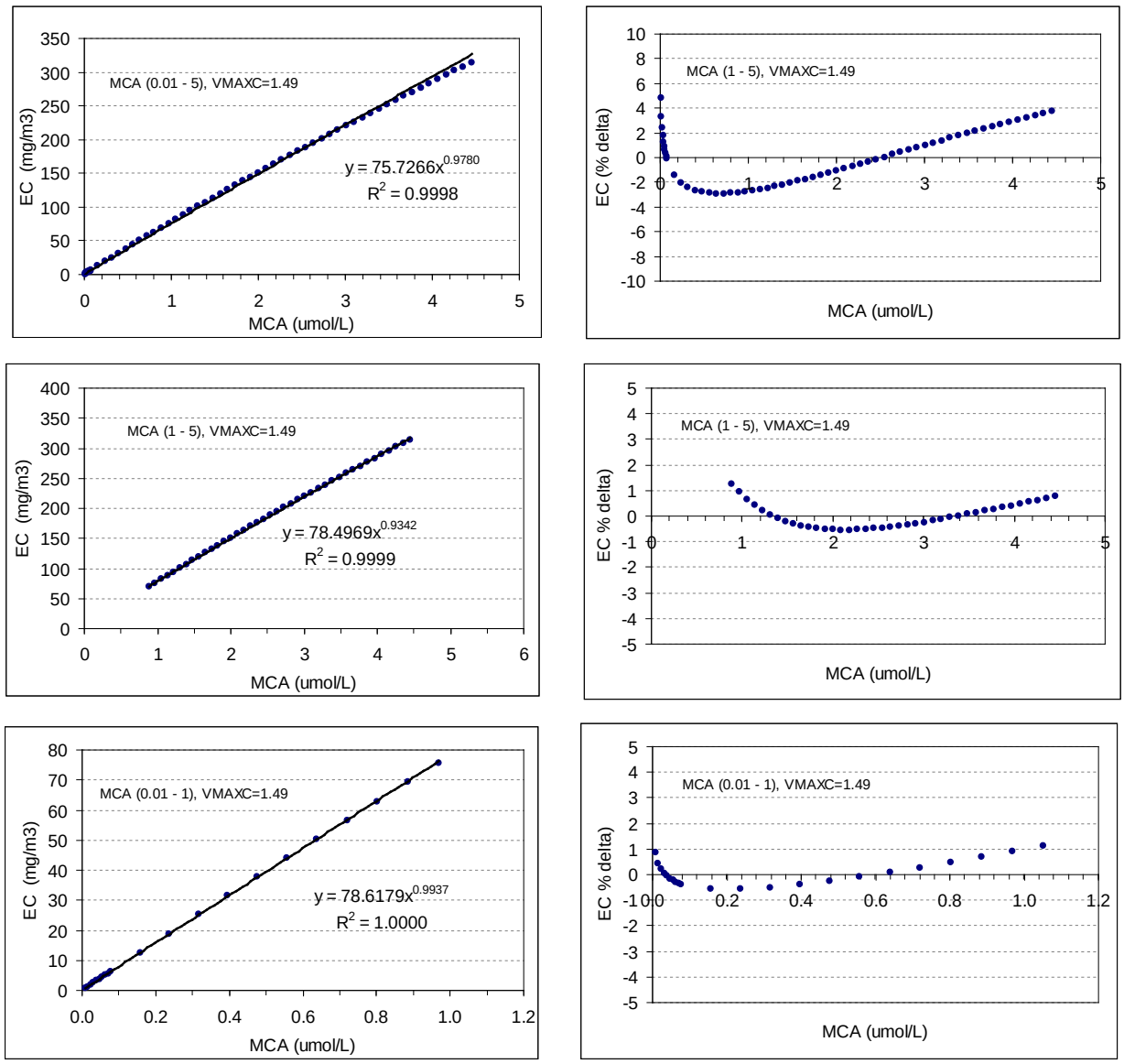


Figure C-6. Relationship between internal dose metric MCA and equivalent EC (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 1.49 mg/hour/kg $BW^{0.70}$.

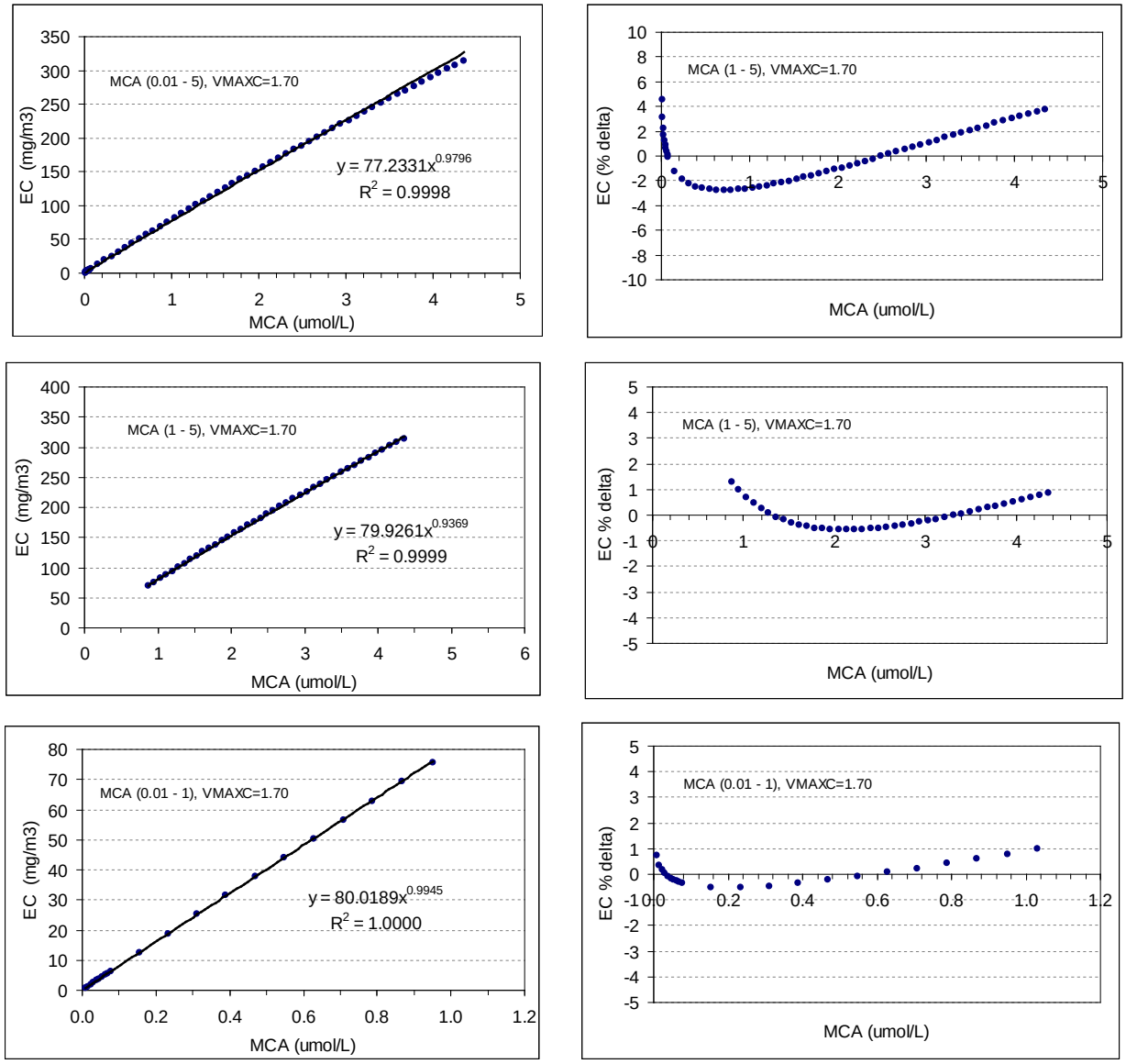


Figure C-7. Relationship between internal dose metric MCA and equivalent EC (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 1.70 mg/hour/kg $BW^{0.70}$.

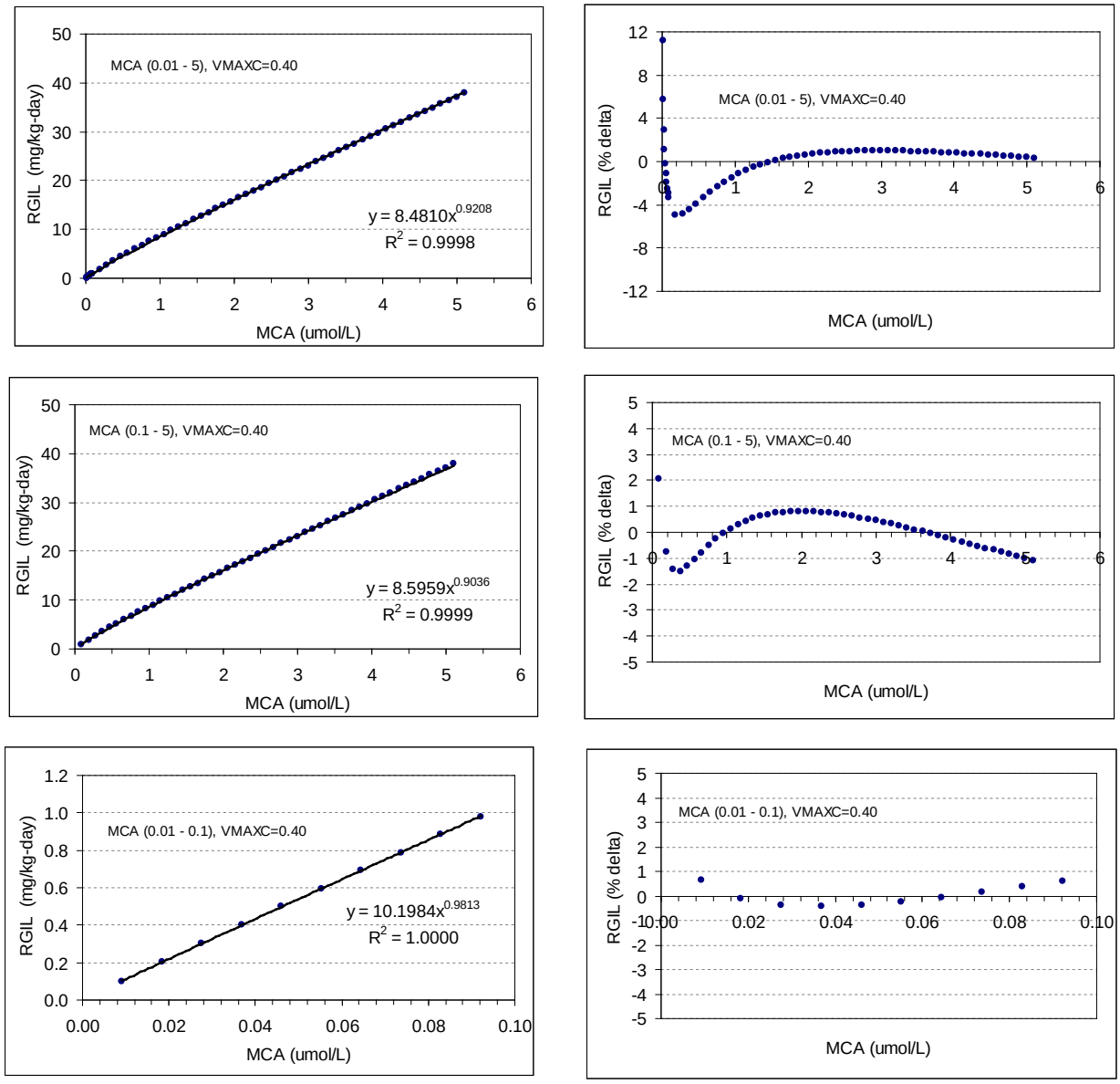


Figure C-8. Relationship between internal dose metric MCA and equivalent RGIL (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 0.40 mg/hour/kgBW$^{0.70}$.

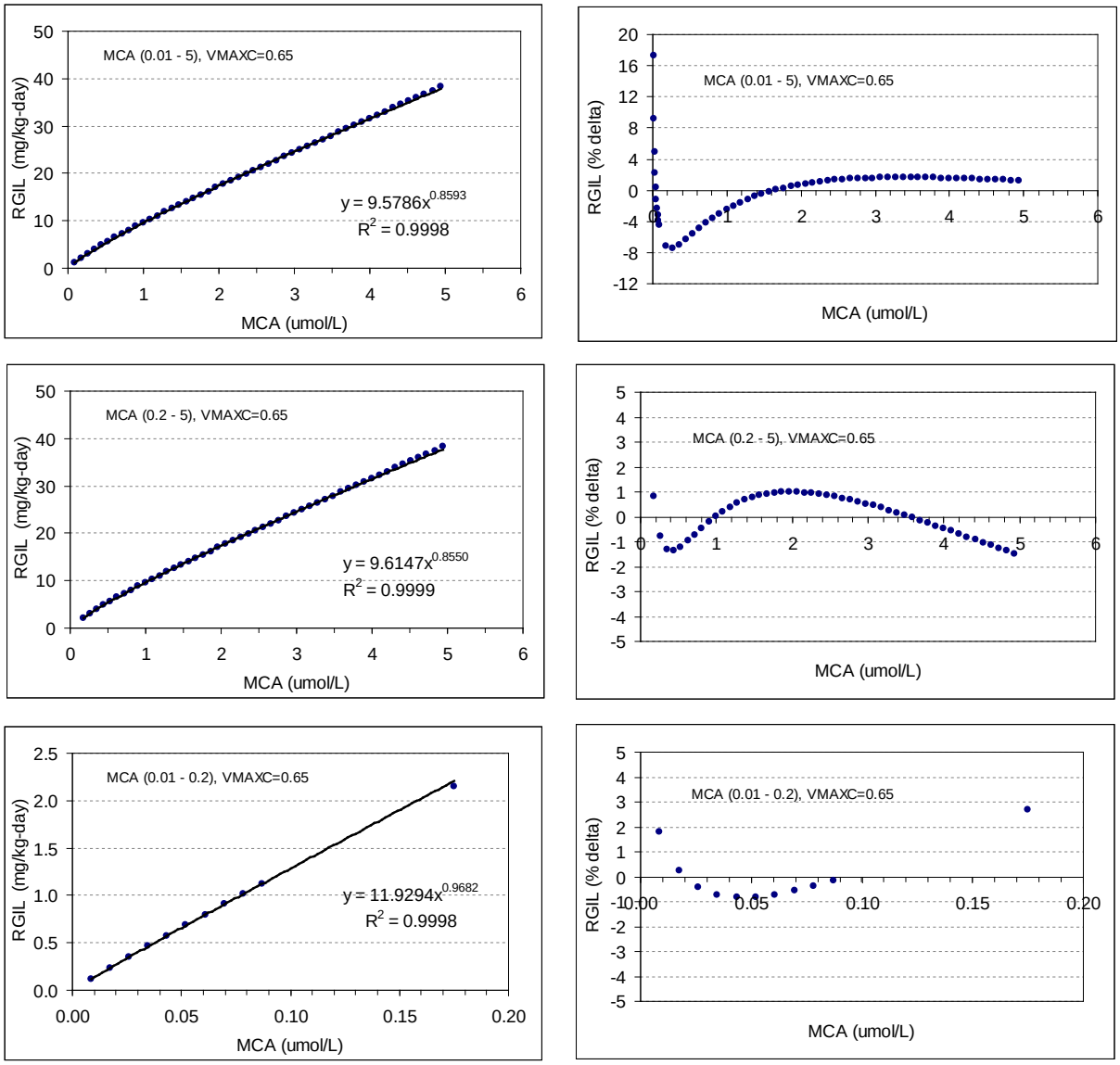


Figure C-9. Relationship between internal dose metric MCA and equivalent RGIL (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 0.65 mg/hour/kg $BW^{0.70}$.

