EPA/635/R-08/011A
External Review Draft

TOXICOLOGICAL REVIEW

OF

Tetrachloroethylene (Perchloroethylene)

(CAS No. 127-18-4)

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

June 2008

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U.S. Environmental Protection Agency
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LIST OF ABBREVIATIONS AND ACRONYMS

8-OHdG	8-hydroxydeoxyguanosine
AAP	alanine aminopeptidase
ALT	alanine transferase
AST	aspartase amino transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area-under-the-curve
BMC	benchmark concentration
BMCL	95% lower bound benchmark dose
BMD	benchmark dose
BMDS	Benchmark Dose Software
BMDU	95% upper bound benchmark dose
BUN	blood urea nitrogen
BW	body weight
CARB	California Air Resources Board
CASRN	Chemical Abstracts Service Registry Number
CCI	Color Confusion Index
CI	confidence interval
CLL	chronic lymphocytic leukemia
CNS	central nervous system
CO_2	carbon dioxide
CT	carbon tetrachloride
CYP	cytochrome P450
CYP P450	cytochrome P450
DCA	dichloroacetic acid
DEHP	di(2-ethylhexyl)phthalate
EEGs	electroencephalograms
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
FMO3	flavin-containing monooxygenase 3
GGT	gamma-glutamyltransferase
GSH	glutathione
GST	glutathione S-transferase
GST*x*	glutathione S-transferase isoform, where *x* denotes different isoforms (such as M, T, P, S, Z)
HEC	human equivalent concentration
HSIA	Halogenated Solvents Industry Alliance
i.p.	intraperitoneal
IAP	intestinal alkaline phosphatase
IARC	International Agency for Research on Cancer
IOM	Institute of Medicine

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
IUGR	intrauterine growth restriction
JISA	Japan Industrial Safety Association
K_m	Michaelis-Menten constant
LEC_{10}s	95% lower confidence limits on the air concentrations associated with a 10% extra risk of cancer incidence
LGL	Large granular lymphocyte
LOAEL	lowest-observed-adverse-effect level
MLE	maximum likelihood estimate (please verify inserted correctly on pg 5-69, MLE was there but not the exact definition)
MCA	monochloroacetic acid
MCL-5	microsomal epoxide hydrolase
MCL	mononuclear cell leukemia
MOA	mode of action
MRL	minimal risk level
NAG	N-acetyl-β-D-glucosaminidase
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NIOSH	National Institutes of Occupational Safety and Health
NK	natural killer
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
NYS DOH	New York State Department of Health
NYS OAG	New York State Office of Attorney General
OR	odds ratio
P450	cytochrome P450
PBPK	physiologically based pharmacokinetic
PCO	palmitoyl CoA oxidation
PHG	public health goal
POD	point of departure
PPAR	peroxisome proliferater activated receptor
PPAR-α	peroxisome proliferater activated receptor, alpha isoform
PPAR-δ	peroxisome proliferater activated receptor, delta isoform
RBP	retinol binding protein
REAL	revised European-American Lymphoma
RfC	reference concentration
RfD	reference dose
RfV	reference value
RR	relative risk
SAP	Scientific Advisory Panel
SCE	sister chromatid exchange

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

SES	socio-economic status
SGA	small for gestational age
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SSB	single-strand breaks
TCA	trichloroacetic acid
TCE	trichloroethylene
TCOH	Trichloroethanol
TCVC	S-(1,2,2,-trichlorovinyl)-L-cysteine
TCVCSO	S-(1,2,2,-trichlorovinyl)-L-cysteine sulfoxide
TCVG	S-(1,2,2-trichlorovinyl) glutathione
TNAP	tissue non-specific alkaline phosphatase
TWA	time-weighted average
U/L	international units per liter
UDS	unscheduled DNA synthesis
UF	uncertainty factor
VCS	visual contrast sensitivity
V_E	ventilation rate
VEP	visually evoked potential
V_{max}	maximum velocity
WHO	World Health Organization

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

In Chapter 6, *Characterization of Hazard and Dose-Response*, the United States Environmental Protection Agency (EPA) has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

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ACKNOWLEDGMENTS

The authors would like to acknowledge the contributions of the following individuals: Terri Konoza of NCEA who managed the document production activities; Cristopher Broyles of Intellitech Systems, Inc. who provided editing support; Lana Wood of Intellitech Systems, Inc.

who provided word processing support; Patricia von Brook of KBM Group who provided editing support for the previous draft; and Christine Chang of KBM Group who provided word processing support for the previous draft.

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 tetrachloroethylene. 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 RfC is defined as an estimate, with uncertainty spanning perhaps an order of magnitude, of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious non-cancer effects during a lifetime. The 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 hrs), short-term (>24 hrs 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. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for tetrachloroethylene has followed the general guidelines for risk assessment set forth by the National Research Council (NRC, 1983, 1994). U.S. Environmental Protection Agency (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, 1996), *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 tetrachloroethylene 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. A comprehensive literature review was carried out through July 2004. In addition, a number of relevant publications since that time have been considered and incorporated in the document.

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2. BACKGROUND

2.1. USES AND PHYSICAL/CHEMICAL PROPERTIES

Tetrachloroethylene is a widely used solvent that is produced commercially for use in dry cleaning, textile processing, and metal-cleaning operations. It has the following use pattern: 55% as a chemical intermediate, 25% for metal cleaning and vapor degreasing, 15% for dry cleaning and textile processing, and 5% for other unspecified uses (ATSDR, 1997).

Table 2-1 lists the physical and chemical properties of tetrachloroethylene (ATSDR, 1997). The reference citations can be found in the Agency for Toxic Substances and Disease Registry (ATSDR) document and are not included in the reference list for this document.

2.2. OCCURRENCE AND EXPOSURE

Tetrachloroethylene has been detected in ground water and surface water as well as in air, soil, food, and breast milk. The primary exposure routes of concern are inhalation of vapor and ingestion of contaminated water. Although dermal exposure is possible via contaminated tap water during showering, bathing, or swimming, this is generally not considered a major route of exposure.

2.2.1. Air

Because of its high volatility, there is considerable potential for release of tetrachloroethylene into the atmosphere. Once in the air, it is not susceptible to wet deposition because of its hydrophobicity. The primary method for removal is photooxidation to trichloroacetyl chloride, trichloroacetic acid (TCA), carbon monoxide, ozone, and phosgene (U.S. EPA, 1982). However, this reaction is very slow, so tetrachloroethylene is not implicated in the buildup of any of the reaction products in the troposphere. Though the half-life of perchloroethylene can vary based on season and environmental conditions, it has been estimated at 96 days under typical conditions (ATSDR, 1997).

Ambient tetrachloroethylene concentrations vary from source to source and with proximity to the source. It should be noted that outdoor concentrations can vary widely within a period of a few hours as a function of wind velocity and direction, precipitation, humidity, and sunlight. ATSDR (1997) reported mean tetrachloroethylene concentrations of 8.8 $\mu g/m^3$ in areas close to points of release.

Table 2-1. Physical and chemical properties of tetrachloroethylene

Property	Information	Reference
Molecular weight	165.83	Lide (1990)
Color	Colorless	Sax and Lewis (1987)
Physical state	Liquid (at room temperature)	Sax and Lewis (1987)
Melting point	-19EC	Lide (1990)
Boiling point	121EC	Lide (1990)
Density at 20EC	1.6227 g/mL	Lide (1990)
Density at 25EC	No data	
Odor	Ethereal	HSDB (1996)
Odor threshold: water	0.3 ppm	U.S. EPA (1987b)
Odor threshold: air	1 ppm	U.S. EPA (1987b)
Solubility: water at 25EC	150 mg/L	HSDB (1996)
Solubility: organic solvent(s)	Miscible with alcohol, ether, chloroform, benzene, solvent hexane, and most of the fixed and volatile oils	HSDB (1996)
Partition coefficients: Log K_{OW}	3.4	HSDB (1996)
Partition coefficients: Log K_{OC}	2.2B2.7	Seip et al. (1986) Zytner et al. (1989a)
Vapor pressure at 25EC	18.47 mm Hg	HSDB (1996)
Henry's law constant at 25EC	1.8 H 10^{-2} atm-m^3/mol	Gossett (1987)
Autoignition temperature	No data	
Flashpoint	None	HSDB (1996)
Flammability limits	Nonflammable	HSDB (1996)
Conversion factors, air	1 mg/L = 141.4 ppm 1 ppm = 6.78 mg/m^3	HSDB (1996)
Explosive limits	No data	

Source: ATSDR (1997).

EPA has carried out modeling to characterize the geographic distribution of tetrachloroethylene for its National-Scale Air Toxics Assessment database (U.S. EPA, 1996). Median census tract-based tetrachloroethylene concentrations across the United States were estimated at about 0.3 μg/m^3 for urban areas and 0.1 μg/m^3 for rural areas (75% upper percentiles of 0.4 and 0.2 μg/m^3, respectively). The California Air Resources Board (CARB, 1998) reported a statewide median air concentration of 0.3 μg/m^3 in 2001, which represents the lowest value in what has been a decreasing trend since 1990. Note that these averages, which are based on geographic areas, only characterize the likely exposure of individuals who spend an equal amount of time in all parts of the defined area, and they may, therefore, significantly underestimate the exposure of individuals who consistently spend time in subareas that have higher tetrachloroethylene concentrations.

Near points of use, such as dry cleaners or industrial facilities, indoor exposure to tetrachloroethylene is more significant than outdoor exposure (U.S. EPA, 2001). Indoor air concentrations in an apartment above a dry cleaning shop have been measured at up to 4.9 mg/m^3 (Verbek and Scheffers, 1980), whereas mean concentrations inside dry cleaning facilities have been found to vary from 48 mg/m^3 to 200 mg/m^3, depending on type of facility (Solet et al., 1990). Concentrations in facilities with post-1990 equipment are likely to be lower (U.S. EPA, 1998).

The off-gassing of garments that have recently been dry-cleaned may be of concern (Tichenor et al., 1990). In the home, tetrachloroethylene vapors may off-gas from the clothes of occupationally exposed individuals, or they may come directly from the exhaled breath of exposed workers (ATSDR, 1997). Relatively high tetrachloroethylene air concentrations have been measured in the proximity of freshly dry-cleaned clothing stored in small, close spaces. A residential closet storing newly dry-cleaned clothing had an air concentration of 2.9 mg/m^3 after 1 day, which rapidly declined to 0.5 mg/m^3 and persisted for several days (Tichenor et al., 1990). There is one documented mortality case: a 2-year-old boy was found dead after being put to sleep in a room with curtains that had been incorrectly dry-cleaned (Garnier et al., 1996).

Dry-cleaned garments transported in an automobile may also lead to unexpectedly high levels of exposure. Park et al. (1998) used simulated driving cycles to estimate the concentrations of several contaminants emitted from in-vehicle sources. Using dry-cleaned clothes as a source, tetrachloroethylene levels inside a stationary vehicle after 30 minutes reached 0.230 mg/m^3. Approximating these exposures is not easy because specific exposure levels would depend on many factors: car velocity, wind speed, ventilation, and time spent in the automobile. Another study demonstrating exposure in a car found that transporting a freshly dry-cleaned

down jacket in a car resulted in a cabin air concentration of 24.8 mg/m^3 after 108 minutes (Chien, 1997).

Air exposure may also occur during showering or bathing as dissolved tetrachloroethylene in the warm tap water is volatilized. Rao and Brown (1993) used an adult physiologically based pharmacokinetic (PBPK) model combined with a microenvironmental exposure model to estimate the dose received by inhalation exposure during showering and bathing as well as by dermal exposure to the water. The tap water concentration of tetrachloroethylene was 1 mg/L, which is probably a higher concentration than exists in most water supplies. They also demonstrated that a majority of the tetrachloroethylene in the blood, as a result of their bathing scenario, resulted from inhalation exposure, while about 15% resulted from dermal absorption.

2.2.2. Water

Because of its relatively low aqueous solubility (see Table 2-1), it is not likely that volatilized tetrachloroethylene will enter surface or rain water. However, it has been detected in drinking water, ground water, and surface water (U.S. EPA, 2001; ATSDR, 1997). Most of this contamination is probably due to release in water following industrial use or by public use of consumer products.

Unless a surface water body is in the vicinity of a highly contaminated site, surface waters are expected to have a lower concentration of tetrachloroethylene than ground water. In an estimate of drinking water contamination in California, McKone and Bogen (1992) assumed that surface water would have a negligible contribution to the concentration of tetrachloroethylene measured in drinking water. Based on data from wells in California, they estimated an average drinking water concentration of 0.3 µg/L, with a standard deviation of 0.35 µg/L.

In areas near sources of contamination, ground water, and surface water concentrations can be considerably higher than average. Because the density of tetrachloroethylene is about 60% higher than that of water, tetrachloroethylene is expected to accumulate near the bottom of a stagnant receiving water body after a large-volume point discharge. Water samples collected near the bottom of the St. Clair River near Sarnia, Ontario, downstream from several petroleum-based production facilities, contained tetrachloroethylene concentrations ranging from 0.002 to 34.6 µg/L (EC, 1993). The concentrations in 17 samples of surface water from the lower Niagara River in New York State in 1981 averaged 0.036 µg/L (with a maximum of 0.134 µg/L; EC, 1993).

Exposure models have been developed to predict the fate and transport of organic compounds such as tetrachloroethylene in environmental media, including air, water, and soil. The outputs from two similar but independently developed environmental exposure models, CalTOX and Fug3ONT, were compared for a scenario designed to reproduce a residential area near an industrial contamination site (Maddalena et al., 1995), in which 75 moles/day are released into the air and 0.7 moles/day are released into surface water. Although the soil predictions differed, the predictions of tetrachloroethylene in air and ground water were similar, with the concentration of air predicted by CalTOX approximately 6 $\mu g/m^3$ and the surface water concentration 82 µg/L. It should be noted that agreement of the models does not confirm the validity of either one, but lends some support to the usefulness of the results.

The off-gassing of tetrachloroethylene from a drinking water supply can result in exposure. In 1976, EPA measured tetrachloroethylene levels ranging from 800 to 2,000 µg/L in drinking water samples in Massachusetts (Paulu et al., 1999). Similar levels were reported elsewhere in New England. These concentrations were attributed to the vinyl-lined asbestos-cement pipes that were used to carry water in this area (Webler and Brown, 1993). Letkiewicz et al. (1982) estimated that 53% of newborn infants are formula-fed from drinking water sources and the other 47% receive all of their fluid from breast milk. Taking into account volatilization during boiling of water, they indicate that the uptake of tetrachloroethylene in formula-fed infants on a mg/kg-day basis is 10 times higher than in adults with the same level of drinking water contamination.

Although dermal exposure is possible via contaminated tap water during showering, bathing, or swimming, this is generally not considered a major route of exposure. Rao and Brown (1993) demonstrated that only 15% of the tetrachloroethylene in the blood resulted from dermal exposure as compared to inhalation of vapors.

2.2.3. Food

Certain foods have been found to be contaminated with tetrachloroethylene (U.S. EPA, 2001). Because of the lipophilic nature of tetrachloroethylene, it may bind to lipid molecules in such foods as margarine, oils, meats, and other fatty foods stored in areas where there is tetrachloroethylene in the air. In 1988, elevated tetrachloroethylene levels were seen in margarine and butter samples obtained from grocery stores located near dry cleaning facilities (Entz and Diachenko, 1988). Further studies confirmed that close proximity to a dry cleaning facility was associated with elevated tetrachloroethylene levels in butter samples (Kacew and Lambert, 1997). Nonetheless, food is not considered to be a major exposure pathway. Other

sources of information about tetrachloroethylene in foods are the Food and Drug Administration (FDA, 2003) and Fleming-Jones and Smith (2003).

2.2.4. Breast Milk

Due to its lipid solubility, tetrachloroethylene can concentrate in milk (NYS DOH, 2000; Schreiber, 1993; Sheldon et al., 1985). Breast milk can contain high concentrations of tetrachloroethylene and some of its toxic metabolites. Reported levels of tetrachloroethylene in breast milk have ranged up to 43 µg/L in the general population (U.S. EPA, 2001).

Schreiber (1993) used a PBPK model to estimate the dose a nursing infant might receive from an exposed mother's breast milk. This study showed that it is possible for the dose an infant receives through breast milk to approach levels that could result in adverse health effects and exceed the 1988 EPA RfD of 0.01 mg/kg-day (U.S. EPA, 1988). Actual indoor air concentrations (24-hr average), as measured in apartments in New York State, were used to predict potential levels in breast milk in these modeling scenarios. The apartments included one located above a dry cleaning facility that used an old dry-to-dry machine (average concentration, 45.8mg/m^3), three located above facilities that used transfer machines (average concentration, 7.7mg/m^3), and two located above facilities that used newer dry-to-dry machines (average concentration, 0.25 mg/m^3; Schreiber, 1993). The predicted breast milk concentrations in these scenarios ranged from 16 to 3,000 µg/L. Assuming that a 7.2 kg infant ingests 700 mL of breast milk per day, Schreiber (1993) determined that the infant dose from milk could range from 0.0015 to 0.3 mg/kg-day.