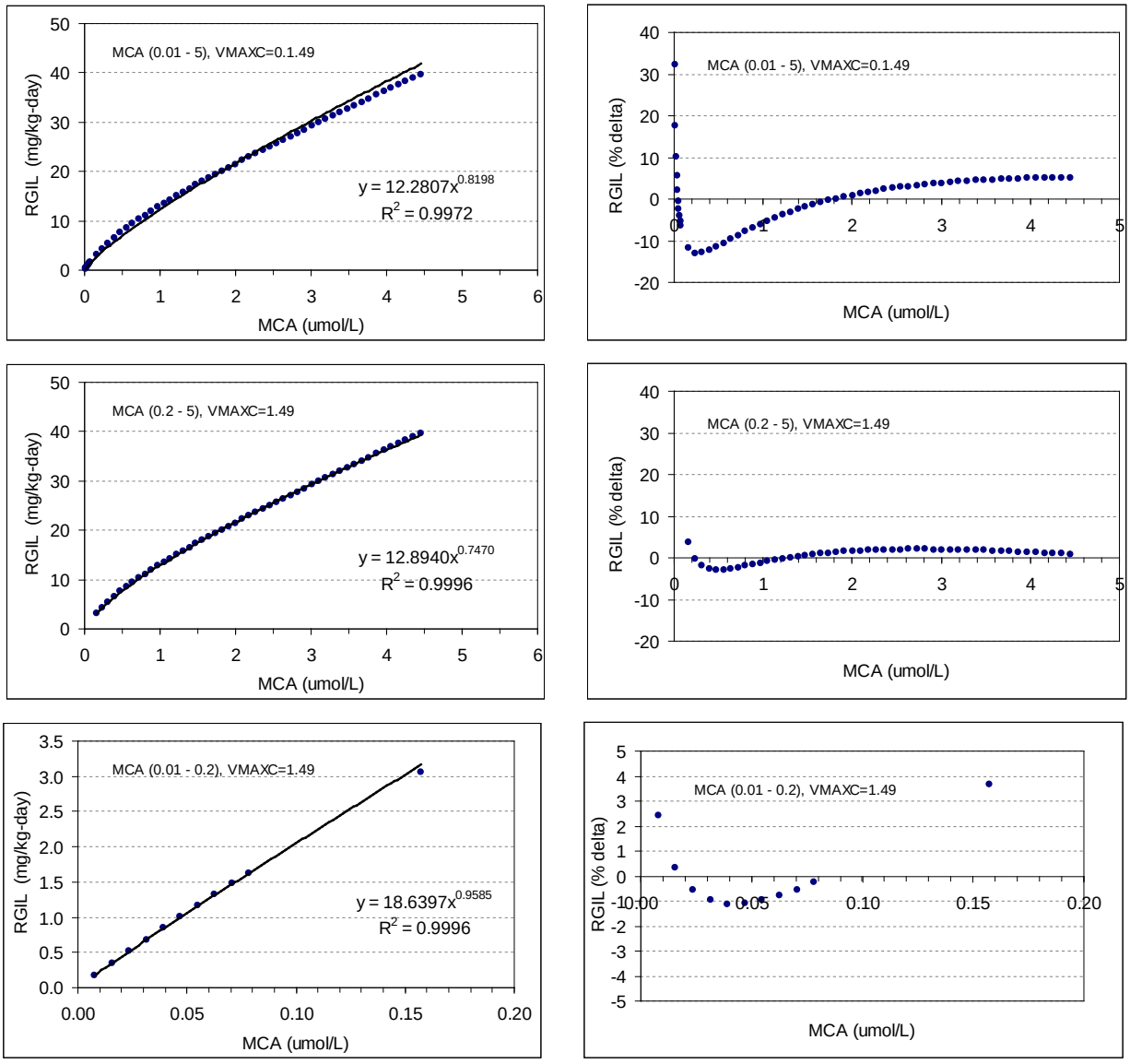


Figure C-10. Relationship between internal dose metric MCA and equivalent RGIL (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 1.49 mg/hour/kg $BW^{0.70}$.

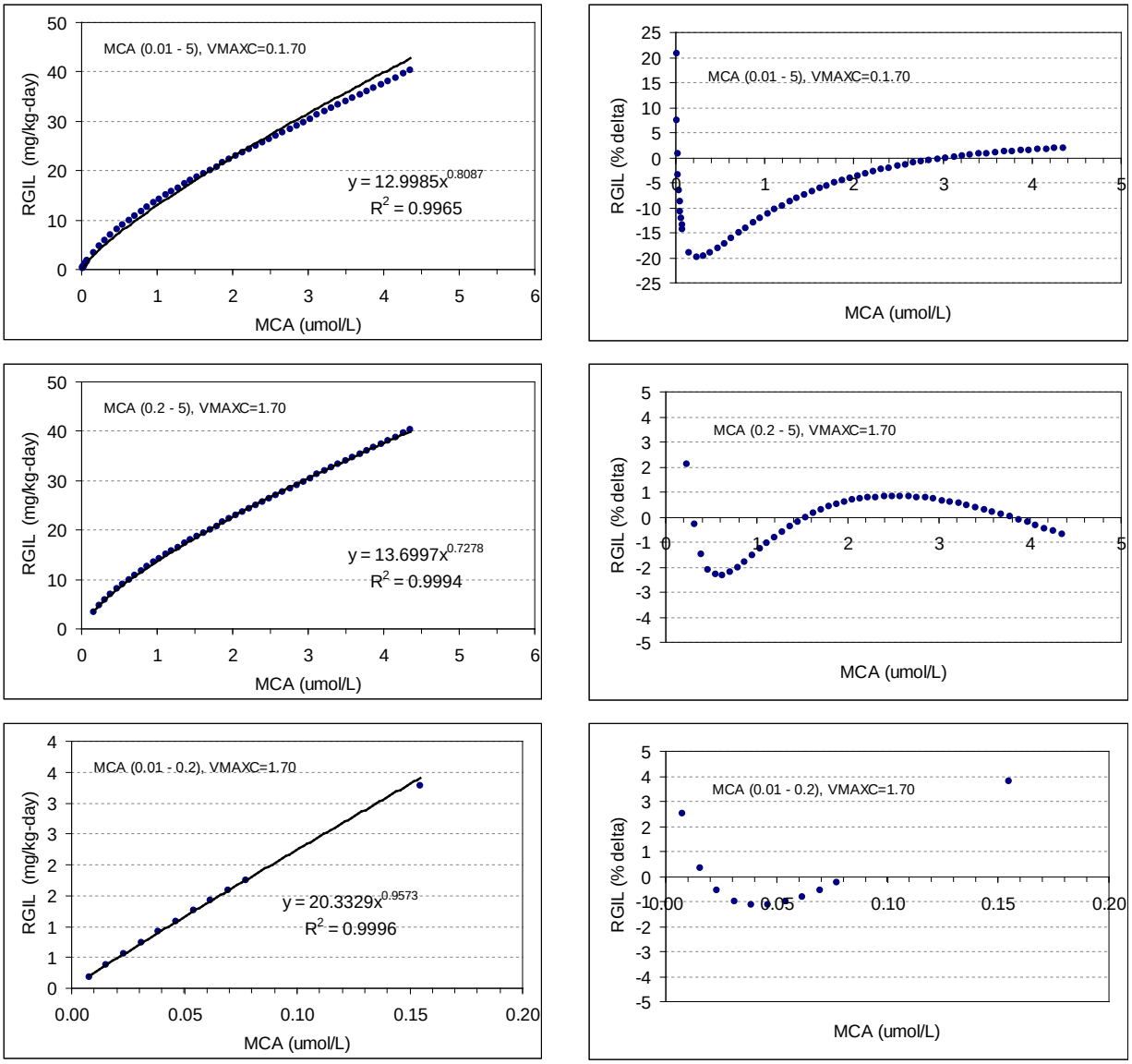


Figure C-11. Relationship between internal dose metric MCA and equivalent RGIL (left panel) and values for percent delta for trend lines (right panel). V_{maxC} = 1.70 mg/hour/kg $BW^{0.70}$.

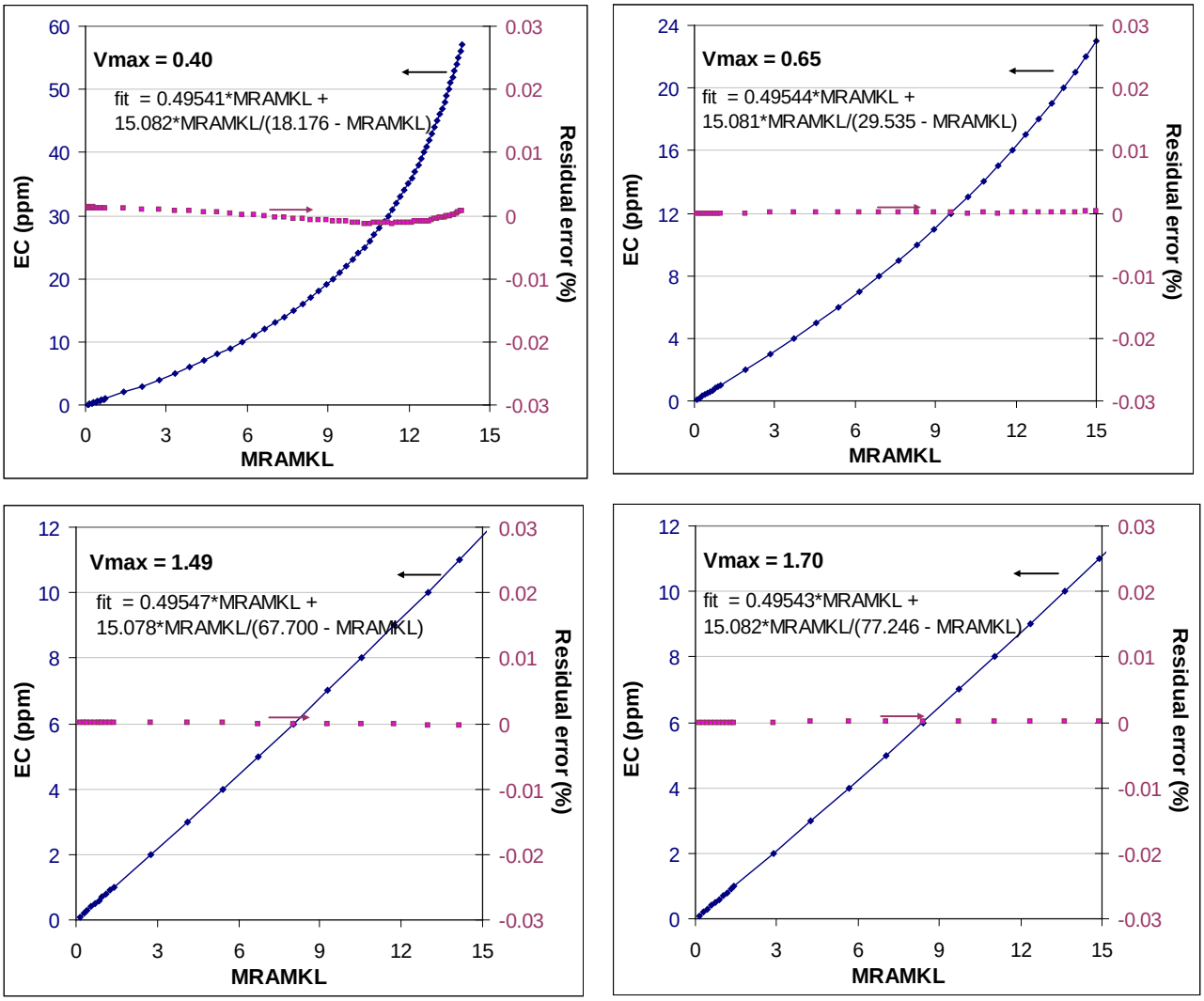


Figure C-12. Relationship between internal dose metric MRAMKL and equivalent EC and values for percent delta for trend lines.

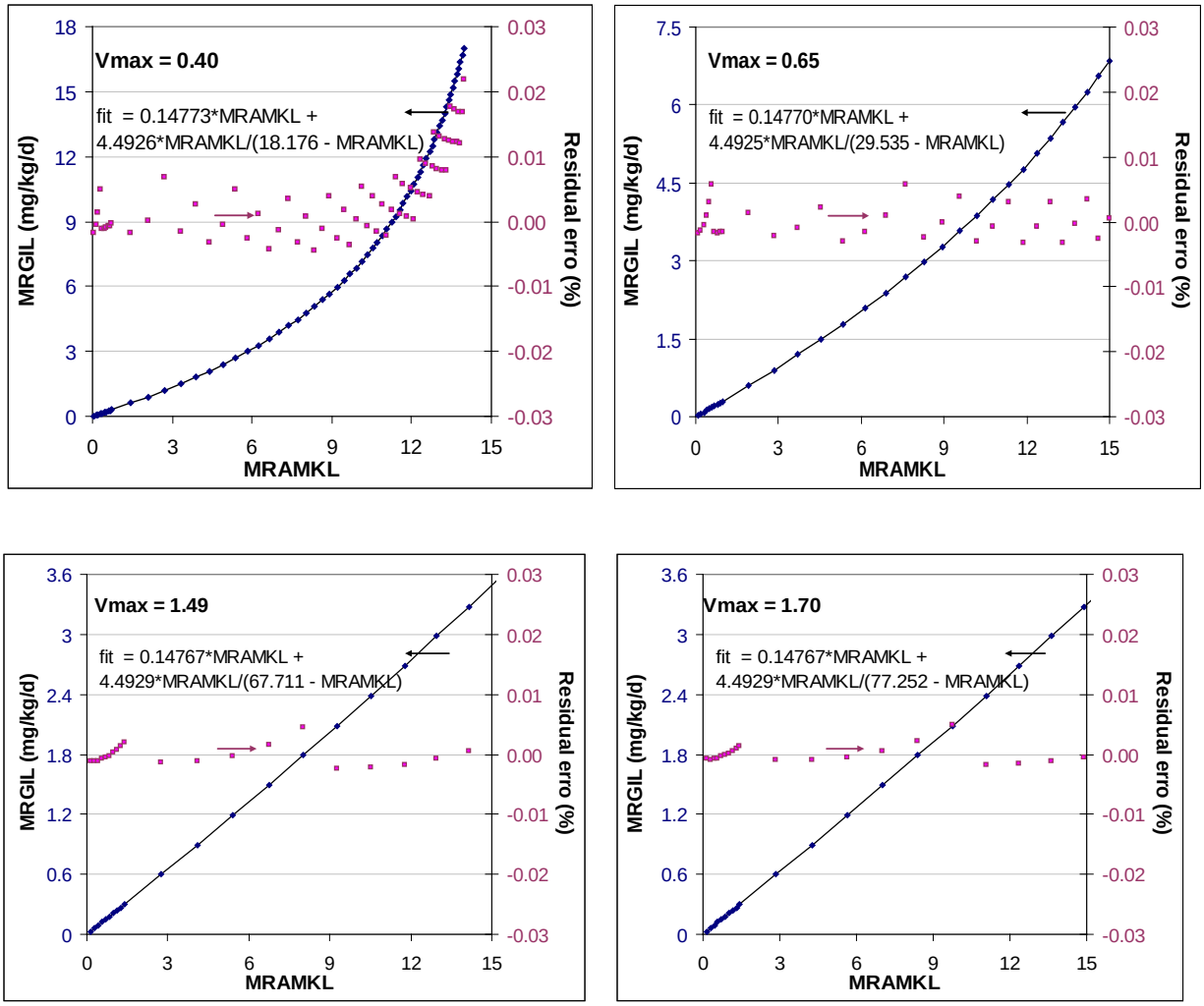


Figure C-13. Relationship between internal dose metric MRAMKL and equivalent RGIL and values for percent delta for trend lines.

C.4. Sensitivity Analysis

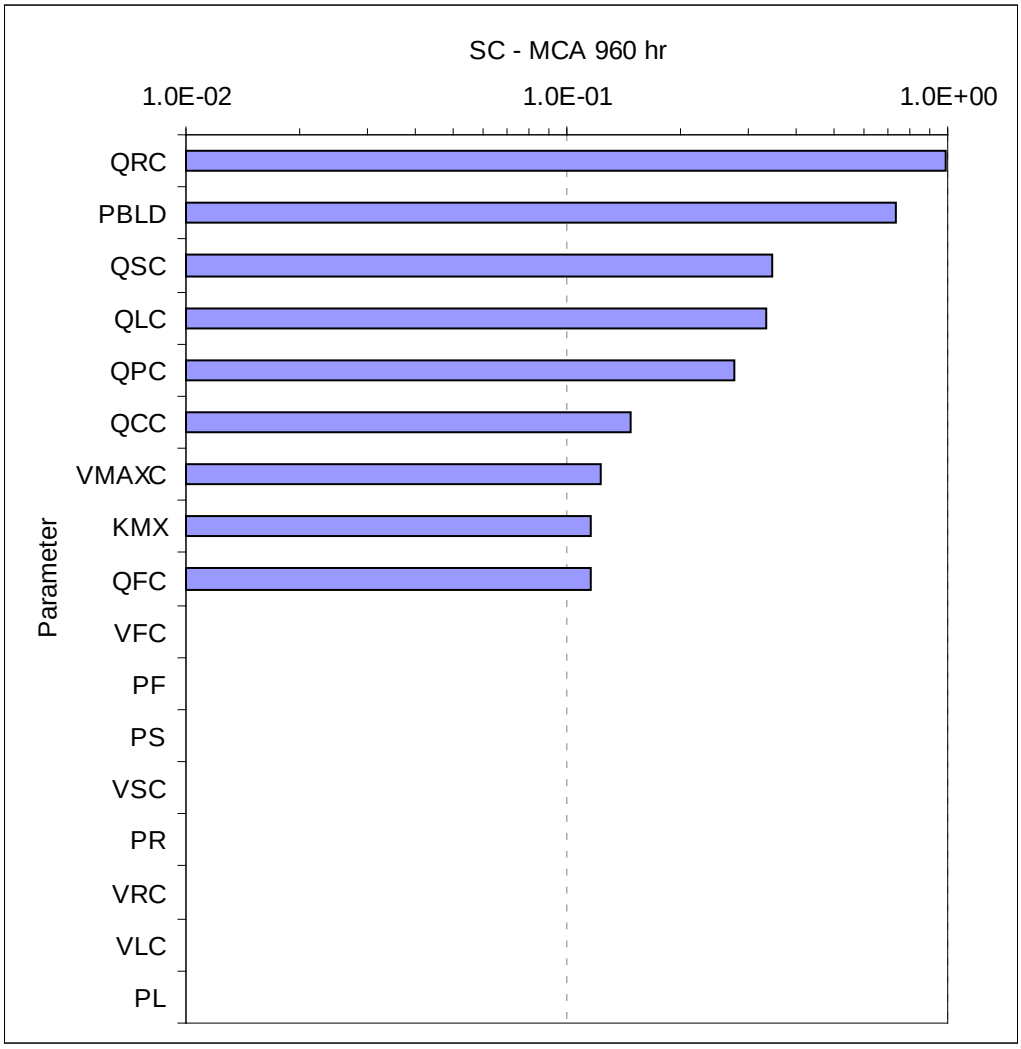
Univariate sensitivity analysis consisted of running the model after perturbing values for single parameters by a factor of 0.01, in the up and down directions. Parameter sensitivities were assessed from comparison of standardized sensitivity coefficients:

$$SC = f'(x) = \frac{f(x+\Delta x) - f(x-\Delta)}{2\Delta x} \cdot \frac{x}{f(x)}$$

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-14 and C-15 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.

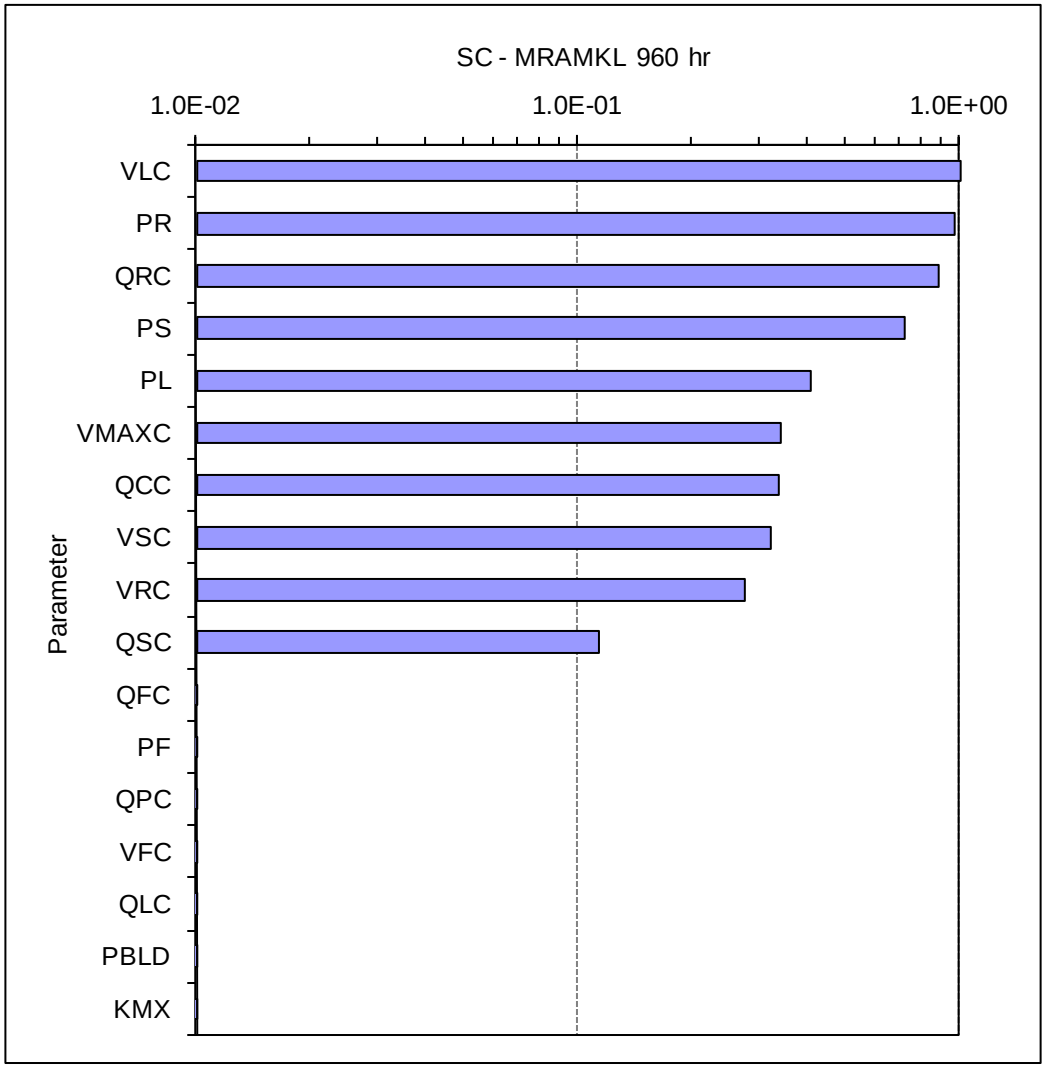
Table C-12. Sensitive parameters (indicated with +) in the human model

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/hr-kg BW)	+	+
K_m	Michaelis-Menten coefficient for metabolism (mg/L)	+	



Absolute values of coefficients ≥0.01 are shown. The simulation was of a continuous exposure to 2.5 ppm for 980 hrs (rank order of sensitivity coefficients was not dependent on exposure time).

Figure C-14. 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 hrs (rank order of sensitivity coefficients was not dependent on exposure time).

Figure C-15. Standardized sensitivity coefficients for the MRAMKL dose metric (average rate of metabolism of carbon tetrachloride µmol/hour/kg liver) simulated with the human carbon tetrachloride PBPK model.

APPENDIX D. BENCHMARK DOSE MODELING FOR DERIVING THE REFERENCE CONCENTRATION

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)
Exposure concentrations modeled: ***0, 5, 25, 125 ppm***
BMR = 10%

	V_{max} = 0.4				V_{max} = 0.65			
Model	**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.
[e]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)
Exposure concentrations modeled: ***0, 5, 25, 125 ppm***
BMR = 10%

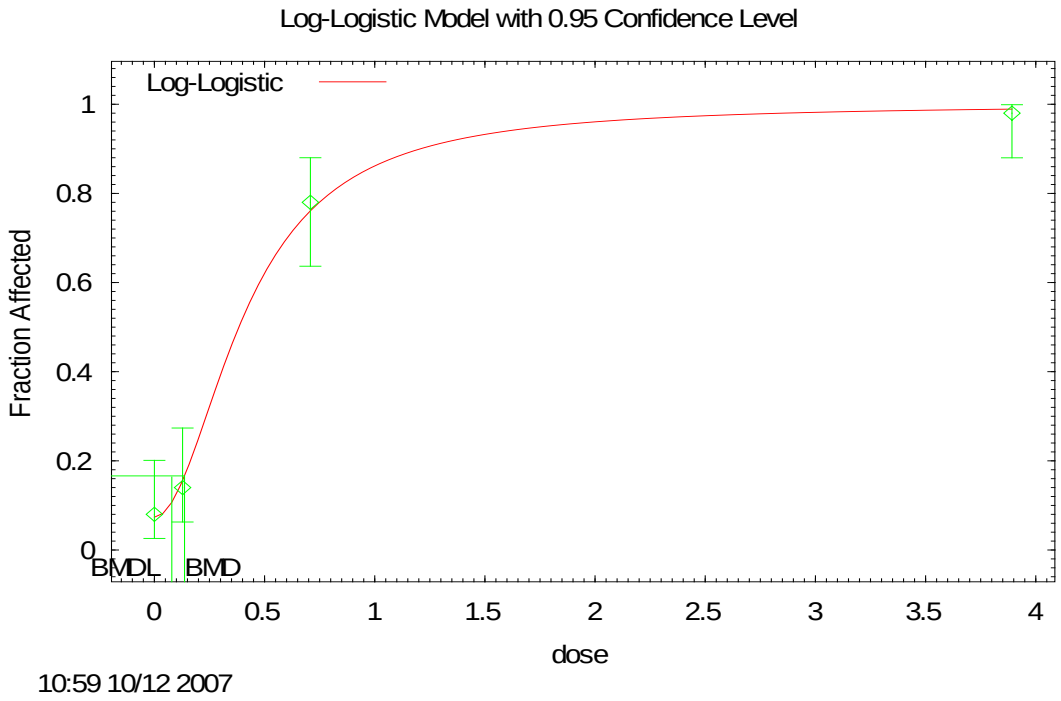
None of the models in BMDS provided an adequate fit of the female rat data.