Using the same exposure conditions as Schreiber (1993), Byczkowski et al. (1994) predicted lower doses to the infant (0.0009–0.202 mg/kg-day), although these doses approached levels that could result in adverse health effects. Exceedances of the RfD were seen only in those apartments above old dry-to-dry machines (0.202 mg/kg-day) or above transfer machines (0.029 mg/kg-day). Ingestion through breast milk and infant exposures is discussed further in Section 4.8. However, Schreiber (1997) has suggested that if infants live adjacent to or in close proximity to dry cleaning facilitates, the dose received through breast milk ingestion will be insignificant when compared with that from their inhalation exposure.

In one case study, the breast milk of a woman was found to contain 10 mg/L of tetrachloroethylene 1 hr following a visit to her husband at his work in a dry cleaning establishment. This concentration dropped to 3 mg/L after 24 hrs. Her child suffered from obstructive jaundice and hepatomegaly, but these conditions improved when breastfeeding was discontinued (Bagnell and Ellenberger, 1977).

2.2.5. Direct Ingestion

In rare circumstances, direct ingestion of tetrachloroethylene has been documented. A 6-year-old boy who directly ingested 12–16 g tetrachloroethylene experienced drowsiness, vertigo, agitation, and hallucinations. He then lost consciousness and went into a coma, and later recovered (Koppel et al., 1985). Follow-up testing on the boy was not reported, so any potential long-term effects of the exposure are unknown.

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3. TOXICOKINETICS

3.1. ABSORPTION

Tetrachloroethylene is rapidly absorbed into the bloodstream following oral and inhalation exposures. It can also be absorbed across the skin following dermal exposure to either pure or diluted solvent or vapors (Stewart and Dodd, 1964; Nakai et al., 1999; Poet et al., 2002).

3.1.1. Inhalation

The major exposure route for tetrachloroethylene is considered to be inhalation (U.S. EPA, 1985; IARC, 1995). Pulmonary uptake of tetrachloroethylene is rapid; however, complete tissue equilibrium occurs only after several hours. Absorption into the systemic circulation through pulmonary uptake is proportional to the ventilation rate, the duration of exposure, and, at lower ambient concentrations to which humans are likely to be exposed, the concentration in the inspired air (Hake and Stewart, 1977; Monster et al., 1979).

Chiu et al. (2007) reported that peak levels of tetrachloroethylene in venous blood and air occurred near the end of a 6-hr inhalation exposure to 1 ppm and declined thereafter. In the Monster et al. (1979) study, uptake after 4 hrs was 75% of its value at the onset of exposure. Increased physical activity increases uptake but lowers the alveolar partial pressure, thus removing more tetrachloroethylene from the alveoli, resulting in a longer time to reach tissue equilibrium (Pezzagno et al., 1988).

The blood/gas partition coefficient for tetrachloroethylene describes how the chemical will partition itself between the two phases. Specifically, it is the ratio of concentrations at steady state; i.e., when all rates are constant after equilibrium has been reached. Reported values for the coefficient in humans range from around 10 to 20 (e.g., Byczkowski and Fisher, 1994; Reitz et al., 1996; Droz and Guillemin, 1986; Ward et al., 1988; Gearhart et al., 1993; Hattis et al., 1990), meaning that if tetrachloroethylene is in equilibrium, the concentration in blood will be 10 to 20 times higher than the concentration in the alveoli.

Opdam and Smolders (1986) determined concentrations of tetrachloroethylene in alveolar air for 1- to 60-second residence times (the time interval from the beginning of an inhalation to the end of the next inhalation) for six volunteers exposed to 0.5 to 9.8 ppm of chemical for 1 to 60 minutes. These investigators found the concentrations of tetrachloroethylene in alveolar air to decrease with residence times for breaths during exposure periods but to increase during post-exposure for residence times less than 10 seconds. Alveolar air tetrachloroethylene concentration correlated with the concentrations in pulmonary artery mixed venous blood.

Like the studies in humans, inhalation studies in laboratory animals provide clear evidence that tetrachloroethylene is readily absorbed via the lungs into the systemic circulation (e.g., Pegg et al., 1979; Dallas et al., 1994a).

3.1.2. Oral

Gastric absorption of tetrachloroethylene occurs at a relatively rapid rate and is essentially complete. Close to 100% of oral doses are absorbed from the gut, according to reports of several studies conducted in mice, rats, and dogs (Dallas et al., 1994a, 1995; Frantz and Watanabe, 1983; Pegg et al., 1979; Schumann et al., 1980). Absorption into the systemic circulation was indicated by blood tetrachloroethylene levels of 21.5 µg/mL following accidental ingestion of the chemical by a 6-year-old boy (Koppel et al., 1985).

3.1.3. Dermal

Absorption of tetrachloroethylene by humans following dermal exposure to vapors of the chemical has been reported to be relatively insignificant (only 1%) when compared with absorption via inhalation of vapors (Riihimaki and Pfaffli, 1978; Nakai et al., 1999). The amount of chemical absorbed during the immersion of one thumb in liquid tetrachloroethylene is equivalent to the uptake during inhalation of 10 to 15 ppm of the compound for the same time period (Stewart and Dodd, 1964).

Studies in animals confirm that dermal uptake of tetrachloroethylene following vapor exposure is minimal when compared with pulmonary uptake (Tsuruta, 1989; McDougal et al., 1990), whereas dermal uptake is greater following direct skin application (Jakobson et al., 1982). Notably, the conclusions of Bogen et al. (1992), based on the results of their study in hairless guinea pigs, indicate that dermal absorption of tetrachloroethylene from contaminated water supplies could be an important route of exposure for humans. These investigators estimated that a standard 70 kg man with 80% of his body immersed in water would completely absorb the amount of tetrachloroethylene in 2 L of that water.

3.2. DISTRIBUTION AND BODY BURDEN

Once absorbed, tetrachloroethylene is distributed by first-order diffusion processes to all tissues in the mammalian body. The highest concentrations of tetrachloroethylene are found in adipose tissue due to the lipophilicity of the compound (U.S. EPA, 1985). Concentrations of tetrachloroethylene reach higher levels in brain and liver than in many other tissues (Garnier et al., 1996; Levine et al., 1981; Lukaszewski, 1979). Absolute tissue concentrations are directly proportional to the body burden or exposure dose. Due to its lipid solubility, tetrachloroethylene

is also concentrated in milk, and it has been measured in human breast milk (Schreiber, 1993, 1997; Schreiber et al., 2002; NYS DOH, 2000). Higher concentrations occur in milk having higher fat content; e.g., a noticeable difference exists between the milk/blood partition coefficients for rats (12) and for humans (2.8; Byczkowski and Fisher, 1994), reflecting the higher fat content of rat milk. Tetrachloroethylene readily crosses both the blood-brain barrier and the placenta. Partition coefficients for various tissues, relative to blood or air, have been reported by several investigators (Ward et al., 1988; Dallas et al., 1994a, b; Gearhart et al., 1993; Byczkowski and Fisher, 1994). Section 3.5 presents examples of these.

Repeated daily inhalation exposures of human volunteers to tetrachloroethylene indicate accumulation of the compound in the body, which is thought to be due to its high lipid solubility. Because of its long residence time in adipose tissue, repeated daily exposure results in an accumulated concentration; tetrachloroethylene from new exposures adds to the residual concentration from previous exposures until steady state is reached. Blood levels of tetrachloroethylene increase over several days with continued daily exposures. Following cessation of these exposures, it is still present in the blood. Exhalation of the compound continues over a number of days due to its slow release from the adipose tissue (Stewart et al., 1977; Altmann et al., 1990; Skender et al., 1991). For a given concentration in blood or air, the half-time—the time necessary to equilibrate the adipose tissue to 50% of its final concentration—is about 25 hrs (Monster, 1979; Fernandez et al., 1976). Therefore, during a single 8-hr exposure, adipose tissue does not reach steady-state equilibrium.

Tetrachloroethylene uptake by fatty tissue during the working hours of the week is countered by the elimination that occurs during nonexposure times of nights and weekends; thus, for persons exposed to tetrachloroethylene on a five-day-a-week work schedule, an equilibrium is eventually established, but it requires a time period of 3 to 4 weeks of exposure for adipose tissue to reach plateau concentrations (U.S. EPA, 1985).

Animal studies provide clear evidence that tetrachloroethylene distributes widely to all tissues of the body, readily crossing the blood-brain barrier and the placenta (Schumann et al., 1980; Ghantous et al., 1986; Savolainen et al., 1977; Dallas et al., 1994b). Following exposure of rats to tetrachloroethylene, the compound has been measured in blood, fat, brain, lungs, liver, kidneys, heart, and skeletal muscle (Savolainen et al., 1977; Dallas et al., 1994b). Highest tissue concentrations were found in adipose tissue (60 or more times blood level) and in brain and liver (4 and 5 times blood level, respectively), as can be calculated from the rat tissue distribution data of Savolainen et al. (1977). Dallas et al. (1994b) found the concentration in fat to be 9 to 18 times the concentrations found in nonfat tissues. Skeletal muscle contained the lowest concentration. In one human fatality case, the concentration of tetrachloroethylene in the brain

was 120 times higher than concentrations measured in the lung. In another case the concentration in the liver was 8, 3.4, and 3.5 times higher, respectively, than concentrations measured in the lung, kidney, and brain (Levine et al., 1981).

3.3. METABOLISM

This section describes the metabolism of tetrachloroethylene, identifying metabolites thought to be causally associated with toxic responses as well as those used to evaluate the flux of parent compound through the known metabolic pathways. Sex- and species-dependent differences in the metabolism of tetrachloroethylene and potential contributors to interindividual differences are identified. Factors that influence metabolism in humans are mentioned. See Section 4.9 for further discussion of how these factors affect variability and susceptibility.

3.3.1. Introduction

The metabolism of tetrachloroethylene has been studied mostly in mice, rats, and humans (for reviews, see Dekant et al., 1987, 1989; Anders et al., 1988; IARC, 1995; U.S. EPA, 1985, 1986, 1991; Lash and Parker, 2001). Tetrachloroethylene is metabolized in laboratory animals and in humans through at least two distinct pathways: oxidative metabolism via the cytochrome P450 (CYP [also abbreviated as P450 and CYP 450]) mixed-function oxidase system and glutathione (GSH) conjugation followed by subsequent further biotransformation and processing, either through the cysteine conjugate beta lyase pathway or by other enzymes including flavin-containing monooxygenase 3 (FMO3) and CYP3A (Daniel, 1963; Filser and Bolt, 1979; Pegg et al., 1979; Costa and Ivanetich, 1980; Dekant et al., 1987, 1989; Anders et al., 1988; U.S. EPA, 1985, 1991; IARC, 1995; Birner et al., 1996; Lash et al., 1998; Volkel et al., 1998; Lash and Parker, 2001). The conjugative pathway, although the minor route quantitatively, is toxicologically significant because it yields relatively potent toxic metabolites (Vamvakas et al., 1987, 1989a, b, c; Dekant et al., 1986a, b, 1989; Werner et al., 1996; Anders et al., 1988; Lash and Parker, 2001).

3.3.2. Extent of Metabolism

Studies in both animals and humans indicate that overall metabolism of tetrachloroethylene is relatively limited (reviewed in U.S. EPA, 1985, 1991; Lash and Parker, 2001), as evidenced by the high percentage of absorbed dose excreted in the breath as the parent molecule (Stewart et al., 1961, 1970; Monster et al., 1979, 1983; Boettner and Muranko, 1969; Ikeda and Otsuji, 1972; Essing et al., 1973; Fernandez et al., 1976; May, 1976; Ohtsuki et al., 1983; Yllner, 1961; Daniel, 1963; Filser and Bolt, 1979; Pegg et al., 1979; Frantz and Watanabe,

1983; Schumann et al., 1980; Buben and O'Flaherty, 1985; Volkel et al., 1998). Because of its high lipid solubility, tetrachloroethylene can be sequestered in fat and, thus, not all metabolism is evident in short sampling time periods.

The extent of metabolism after inhalation exposure in humans has been estimated by measuring trichloro-compounds excreted in the urine and exhalation of tetrachloroethylene in expired air (Bolanowska and Golacka, 1972; Fernandez et al., 1976; Monster et al., 1979, 1983; Monster and Houtkooper, 1979; Ikeda et al., 1972; Boettner and Muranko, 1969; Essing et al., 1973; May, 1976; Stewart et al., 1961, 1970). Several studies reported only about 1–3% of the estimated amounts inhaled were metabolized to TCA and other chlorinated metabolites, although additional tetrachloroethylene—as much as 20% or more of the dose—may be metabolized over a longer period (Monster et al., 1979; U.S. EPA, 1985, 1991; Bois et al., 1996; Bogen et al., 1992). For example, Chiu et al. (2007) noted that although an average of 0.4% of tetrachloroethylene intake (1 ppm for 6 hrs) was recovered in urine as TCA, total recovery in urine and exhaled air accounted for on average only 82% of intake. This would imply 18% metabolized, but Chiu et al. (2007) noted substantial uncertainty and variability in these calculations and concluded they were consistent with previous studies at higher exposures. Interestingly, Chiu et al. (2007) also noted significant variability among the seven subjects and among the four occasions, contributing to the uncertainty in measurements.

The extent of metabolism in animals has been estimated by conducting excretion-balance studies using isotopically labeled tetrachloroethylene. In rodents, 2–88% of the dose was metabolized, depending on dose level and species: the higher the dose the smaller the percent metabolized. Rats metabolized a lower percent of a given tetrachloroethylene body burden than did mice (Yllner, 1961; Daniel, 1963; Filser and Bolt, 1979; Pegg et al., 1979; Frantz and Watanabe, 1983; Schumann et al., 1980). As an example, using data from the Pegg et al. (1979) and Schumann et al. (1980) studies in rats, EPA calculated that the percent of body burdens excreted were unchanged following exposure to 10 and 600 ppm for 6 hrs, were 68 and 99%, respectively (U.S. EPA, 1985). For comparison, studies in mice exposed to 10 ppm for 6 hrs found pulmonary excretion of only 12%, whereas 83% of the tetrachloroethylene was excreted by the pulmonary route for a body burden of about 11 mg from oral administration (U.S. EPA, 1985). As body burden is increased, the proportion of tetrachloroethylene excreted unchanged increases and the percent metabolized decreases.

3.3.3. Pathways of Metabolism

The two known biotransformation pathways for tetrachloroethylene metabolism are (1) oxidation by cytochrome P450 (CYP) enzymes and (2) conjugation with GSH followed by

further processing of the conjugate through various pathway bifurcation branches. The initial step in the metabolism of tetrachloroethylene may be either epoxidation or chlorine migration for the oxidative pathway or conjugation with GSH for the secondary pathway (Costa and Ivanetich, 1980; Miller and Guengerich, 1982, 1983; Dekant et al., 1986b, 1987, 1998; Lash et al., 1998; Lash and Parker, 2001). It is possible that other as yet unrecognized pathways for tetrachloroethylene exist in humans (Sakamoto, 1976; Monster et al., 1979; U.S. EPA, 1985, 1991; Bois et al., 1996).

3.3.3.1. *Cytochrome P450-Dependent Oxidation*

Oxidative metabolism by the cytochrome P450, or CYP-dependent, pathway is quantitatively the major route of tetrachloroethylene biotransformation (U.S. EPA, 1991; IARC, 1995; Lash and Parker, 2001). This pathway was initially proposed by Powell (1945) for trichloroethylene and was subsequently supported for tetrachloroethylene by the results of Yllner (1961), Daniel (1963), Leibman and Ortiz (1970, 1977), Costa and Ivanetich (1980), and others. The pathway is operative in humans and rodents and leads to several metabolites, some of which are known toxins and carcinogens (U.S. EPA, 1991; IARC, 1995). Figure 3-1 depicts the overall scheme of tetrachloroethylene P450 metabolism. Known metabolites presented in this figure are identified by an asterisk.

The major excretory metabolite of the oxidative pathway, TCA, is excreted in the urine of all species tested. Figure 3-1 identifies many common urinary metabolites, including dichloroacetic acid (DCA), trichloroacetylethanolamide, oxalylethanalamide, and oxalic acid. Trichloroethanol (TCOH) has been measured in some, but not all, studies (Bonse et al., 1975; Bonse and Henschler, 1976; Yllner, 1961; Dmitrieva, 1967; Pegg et al., 1979; Ogata et al., 1962, 1971; Tanaka and Ikeda, 1968; Ikeda and Otsuji, 1972; Ikeda et al., 1972; Monster et al., 1983; Weichardt and Lindner, 1975; Dekant et al., 1986b, 1987; Birner et al., 1996; U.S. EPA, 1985, 1986, 1991). Oxalic acid is a relatively major urinary metabolite in rats (Dmitrieva, 1967; Pegg et al., 1979). Pulmonary excretion of carbon dioxide (CO_2) has been identified in exhaled breath from rodents exposed to ^{14}C-labeled tetrachloroethylene (Pegg et al., 1979; Schumann et al., 1980). Oxalic acid and formic acid plus CO_2 are hypothesized to arise from action of microsomal epoxide hydrase on the initial epoxide intermediate to yield tetrachloroethylene glycol, which may then be further processed via two routes to these aforementioned end products.

Oxidative metabolism of tetrachloroethylene, irrespective of the route of administration, occurs predominantly in the liver but also occurs at other sites. For example, the kidneys exhibit cytochrome P450 enzyme activities, mostly in the proximal tubules, although total activity is

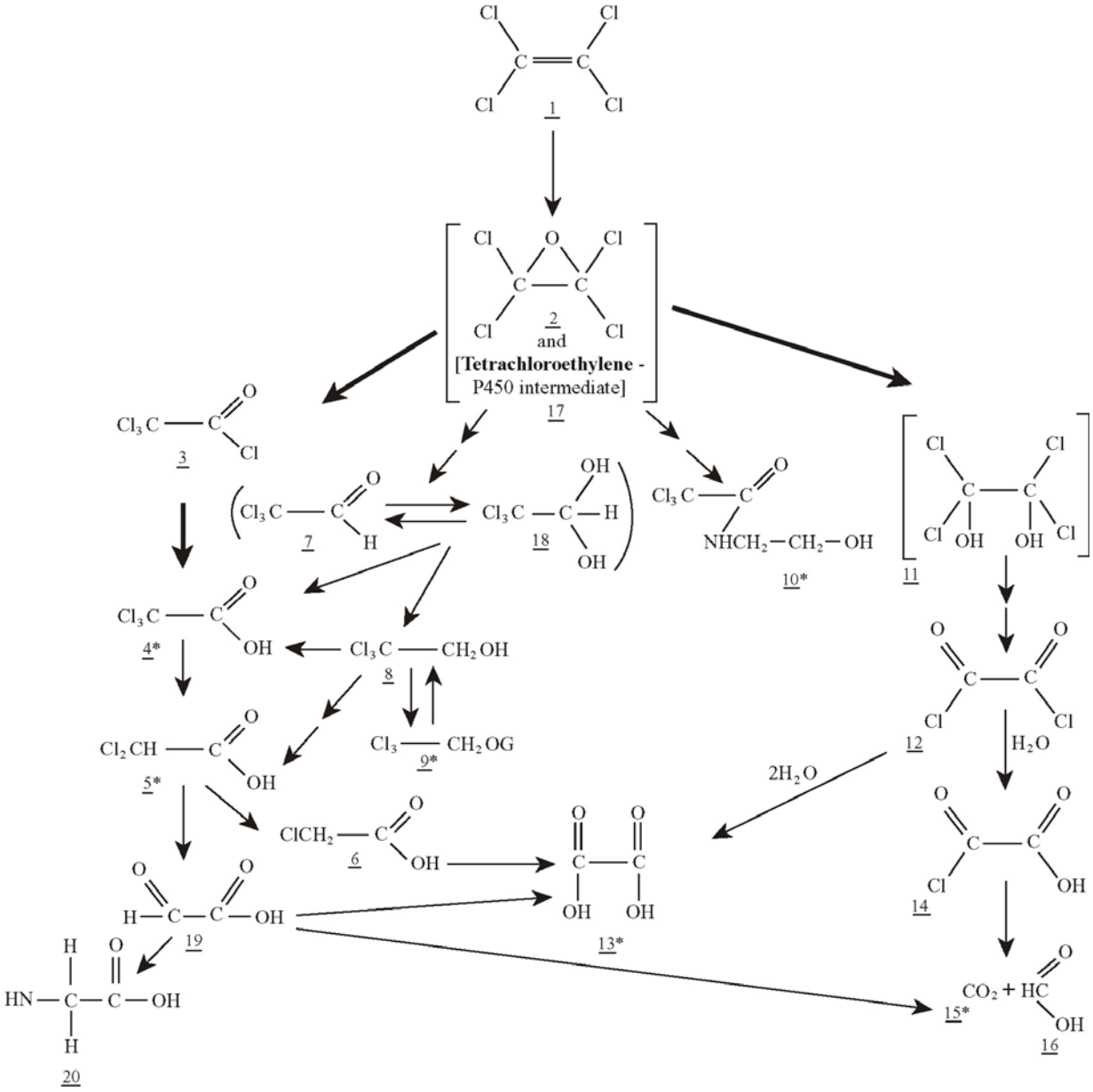

Figure 3-1. Postulated scheme for the metabolism of tetrachloroethylene by the P450 oxidative pathway. Tetrachloroethylene and identified (*) metabolites: 1 tetrachloroethylene, 2 tetrachloroethylene oxide, 3 trichloroacetyl chloride, 4 trichloroacetic acid, 5 dichloroacetic acid, 6 monochloroacetic acid, 7 chloral, 8 trichloroethanol, 9 trichloroethanol glucuronide, 10 trichloroacetyl ethanolamide, 11 tetrachloroethylene glycol, 12 ethandioyl dichloride, 13 oxalic acid, 14 glyoxylic acid chloride, 15 carbon dioxide, 16 formic acid, 17 P450 intermediate, 18 chloral hydrate, 19 glyoxylic acid, and 20 glycine.

Sources: Adapted from Pegg et al. (1979), Costa and Ivanetich (1980), U.S. EPA (1985), Dekant et al. (1986a), Lash and Parker (2001).

markedly less than in the liver (Lash and Parker, 2001; Lash et al., 2001). CYP enzymes occurring in other extrahepatic tissues—brain and lungs, for example—may also contribute to oxidative metabolism of tetrachloroethylene.

3.3.3.1.1. ***Formation of tetrachloroethylene oxide.*** The first step in the oxidation of tetrachloroethylene is hypothesized to yield 1,1,2,2-tetrachloroethylene oxide, a relatively unstable epoxide (Costa and Ivanetich, 1980; Miller and Guengerich, 1982, 1983). Although an initial epoxide metabolite has not been unequivocally demonstrated for tetrachloroethylene, evidence for this epoxide does exist. The epoxide has been chemically synthesized (Frankel et al., 1957; Bonse et al., 1975; Kline et al., 1978). The several potential fates of tetrachloroethylene epoxide include trichloroacetyl chloride, oxalate dichloride through tetrachloroethylene glycol, trichloroacetyl aminoethanol, and possibly chloral hydrate (in equilibrium with chloral; Bonse and Henschler, 1976; Henschler and Bonse, 1977; Pegg et al., 1979; U.S. EPA, 1985, 1986). Formation of trichloroacetyl chloride directly from tetrachloroethylene, without the formation of the epoxide intermediate, via the mechanism of CYP-mediated olefin oxidation has also been postulated (Guengerich and Macdonald, 1984).

3.3.3.1.2. ***Metabolism to Trichloroacetic Acid (TCA) and possibly Trichloroethanol (TCOH).*** Measurement of urinary TCA has been used as a biomarker for tetrachloroethylene exposure (U.S. EPA, 1985; IARC, 1995), although TCA can be a by-product of metabolism of other chemical compounds. TCA, a major tetrachloroethylene urinary metabolite in both humans and laboratory rodents (Yllner, 1961; Daniel, 1963; Leibman and Ortiz, 1970, 1977; Birner et al., 1996; Dekant et al., 1987; Ohtsuki et al., 1983; Volkel et al., 1998), is believed to result primarily from the oxidation of tetrachloroethylene to trichloroacetyl chloride. This oxidation may occur through the epoxide intermediate, with chloride migration leading to the reactive trichloroacetyl chloride, which can then react with amino groups of cellular proteins or undergo hydrolysis to produce the TCA. N-(di- and trichloroacetylated)-L-lysines, formed by interaction of tetrachloroethylene reactive metabolites with protein, have been identified in liver and kidney tissue of rats exposed to tetrachloroethylene (Birner et al., 1994; Pahler et al., 1999a).

The proposed chloral hydrate intermediate is another potential source of TCA, but chloral hydrate can also be further metabolized to TCOH (Sellers et al., 1972; Birner et al., 1996). This latter pathway to TCOH would be the favored reaction, and it is thought to be catalyzed by both alcohol dehydrogenase (Larson and Bull, 1989) and CYP2E1 (Schultz and Weiner, 1979; Ni et al., 1996). The resulting TCOH is then conjugated with glucuronide, a reversible reaction, and both the alcohol and its glucuronide conjugate have been reportedly detected as urinary excretion products following tetrachloroethylene exposures. TCOH has been detected in the urine of

subjects exposed to tetrachloroethylene in some studies (Birner et al., 1996; Ikeda and Otsuji, 1972; Ikeda et al., 1972; Ogata et al., 1962, 1971; Tanaka and Ikeda, 1968; Monster et al., 1983; Weichardt and Lindner, 1975; Schreiber et al., 2002), but could not be identified by others (Fernandez et al., 1976; Hake and Stewart, 1977; Monster et al., 1979; Volkel et al., 1998; Yllner, 1961; Daniel, 1963; Buben and O'Flaherty, 1985; Costa and Ivanetich, 1980). TCOH is thought to be an artifact of the methodology used or could arise due to unknown exposures to other chemicals. Thus, because TCOH is clearly not a significant metabolite for tetrachloroethylene, very little, if any, TCA produced from tetrachloroethylene metabolism is likely to come through chloral, either directly or indirectly through TCOH (Lash and Parker, 2001).

3.3.3.1.3. ***Formation of dichloroacetic acid (DCA) and other products.*** TCA is the major source of DCA from the tetrachloroethylene P450 oxidation pathway. Although DCA has been identified as a tetrachloroethylene urinary metabolite (Yllner, 1961; Dekant et al., 1987; Volkel et al., 1998), it is not clear whether the DCA is a product of further metabolism of TCA, of another pathway originating with GSH conjugation, or both. The major organ site of DCA production is likely to differ for each pathway, with DCA arising from oxidative metabolism primarily in the liver and from GSH-dependent metabolism products mostly in the kidney. The amount of DCA produced from tetrachloroethylene oxidative metabolism may vary across species and is likely to be less than TCA. This is because DCA derived from P450 oxidation comes only from dechlorination of TCA, which is not extensively metabolized, but rather, is mostly excreted unchanged in urine.

The lack of a role for DCA in tetrachloroethylene liver toxicity is supported by the limited findings of Maronpot and his coworkers (Anna et al., 1994; Maronpot et al., 1995), which showed no similarities in mutation spectra between tetrachloroethylene-induced liver tumors and DCA-induced liver tumors. It is interesting to note, however, that the kinetics of metabolism and the sensitivity of target tissue to TCA and DCA and their precursors are likely of key importance to understanding species differences in responsiveness to tetrachloroethylene.

Dechlorination of TCA to DCA is catalyzed by gut contents (ingested food and bacteria) of the rat and mouse (Moghaddam et al., 1996); isolated mouse microflora have been shown to convert TCA to DCA (Moghaddam et al., 1997). DCA can be rather quickly processed to other chemical species, such as monochloroacetic acid (MCA), glycolic acid, glyoxylic acid, and oxalic acid (Abbas and Fisher, 1997; Lash et al., 2000; Bull, 2000; Board et al., 1997; Tong et al., 1998a, b; Lash and Parker, 2001). Conversion to glyoxylic acid is thought to occur by action of the GST zeta (GSTZ in humans) isoform of glutathione S-transferase (GST; Lash et al., 2000). DCA is a mechanism-based inactivator of GSTZ, of which five polymorphic variants exist

(Tzeng et al., 2000). Potent and irreversible inhibition of GSTZ activity by DCA occurs, and the substrate inhibition of the enzyme in vitro differs between rats and humans, with the enzyme being relatively more sensitive to inhibition by DCA in rats. Further degradation of DCA in the liver occurs primarily in hepatic cytosol (Lipscomb et al., 1995). Human liver cytosol is less efficient than either rat or mouse liver cytosol in processing DCA (Lipscomb et al., 1995).

Trichloroacetyl ethanolamide also may be formed from the tetrachloroethylene oxide intermediate or from the alternative chlorine migration in an oxygenated tetrachloroethylene transition state. $^{14}CO_2$ has been recovered from laboratory animals administered ^{14}C-labeled tetrachloroethylene (Frantz and Watanabe, 1983; Pegg et al., 1979; Schumann et al., 1980). A measurable portion of tetrachloroethylene is completely metabolized in a dose-dependent manner to CO_2. The oxalate metabolite excretory product may be derived from DCA or MCA (Tong et al., 1998a, b), although oxalic acid is also produced from the epoxide through tetrachlorodiacetyl chloride and oxalic acid dichloride intermediates (Pegg et al., 1979; Costa and Ivanetich, 1980). The occurrence of oxalic acid and of CO_2 as major metabolites of tetrachloroethylene, at least in rodents, indicates the existence of pathway(s) of metabolism other than the primary TCA pathway.

3.3.3.1.4. ***Species-dependent differences.*** Although thought to be qualitatively similar, there are clear differences among species in the quantitative aspects of tetrachloroethylene metabolism (Schumann et al., 1980; Ikeda and Otsuji, 1972; Volkel et al., 1998; U.S. EPA, 1991; Lash and Parker, 2001). These differences are in the relative yields and kinetic behavior of metabolites (Volkel et al., 1998; Ohtsuki et al., 1983; Green et al., 1990; U.S. EPA, 1985, 1991). Rodents and humans differ in relative rates of tetrachloroethylene metabolism in key target organs, in the doses at which saturation of metabolism occurs, and in the half-times in the body.

The rate of metabolism of tetrachloroethylene is faster in rodents than in humans and higher blood levels of metabolites are obtained in rodents as compared to humans. The higher blood levels of metabolites in rodents are particularly noticeable at the higher tetrachloroethylene exposure levels because saturation is approached at lower exposure levels in humans than in rodents. The half-time in the body of these metabolites is, however, noticeably longer for humans than for rodents (144 hrs in humans vs. approximately 10 hrs or less in rodents; see U.S. EPA, 1985). It is for this reason that examinations of tetrachloroethylene concentration and toxicity associations must reflect both blood concentration and time-integrated dose metrics such as area-under-the-curve.

A study of species differences in tetrachloroethylene metabolism conducted by Dekant and colleagues is presented in Volkel et al. (1998). These investigators compared both oxidative and GSH-dependent metabolism in rats and humans exposed for 6 hrs to 10, 20, or 40 ppm

tetrachloroethylene by inhalation. Rats were also exposed to 400 ppm concentrations. TCA was the major urinary excretion product in both species; however, the elimination half-time was more than four times slower in humans than in rats. Blood plasma concentrations of the metabolite were higher (three- to eight-fold, depending on the dose) in rats than in humans exposed to identical air concentration levels. These observations are in agreement with metabolic rates in general, which are higher in mice than in rats; rats, in turn, have higher metabolic rates than do larger animals, including humans. Dekant and his coworkers also reported urinary excretion of DCA by rats but not humans. They concluded most of the DCA resulted from GSH-dependent metabolism. DCA, however, is further metabolized by P450 enzymes, which, in turn, limits its detectability in urine.

3.3.3.1.5. ***Cytochrome P450 (CYP) isoforms and genetic polymorphisms.*** Relatively few studies provide information about which specific CYP isoforms play a role in tetrachloroethylene oxidative metabolism. CYP2E1 is presumed to have an important role in tetrachloroethylene metabolism (Lash and Parker, 2001); however, the chemical-specific related data are too sparse to provide strong support for this assumption (Doherty et al., 1996). CYP2B1/2 may also be important for the metabolism of tetrachloroethylene. CYP3A isoenzymes may contribute to the generation of reactive sulfoxides from metabolites of the GSH pathway (see below). Costa and Ivanetich (1980) showed increased hepatic metabolism following treatment with agents now known to induce these isoenzymes specifically.

Genetic polymorphisms are DNA sequence variations that result in changes in protein sequence of an enzyme that can alter the enzyme's ability to catalyze a reaction or alter the expression of an allele. Polymorphisms are known for most of the CYP enzymes including CYP2E1 (McCarver et al., 1998; Hu et al., 1999) and CYP3A4 (Sata et al., 2000; Westlind et al., 1999).