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)
Exposure concentrations modeled: ***0, 5, 25 ppm*** (high dose dropped)
BMR = 10%

	V_{max} = 0.4				V_{max} = 0.65			
Model	**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/hour/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=0.4\RAT-FATTYLIVER-MCA-4.(d)
      Gnuplot Plotting File:  G:\CARBON TET\BMD\BMD MODELING 10-2007\RFC RAT LIVER\MALE RAT\MCA-
VMAX=0.4\RAT-FATTYLIVER-MCA-4.plt
                          Fri Oct 12 10:59:34 2007
 =================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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
```

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

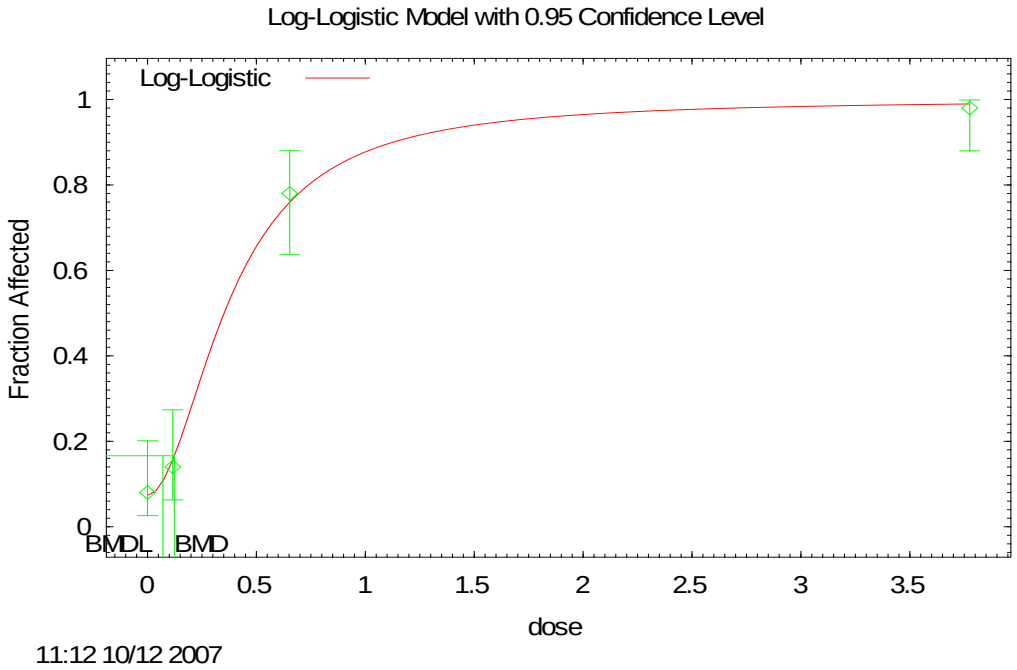
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.136715

BMDL = 0.0790319

Male Rat
Dose metric: MCA
Vmax = 0.65 mg/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

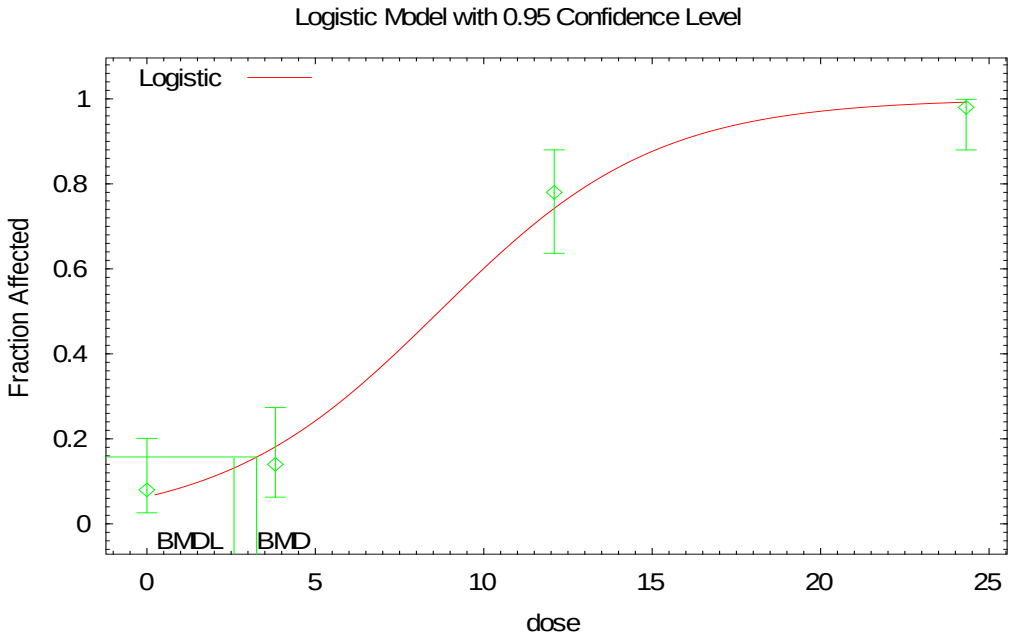
Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.123076

BMDL = 0.0707077

Male Rat
Dose metric: MRAMKL
Vmax = 0.4 mg/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

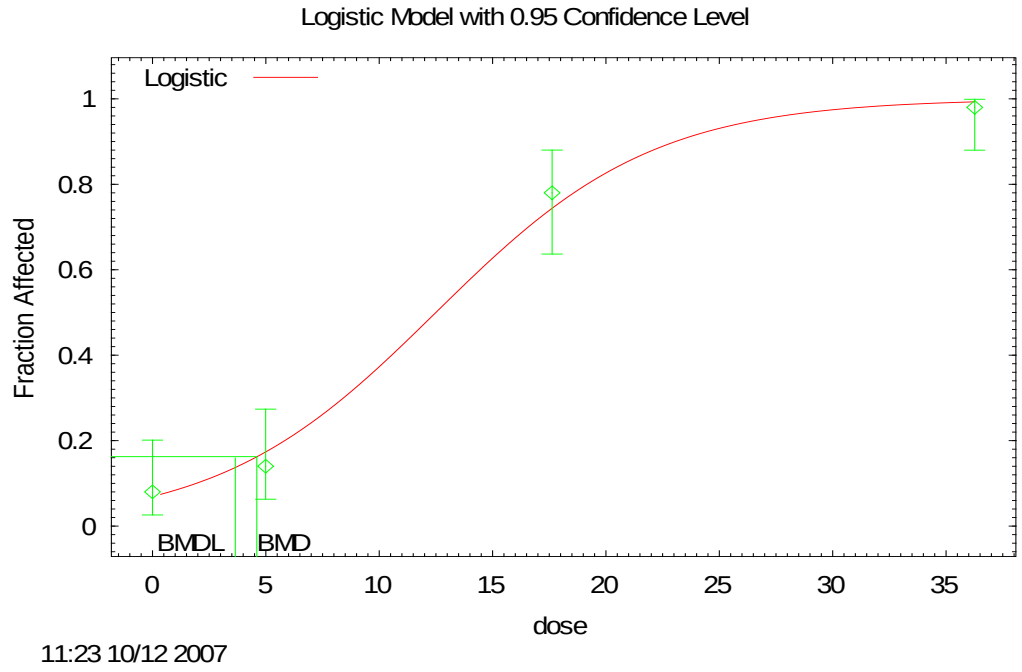
Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.25675

BMDL = 2.58557

Male Rat
Dose metric: MRAMKL
Vmax = 0.65 mg/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

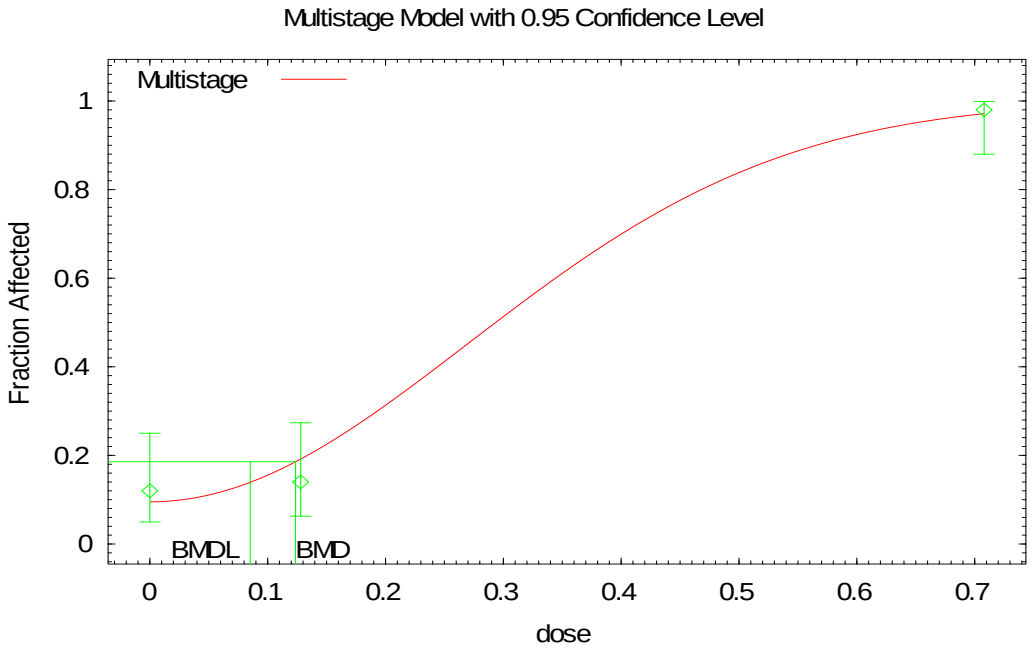
Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.60057

BMDL = 3.65284

Female Rat
Dose metric: MCA
Vmax = 0.4 mg/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

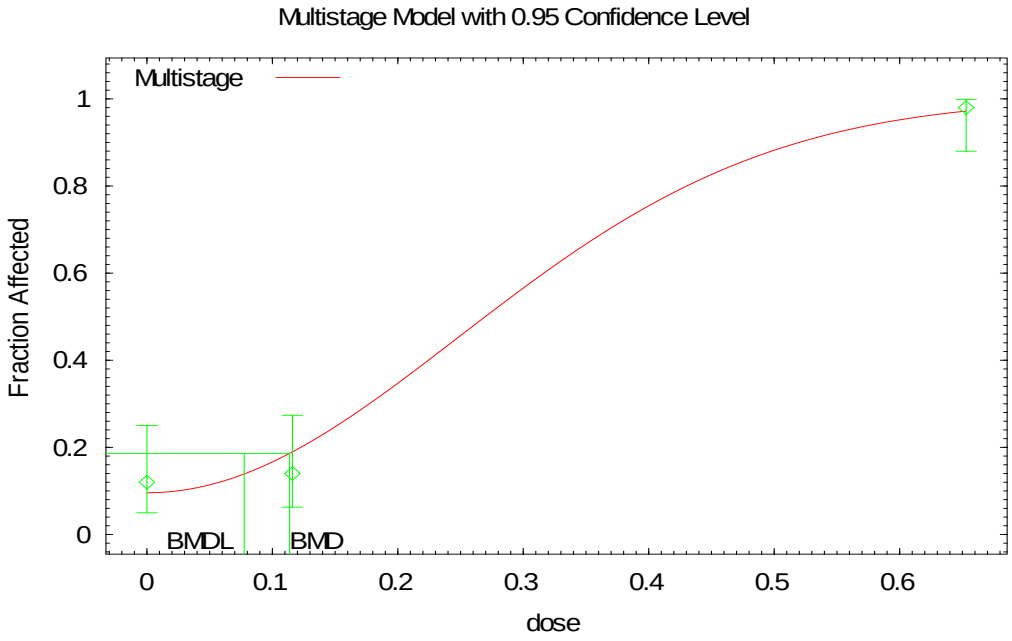
BMD = 0.123631

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/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

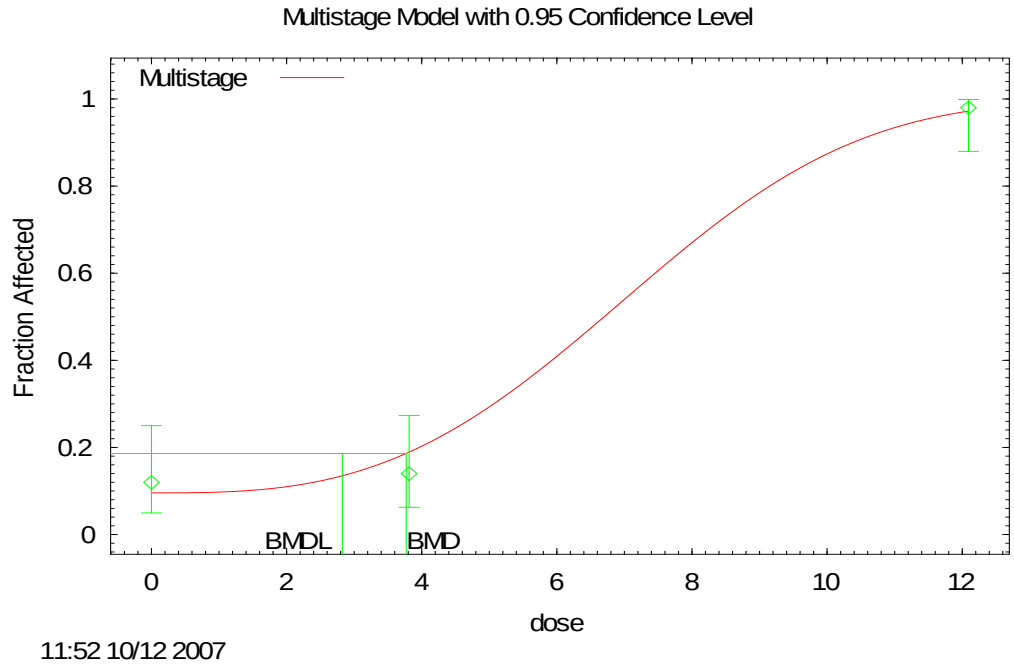
BMD = 0.113721

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/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

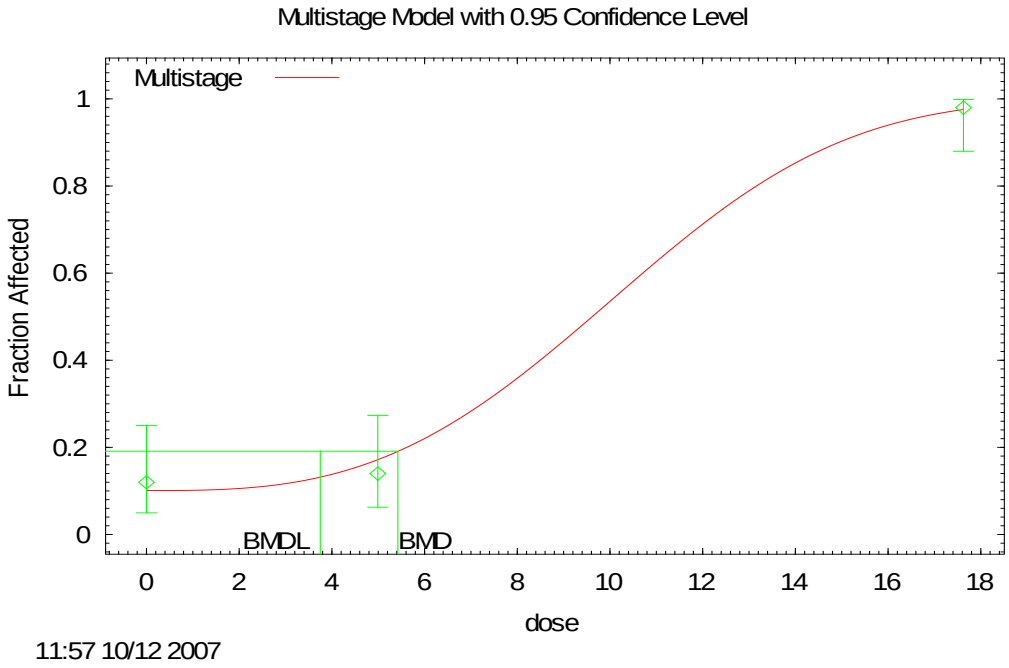
BMD = 3.76974

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/hour/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 or carcinoma)
Female F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Exposure concentrations 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 χ^2 test.

Liver tumors (adenoma or carcinoma)
Female F344 rats exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Exposure concentrations 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 or carcinoma)
Female BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Exposure concentrations 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 χ^2 test.

Note: 3-stage model did not provide a sufficiently improved model fit.

Liver tumors (adenoma or carcinoma)
Female BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Exposure concentrations 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 or carcinoma)