Metabolism of tetrachloroethylene to its putative epoxide is likely affected by CYP enzymes. The metabolism of the putative metabolite chloral hydrate to TCOH and TCA may be catalyzed by both alcohol dehydrogenase and CYP2E1. Oxidation of TCOH is catalyzed by P450 enzymes, with CYP2E1 the likely predominant isoform involved, although other isoenzymes may also play a role, even substituting for CYP2E1 in processing tetrachloroethylene. Rat kidney expresses CYP2E1 (Cummings et al., 1999; Speerschneider and Dekant, 1995), although human kidney has not been shown to do so (Amet et al., 1997; Cummings et al., 2000a). Therefore, renal CYP metabolism by this isoform in rat kidney would be relevant only insofar as the involvement of other isoenzymes in metabolizing tetrachloroethylene via this route.

3.3.3.2. *Glutathione (GSH) Conjugation Pathway*

Figure 3-2 shows the second metabolic pathway for tetrachloroethylene (Dekant et al., 1987, 1988; Sausen and Elfarra, 1990; Volkel et al., 1998). This GSH pathway was subsequently shown to exist in both rodents and humans (Volkel et al., 1998).

The GSH pathway is initiated by the conjugation of the parent tetrachloroethylene molecule with GSH to form S-(1,2,2-trichlorovinyl)glutathione (trichlorovinyl glutathione, or TCVG). This reaction, which is catalyzed by the GSH-S-transferase enzymes (GSTs), a group of enzyme isoforms, was traditionally considered to be a detoxification reaction, leading to more water-soluble compounds that are more readily excreted. In many cases, however, as with certain halogenated alkanes and alkenes such as tetrachloroethylene, GSH conjugation can be important for bioactivation. The critical step for the alkenes would occur after the enzymatic removal of the glutamyl and glycine residues from the GSH conjugate to yield the corresponding cysteine S-conjugate, which in the case of tetrachloroethylene would be S-(1,2,2-trichlorovinyl) cysteine (trichlorovinyl cysteine, or TCVC; Dekant et al., 1987, 1989; Anders et al., 1988; Green et al., 1990; Vamvakas et al., 1987, 1989a, b, c).

Tetrachloroethylene conjugation with GSH is thought to primarily occur through an interorgan process. GSH conjugation occurs predominantly in the liver to form TCVG, which is then further metabolized to the corresponding cysteine conjugate, TCVC, by the enzymes gamma-glutamyltransferase (GGT) and cysteinylglycine dipeptidase. TCVC acts as a substrate for several enzymes. Beta lyase cleaves TCVC to yield an unstable thiol, giving rise to cytotoxic and mutagenic products, particularly the reactive thioketene. TCVC may also be activated by cysteine conjugate S-oxidase activity, which also can rearrange to form the reactive thioketene. Although Green et al. (1990) hypothesized that GSH conjugation and subsequent activation of tetrachloroethylene did not occur in humans, the N-acetyl urinary metabolite has subsequently been clearly identified in humans exposed to tetrachloroethylene in occupational settings, in laboratory studies, and in residential buildings (Birner et al., 1996; Volkel et al., 1998; Schreiber et al., 2002). Therefore, this pathway is now known to operate in humans as well as in rodents.

This GSH conjugation pathway was recognized much later than was the oxidative pathway, probably because it is relatively minor quantitatively compared with the CYP pathway, yet it may be toxicologically influential (U.S. EPA, 1991; IARC, 1995; Lash and Parker, 2001). The evidence for this is based on in vitro kinetics and the relatively low recovery of urinary mercapturates as compared with urinary TCA and other CYP-derived metabolites (Green et al., 1990; Birner et al., 1996). Urinary mercapturates comprise from 1% to as little as 0.03% of total recovered urinary metabolites, but this does not reflect the total flux through the GSH pathway but rather only the portion that is excreted. In particular, the amount of the mercapturate product

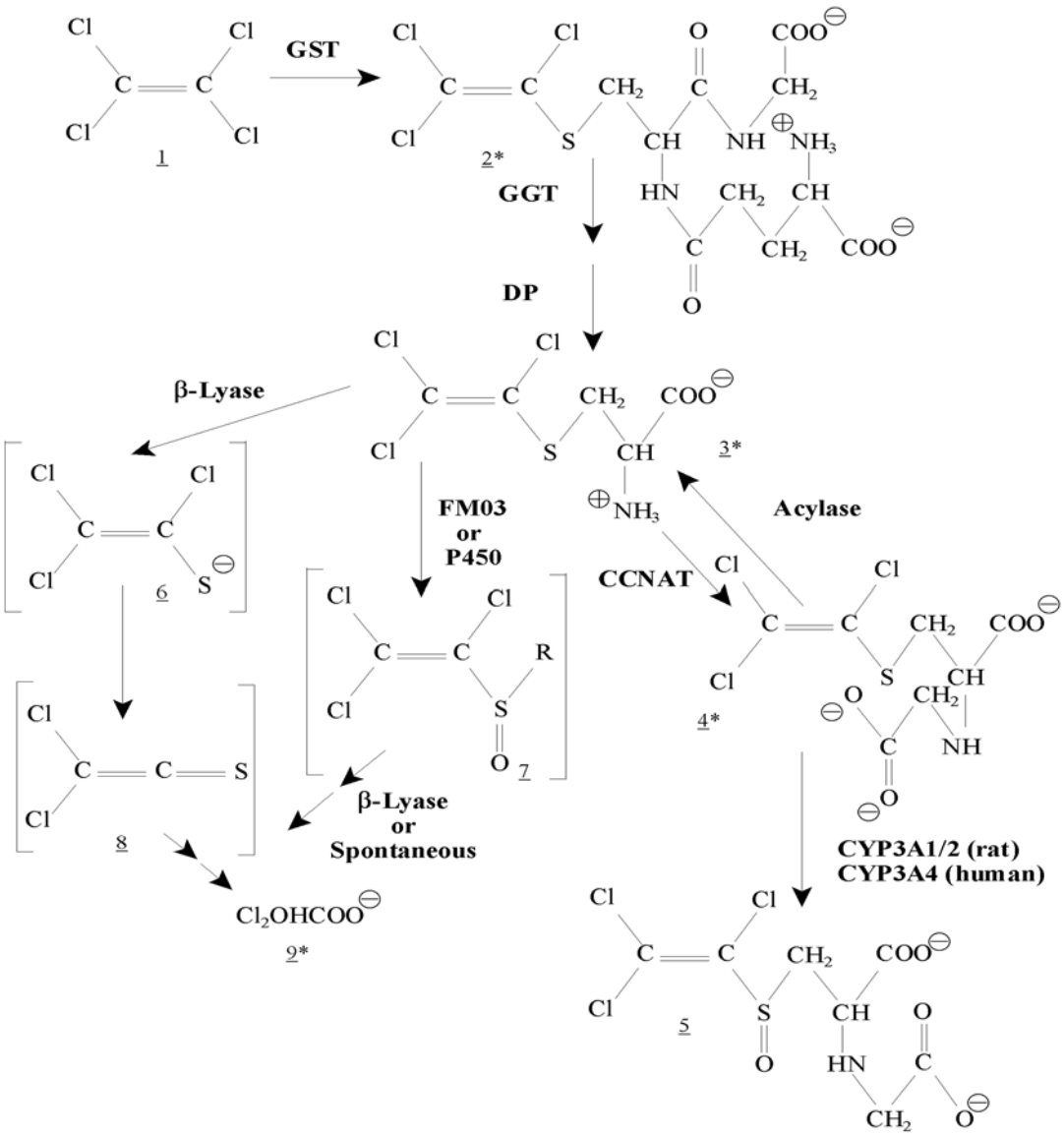

Figure 3-2. Metabolism of tetrachloroethylene by the glutathione conjugation pathway. Tetrachloroethylene and identified (*) metabolites: 1 tetrachloroethylene, 2 S-1,2,2-trichlorovinyl) glutathione (TCVG), 3 S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), 4 N-acetyl trichlorovinyl cysteine (NAcTCVC), 5 NAcTCVC sulfoxide, 6 1,2,2-trichlorovinylthiol, 7 TCVCSO, 8 2,2-dichlorothioketene, and 9 dichloroacetate. Enzymes: glutathione-S-transferase (GST), gamma-glutamyltransferase (GGT), dipeptidase (DP), beta lyase, FMO3, CCNAT, acylase, CYP3A1/2, and CYP3A4. Unstable reactive metabolites are shown in brackets.

Source: Lash and Parker (2001).

excreted in the urine also does not reflect the amount of the more important portion that is converted to toxic by-products through further metabolism.

For tetrachloroethylene, the GSH pathway is associated with renal toxicity (Anders et al., 1988; Dekant et al., 1989; U.S. EPA, 1991; IARC, 1995; Lash et al., 2000; Lash and Parker, 2001). The initial conjugation with GSH occurs mainly in the liver (Dekant et al., 1987; Green et al., 1990; Vamvakas et al., 1987, 1989a), with transport of the conjugate and its cysteine counterpart to the kidney target organ for further processing. This first step also occurs within the kidney (Lash et al., 1998). As shown in Figure 3-2, tetrachloroethylene is initially conjugated with GSH to form TCVG. This reaction is catalyzed by cytosolic and microsomal GSTs. TCVG is then processed through the cysteinylglycine conjugate S-(1,2,2,trichlorovinyl)-L-cysteinylglycine to TCVC by the enzymatic removal of glutamyl and glycine residues by GGT and various membrane-bound dipeptidases known as cysteinylglycine dipeptidase (reviewed by Anders et al., 1988; Dekant et al., 1989; U.S. EPA, 1991; Lash and Parker, 2001). These enzymes reside in tissues other than the kidneys (e.g., the brain), indicating a potential for toxic reactive metabolite formation in those tissues as well. Conversion of TCVG to TCVC by these cleavage enzymes leads to a critical bifurcation point of the GSH pathway because the TCVC may be processed by certain enzymes to yield reactive, toxic chemical species, although it may be metabolized via a different route to yield an excretory product (Lash and Parker, 2001).

Importantly, the TCVC metabolite may also act as a substrate for renal beta lyases (Dekant et al., 1988 reviewed by Anders et al., 1988; Dekant et al., 1989; U.S. EPA, 1991; Lash et al., 2000; Lash and Parker, 2001). Renal beta lyases are known to cleave TCVC to yield an unstable thiol, 1,2,2-trichlorovinylthiol, that may give rise to a reactive chemical species that can form covalent adducts with cellular nucleophiles, including DNA and proteins (Volkel et al., 1999). Beta lyases are a family of pyridoxal phosphate-containing enzymes that are located in several tissues besides the kidneys, including liver and brain, and in intestinal flora, although their substrate specificities may vary. Hepatic beta lyase is distinct from renal beta lyase and has not been found to have a role in TCVC metabolism. Beta lyase activity is higher in rat kidney than in human kidney (Cooper, 1994; Lash et al., 1990), which is consistent with overall metabolic rates being higher in smaller versus larger mammalian species.

In addition to activation by beta lyases, TCVC may be metabolized by a flavin-containing monooxygenase, FMO3, or CYP enzymes to TCVC sulfoxide (TCVCSO), another reactive metabolite (Ripp et al., 1997). TCVSO is a more potent nephrotoxicant than TCVC (Elfarra and Krause 2007). These TCVC sulfoxide and beta lyase cleavage products rearrange, forming a thioketene (Dekant et al., 1988; Ripp et al., 1997), which is a potent acylating agent capable of binding to cellular macromolecules, including DNA (Birner et al., 1996; Pahler et al., 1999a, b; Volkel et al., 1999). Interestingly, the thioketene can degrade to form DCA, potentially making

this metabolite a product of both tetrachloroethylene metabolism pathways (Dekant et al., 1987; Volkel et al., 1998).

In addition to beta lyase and FMO3/CYP activation of TCVC, reactive sulfoxides can also be produced by further CYP3A metabolism of N-acetyl-S-(1,2,2 -trichlorovinyl)-L-cysteine (NAcTCVC; Werner et al., 1996). This tetrachloroethylene-derived mercapturate metabolite results from TCVC being acetylated via a reversible reaction (Bartels, 1994; Birner et al., 1996; Duffel and Jakoby, 1982). N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine may be excreted in the urine. However, in addition to its activation to sulfoxides via CYP3A metabolism, it can also be transported to other organs and deactylated intracellularly, regenerating the cysteine conjugate TCVC and thus making it available to other enzymes for activation (Uttamsingh et al., 1998). It should be noted that the N-acetylation reaction is catalyzed by an enzyme located in the endoplasmic reticulum that is distinct from the cytosolic enzymes that are polymorphic in humans (Lash and Parker, 2001).

3.3.3.2.1. ***Glutathione S-transferase (GST) isoenzymes/polymorphisms.*** GSTs are a family of isoenzymes (Mannervik, 1985) found in cytoplasm. A distinct microsomal GST isoenzyme also exists in most mammalian tissues (Otieno et al., 1997). Although GST activity occurs in most cell types, the liver is by far the predominant site of GSH conjugation. GST alpha, designated as GSTA in humans, is the predominate isoenzyme expressed in normal kidney from rodents and humans (Campbell et al., 1991; Overby et al., 1994; Mitchell et al., 1997; Rodilla et al., 1998; Cummings et al., 2000b). Available data thus far do not indicate that variability in activity of this isoenzyme is important to differences in individual susceptibility to toxicity. GSTZ catalyzes the oxidative metabolism of DCA to glyoxylate (Board et al., 1997; Tong et al., 1998a, b), however, the tetrachloroethylene metabolite DCA has been shown to be a potent, irreversible inhibitor of GSTZ activity (Tzeng et al., 2000).

There are five human polymorphic variants of this GSTZ isoenzyme (Tzeng et al., 2000; U.S. EPA, 1998). These genetic polymorphisms may influence tetrachloroethylene metabolism although human data regarding this hypothesis are lacking. There are some species differences in the other three cytoplasmic GSTs relevant to liver and kidney. GSTP expression is the most variable and appears to be polymorphic in humans (Rodilla et al., 1998). It has been found in rat liver (Cummings et al., 1999), but only in biliary ducts in humans (Terrier et al., 1990; Campbell et al., 1991). GSTP has been detected within the human kidney in various cell types (Terrier et al., 1990) but has not been isolated from rat kidney cells (Cummings et al., 1999), although GSTP has also been detected in rabbit kidney (Cummings et al., 1999).

Two homodimeric GST theta (GSTT) isoenzymes have been identified in human kidney (Veitch et al., 1997; Cummings et al., 2000a). GSTT has been detected in rat and mouse liver

and in mouse but not rat kidney (Cummings et al., 1999; Quondamatteo et al., 1998). GST mu (GSTM) has been detected in rat kidney distal tubule cells (Cummings et al., 2000b) and in mouse and rabbit liver and kidney (Overby et al., 1994; Mitchell et al., 1997), but it was not detected in human kidney (Cummings et al., 2000a). It is not clear just how the differences in these isoenzymes are related to species differences in tetrachloroethylene toxicity because the isoenzyme specificity and reaction rates have not yet been studied with regard to tetrachloroethylene (Lash and Parker, 2001).

Some controversy surrounds the importance of the GSH conjugation pathway with regard to tetrachloroethylene metabolism in humans. As noted above, the GSH pathway for tetrachloroethylene was originally demonstrated only in rodents, and interpretation of the then-existing data led some scientists to conclude that the pathway was not operative in humans (Green et al., 1990; U.S. EPA, 1991). More recent data clearly demonstrate the existence of the pathway in humans (Birner et al., 1996; Volkel et al., 1998; Schreiber et al., 2002). There are discrepancies regarding reported rates of tetrachloroethylene GSH metabolism, however (Green et al., 1990; Dekant et al., 1987; Lash et al., 1998; Lash and Parker, 2001). These differences may be due, in part, to different chemical assay methodology or to problems resulting from the stability of the chemical product being measured or both (Lash and Parker, 2001). Some of the published findings concerning TCVG production would not predict any less susceptibility for humans than for rodents with regard to renal toxicity (Lash et al., 1998).

3.3.3.2.2. ***Gamma-glutamyltransferase (GGT).*** Species-dependent differences in GGT (Hinchman and Ballatori, 1990) also are not thought to be limiting, because renal activity is present at high enough levels even in humans so that GGT activity is not the rate-limiting step in the metabolism. Species-dependent differences in this enzyme (described below) would have only a very small quantitative effect on the overall metabolism of TCVG and other similar GSH conjugates. Species differences in GGT activities, therefore, would not have a major role in species differences in renal toxicity (Lash and Parker, 2001) in affecting transformation of TCVG to TCVC, and thus, should not be important to differences in susceptibility to tetrachloroethylene-induced renal toxicity.

GGT is the only enzyme that can split the gamma-glutamyl bond in the GSH conjugates to form cysteine conjugates (Lash and Parker, 2001). It is this reaction that creates TCVC, the substrate for the enzymes that generate the toxic metabolites. Therefore, the distribution of GGT is important. Renal proximal tubular cells have the highest activities of GGT of all tissues, although GGT activity also occurs in the liver, and the kidney-to-liver ratio of this enzyme varies among species. In the rat, the specific activity ratio is 875 (Hinchman and Ballatori, 1990). The ratio is lower in other species that have been studied. The tissue distribution and relative activity

have not been fully studied in humans, but it is known that GGT activity is considerably higher in human liver than in rodent liver (Lash and Parker, 2001). The kidney-to-liver ratio of GGT for humans is thought to be closer to those of pigs (2) and Macaques (5) than to those of rats or mice (423). For this reason, use of a rodent model for the processing of the tetrachloroethylene GSH conjugate to the corresponding cysteine conjugate would overestimate the contribution of the kidneys and underestimate the contribution of the liver in cleaving TCVG to TCVC. Even so, the liver excretes most of the cysteine conjugates such as TCVC into the bile or plasma, where it is cycled to the kidneys and taken up into renal epithelial cells. So, the TCVC will still end up in the kidneys.

3.3.3.2.3. ***Beta lyase.*** The beta lyase enzyme is among the most important activator of toxic products in the conjugation pathway, a fact particularly well documented in the kidney. There are some data, however, that indicate that renal beta lyase-dependent metabolism is greater in rats than in mice or in humans and greater in male than in female rats (Green et al., 1990; Lash et al., 1990; Volkel et al., 1998). This is not entirely in keeping with metabolic rates in general, which are higher in mice than in rats, and rats, in turn have higher metabolic rates than do larger animals, including humans. Studies that measured only cytoplasmic beta lyase activity did not consider the importance of mitochondrial beta lyase activity, which may be key to tetrachloroethylene metabolite toxicity (Lash et al., 2001).

In contrast, it must also be noted that species comparisons of tetrachloroethylene metabolism in chronic exposures on a surface area- or metabolic-rate basis rather than on a direct body-weight basis, particularly when including the total area-under-the-curve (AUC) for amount metabolized, indicate that metabolite production in rats and humans may not differ significantly (U.S. EPA, 1986; Rhomberg, 1992; Calabrese, 1983). The fact is that metabolic rates and the amounts metabolized are not the same thing. Metabolic rates are always faster in smaller species. Total AUC may or may not be similar among species. Even if AUC is the same, the peak blood levels may differ greatly from species to species. In other words, the pharmacokinetics are not the same.

The higher percentage of mercapturate found in rat versus human urine does not indicate a higher level of production of toxic products in the rat, because excreted mercapturate allows no estimate of the amount of TCVC or N-acetyl TCVC being processed through alternate routes (Lash and Parker, 2001). The relatively higher percentage of DCA in the urine may, however, indicate a relatively higher beta lyase enzyme activity and a higher thioketene production in rats if the DCA is indeed largely the product of the GST pathway rather than the oxidative pathway (Volkel et al., 1998). It is not known whether sex-dependent variation of beta lyase activity exists in humans as it does in rats (Volkel et al., 1998).

And finally, it is important to note that because the enzymes involved in this activation pathway are also present in other tissues (Dohn and Anders, 1982; Stevens, 1985; Stevens and Jacoby, 1983; Tateishi et al., 1978; Tomisawa et al., 1984, 1986; Larsen, 1985; Larsen and Stevens, 1986; Alberati-Giani et al., 1995; Malherbe et al., 1995), there exists a potential for formation of the reactive metabolites at sites other than the kidney, e.g., in the brain. In one carcinogenicity bioassay of tetrachloroethylene, a biologically significant elevation of gliomas in the rat brain was reported (NTP, 1986). Whether or not toxic metabolites resulting from beta lyase activity in the brain play a role in the development of the gliomas in the rat has not been studied. The possibility that such tetrachloroethylene metabolites could be involved in the mode of tumorigenic action producing gliomas is not unrealistic.

3.3.3.3. *Relative Roles of the Cytochrome P450 (CYP) and Glutathione (GSH) Pathways*

Although it is clear that the oxidative CYP pathway is quantitatively more important than the GSH conjugation pathway, the interorgan patterns for some of the intermediate metabolites, as well as the relative toxicity of certain key metabolites generated from these pathways, influence the relative importance of the two pathways in determining toxicity. It is still not certain which metabolites, alone or in combination, are explicitly responsible for specific tetrachloroethylene toxicities, and it is likely that different metabolites contribute to toxicity at different target sites. In general, CYP metabolism is associated with tetrachloroethylene-induced liver toxicity, whereas GSH conjugation followed by further processing by beta lyase and other enzymes is associated with tetrachloroethylene-induced renal toxicity. There is a possibility that beta lyase products could contribute to toxicity in the brain, for example, and be a factor in the gliomas observed in rats. The parent compound itself is also likely to be a contributing factor to tetrachloroethylene neurotoxicity, particularly central nervous system (CNS) effects.

Data from experiments designed to assess the effects of enzyme modulation suggest competition between the two pathways (Dekant et al., 1987; Lash et al., 1999; Volkel et al., 1998; Lash et al., 2001). Other data show relatively low urinary excretion of mercapturates as compared to CYP-derived products. On the basis of these findings, some scientists have concluded that there is a lack of toxicological significance for the low-affinity, low-activity GSH pathway except when the high-affinity CYP pathway approaches saturation (Green et al., 1990, 1997; Volkel et al., 1998). However, this conclusion does not consider the relative toxicological potency or chemical reactivity of the metabolites from the two pathways or the fact that the amount of mercapturate excreted is not a valid quantitative indicator of the extent of conjugative pathway metabolism (Lash and Parker, 2001).

Specific tetrachloroethylene metabolites are known to be associated with certain toxicities when they are administered directly. Exactly how these same compounds—as

metabolites of tetrachloroethylene—contribute to the various toxicities associated with exposure to the parent compound is not yet well understood.

3.3.4. Susceptibility

Differences in enzyme activity may lead to variations among individuals in their sensitivity to tetrachloroethylene toxicities. A 10-fold difference in CYP enzyme metabolic capacity among humans is a generally accepted norm. Although individual variations in the CYP2E1 enzymatic activity as high as 20- to 50-fold have been reported (Stephens et al., 1994; Yoo et al., 1988; Lieber, 1997), these in vitro measurements would be taken out of physiological context if used to estimate in vivo interindividual variations. Measurable and obvious differences in CYP enzymatic activity are observed among various ethnic groups and age groups (Goldstein et al., 1969; Raunio et al., 1995). No chemical-specific data regarding the manner in which CYP enzyme isoforms might affect susceptibility to adverse effects are available for tetrachloroethylene.

Diagnosis of polymorphisms in carcinogen-activating and -inactivating enzymes and cancer susceptibility have been noted (Stephens et al., 1994; Yoo et al., 1988; Raucy, 1995). Potential strain-dependent differences among rodents and human genetic polymorphisms in metabolizing enzymes involved in biotransformation of tetrachloroethylene are now known to exist. Whether CYP polymorphisms could account for interindividual variation in tetrachloroethylene metabolism among humans–and thus differences in susceptibility to tetrachloroethylene-induced toxicities–is not known.

The GSTs involved in tetrachloroethylene metabolism are described in Section 3.3.2. A potential exists for interindividual variation to occur in tetrachloroethylene metabolism as a result of variability in GST enzyme expression. It is important to note that GST polymorphism has been associated with increased risk of kidney cancer in people exposed to trichloroethylene. This information is discussed in EPA's draft health assessment report on trichloroethylene (U.S. EPA, 2001). There are no direct, chemical-distinctive data with regard to the specific isoenzyme family responsible for TCVG formation in metabolism of tetrachloroethylene. There are species-dependent differences as to which isozymes occur in liver and kidney, although it is unknown how the various enzymes are related to differences in metabolism of tetrachloroethylene. The compound is likely a good substrate for GSTA (Lash and Parker, 2001). GSTT and GSTP occur in human kidney, as does GSTA, the primary isozyme in human kidney, meaning that there is a potential for differences in the ability to produce TCVG. GSTZ transforms the tetrachloroethylene metabolite DCA. DCA has also been shown to have a potent irreversible inhibitory effect on the GSTZ isoenzyme, which is known to have at least four polymorphic variations.

Inhibition or induction of the enzymes responsible for tetrachloroethylene metabolism can, and likely does, alter susceptibility to toxicity (U.S. EPA, 1985; IARC, 1995; Lash and Parker, 2001). Numerous environmental pollutants and therapeutic agents alike have the potential to induce or inhibit tetrachloroethylene-metabolizing enzymes. For example, tetrachloroethylene metabolism is increased by inducers of cytochrome CYP enzymes such as toluene, phenobarbital, and pregnenolone-16 alpha-carbonitrile, whereas CYP inhibitors such as SKF 525A, metyrapone, and carbon monoxide decrease tetrachloroethylene metabolism (Moslen et al., 1977; Ikeda and Imanura, 1973; Costa and Ivanetich, 1980). Chronic exposure to tetrachloroethylene has been shown to cause self-induction of metabolism (Kaemmerer et al., 1982; Savolainen et al., 1977; Vainio et al., 1976). Other factors, such as health status or disease state, activity patterns, or concomitant exposure to other chemicals, can potentially influence tetrachloroethylene metabolism and its resulting toxicity. Section 4.9 addresses issues coexposures and cumulative risk in greater detail.

3.3.5. Comparison of Tetrachloroethylene Metabolism with Trichloroethylene Metabolism

Tetrachloroethylene is structurally related to trichloroethylene, and these two compounds cause similar adverse health effects. The toxic effects, with the possible exception of neurotoxicity, are attributed to metabolites. TCA, DCA, chloral, and TCOH are reported P450 biotransformation products of tetrachloroethylene and trichloroethylene; however, both the relative amounts of these metabolites produced and the precursor intermediates in the oxidative pathways are different for the two compounds. Interestingly, although tetrachloroethylene is not as extensively biotransformed as trichloroethylene, it is slightly more toxic. Differences in pharmacodynamics of precursors to P450 metabolic products as well as pharmacokinetic differences between the two parent compounds may be related to their pharmacologic potencies (Buben and O'Flaherty, 1985). Excretion of urinary mercapturates indicates that, relative to P450 oxidation, tetrachloroethylene is more extensively metabolized via GSH conjugation than is trichloroethylene. However, these urinary excretion products do not reflect the total flux through the GSH pathway since the resulting glutathione and cysteine conjugates have been shown to undergo further processing to products that are highly reactive. The Appendix for Chapter 3 provides additional discussion of tetrachloroethylene/trichloroethylene comparative metabolism.

3.4. EXCRETION

Tetrachloroethylene is eliminated from the body by two major processes: pulmonary excretion and rate-limited metabolism. Tetrachloroethylene that is not metabolized is exhaled unchanged, and this elimination process is the primary pathway of tetrachloroethylene excretion

in humans for all routes of administration (Monster et al., 1979; Stewart et al., 1961, 1970, 1974, 1977; Guberan and Fernandez, 1974; Opdam and Smolders, 1986; Koppel et al., 1985; Stewart and Dodd, 1964). Pulmonary elimination of (unchanged) parent compound is also important to tetrachloroethylene excretion by animals (Pegg et al., 1979; Yllner, 1961; Frantz and Watanabe, 1983; Schumann et al., 1980; Bogen et al., 1992). A very small amount of tetrachloroethylene has been shown to be eliminated through the skin (Bolanowska and Golacka, 1972); however, it represents an insignificant percent of total tetrachloroethylene elimination.

Pulmonary elimination of unchanged tetrachloroethylene and other volatile compounds is related to ventilation rate, cardiac output, and the solubility of the compound in blood and tissue. The lung clearance of tetrachloroethylene in six adults exposed at rest to 72 ppm and 144 ppm of tetrachloroethylene averaged 6.1 L/min initially and decreased to 3.8 L/min after 4 hrs (Monster et al., 1979). Lung clearance represents the volume of air from which all tetrachloroethylene can be removed per unit time. Normal ventilation rates in adults range from 5–8 L air/min at rest. Pulmonary elimination of unchanged tetrachloroethylene at the end of exposure is a first-order diffusion process across the lungs from blood into alveolar air, and it can be thought of as the inverted equivalent of its uptake from the lungs. Pulmonary excretion occurs in three first-order phases of desaturation of blood vessel-rich tissues, muscle tissue, and adipose tissues (Monster et al., 1979; Guberan and Fernandez, 1974). For humans, the half-times of elimination from these three tissue groups are 12 to 16 hrs, 30 to 40 hrs, and 55 to 65 hrs, respectively (Monster et al., 1979).

The long half-time of tetrachloroethylene elimination from adipose tissue, due to the high adipose tissue/blood partition coefficient and the low rate of blood perfusion of the fat tissue (Eger, 1963), is independent of the body burden of tetrachloroethylene, indicated by parallel blood and exhaled air concentration decay curves (U.S. EPA, 1985). However, the exhaled air or end alveolar air concentrations and the blood concentrations after exposure and throughout desaturation are proportional to the acquired body burden or exposure concentration and duration, and they can serve as a means of estimating body burdens. The half-life of tetrachloroethylene in the human body, measured as the inverse of the slope of the log-concentration versus time curve of the exhaled chemical, varies from 5 to 20 minutes for the first phase of elimination up to approximately 50 hrs during its extended phase (Chien, 1997; Monster et al., 1979). The long half-time of tetrachloroethylene pulmonary excretion indicates that a considerable time is necessary to completely eliminate the compound. This time is greater than five times the half-life, or about 2 weeks for humans. For the rat, the half-time of pulmonary elimination is about 7 hrs.

Metabolism of tetrachloroethylene provides another means of elimination of the parent compound. Metabolism in humans is not considered to be as important to tetrachloroethylene

excretion as is pulmonary excretion; however, at low ambient exposure concentrations, this may not be the case. The rationale for assigning greater importance to elimination by metabolism in humans is discussed later in this section. The biotransformation process is well accepted as being important to elimination of tetrachloroethylene in rodents (see Metabolism, Section 3.3).

The mean half-time of elimination for total trichloro-compounds for 13 subjects exposed to tetrachloroethylene was determined to be 144 hrs (Ikeda and Imamura, 1973). When TCA is administered directly, however, the half-life is not that long. The longer half-life of TCA from tetrachloroethylene metabolism is likely due to constant metabolic conversion of the parent compound to TCA as tetrachloroethylene is cycled to the liver over the period of time it is released from adipose tissue.

The urinary excretion of tetrachloroethylene biotransformation products, primarily TCA, has been thought to represent only a small percent of the total absorbed dose of tetrachloroethylene in humans (U.S. EPA, 1985; ATSDR, 1997). Urinary excretion of TCA (or total trichloro-compounds) was estimated to be only 1 to 3% in balance studies conducted in humans (Stewart et al., 1961, 1970; Monster et al., 1979, 1983; Monster and Houtkooper, 1979; Boettner and Muranko, 1969; Ikeda et al., 1972; Essing et al., 1973; Fernandez et al., 1976; May, 1976). The shortcomings of the human balance studies include the lack of follow-up of the subjects over a long time period. It is highly likely that a larger percent of the tetrachloroethylene dose was eventually metabolized. Not all of the dose was accounted for in these studies, indicating that more of the dose may be metabolized. Part of the dose may be metabolized to biotransformation products, such as oxalic acid, that were not measured. It is important to note that estimates of risk calculated directly from the data from such studies would seriously underestimate risk of exposure, because the tetrachloroethylene dose in some of these studies does not likely reflect low-dose exposure metabolism (U.S. EPA, 1985, 1991; Bois et al., 1996).

A literature review published by Hattis et al. (1990) reported estimates of the fraction of tetrachloroethylene metabolized at a low dose of 1 ppm to range from 2 to 86%. Based on data from the 1979 Monster et al. study, Bois and his colleagues (Bois et al., 1996) determined that at exposure levels above the current occupational standards, a median of approximately 1.5% of inhaled tetrachloroethylene would be metabolized, whereas at ambient air levels (0.001 ppm) the median estimate of the fraction of inhaled dose that would be metabolized is 36%, a considerably higher fraction of the dose.