Male BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Exposure concentrations 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)
Exposure concentrations 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)
Exposure concentrations 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.

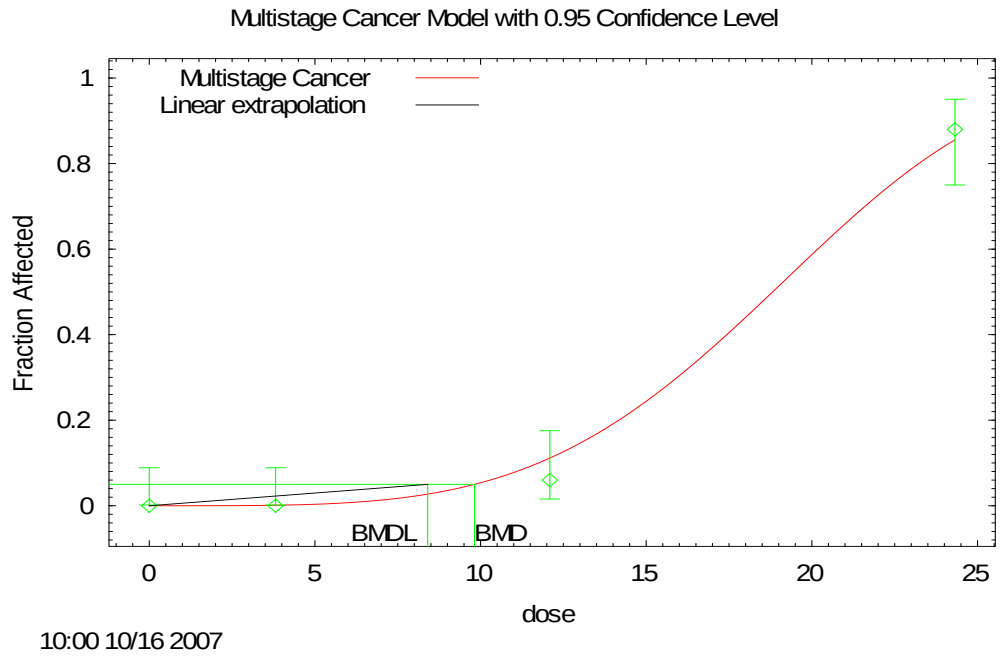
Pheochromocytomas
Male BDF1 mouse exposed to carbon tetrachloride vapor for 104 weeks (6 hours/day, 5 days/week)
Exposure concentrations modeled: ***0, 5, 25, 125 ppm***
Models other than Multistage
BMR = 0.1

	Fisher					Thrall				
Model	**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

[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 or carcinomas (0, 5, 25, 125 ppm dose groups)

Dose metric: MRAMKL
Vmax = 0.4 mg/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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

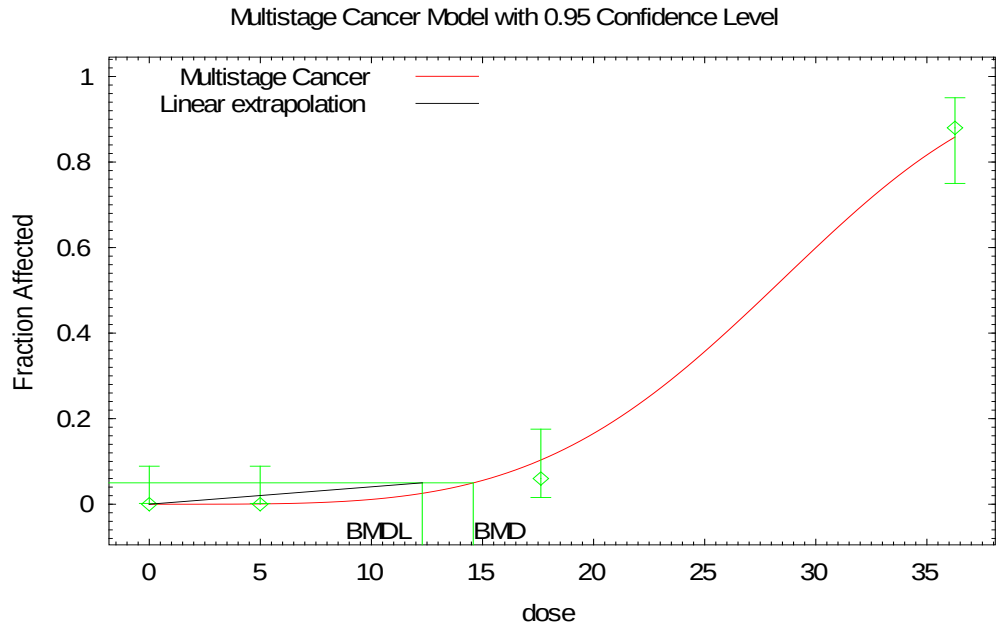
BMDL = 8.40334

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/hour/kg $BW^{0.07}$



13:09 12/14 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
                           Tue Oct 16 10:05:09 2007
 =================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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

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)	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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

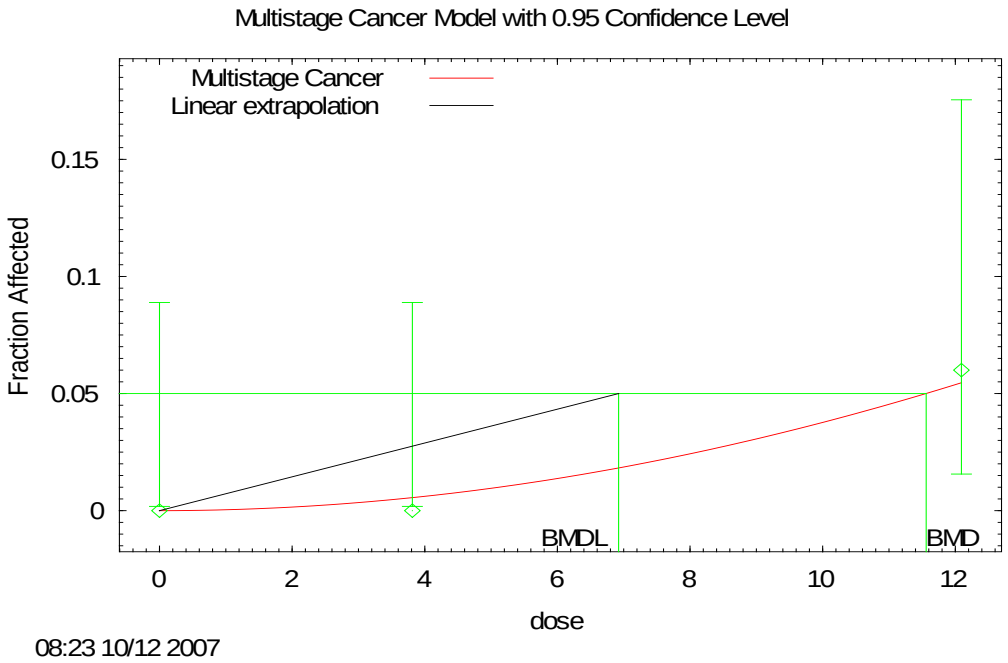
BMDU = 15.6526

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 or carcinomas (0, 5, 25 ppm dose groups)

Dose metric: MRAMKL
Vmax = 0.4 mg/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

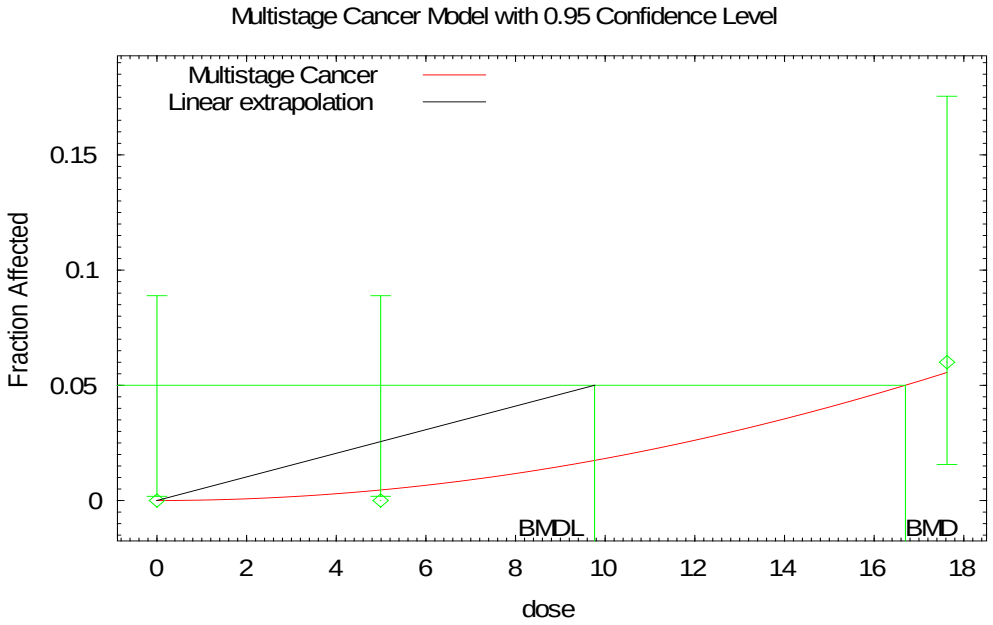
BMDL = 6.92352

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/hour/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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

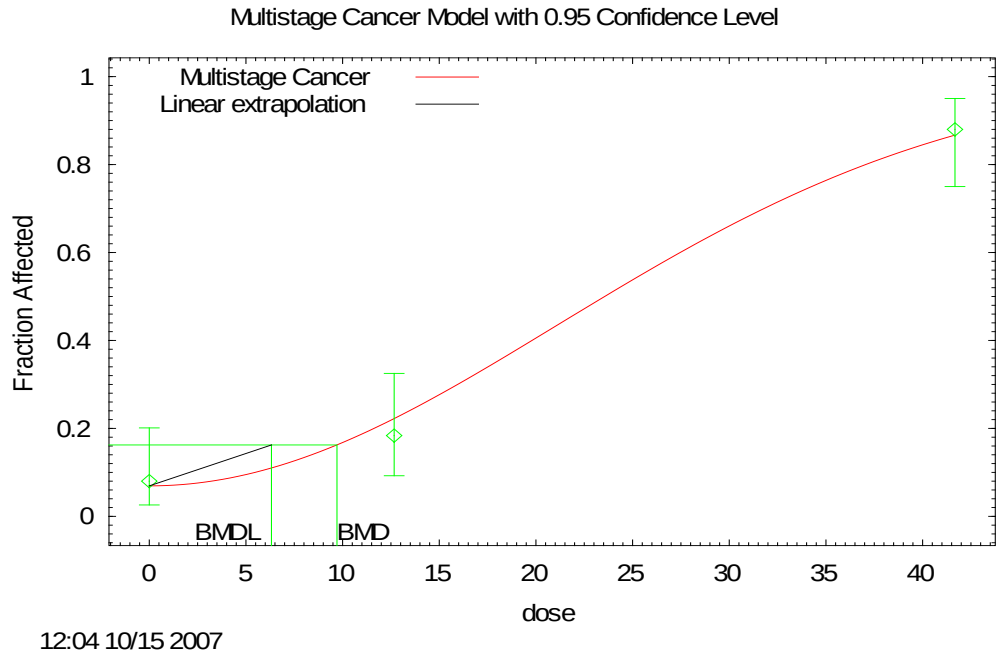
Taken together, (9.76339, 43.9237) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00512117

Female BDF1 mouse – hepatocellular adenomas or carcinomas (0, 5, 25 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 08:54:44 2007
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

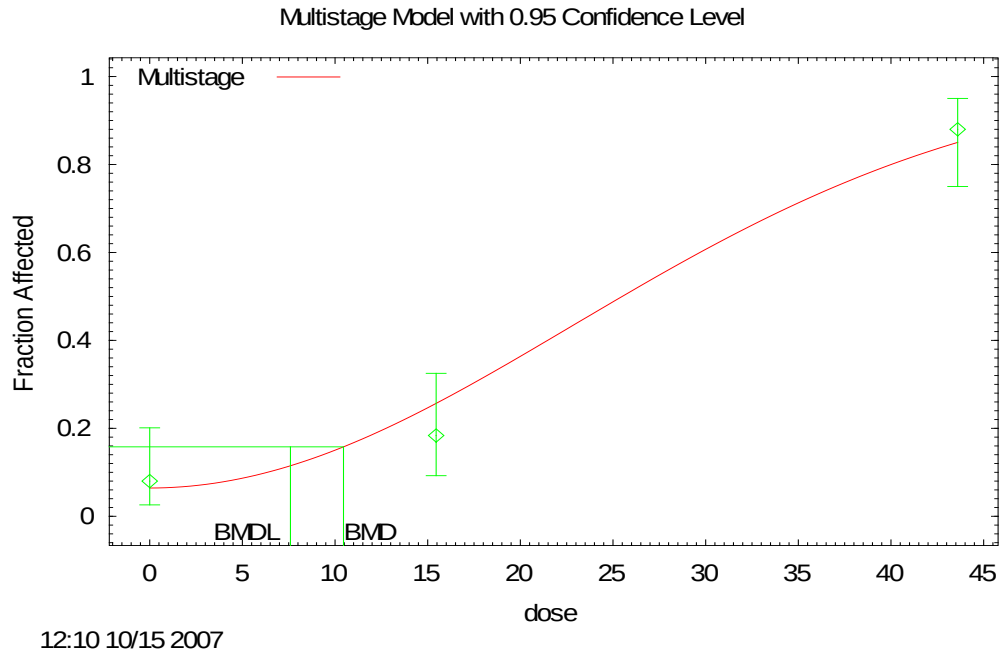
BMDL = 6.3204

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

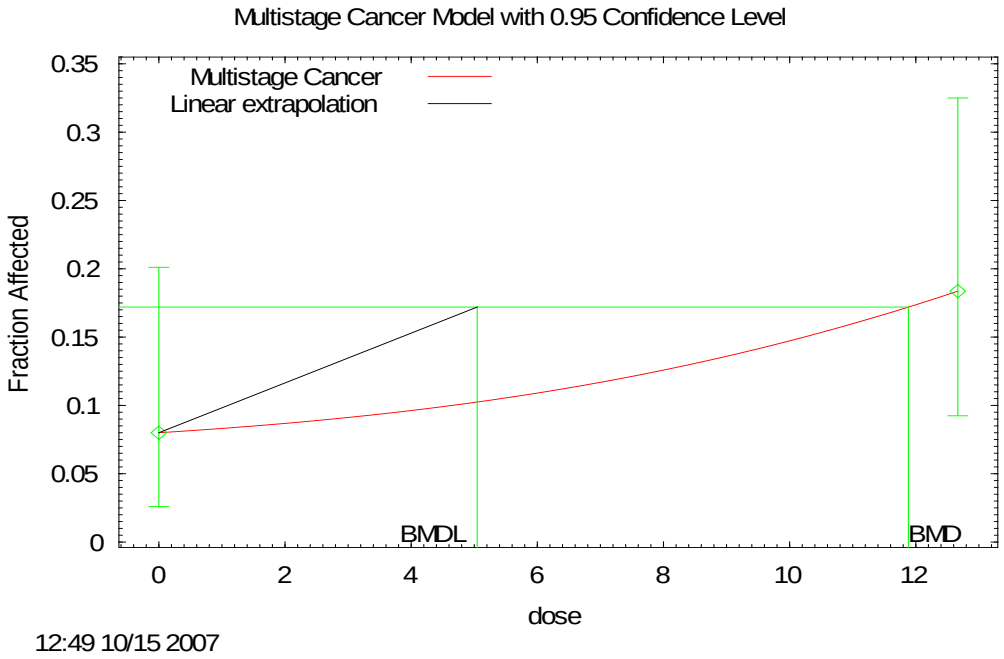
BMDL = 7.59255

BMDU = 12.107

Taken together, (7.59255, 12.107) is a 90 % two-sided confidence interval for the BMD

Female BDF1 mouse – hepatocellular adenomas or 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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

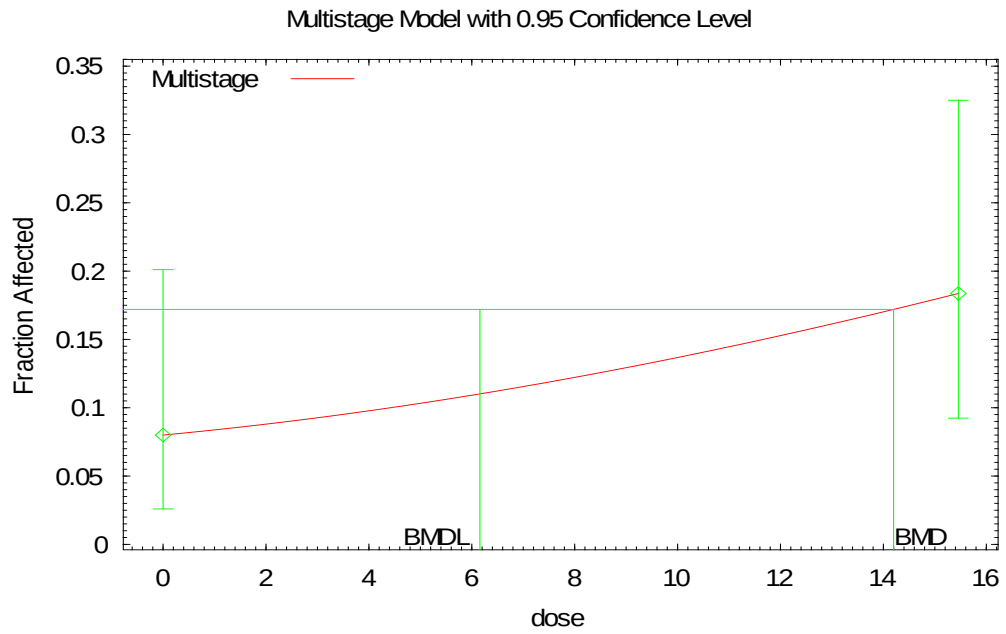
BMD = 11.6352

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

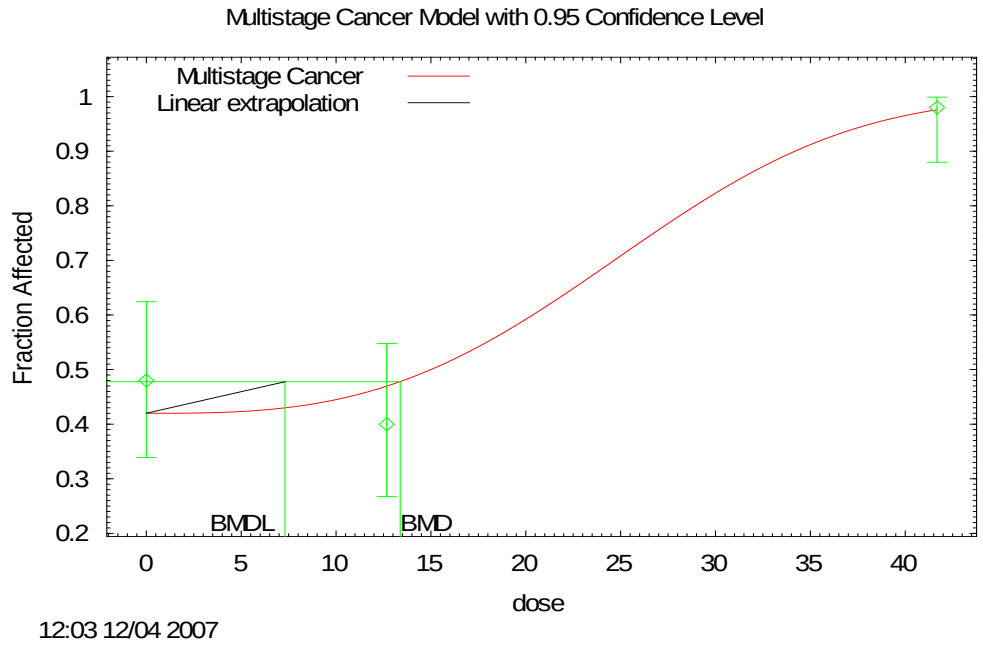