Metabolism of tetrachloroethylene has generally been reported to contribute more to its elimination in rats and mice than in humans. The relative importance of metabolism elimination of tetrachloroethylene in rodents depends on the species and the dose (Pegg et al., 1979; Schumann et al., 1980; Dallas et al., 1994a; Bogen et al., 1992). As the body burden of

tetrachloroethylene is increased in the rat or mouse, the percentage excreted as unchanged parent compound increases. Conversely, as metabolism is the other principal route of elimination of tetrachloroethylene, when the body burden increases, the percentage of the burden metabolized decreases, although the absolute amount metabolized increases (Pegg et al., 1979; Schumann et al., 1980). These observations suggest that, in the rodent, metabolism of tetrachloroethylene and urinary excretion of its metabolites are rate limited and dose dependent, whereas pulmonary excretion is a first-order process and is dose independent, with half-time and rate constant being independent of the dose. Data from studies by Filser and Bolt (1979) and Buben and O'Flaherty (1985) suggest that elimination of tetrachloroethylene by metabolism is greater in mice than in rats**.**

3.5. PHYSIOLOGICALLY BASED AND OTHER TOXICOKINETIC MODELING

Most of the understanding of the pharmacokinetics of tetrachloroethylene in humans is based on a limited number of human data sets (Monster et al., 1979; Fernandez et al., 1976; Volkel et al., 1998) and on extrapolations from animal data to humans using PBPK modeling. PBPK models can provide estimates of tissue concentration as well as total metabolism of tetrachloroethylene. Models that incorporate transfer into milk and subsequent infant exposure have also been validated using measured human milk concentrations of tetrachloroethylene (Byczkowski and Fisher, 1994). In addition, researchers have looked at the variability in the measured and estimated PBPK model parameters and the implications for applying the models to risk assessment. The critical difference among the various models is in their different approaches to estimating the metabolic parameters.

3.5.1. Various Physiologically Based Pharmacokinetic (PBPK) Models

Chen and Blancato (1987) developed a PBPK model for rats, mice, and humans. The metabolic parameters maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) were derived by fitting the model to the total amount of metabolized tetrachloroethylene. Experimental data on total metabolite were available for rodents. However, for humans, it was assumed that the urinary metabolite TCA, as measured by Monster et al. (1979), accounted for 30% of the total metabolite. This percentage was chosen because it resulted in a better fit. The model consisted of five compartments: lung, fat tissue, richly perfused tissue, poorly perfused tissue, and liver. The model was used to estimate cancer risk from inhalation and drinking water exposures, based on total daily absorbed tetrachloroethylene.

Reitz et al. (1996) developed a PBPK model for rats, mice, and humans that describes the total metabolism of tetrachloroethylene using Michaelis-Menten kinetics. The partition coefficients for the five tissue compartments were measured independently and were similar to

those used by Chen and Blancato (1987), giving confidence in the reasonableness of both sets of numbers. For rats and mice, the metabolic parameters V_{max} and K_m, as well as the volume and blood flow rates of the fat compartment, were obtained by simultaneously optimizing the fit to three sets of in vivo data gathered in 6-hr inhalation radiolabeled tetrachloroethylene exposure studies. These data were (a) concentration of tetrachloroethylene in exhaled breath, (b) radioactive body burden present in animals at end of exposure, and (c) total post-exposure radioactive metabolites recovered from all excreta and carcass homogenates.

The metabolic parameters for humans were estimated as follows using a "parallelogram approach" (Reitz et al., 1989). First-order constants for the rate of metabolism were measured in vitro using isolated liver microsomes of all three species. The ratio of these in vivo and in vitro metabolic rates was assumed to be nearly constant across species, as was found to be the case for rats and mice. Using this constant ratio, the human in vivo metabolic rate constant per gram of liver could be determined from the human in vitro value. K_m was assumed to be invariant across species because it is derived solely from the reaction rate constants for the enzyme-catalyzed metabolic reactions. In contrast, Chen and Blancato (1987) found very different values of K_m for each species because this was a fitted parameter in their model. V_{max}, on the other hand, depends on the concentration of the enzyme (substrate) present and is likely to exhibit large inter- and intraspecies variability. As also noted by Reitz et al. (1996), there are inherent uncertainties in estimates from in vitro studies.

Reitz et al. (1996) also used a second method for estimating V_{max}, which was based on extrapolation from in vivo animal studies of other chemicals metabolized by cytochrome P450 enzymes. V_{max}, so estimated, was allometrically scaled to humans. The values obtained by Reitz et al. (1996) through both these independent methods were comparable. The overall average value of 32.9 mg/hr was then used in the PBPK model. This value compares with the value of 42.2 mg/hr used by Chen and Blancato (1987). The Chen and Blancato (1987) and Reitz et al. (1996) models differ considerably in the values of K_m for humans: 4.66 mg/L and 32.04 mg/L, respectively. Chen and Blancato (1987) also demonstrated that because tetrachloroethylene is so poorly metabolized, the levels of tetrachloroethylene in blood and tissues are not extremely sensitive to the values of V_{max} and $K_{m.}$

A human PBPK model was developed for the purpose of investigating neurotoxicological endpoints (Rao and Brown, 1993). In this case, tetrachloroethylene, not its metabolites, is of toxicological interest. This model was similar to the others previously discussed except that it included a skin compartment to allow for dermal absorption of tetrachloroethylene from shower water and a brain compartment so that the researchers could evaluate tetrachloroethylene concentrations in this organ. The model was coupled with an exposure model that predicted the

amount of tetrachloroethylene a human would be exposed to from water during showering and bathing.

The values for V_{max} and K_m in Rao and Brown (1993) were estimated using the method of Reitz and Nolan (1986). The predictions of the model were fit to total metabolite levels measured in rats and mice (Schumann et al., 1980; Pegg et al., 1979) to obtain the maximum rate of metabolism, V_{max} (which varied across species and was allometrically related to body weight raised to 0.74 power), and K_m (considered invariant across species). Other parameters for tetrachloroethylene were derived from various experimental data reported in the literature. The value of V_{max} for humans was determined by fitting the predicted total metabolite level to that estimated from urinary metabolite measurements in humans (Monster et al., 1979, and Fernandez et al., 1976, combined), assuming that the ratio of urinary to total metabolites would be the same in humans as that observed in rats (equal to 0.71). Although the value of K_m for humans in the Rao and Brown (1993) and Reitz et al. (1996) models were similar, their values for V_{max} differed significantly (see Table 3-1). Rao and Brown (1993) also provided parameter estimates for 6- and 10-year-old children.

Table 3-1. Comparison of V_{max}/K_m ratios[a]

Model	V_{max} (mg/hr)[b]	K_m (mg/L)	V_{max}/K_m (L/hr)
Rao and Brown	6.77	4.56	1.48
Reitz	32.9	4.66	7.06
Bois et al.	1.86	0.133[c]	14
Gearhart et al.	5.48	7.7	0.712

[a] Ratios are for the human models used for animal-to-human extrapolations in this assessment and those of Gearhart et al. (1993), which accurately predicted production of major metabolite, TCA, in urine.
[b] 70 kg human.
[c] The "posterior" value for K_m in Bois et al. (1996) was multiplied by the liver volume and the liver tissue/blood partition coefficient in order to conform to the format of the pharmacokinetic equations in this document.

Bois et al. (1996) used a Bayesian analysis in conjunction with a PBPK model that was structurally similar to that used by Reitz et al. (1996). The analysis used "prior" empirically determined distributions for the parameters in the model that were based on values in the literature and other previously conducted PBPK analyses. The Markov Chain Monte Carlo method was used with the PBPK model to compute updated "posterior" population distributions of these parameters that provided optimal fits to the individual blood and exhaled

tetrachloroethylene concentrations of subjects in Monster et al. (1979). Due to lack of good prior knowledge about the population variability of physiological parameters, standard reference values were assumed and standard deviations were selected by "reasonable guess" to generate a relatively diffuse prior.

The Michaelis-Menten parameter, V_{max}, was obtained first for rats and mice by fitting the model to in vivo data on the rate of total metabolite formation (Bois et al., 1990). The investigators then estimated human values by using the ratio of human and animal values of V_{max}, as determined by Reitz (1992). The geometric mean of these values was in agreement with that obtained by extrapolation from the animal values, based on allometric scaling by body weight. This value was used as the "prior" estimate of V_{max}. K_m was treated as invariant across species, and the geometric mean of values determined for rats and mice was used as the "prior" estimate. The Monte Carlo simulation was run for 10,000 iterations, after which time the parameter distributions converged. Tetrachloroethylene concentrations in blood and exhaled air were fit extremely well to the Monster et al. (1979) data. The shape of the prior distribution was seen to have little impact on final results. Model predictions were compared against alveolar concentrations of subjects in the Opdam and Smolders (1986) study, and all data points were seen to fall within the 95^{th} percentile envelope of predictions. The exposure concentrations in this study were 5 to 100 times lower than those used in the Monster et al. (1979) study; thus, this comparison provides further weight to the strength of the model.

The mean value for the posterior estimate of V_{max} was 20 times lower than the prior estimate. Thus, the Bois et al. (1990) results imply that the maximum rate of tetrachloroethylene metabolism in humans is much lower than that extrapolated by body weight raised to 3/4 power allometric scaling from rodents.

Other authors have developed models for tetrachloroethylene that specifically describe the kinetics of its major metabolite, TCA. Gearhart et al. (1993) developed a model for tetrachloroethylene that also included the kinetics of TCA, assuming that TCA comprised 60% of the total tetrachloroethylene metabolized in the rodent and using similar parameters for TCA as in a model for trichloroethylene. Tetrachloroethylene metabolism parameters for mice were estimated by fitting the model to the time course of decrease in chamber concentration of tetrachloroethylene in gas uptake studies. The model was independently validated at low oral doses (acute oral gavage of tetrachloroethylene in corn oil) by comparing the time course of blood concentrations of tetrachloroethylene and TCA in mice. Details pertaining to the derivation of parameters for metabolism in humans are not provided in the original paper but are available in the review by Clewell et al. (2005).

The parameters for describing tetrachloroethylene metabolism in humans were derived by fitting the model to urinary excretion of TCA in two subjects in a study by Fernandez et al.

(1976), assuming the same ratio of TCA to total metabolite as in the rodent. This value was set to 0.6 and attributed to Dekant et al. (1986b). The validity of using this value for humans has not been evaluated. Reitz et al. (1996), in their radiolabeled tetrachloroethylene studies, determined the fraction of urinary to total metabolites to range from 0.49 to 0.59 in rats and from 0.56 to 0.66 in mice for exposure concentrations that varied by two orders of magnitude.

Clewell et al. (2005) evaluated the Gearhart et al. (1993) model further, comparing its predictions against the more recently available urinary and blood TCA data gathered by Volkel et al. (1998) on human subjects exposed to tetrachloroethylene concentrations of 10 to 40 ppm for 6 hrs. The predicted blood TCA concentrations were in general agreement with the experimental data, but the rate of urinary excretion of TCA was overpredicted by roughly a factor of 2. Clewell et al. (2005) extended the Gearhart et al. (1993) model to include metabolism of tetrachloroethylene in the kidney, allowing for excretion directly into urine. Assuming metabolism in this organ to be at 10% of the capacity of the liver, substantial improvement was noted in the agreement with experimental data. An advantage in using the Volkel et al. (1998) data is that they pertain to exposure concentrations that are lower than those in other studies (e.g., 72 to 144 ppm in the Monster et al., 1979, study). In addition to developing this refined model, the Clewell et al. (2005) work provides an extensive review and evaluation of available PBPK models for tetrachloroethylene.

Loizou (2001) used a PBPK model that was structurally similar to that of Gearhart et al. (1993). The model assumes a 15% stoichiometric yield for the total metabolite produced across various dose levels (i.e., 15% of the parent compound in the liver is metabolized), but the basis for these assumptions is not substantiated. The above yield is also assumed to hold for the production of TCA because it is the major metabolite (E-mail dated June 26, 2002, from G. Loizou, Health and Safety Laboratory, UK, to R. Subramaniam, U.S. EPA). Elimination rates of TCA through blood and urine were chosen by calibrating the model to fit blood and urinary TCA kinetics and exhaled tetrachloroethylene TCA concentration levels obtained from Monster et al. (1979).

Other compartments have been added to human PBPK models to answer specific questions. One model was developed for the purpose of predicting cancer risk in breastfed infants (Byczkowski and Fisher, 1995). This model did not include a brain compartment but instead included a milk compartment for the mother. Hence, milk concentrations were predicted on a real-time basis, and the daily dose to a nursing infant could be computed. To assess cancer risk, the authors used a standard method based on intake dose of the parent compound (U.S. EPA, 1989).

This document has described three human PBPK models—those of Rao and Brown (1993), Reitz et al. (1996), and Bois et al. (1996)—in order to extrapolate health risk from

laboratory animals to human. The rationale for the selection of these models is discussed in Section 3.5.3.

3.5.2. Variability and Uncertainty

A number of models and studies have been discussed in the preceding sections on animal and human pharmacokinetics, and other models have appeared in older literature (see Hattis et al., 1990; Clewell et al., 2005). These models can be shown to adequately predict human data on concentrations of the parent tetrachloroethylene compound in blood and exhaled air and have been used to varying degrees along with cancer risk models to attempt to predict the risk of tetrachloroethylene exposure. There is likely to be considerable biological variability in many of these parameters, and the uncertainties about the values and their interpretations are significant.

Variability in pharmacokinetic measurements can exist within an individual over repeated measurements (intra-individual variability) and between different individuals in a population (inter-individual variability). Although this variability can introduce uncertainty into risk assessments that are based on single-point estimates, it is also a factor that can be explored using physiologically based mathematical modeling (including PBPK models) along with statistical techniques to estimate parameter distributions. Some studies have attempted to examine how variability among individuals affects risk (Bois et al., 1990, 1996; Gearhart et al., 1993; Isukapalli et al., 1998).

Bois et al. (1996) used Markov Chain Monte Carlo analysis to investigate the sensitivity of model output to changes in parameters and to determine the parameter distributions needed to explain the intersubject variability in humans. In simple Monte Carlo analysis, parameter values are selected from predetermined empirical or experimental distributions to investigate variability in model output. The Markov chain technique takes into account prior knowledge and collected data to modify the parameter distributions. In this case, population distributions were developed using knowledge of the six subjects in the Monster et al. (1979) data set for which a fit was desired. The new (posterior) parameter distributions were then used to estimate the amount of tetrachloroethylene metabolized during ambient exposures of approximately 1 ppb inhaled dose. The investigators estimated that a median value of 36% was metabolized, with 95% confidence bounds of 15% and 58% at low inhalation exposure concentrations of 0.001 ppm, in contrast to a median value of 1.7% metabolized at a 50 ppm concentration.

In an earlier study, Bois et al. (1990) used the conventional Monte Carlo method in conjunction with PBPK modeling to consider the effect of pharmacokinetic parameter uncertainties on the precision in model predictions and, subsequently, on cancer risk estimates. Empirically reasonable probability distributions were assigned to the scaling coefficients associated with each parameter. It was acknowledged that certain parameters would co-vary (for

example, body weight and liver weight), so these parameters would not be altered independently in the Monte Carlo simulation. The metabolic parameters were seen to be the most important determinants in the sensitivity of the results, as also observed by Reitz et al. (1996). The investigators calculated a median rate of metabolism in humans of 58 ng/day/kg$^{2/3}$, with 5th and 95th percentiles of 34 and 104 ng/day/kg$^{2/3}$, upon continuous exposure to 1 ng/L of tetrachloroethylene. The variability in the rate of metabolism was estimated to be much lower than that expected from the variability in V_{max} and K_m due to the covariance of these two parameters.

To assess variability in uptake and elimination within a single individual over multiple exposures under different exposure patterns, Chien (1997) collected exhaled breath measurements on a single individual following four different exposure scenarios in a controlled environmental facility (three replicates per scenario for a total of 12 exposures) and following tetrachloroethylene exposure in 22 dry cleaning facilities, where ambient levels of tetrachloroethylene were recorded and exposures were carefully timed. Hence, the variability in exhaled breath as a biomarker measurement and surrogate for internal dose could be evaluated in the same individual under clinical conditions in which exposure magnitude, duration, and pattern were carefully controlled and in a field environment where only the duration of exposure was controlled. The controlled exposures occurred for either 30 minutes or 90 minutes, with exposure concentrations ranging from 0.5 to 3 ppm. The experiments were designed to result in potential inhalation exposures of 297 μg/L-min. Differences in percent uptake and elimination half-life between exposure sessions at the same environmental concentration were statistically insignificant. However, percent uptake was dependent on environmental concentration.

Gearhart et al. (1993) performed 600 runs of a PBPK model in Monte Carlo fashion to produce a distribution of output results and attempted to look at the effect of the variation in the values of partition coefficients on the prediction of different dose surrogates such as area under the blood time curve for metabolites in the liver. For this dose surrogate, the investigators determined that the coefficient of variation was 25% and that the maximum was less than twice the mean. They concluded that parameter uncertainty in the models does not constitute a significant source of variability in using PBPK models for risk assessment. It must be noted that variation of the metabolic parameters was not included in their exercise.

The quantity of metabolite produced was observed to be very sensitive to V_{max} and K_m, parameters that vary significantly across models. On the other hand, the concentration of tetrachloroethylene in the blood is relatively insensitive to these parameters. Tables 3-1 and 3-2 indicate the range in the values of these parameters reported in the literature for humans and laboratory animals.

Table 3-2. Variation in values of metabolic parameters for tetrachloroethylene, as seen in the literature

Subject	Reference	V_{max} (mg/hr)	K_m (mg/L)	Comment
Animal				
Sprague-Dawley rat	Chen and Blancato (1987)	0.35	2.94	Fit model to 10 and 600 ppm inhalation exposure from Pegg et al. (1979).
B6C3F1 mouse	Chen and Blancato (1987)	0.18	1.47	Least squares fit to total metabolized from a gavage and inhalation study of Pegg et al. (1979).
F344 rat	Reitz et al. (1996)	0.325	5.62	Optimization to fit entire data set collected and reported in this study for 6 hr inhalation exposures. Metabolic parameters and size/perfusion rate of fat compartment were fit.
B6C3F1 mouse	Reitz et al. (1996)	0.355	3.69	Same as F344 rats in this paper (above).
Sprague-Dawley rat	Dallas et al. (1994a, b)	0.009	0.019	Metabolic parameters and blood/air partition coefficient estimated via nonlinear regression to an intra-arterial injection of 10 mg/kg in the rats.
	Byczkowski and Fisher (1994)	0.012	0.32	Fit model to exhaled tetrachloroethylene from closed chamber inhalation studies.
Humans				
	Chen and Blancato (1987)	42.2	32	Least squares fit to urinary TCA data from Monster et al. (1979) and Fernandez et al. (1976), assuming that TCA represents 30% of overall metabolism.
	Reitz et al. (1996)	32.9	4.66	Determined from in vitro studies with human liver cells and in vitro and in vivo studies in animals.
	Rao and Brown (1993)	6.77	4.56	Published literature.
	Bois et al. (1996)	1.86	0.133[a]	Central estimate, Markov Chain Monte Carlo posterior distribution fit to Monster et al. (1979) exhaled and venous blood concentrations.
10-year-old child	Rao and Brown (1993)	4.25	4.56	Published literature.
3-year-old child	Rao and Brown (1993)	2.64	4.56	Published literature.
	Byczkowski and Fisher (1995)	2.94	0.32	Allometric scaling from model optimizations based on the exhaled breath of rats during closed chamber inhalation exposure.

[a] See text and footnote in Table 3-1.

Age and gender-specific differences in pharmacokinetics can have a significant impact on tissue dosimetry. The immaturity of metabolic enzyme systems in the perinatal period may lead to decreased clearance of toxic chemicals as well as decreased production of reactive metabolites. Clewell et al. (2004) examined these differences for various stages in life using PBPK modeling for tetrachloroethylene and five other chemicals that differed considerably in their physicochemical (lipophilicity, solubility, and volatility) and metabolic characteristics. Parameters describing growth of various tissues were taken from the literature, and blood flow changes with age were assumed to change proportionally with tissue volumes. For tetrachloroethylene, only oxidative metabolism—specifically the production of TCA—was considered. Data on age-dependent development of CYP2E1 was used for this purpose (Vieira et al., 1996). The parameters for tetrachloroethylene were taken from the Gearhart et al. (1993) model, and the age-dependence of metabolism was based on the CYP2E1 data. The Gearhart et al. (1993) model describes the amount of TCA produced as 60% of the total metabolized tetrachloroethylene; this was fixed in the life-stage model.

The dose metrics examined were blood concentrations of the parent compound and the metabolite TCA. Continuous lifetime oral exposure was simulated at a daily dose rate of 1 µg/kg/day. Table 3-3 provides the average daily dose during different life stages of a male expressed relative to that of a 25-year-old adult male. The gender and age differences in tetrachloroethylene and TCA blood concentrations are detailed further in Figure 3-3.

Table 3-3. Ratio of average daily dose at various life stages to the average daily dose for a 25-year-old adult: PBPK simulations

Dose metric	Life stage			
	0–6 months	**0.5–5 years**	**5–25 years**	**25–75 years**
Tetrachloroethylene blood concentration	0.33	0.42	0.76	1.2
TCA blood concentration	0.057	0.16	0.59	1.4

Source: Clewell et al. (2004).

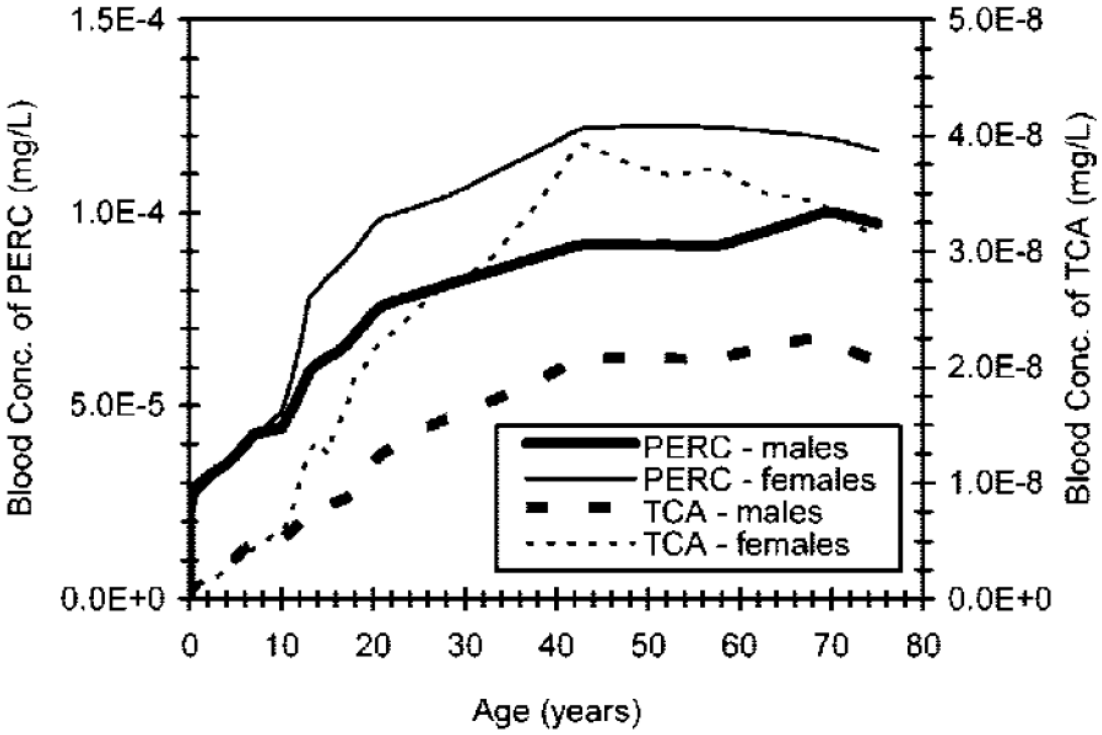

Figure 3-3. PBPK simulations of variations with age and gender in blood concentrations of tetrachloroethylene and its main metabolite trichloroacetic acid (TCA). Simulations are for continuous lifetime oral exposure at a constant daily intake of 1 µg/kg/day.

Source: Clewell et al. (2004).

Considerable gender differences in blood concentrations of TCA and tetrachloroethylene were seen in these predictions. Internal dose during infancy differed most from the corresponding dose in a 25-year-old. Tetrachloroethylene and TCA blood concentrations increased with age, which the authors attributed to the lower metabolic and pulmonary clearance of tetrachloroethylene when compared with other volatiles as well as its higher lipophilicity, both resulting in storage of the compound in fat and other tissues. These age and gender differences in pharmacokinetic sensitivity are significant, but they need to be considered together with pharmacodynamic considerations in determining the contribution of exposure at a life stage to lifetime risk.

The same group of authors (Gentry et al., 2003) developed a PBPK model for tetrachloroethylene that compared maternal and fetal/neonatal blood and tissue dose metrics during pregnancy and lactation. The manuscript contains the details on the structure of the model. Oxidative metabolism (TCA) in the mother and lactating infant was modeled using data

for CYP2E1 (Vieira et al., 1996); metabolism in the fetus was not included due to lack of information pertaining to the development of this pathway during gestation. The dose metrics were the fetal and infant blood concentrations of tetrachloroethylene and TCA. Changes in fetal blood concentrations were not pronounced because changes in tissue composition occurred in both mother and fetus during pregnancy (Gentry et al., 2003). A decrease of nearly three orders of magnitude of blood concentrations in the lactating infant when compared with that of the fetus was calculated. This decrease was attributed to the lower exposure rate during lactation as compared with placental exposure. Concentrations in the lactating infant were considerably lower, by more than two orders of magnitude, than in the mother. The largest variation in blood concentration occurred in the early postnatal period.

As the authors indicated, validation of the results in the Clewell et al. (2004) and Gentry et al. (2003) work and further refinement of the parameters in the models are necessary. It would therefore be premature to consider the results of such analyses for use in risk assessment. Further investigation of variability in the parameters used in the Clewell et al. (2004) analysis is needed before the results from Table 3-3 can be used to weigh upon considerations of a pharmacokinetic uncertainty factor for age and gender variability. Nonetheless, these models will enable future studies to focus on the key factors that are likely to influence pharmacokinetic susceptibility.

3.5.3. Animal-to-Human Extrapolation Using a Physiologically Based Pharmacokinetic (PBPK) Model

3.5.3.1. *Choice of Physiologically Based Pharmacokinetic (PBPK) Model*

As explained above, the evidence suggests that by-products of tetrachloroethylene metabolism are implicated in carcinogenesis in both rodent species. Inhaled concentration of the parent compound is therefore not an appropriate dosimeter. The use of pharmacokinetic modeling is expected to be useful in this regard. Various dose metrics are explored in detail in Chapter 4. Because the choice of the most appropriate dose metric has bearing on our selection of PBPK models, the issues are briefly summariuzed here.

Both the oxidative and GSH-dependent pathways of tetrachloroethylene metabolism are known to be involved significantly in the various tumors. Tetrachloroethylene hepatotoxicity is associated with cytochrome P450 metabolism occurring in the liver. TCA is considered to be the predominant metabolite associated with this P450 oxidation pathway. However, TCA may not be the sole contributory metabolite to tetrachloroethylene-induced hepatotoxicity and cancer, and reactive intermediates such as tetrachloroethylene oxide and trichloroacetyl chloride may also be involved. In the case of renal toxicity, GSH conjugates formed in the liver and transported to the

kidney are thought to be the primary agents. The GSH pathway is also implicated in the mode of action for leukemia (see Chapter 4).

Tetrachloroethylene is a chemical that has generated prolific pharmacokinetic modeling endeavors. A consideration in determining appropriate PBPK model structures for use in risk assessment is the ability to use the same model to predict dose metrics for all the endpoints. Although many models have been developed to predict concentrations of the parent compound and total metabolite levels, only the model by Gearhart et al. (1993) and its variations, developed by Clewell et al. (2005) and Loizou (2001), predict both tetrachloroethylene and TCA concentrations. These models were reviewed in previous sections. As noted in Chapter 4, there is no reliable quantitative data on GSH conjugates formed by tetrachloroethylene; accordingly, there are no models that can specifically predict these metabolite levels.

Various uncertainties are associated with the use of PBPK models developed to predict the kinetics of TCA produced as a result of tetrachloroethylene metabolism. One assumption pertains to the fraction of tetrachloroethylene metabolized to TCA in humans. Loizou (2001) made the assumption that 15% of tetrachloroethylene reaching the liver is metabolized. In related models, Gearhart et al. (1993) and Clewell et al. (2005) estimated their parameters on this assumption that urinary TCA in humans accounts for 60% of the total metabolism of tetrachloroethylene. This percentage was assumed to be independent of dose and was based on a range around the value seen in rodents (Reitz et al., 1996; Clewell et al., 2005); however, its reliability for humans is not known.

At an exposure concentration of 72 ppm in humans, Monster et al. (1979) determined that 95% of inhaled tetrachloroethylene was exhaled unmetabolized, 2% was excreted as TCA in urine, and 1% of TCA remained in systemic circulation. Thus, these data suggest that urinary TCA may comprise roughly 40% of the total metabolite in humans. It may be noted that other tetrachloroethylene metabolites are also known to be excreted in the urine of exposed humans (Ikeda and Ohtsuji, 1972).

The measurement of urine levels of TCA using the photometric Fujiwara reaction method, which was used in the Fernandez et al. (1976) and Monster et al. (1979) studies, is hindered by analytical or methodological problems, such as providing information only on the total trichloro content and blank levels being significantly high in unexposed subjects (Reitz et al., 1996). The TCA measurements in the Fernandez et al. (1976) study were used in estimating the metabolic parameters in the Gearhart et al. (1993) model. Other problems include the half-life of TCA in humans being long—in the neighborhood of 100 hrs (Muller et al., 1974).

TCA alone may not be an adequate dose metric for liver tumors. Buben and O'Flaherty (1985) compared various indices of liver toxicity for tetrachloroethylene and trichloroethylene, finding tetrachloroethylene to be at least twice as potent as trichloroethylene on a molar basis for

equivalent amounts of total metabolite generated. They concluded that the toxic metabolite in tetrachloroethylene is considerably more toxic than that in trichloroethylene with regard to liver toxicity. In Appendix 4A, we compare the potency of liver tumors in the TCA and tetrachloroethylene bioassays and conclude that TCA (produced in the metabolism of tetrachloroethylene) alone does not appear to be sufficient to account for the tumorigenicity of tetrachloroethylene for the exposures that were examined. Clewell et al. (2005) had similar conclusions and suggest a combination of metabolites as the responsible agents.

All the above factors combined led to the use of the rate of overall metabolism (the total amount of tetrachloroethylene metabolized per day) as a surrogate for the toxic dose in the route-to-route and animal-to-human extrapolations for liver tumors, leukemia, and kidney tumors. This is more reasonable than using the parent chemical, even though total metabolized dose is not a perfect dose metric in that it does not actually estimate the tissue concentration of toxic metabolites. Inhaled concentration of the parent chemical was used as the basis for extrapolations for other cancers.

In this assessment, three of the most recently developed human PBPK models that predict total metabolite levels were considered: those of Rao and Brown (1993; the Rao and Brown model), Reitz et al. (1996; the Reitz model), and Bois et al. (1996; the Bois model). These three models were chosen to allow cancer risk estimates to reflect uncertainties that arise from using different data and methods to calibrate human PBPK models. This enables provision of a range of values for extrapolation from laboratory animals to human. In later sections, these models are compared with each other and with experimental data. The three models were chosen on the basis of their different approaches to estimating metabolic parameters, as summarized in the previous section. Although the models describe the overall metabolism of the parent compound, they do not describe the kinetics of the metabolites.

The Reitz model used in vivo rodent data on total metabolism and parent compound concentrations in blood and exhaled breath. The development of a human model used a "parallelogram" approach wherein in vivo metabolic rate constants were related to experimentally determined in vitro values by assuming the relationship of in vivo to in vitro metabolic rates to be invariant across species. The Bois et al. model, on the other hand, used Bayesian inference methods to fit model predictions to laboratory data on exhaled air and blood concentrations of tetrachloroethylene in human volunteers. The Rao and Brown model used the same human study but assumed the ratio of urinary TCA to total metabolite levels to be equal to 0.71 in order to derive metabolic parameters. The Rao and Brown model was included to permit examination of the range in risk values that would arise if metabolic parameters are derived from

urinary TCA data extrapolated to total metabolites with this assumption.[1] These and other PBPK models generally seem to predict parent concentrations well; however, they differ considerably in their predictions of the amount metabolized. As shown in Tables 3-1 and 3-2, there are large differences in the metabolic parameters used by various authors.

The human PBPK models chosen suffer from the limitation that their predictions of total metabolite cannot be accurately evaluated because such data are not available. The models predict only total metabolite levels, so it is not possible to validate them against specific metabolites, such as TCA, that have been measured in experiments. These models have been validated against concentrations of the parent compound in blood and exhaled breath. However, because the metabolism of tetrachloroethylene is slow (particularly in humans, with roughly 95% of tetrachloroethylene being exhaled unmetabolized), the concentrations of the parent compound are not sensitive to the values of the metabolic parameters. A similar argument applies to our use of the Bois model. The posterior distributions of parameters in this model were obtained by fitting to the parent compound concentrations in the Monster et al. (1979) study. Further, as explained above, these three models are limited in different ways.