```
 ======================================================================
      Multistage Model. (Version: 2.8;  Date: 02/20/2007)
      Input Data File: G:\CARBON TET\BMD\BMD MODELING 10-2007\TUMORS FEMALE MOUSE LIVER\MRAMKL-
THRALL\FMOUSE_LIVER_ADCAR_MRAMKL-THRALL.(d)
      Gnuplot Plotting File:  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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     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
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       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 or 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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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

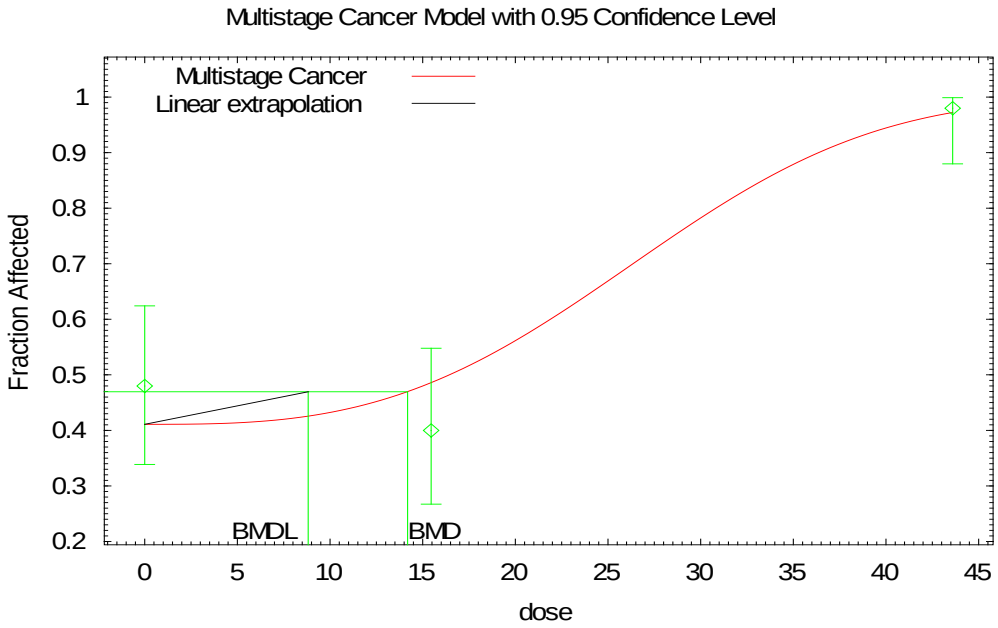
BMDL = 7.30705

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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

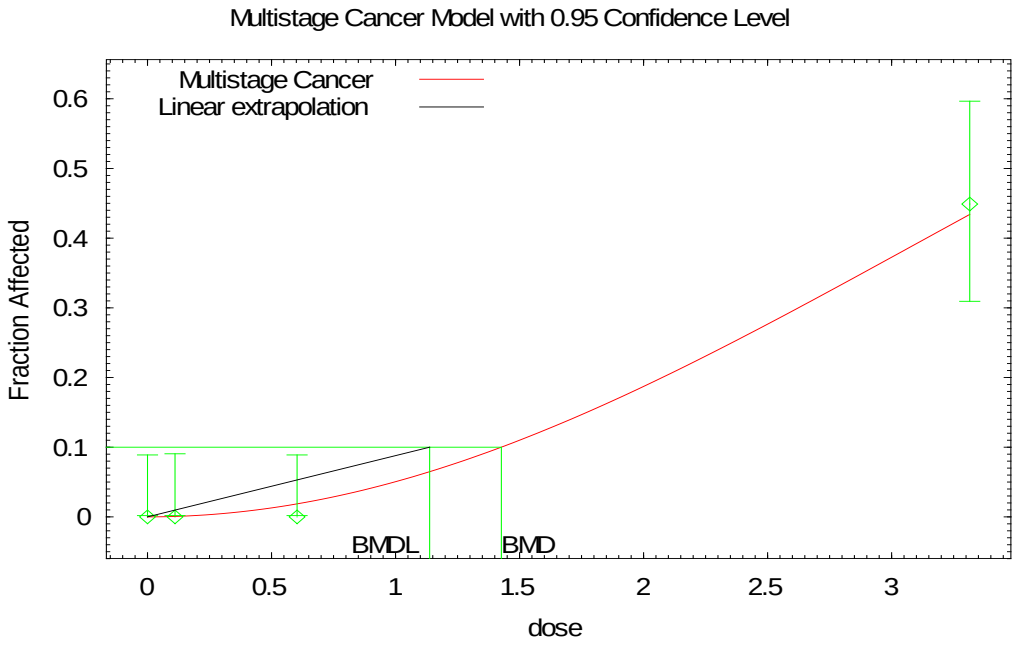
BMDL = 8.82145

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

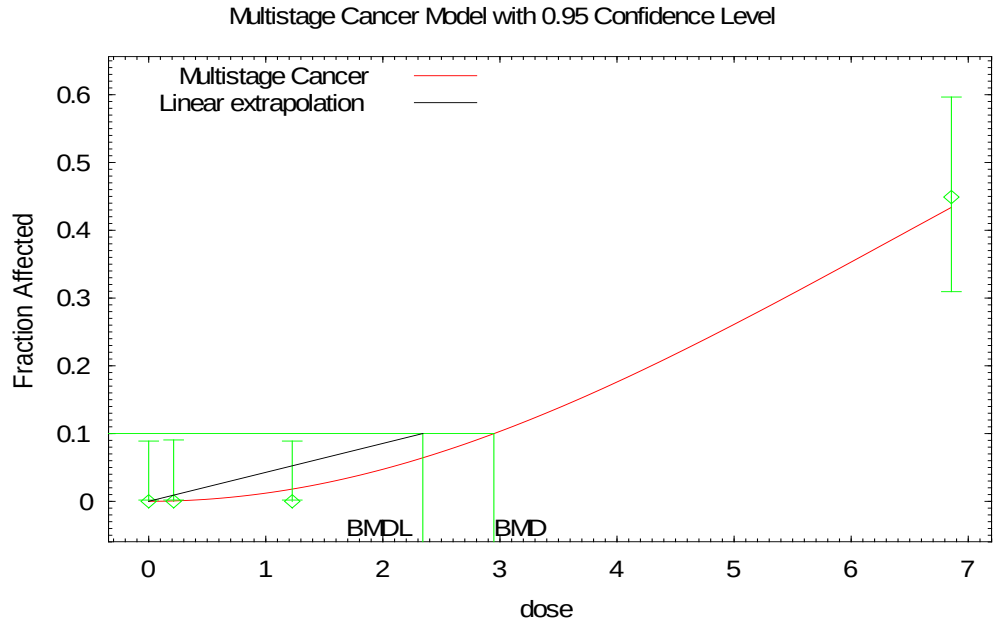
BMDL = 1.13753

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

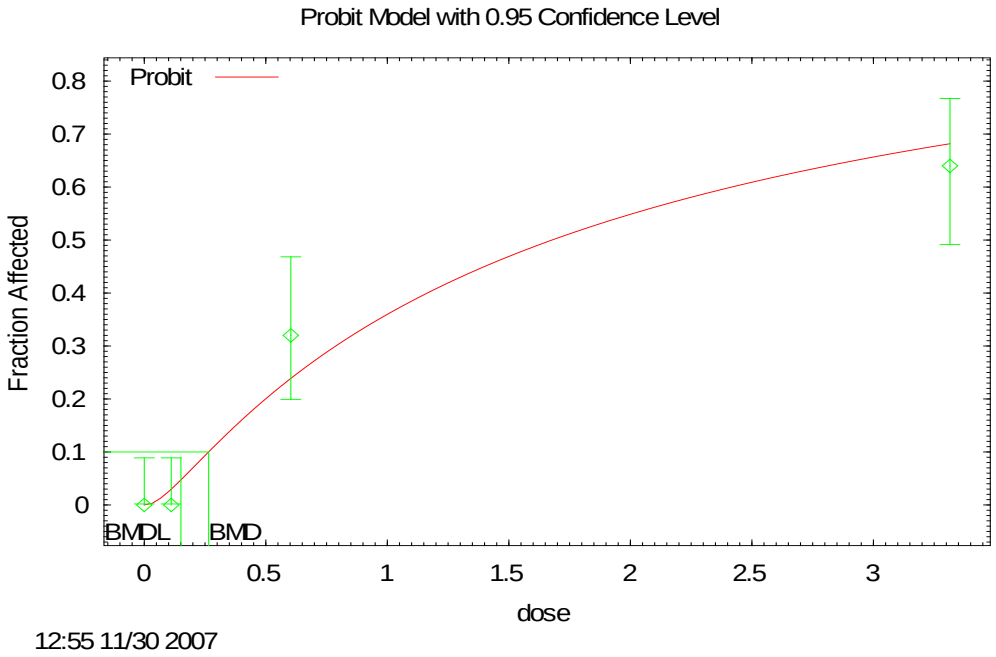
BMDU = 3.55893

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

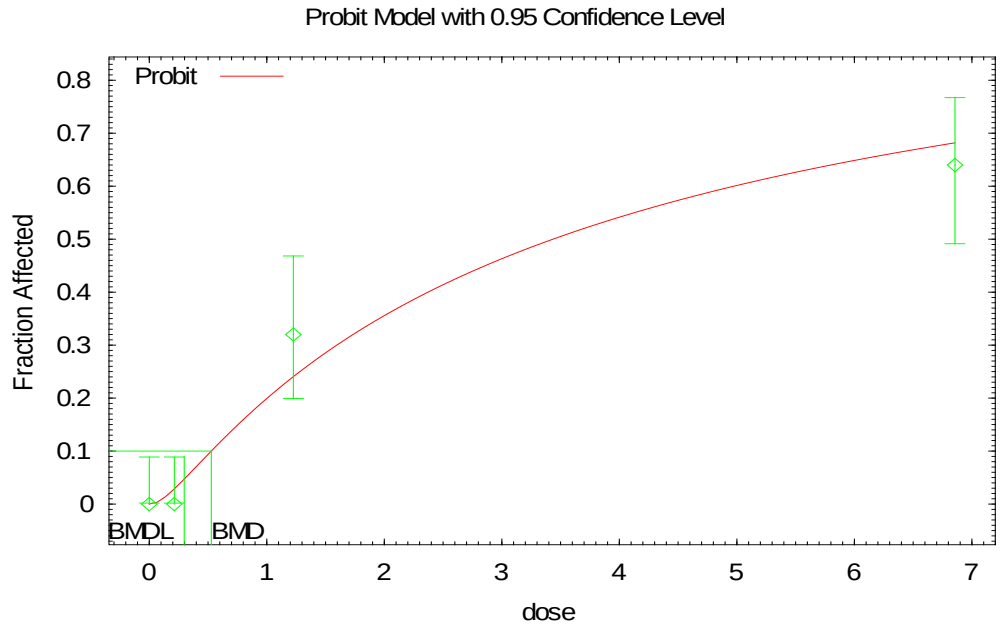
Risk Type = Extra risk

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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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 and 0.3177 μmol/L, respectively; these values are 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 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 <1, whereas in BMDS, the slope parameter for the log-probit model is typically constrained to be >1. Clearly, constraining the slope parameter in this situation leads to misspecifying the statistical model and should be avoided.

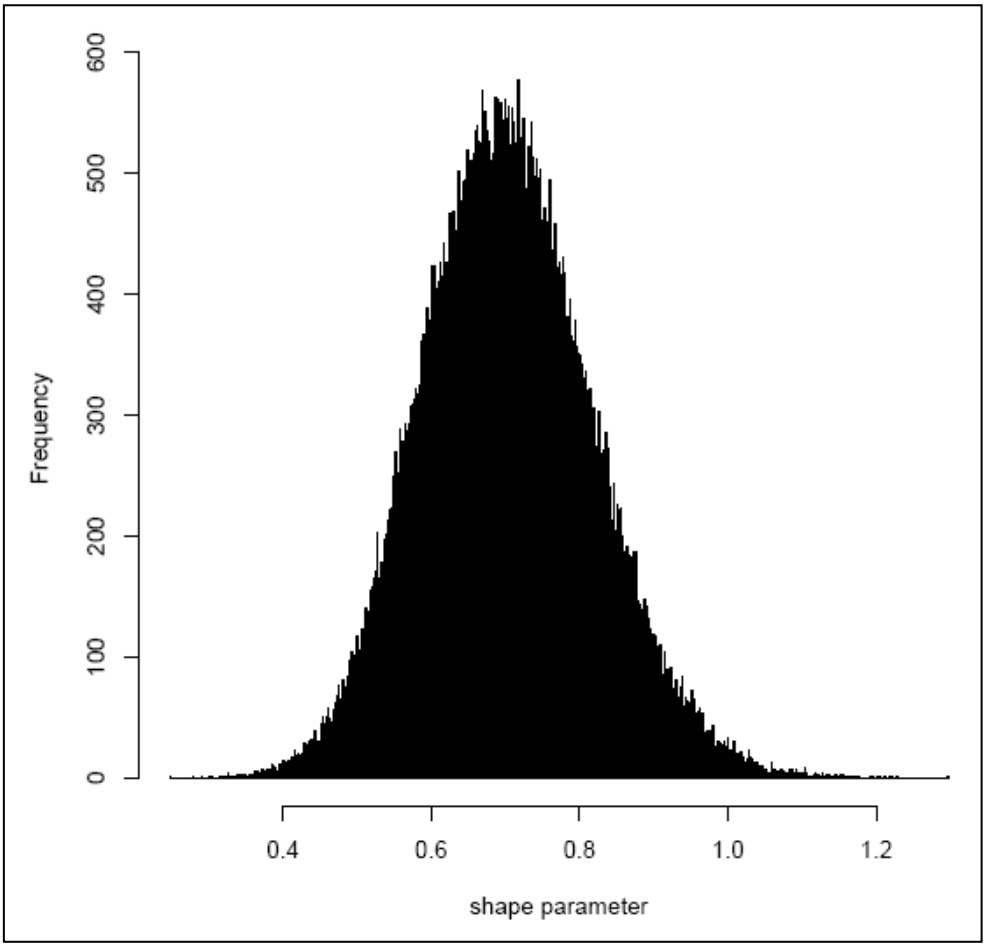


Figure E-1. Histogram of the shape parameter.

APPENDIX F. SOURCE CODE FOR PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

```
% ----------------------------------------------------------------------
%
% File: HUMINH.M
%
% Programmed by Gary Diamond
% Syracuse Research Corporation, 02/2005
%
%This run time file implements CCL4.CSL for inhalation exposure
%(human parameters)
% ----------------------------------------------------------------------
%Prepare time history variables
prepare @clear @all

%Set communication interval
CINT=1.;

%Set simulation stop (hr)
TSTOP=17250.;

%Integration error check
!!SET WESITG=.F.

%Air Exposure Parameters
AIRC=2.27;  %ppm
AIRON=0.; %hr
AIROFF=1000000.; %17520.; %hr
CIOFF=0.; %ppm
APER1=24.; %hr
AWID1=24.; %hr
APER2=168.; %hr
AWID2=168.; %hr

%Oral Exposure Parameters
RGIL=0.0;
FGIL=1.0;

%Human Parameters
BW=70.;
VLC=0.04;
VFC=0.30;  %Revised from 0.1 (03/2007)
VSC=0.62;
VRC=0.05;
```

```
QCC=15.;
QPC=15.;
QSF=0.74;
QLC=0.25;
QFC=0.06;
QSC=0.18;
QRC=0.51;

PBLD=2.64;
PL=3.14;
PF=79.42;
PS=1.0;
PR=3.14;

%Chemical Parameters
MW=153.8;
VMAXC=1.49;
KMX = 0.25;
VMAXSF=0.7;
A1=0.065;
A2=0.095;
A3=0.84;
K1=0.25;
K2=0.03;
K3=0.025;
K4=0.;
K5=0.0004;

!! START /NC

%Output
HUM1=[_time _day _air _ca _ramkb _mcl _cf _af, _cl];

HUM2=rot90(fliplr(HUM1));
out=fopen('HUMAN.out','w');
fprintf(out,"%f,%f,%f,%e,%e,%e,%e,%e,%e\n",HUM2);
fclose(out);
```

```
% ----------------------------------------------------------------------
%
% File: HUMOR.M
%
% Programmed by Gary Diamond
% Syracuse Research Corporation, 10/2007
%
%This run time file implements CCL4.CSL for "oral" exposure (RGIL)
%(human parameters)
% ----------------------------------------------------------------------

%Prepare time history variables
prepare @clear @all

%Set communication interval
CINT=24.;

%Set simulation stop (hr)
TSTOP=5000.;

%Integration error check
!!SET WESITG=.F.

%Air Exposure Parameters
AIRC=0.;      %ppm
AIRON=0.; %hr
AIROFF=1000000.; %17520.; %hr
CIOFF=0.; %ppm
APER1=24.; %hr
AWID1=24.; %hr
APER2=168.; %hr
AWID2=168.; %hr

%Oral Exposure Parameters
RGILC=10.0;
FGIL=1.0;

%Human Parameters
BW=70.;
VLC=0.04;
VFC=0.30;  %Revised from 0.1
VSC=0.62;
VRC=0.05;

QCC=15.;
QPC=15.;
QSF=0.74;
```

```
QLC=0.25;
QFC=0.06;
QSC=0.18;
QRC=0.51;

PBLD=2.64;
PL=3.14;
PF=79.42;
PS=1.0;
PR=3.14;

%Chemical Parameters
MW=153.8;
VMAXC=0.40;
KMX = 0.25;
VMAXSF=0.7;
A1=0.065;
A2=0.095;
A3=0.84;
K1=0.25;
K2=0.03;
K3=0.025;
K4=0.;
K5=0.0004;

!! START /NC

%Output
HUM1=[_time _rgil _mca _mramkl];

HUM2=rot90(fliplr(HUM1));
out=fopen('HUMAN.out','w');
fprintf(out,"%e,%e,%e,%e\n",HUM2);
fclose(out);
```

```
% ----------------------------------------------------------------------
%
% File: MOUINH_JF.M
%
% Programmed by Gary Diamond
% Syracuse Research Corporation, 10/2007
%
%This run time file implements CCL4.CSL for inhlation exposure
%(mouse parameters, Fisher et al. 2004)
% ----------------------------------------------------------------------
%Prepare time history variables
prepare @clear @all

%Set communication interval
CINT=24.;

%Set simulation stop (hr)
TSTOP=17520.;

%Integration error check
!!SET WESITG=.F.

%Air Exposure Parameters:
AIRC=125.;  %ppm
AIRON=0.; %hr
AIROFF=17520; %hr
CIOFF=0.; %ppm
APER1=24.; %hr
AWID1=6.; %hr
APER2=168.; %hr
AWID2=120.; %hr

%Mouse Parameters:
BW=0.036;
VLC=0.04;
VFC=0.04;
VSC=0.69;
VRC=0.14;

QCC=30.;
QPC=30.;
QSF=0.75;
QLC=0.24;
QFC=0.05;
QSC=0.17;
QRC=0.54;
```

```
PBLD=3.8;
PL=4.8;
PF=91.4;
PS=2.5;
PR=4.8;

%Chemical Parameters:
MW=153.8;
VMAXC=1.;
KMX = 0.3;
VMAXSF=0.75;

%From Thrall et al 2000
A1=0.065;
A2=0.095;
A3=0.84;
K1=0.25;
K2=0.03;
K3=0.025;
K4=0.;
K5=0.00042;

!! START /NC
%Output
%MOU=[AIR MCA MRAMKB MCL MRAMKL]
MOU1=[_time _day _air _mramkl _mca _mramkb _mcl _mramkl];  %output matrix

%formating of output for printed comma-dilimited file:
MOU2=rot90(fliplr(MOU1));
out=fopen('MOU.out','w');
%fprintf(out,"%f,%f,%f,%f,%f,%f,%f\n",MOU2);
%fprintf(out,"%f,%f,%f,%e,%e,%e,%e,%e\n",MOU2);
fprintf(out,"%e\n",MOU);
fclose(out);
```

```
% ----------------------------------------------------------------------
%
% File: MOUINH_KT.M
%
% Programmed by Gary Diamond
% Syracuse Research Corporation, 3/2007
%
%This run time file implements CCL4.CSL for inhlation exposure
%(mouse parameters, Thrall et al. 2000)
% ----------------------------------------------------------------------
%Prepare time history variables
prepare @clear @all

%Set communication interval
CINT=24.;

%Set simulation stop (hr)
TSTOP=17520.;

%Integration error check
!!SET WESITG=.F.

%Air Exposure Parameters:
AIRC=2.5;    %ppm
AIRON=0.; %hr
AIROFF=17520; %hr
CIOFF=0.; %ppm
APER1=24.; %hr
AWID1=6.; %hr
APER2=168.; %hr
AWID2=120.; %hr

%Mouse Parameters:
BW=0.036;
VLC=0.04;
VFC=0.04;
VSC=0.78;
VRC=0.05;

QCC=28.;
QPC=28.;
QSF=0.74;
QLC=0.24;
QFC=0.05;
QSC=0.19;
QRC=0.52;
```

```
PBLD=7.83;
PL=2.08;
PF=23.0;
PS=0.61;
PR=2.08;

%Chemical Parameters:
MW=153.8;
VMAXC=0.79;
KMX = 0.46;
VMAXSF=0.7;
A1=0.065;
A2=0.095;
A3=0.84;
K1=0.25;
K2=0.03;
K3=0.025;
K4=0.;
K5=0.00042;

!! START /NC

%Output
%MOU=[AIR MCA MRAMKB MCL MRAMKL]
MOU1=[_time _day _air _mramkl _mca _mcl];  %output matrix

%formating of output for printed comma-dilimited file:
MOU2=rot90(fliplr(MOU1));
out=fopen('MOU.out','w');
fprintf(out,"%f,%f,%e,%e,%e,%e\n",MOU2);
fprintf(out,"%e\n",MOU);
fclose(out);
```

```
% ------------------------------------------------------------------
%
% File: RATINH.M
%
% Programmed by Gary Diamond
% Syracuse Research Corporation, 02/2005
%
%This run time file implements CCL4.CSL for inhalation exposure
%(rat parameters)
% ------------------------------------------------------------------

%Prepare time history variables
prepare @clear @all

%Set communication interval
CINT=1.;

%Set simulation stop (hr)
TSTOP=17250.;

%Integration error check
!!SET WESITG=.F.

%Air Exposure Parameters:
AIRC=4.;      %ppm
AIRON=0.; %hr
AIROFF=17520; %hr
CIOFF=0.; %ppm
APER1=24.; %hr
AWID1=6.; %6.; %hr
APER2=168.; %hr
AWID2=120.; %120.; %hr

%Rat Parameters:
BW=0.452;
VLC=0.04;
VFC=0.08;
VSC=0.74;
VRC=0.05;

QCC=15.;
QPC=15.;
QSF=0.74;
QLC=0.25;
QFC=0.04;
QSC=0.20;
QRC=0.51;
```

```
PBLD=4.52;
PL=3.14;
PF=79.42;
PS=1.0;
PR=3.14;

%Chemical Parameters:
MW=153.8;
VMAXC=0.4;
KMX = 0.25;
VMAXSF=0.7;
A1=0.065;
A2=0.095;
A3=0.84;
K1=0.25;
K2=0.03;
K3=0.025;
K4=0.;
K5=0.00042;


!! START /NC

%Output
RAT1=[_time _day _air _mca _mramkl _mcl];  %output matrix

%formating of output for printed comma-dilimited file:
%RAT2=rot90(fliplr(RAT1));
%out=fopen('RAT.out','w');
%fprintf(out,"%f,%f,%f,%e,%e,%e\n",RAT2);
%fclose(out);
```

```
PROGRAM: CCL4R

      !This program simulates the pharmacokinetics of carbon tetrachloride
      !The program is based on ITRICCL4.ACSL, developed by KD THRALL 9/98; ACSL code provided to
GDiamond, 04/2004
      !The above code was translated with minor modifications, by GDiamond, 05/2004

INITIAL
      VARIABLE TIME = 0.0 !Set independent variable to be TIME
  ALGORITHM IALG = 2  !Numerical integration algorithm - Gear for stiff systems
      CINTERVAL CINT=100. !Communication interval
      NSTP = 1000 !Set initital integration cycle length at CINT/1000
      MERROR AL=0.0001 !Set error tolerance for Gear

  !*****BODY AND TISSUE MASSES*****
      CONSTANT BW = 0.2 !Body weight (kg)
  CONSTANT VLC = 0.04 !Liver fraction of body weight
      CONSTANT VFC = 0.08 !Adipose fraction of body weight
      CONSTANT VSC = 0.74 !Slowly-perfused fraction of body weight
  CONSTANT VRC = 0.05 !Rapdily-perfused fraction of body weight

      VL=VLC*BW  !Liver (kg
   VF=VFC*BW  !Adipose (kg)
   VS=VSC*BW  !Slowly-perfused (kg)
   VR=VRC*BW !Rapidly-perfused (kg)

      !*****BLOOD FLOWS*****
      CONSTANT QCC=14  !Cardiac output (L/hr-BW^SF)
      CONSTANT QPC=14  !Alveolar ventilation (L/hr-BW^SF)
      CONSTANT QLC = 0.25  !Liver fraction of cardiac output
  CONSTANT QFC = 0.09       !Adipose fraction of cardiac output
  CONSTANT QSC = 0.15       !Slowly-perfused fraction of cardiac output
  CONSTANT QRC = 0.51       !Rapidly-perfused fraction of cardiac output

      CONSTANT QSF = 0.74 !QC and QP scaling factor (SF)
      QC=QCC*BW**QSF!Cardiac output (L/hr)
      QP=QPC*BW**QSF !Alveolar ventilation (L/hr)

      QL = QLC*QC    !Liver (L/hr)
  QF = QFC*QC  !Adipose (L/hr)
  QS = QSC*QC  !Slowly-perfused (L/hr)
  QR = QRC*QC !Rapidly-perfused (L/hr)

      !*****PARTITION COEFFICIENTS*****
      CONSTANT PBLD = 4.52 !Blood:air partition coefficient
```

```
 CONSTANT PL = 3.14   !Liver:blood partition coefficient
 CONSTANT PF = 79.42 !Adipose:blood partition coefficient
    CONSTANT PS = 1.0       !Slowly-perfused:blood partition coefficient
 CONSTANT PR = 3.14  !Rapidly-perfused:blood partition coefficient

    !*****METABOLISM and EXCRETION*****
    CONSTANT MW=153.8 !Molecular weight of CCl4
    CONSTANT VMAXC = 0.40 !VMAX for metabolism in liver (mg/hr-BW^SF)
    CONSTANT KMX = 0.25 !KM for metabolism in liver (mg/L)
    CONSTANT VMAXSF=0.7  !Scaling factor for VMAXC (SF)
    CONSTANT A1=0.085  !Fraction of metabolism rate to M1 pool
    CONSTANT A2=0.095  !Fraction of metabolism rate to M2 pool
    CONSTANT A3=0.84  !Fraction of metabolism rate to M3 pool
    CONSTANT K1=0.123     !Rate constant for conversion of M1 to exhaled metabolite (CO2) (hr-1)
    CONSTANT K2=0.03 !Rate constant for conversion of M2 to urinary metabolite (hr-1)
    CONSTANT K3=0.0252 !Rate constant for conversion of M3 to fecal metabolite (hr-1)
    CONSTANT K4=0.  !Rate constant for conversion of M2 to M1 (hr-1)
    CONSTANT K5=0.00042 !Rate constant for conversion of M3 to M1 (hr-1)

    VMAX = 1000*VMAXC*BW**VMAXSF/MW!Maximum rate of metabolism in liver (umol/hr)
    KM = 1000*KMX/MW !Michaelis constant for metabolism in liver (umol/L)

    !*****EXPOSURE - AIR*****
    CONSTANT AIRC = 1. !Air exposure concentration (ppm)
    AIR = AIRC; !Air exposure concentration (ppm)
 AIRCM = AIR/24.45  !Air exposure (umol/L)
    mgAIR = AIR*MW/24.45  !Air exposure (mg/m3)
    ugAIR=AIR*MW/24.45    !Air exposure concentration (ug/L)

    CONSTANT TSTOP = 700.  !Length of simulaion (hr)
    CONSTANT AIRON=0.  !Time air exposure starts (hr)
    CONSTANT AIROFF=700. !Time air exposure stops (hr)
    CONSTANT CIOFF=0.  !Concentration in inhaled air when exposure is off (ppm)
    CONSTANT APER1=24.  !Pulse period 1 for air exposure (e.g., hours in a day)
    CONSTANT AWID1=24.  !Pulse width 1 for air exposure (e.g., 6 hours each day)
    CONSTANT APER2=168.  !Pulse period 2 for air exposure (e.g., hours in a day)
    CONSTANT AWID2=168.  !Pulse width 2 for air exposure (e.g., 6 hours each day)

!*****EXPOSURE - ORAL ******GD 08/2007
    CONSTANT RGILC=0. !Rate of uptake from GI to liver (umol/hr)
 RGIL=RGILC !Rate of uptake from GI to liver (umol/hr)
                                        !Use for simuulating constant rate of uptake from GI-tract
    MGRGIL=RGIL*MW/1000    !Rate of uptake from GI to liver (mg/hr)
    MGRGILKGD=RGIL*24*MW/(1000*BW)!Rate of uptake to liver (mg/kg-day)
    CONSTANT GILF=1.      !Absorption fraction
    !CONSTANT POINTS = 96.
    !CINT = TSTOP/POINTS !Sets communication for 96 times in the simulation
```

```
END      !of INITIAL section of program

DYNAMIC

DERIVATIVE

      DAY=TIME/24
      YEAR=DAY/365

      !*****CONCENTRATION IN INHALED AIR (umol/L)*****
      CION = AIRCM*PULSE(AIRON,APER1,AWID1)*PULSE(AIRON,APER2,AWID2)
      CI = RSW(TIME.LE.AIROFF,CION,CIOFF)
      RAI = QP*(CA/PBLD-CI)!Rate inhaled (umol/hr)
      CP = CI*24.45 !Concentration in chamber (ppm)

      !*****AMOUNT TAKEN IN BY ONE ANIMAL (umol)*****
      RIN = QP*CI
      AIN = INTEG(RIN,0.0)

      !*****LIVER*****
      !Use for simulation of constant rate of uptake from GI-tract (GD 08/2007)
      RAL = QL*(CA-CVL)+RGIL-RAM      !Rate of change in amount (umol/hr)

      !Use for simulation of inhalation exposure
      !RAL = QL*(CA-CVL)-RAM  !Rate of change in amount (umol/hr)
      AL = INTEG(RAL, 0.0)    !Amount (umol)
      CVL = CL/PL !Concentration in venous blood (umol/L)
      CL = AL/VL !Concentration (umol/L)
      AUCCL = INTEG(CL,0.)  !AUC concentration (umol/L x hr)

      !Average concentration in liver (umol/L) - MCL(GD 05/2004)
      IF (TIME .GT. 0.) THEN
      MCL = AUCCL/TIME
      ELSE
      MCL= 0.
      END IF

      ugCL = CL*MW/1000      !Concentration (ug/g)
      MugCL = MCL*MW/1000      !Average concentration (ug/g)
      AUCugCL = AUCCL*MW/1000    !AUC concentration (ug/g x hr)

      !*****METABOLIZED*****
      RAM = (VMAX*CVL)/(KM+CVL)      !Rate, total (umol/hr)
      RAMKB= RAM/BW  !Rate, total (umol/hr x kq body)
      RAMKL=RAM/VL  !Rate, total (umol/hr x kg liver)
      AUCRAM=INTEG(RAM,0.0)  !AUC rate (umol/hr x hr)
```

```
    !Average rate of metabolism (umol/hr) - MRAM (GD 05/2004)
    IF (TIME .GT. 0.) THEN
    MRAM = AUCRAM/TIME
    ELSE
    MRAM= 0.
    END IF

    MRAMKB=MRAM/BW  !Average rate, total (umol/hr x kg body) (GD 05/2004)
    MRAMKL=MRAM/VL  !Average rate, total (umol/hr x kg liver) (GD 05/2004

    RAXA = RAM*A1-K1*AXA+K4*AXU+K5*AXF   !Rate to air pool (umol/hr)
    RAXF = RAM*A3-K3*AXF-K5*AXF  !Rate to feces pool (umol/hr)
    RAXU = RAM*A2-K2*AXU-K4*AXU  !Rate to urine pool(umol/hr)
    AM = INTEG(RAM,0.) !Amount total (umol)
    AMK = AM/BW  !Amount total (umol/kg bw)
    AMKL=AM/VL  !Amount total (umol/kg liver)
    AXA = INTEG(RAXA,0.)  !Amount in air pool (umol)
    AXF = INTEG(RAXF,0.)  !Amount in feces pool (umol)
    AXU = INTEG(RAXU,0.)  !Amount in urine pool (umol)

    RA = AXA*k1  !Rate to air (umol/hr)
    RU = AXU*k2  !Rate to urine (umol/hr)
    RF = AXF*k3  !Rate to feces (umol/hr)
    ugRA = RA*MW  !Rate to air (ug/hr)
    ugRU = RU*MW  !Rate to urine (ug/hr)
    ugRF = RF*MW  !Rate to feces (ug/hr)
    CAX = RA/((3/2)*QP)  !Concentration of metabolite in exhaled air (umol/L)
    CAXM = CAX*24.45  !Concentration of metabolite in exhaled air (ppm)

    !*****FAT*****
RAF = QF*(CA-CVF) !Rate of change in amount (umol/hr)
AF = INTEG(RAF, 0.0) !Amount (umol)
CVF = CF/PF  !Concentration in venous blood (umol/L)
CF = AF/VF  !Concentration (umol/L)
    AUCCF = INTEG(CF, 0.) !AUC concentration (umol/L x hr)

    !Average concentration in fat (umol/L) - MCF (GD 03/2007)
    IF (TIME .GT. 0.) THEN
    MCF = AUCCF/TIME
    ELSE
    MCF=0.
    END IF

ugCF = CF*MW/1000    !Concentration(ug/g)
    MugCF = MCF*MW/1000      !Average concentration (ug/g)
    AUCugCF = AUCCF*MW/1000    !AUC concentration (ug/g x hr)
```

```
    !*****SLOWLY PERFUSED TISSUES*****
RAS = QS*(CA-CVS)  !Rate of change in amount (umol/hr)
AS = INTEG(RAS, 0.0) !Amount (umol)
CVS = CS/PS  !Concentration in venous blood (umol/L)
CS = AS/VS  !Concentration in umol/L
ugCS = CS*MW/1000  !Concentration in ug/g

!*****RICHLY PERFUSED TISSUES*****
RAR = QR*(CA-CVR)   !Rate of change in amount (umol/hr)
AR = INTEG(RAR,0.0)  !Amount (umol)
CVR = CR/PR  !Concentration in venous blood (umol/L)
CR = AR/VR  !Concentration in umol/L
ugCR = CR*MW/1000   !Concentration in ug/g

!*****MIXED VENOUS BLOOD*****
CV = (QF*CVF+QL*CVL+QS*CVS+QR*CVR)/QC     !Concentration (umol/L)
    AUCCV=INTEG(CV,0.)   !AUC concentration (umol/L x hr)


    !Average concentration in venous blood (umol/L) - MCV (GD 05/2004)
    IF (TIME .GT. 0.) THEN
    MCV = AUCCV/TIME
    ELSE
    MCV=0.
    END IF

ugCV = CV*MW/1000   !Concentration (ug/g)
    MugCV = MCV*MW/1000     !Average concentration (ug/g)
    AUCugCV = AUCCV*MW/1000   !AUC concentration (ug/g x hr)

!*****ARTERIAL BLOOD*****
CA = (QC*CV+QP*CI)/(QC+(QP/PBLD))  !Concentration(umol/L)
    AUCCA=INTEG(CA,0.)  !AUC concentration (umol/L x hr)


    !Average concentration in atrerial blood (umol/L) - MCA (GD 05/2004)
    IF (TIME .GT. 0.) THEN
    MCA = AUCCA/TIME
    ELSE
    MCA=0.
    END IF

    ugCA=CA*MW/1000  !Concentration (ug/g)
    MugCA=MCA*MW/1000  !Average concentration (ug/g)
    AUCugCA=AUCCA*MW/1000     !AUC concentration (ug/g x hr)
```

```
  !*****AMOUNT EXHALED*****
  CX=CA/PBLD   !Concentration in alveolar air (umol/L)
  CXPPM = (0.7*CX+0.3*CI)*24.45  !Concentration in exhaled air (ppm)
                                              !Total ventilation is 0.7 of alveolar ventilation
  RAX= QP*CX  !Rae of change in amount (umol/hr)
  AX=INTEG(RAX,0.)  !Amount (umol)

      !*****NET AMOUNT ABOSORBED*****
      DOSEX = AIN-AX  !Net amount absorbed(umol)
      BODY=AL+AF+AS+AR  !Amount in body (umol)
      MASSB = BODY+AM+AX  !Mass balance (umol)

END  !of DERIVATIVE section of program

TERMT(TIME .GE. TSTOP)  !Termination condition

END      !of DYNAMIC section of program

TERMINAL

END  !of TEMINAL section of program

END      !of program
```