3.5.3.2. *Implementation of Physiologically Based Pharmacokinetic (PBPK) Models*

Implementation of the Rao and Brown, Reitz, and Bois models follows the PBPK model structure of Ramsey and Andersen (1984). The Reitz and Bois models are composed of four compartments: poorly perfused tissues, well-perfused tissues, fat, and liver. The Rao and Brown model contains, in addition, a separate compartment for the brain. In the implementation of the Rao and Brown model herein, there is no separate skin compartment. The compartments are assumed to be homogeneous, and distribution is limited by blood flow. The metabolism of tetrachloroethylene is modeled by a Michaelis-Menten term in the differential equation for the liver compartment. The simulation is represented by the following equations:

$$\frac{dM_i}{dt} = Q_i (C_{art} - C_{vi})$$

$$\frac{dM_l}{dt} = Q_l (C_{art} - C_{vl}) - \frac{V_{\max}}{K_m + C_{vl}} C_{vl}$$

[1]Another approach is to adjust the urinary TCA predicted by Clewell et al. (2005) by the inverse of this factor to derive total metabolite levels. A preprint of this in-press manuscript was not received in time to be able to exercise the Clewell et al. (2005) model for the purposes of this document.

where:

I = compartments other than liver

l = liver

M_i = mass of tetrachloroethylene in the ith compartment

C_{vi} = venous concentration of tetrachloroethylene at the exit from compartment I

C_{art} = arterial concentration of tetrachloroethylene

Q_i = blood flow rate into the ith compartment

V_{max}, K_m = Michaelis-Menten constants.

Pulmonary exchange is represented by:

$$Q_{alv}\,(C_{inh} - C_{alv}) = Q_{tot}\,(C_{art} - C_{ven})$$

$$C_{art} = h_{ba}\,C_{alv}$$

$$C_{exh} = 0.67C_{alv} \,\%\, 0.33C_{inh}$$

where:

Q_{alv} = alveolar ventilation rate (which is different from the inspiratory flow rate because of the respiratory dead space). The alveolar ventilation rate and cardiac output (the ratio that is referred to as the ventilation-to-perfusion ratio) increase with activity but at different rates

C_{inh} = inhaled concentrations of the chemical

C_{alv} = alveolar concentrations of the chemical

Q_{tot} = total blood flow rate (equal to the cardiac output)

C_{art} = arterial concentrations of the chemical

C_{ven} = venous concentrations of the chemical

h_{ba} = blood/air partition coefficient

C_{exh} = exhaled concentrations of the chemical

For oral exposures, the gastric route was added by assuming "first-pass" metabolism—by assuming that all tetrachloroethylene ingested is transported directly to the liver, the metabolizing organ. A separate PBPK compartment for the stomach was, therefore, not necessary. The absorption of tetrachloroethylene in the stomach was modeled as a first-order

process with an absorption rate constant, k_a. Then the mass balance equation for the liver may be modified to have an additional source term as follows:

$$\frac{dM_l}{dt} = Q_l(C_{art} - C_{vl}) - \frac{V_{max}}{K_m + C_{vl}} C_{vl} + k_a M_0(t)\exp(-k_a t)$$

M_0 is amount of tetrachloroethylene ingested and is itself a function of time. In these simulations, tetrachloroethylene was administered via drinking water as a series of boluses.

These model equations were solved using the Simulink Module of the MATLAB computational software package (The Mathworks, Natick, MA) and the single-point estimation module in the software package MCSIM (Bois et al., 1996). It was verified that both packages produced the same results when applied to the same set of equations and parameters.

A note is in order regarding this implementation of the Bois model. Bois's Bayesian approach produces a (posterior) distribution of parameters and, therefore, a distribution rather than a point estimate of dose. However, this assessment is not carried out within such a statistical framework. The central estimate of the parameters in the Bois et al. posterior distribution was used to provide point PBPK estimates of internal dose of tetrachloroethylene and of its overall metabolic rate. The point estimates obtained in this manner reproduce (coincide with) the median population estimated by Bois et al. for the amount of tetrachloroethylene metabolized for a large range of exposure concentration, 0.001 to 50 ppm. It is therefore reasonable to use the central estimates of Bois's posterior distribution of parameters to provide point estimates of dose for extrapolation purposes.

Most human PBPK models have been implemented to investigate inhalation exposure and do not incorporate gastric absorption rate constants. Values in the literature for the gastric absorption rate vary widely. Ward et al. (1988) reported a gastric absorption rate constant in mice of 0.5 L/hr. Dallas et al. (1995) reported oral absorption rate constants in rats and dogs as 1.5 and 20.4 L/hr, respectively, obtained by fitting blood concentrations following oral gavage. For modeling purposes, a gastric absorption rate constant of 1.6 L/hr was chosen. This predicts a reasonably rapid gastric absorption consistent with the data. It was determined that the resulting blood concentrations of tetrachloroethylene are not particularly sensitive to larger values of this parameter. Simulations of gastric absorption of tetrachloroethylene were carried out for humans for use in route-to-route extrapolation. Because these simulations were at low exposures, and because of first-pass metabolism effects, the uncertainty in the gastric absorption rate constant is not likely to significantly affect the results of the extrapolation. Increasing the gastric absorption rate constant to 20 L/hr results in an approximately twofold increase in peak blood concentration.

Changing this parameter does not substantially impact the elimination profile. Table 3-4 shows the parameter sets used in this modeling effort.

For inhalation exposures, ventilation rate is a key parameter. In rodents, ventilation rate (V_E) was calculated as a function of body weight using the following equations (U.S. EPA, 1994):

$$\text{For mice: } V_E(L/min) = e^{0.326+1.05\ ln(w)}$$

$$\text{For rats: } V_E(L/min) = e^{-0.578+0.821\ ln(w)}$$

where w is body weight in kilograms and ln represents the natural log operation. These equations provide total ventilation rate. The alveolar ventilation rate is the total ventilation rate less the volume of air that is inhaled through the physiological dead space (total effective volume not involved in gas exchange) in a given time. For the rats and mice and for resting inspiratory rates (7.5 L/min) in humans, Q_{alv} . 0.67 V_E (Brown et al., 1997). For the exercising individual (24 to 49 L/min), Q_{alv} increases up to 0.8 V_E (Brown et al., 1997). For the ventilation rates covered in this document, it was considered reasonable to use the relationship Q_{alv} . 0.67 V_E throughout. These values represent reasonable physiological values, recognizing that there is substantial variation. The alveolar ventilation rate corresponding to the resting inhaled minute volume is 5.5 L/min. However, the EPA typically assumes a total ventilation rate of 13.8 L/min for a 70 kg human. Thus, unless otherwise stated, the calculations presented in this assessment assume an alveolar ventilation rate of 9.3 L/min.

In order to extrapolate between equivalent metabolized doses in animals and humans, the PBPK structure of the Reitz model was used for rats and mice, and all three PBPK models (Rao and Brown, Reitz, and Bois) were used for humans. The animal PBPK models were run to simulate the exposure conditions of the animal bioassay studies. During the human equivalent exposures, the model was run to simulate continuous low-level chronic exposure at steady-state conditions. Chapter 5 discusses the details and results of the extrapolation.

3.5.4. Comparison of Physiologically Based Pharmacokinetic (PBPK) Simulations With Experimental Data

The models were run to simulate various experimental and clinical exposure scenarios from the literature. Simulated concentration levels of tetrachloroethylene in the blood and in exhaled air were compared with measured values. As discussed in a previous section, it was not feasible within the constraints of these models to make credible quantitative comparisons with data on urinary or blood levels of major tetrachloroethylene metabolites.

Table 3-4. Parameters for tetrachloroethylene PBPK modeling

Parameter	Mouse	Rat	Human model		
			Rao and Brown	Reitz	Bois
BW (kg)	variable[a]	variable[a]	70	70	70
Cardiac output (L/hr)[b]	0.275 x 60 x $(BW)^{0.75}$	0.275 x 60 x $(BW)^{0.75}$	430	430	430
Alveolar ventilation (L/hr)[b]	0.67 x V_E[c]	0.67 x V_E[d]	558	558	558
Tissue volumes[e] (%)					
Rapidly perfused	4.5	4.5	1.7	3.71	15.1
Slowly perfused	79.5	75.7	57	57	49.4
Fat	9.18	6.8	23.1	23.1	23.1
Brain	1	1	2		
Liver	5.86	2.53	3.4	3.14	2.54
Blood flow (% cardiac output)					
Rapidly perfused	44	39.2	41	52	63.7
Slowly perfused	19	19	19	19	12.9
Fat	1.98	6.84	5	5	4.88
Brain	10	10	11		
Liver	25	25	24	24	17.9
Partition (tissue/blood)					
Rapidly perfused	3	3.73	3.72	5.88	1.92
Slowly perfused	2.59	1.06	1.06	3.1	2.9
Fat	48.3	86.9	86.6	119.13	84.1
Brain	3	3.73	3.72		
Liver	3	3.73	3.72	5.88	3.08
Blood/air	16.9	18.9	10.3	10.3	16
Metabolic parameters					
V_{max} (mg/hr)	0.355	0.325	6.77	32.9	1.86
K_m (mg/L)	3.69	5.62	4.56	4.66	0.133[f]
Gastric absorption rate					
K_a (1/hr)	1.6	1.6	1.6	1.6	1.6

[a] The simulations in this document use 0.03 kg and 0.3 kg for the mouse and rat, respectively.
[b] $V_E(L/hr) = 60 \times e^{0.326+1.05\ln(BW)}$, where V_E is the minute ventilation.
[c] $V_E(L/hr) = 60 \times e^{-0.578+0.821\ln(BW)}$.
[d] Values used in the animal-to-human extrapolation.
[e] A density of 0.92 and 1 g/cc was used for fat and for other compartments, respectively.
[f] The "posterior" value for K_m in Bois et al. (1996) was multiplied by the liver volume and the liver tissue/blood partition coefficient in order to conform to the format of the pharmacokinetic equations in this document.
BW = body weight.

Figure 3-4 shows a comparison of the blood concentration levels predicted by the three models considered in this assessment with clinical data from the human inhalation study by Monster et al. (1979). In that study, six male volunteers breathed 72 ppm or 144 ppm tetrachloroethylene at rest. In a third session, they breathed 142 ppm tetrachloroethylene at rest with two intermittent 30-minute exercise excursions. All exposures lasted a total of 4 hrs. The researchers measured tetrachloroethylene in blood and exhaled air after exposure until almost no tetrachloroethylene remained. They monitored TCA in blood and urine for up to 100 hrs after exposure. Their data set is widely cited and is perhaps the most complete in terms of human distribution and in vivo metabolism data. The concentration of tetrachloroethylene in exhaled air and blood as measured in the Monster experiments have been used to validate several human PBPK models. In the comparison presented here, only tetrachloroethylene exposure at 72 ppm was considered, and simulations were carried out at two different ventilation-to-perfusion ratios (ratio of alveolar ventilation rate to cardiac output), corresponding to occupational activity levels.

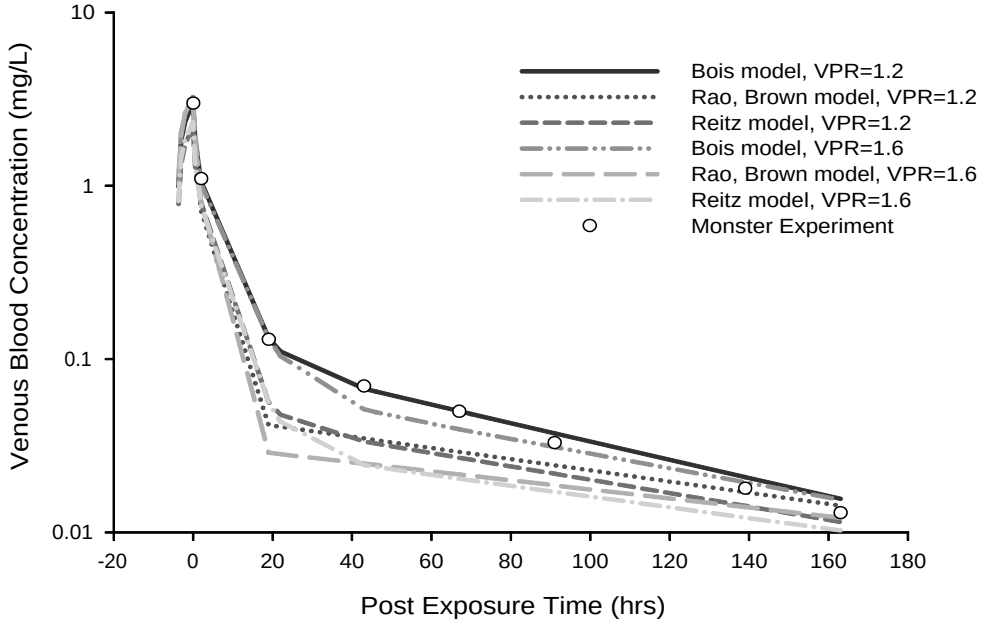

Figure 3-4. Comparison of model predictions for blood concentration with inhalation experiment. Tetrachloroethylene was inhaled at a concentration of 72 ppm. Simulations were performed at different ventilation-to-perfusion ratios (VPR) and at an alveolar ventilation rate of 7 L/min (the geometric mean of values in the Monster experiment). Standard deviations on the experimental data were very small (e.g., 0.025 mg/L and 0.003 mg/L at 20 and 140 hrs, respectively).

Source: Adapted from Monster et al. (1979).

For any particular model, the increase in ventilation-to-perfusion ratio from 1.2 to 1.6 does not appear to make much difference, as shown in Figure 3-4. The much closer correspondence of the Bois model predictions to the Monster data is to be expected because the model's posterior distribution of parameters was arrived at by fitting to the Monster data. The Rao and Brown and Reitz model predictions are less than the experimental values, generally within a factor of 2 and 3, respectively. These two models do not differ much in their predictions of tetrachloroethylene blood concentrations.

Stewart et al. (1970) analyzed the expired breath of subjects repeatedly exposed to 100 ppm tetrachloroethylene (7 hrs/day for 5 days) using gas chromatography and infrared spectrometry. Figure 3-5 compares the mean alveolar concentration of tetrachloroethylene in these subjects with the results of model simulations. The subjects were assumed to be at rest (alveolar ventilation rate of 5.02 L/min and a ventilation-to-perfusion ratio of 1). All three models agree reasonably well with the experimental data; the Bois et al. model differs the least from the experimental result.

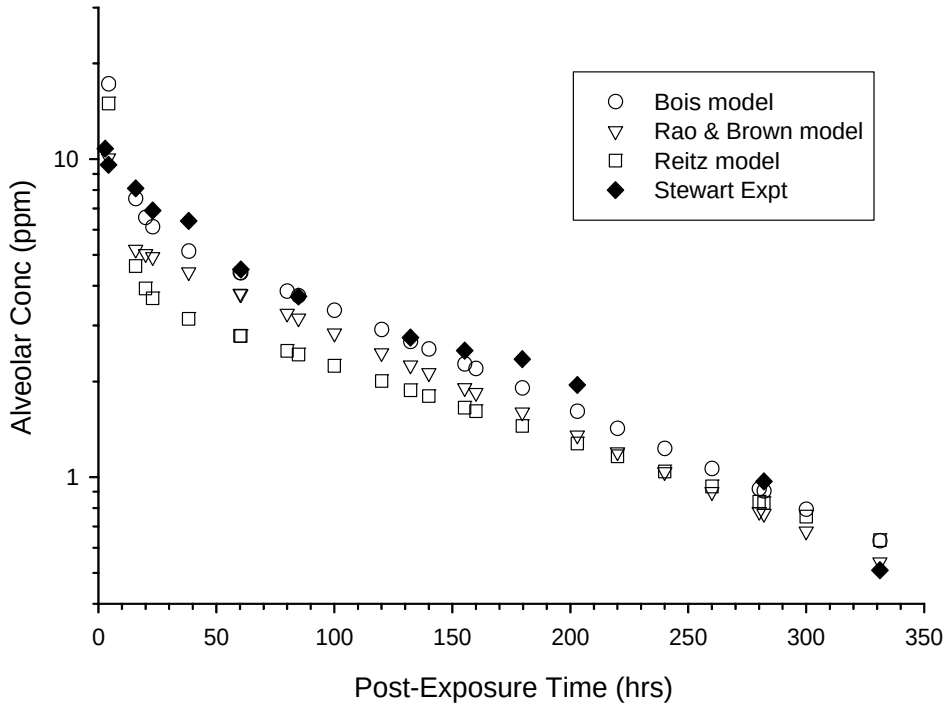

Figure 3-5. Comparison of model predictions for alveolar concentration of tetrachloroethylene with experimental data on humans. Tetrachloroethylene was inhaled at a concentration of 100 ppm, 7 hrs/day for 5 days. The experimental data Stewart et al. (1970) show the mean alveolar concentration of tetrachloroethylene in these subjects. Resting breathing conditions (alveolar ventilation rate of 5.02 L/min and a ventilation-to-perfusion ratio of 1.0) were assumed. Some points early in the time course were deleted because of difficulty in obtaining numerical values from the author's plot.

Opdam and Smolders (1986) exposed six human subjects to constant levels of tetrachloroethylene ranging from 0.5 to 9 ppm and measured the concentration of tetrachloroethylene in their exhaled breath (from which their alveolar concentrations could be deduced) during exposure up to 50 to 60 minutes. Separate data were gathered on males and females. Figures 3-6a and 3-6b compare their results for the ratio of alveolar to inhaled concentrations of tetrachloroethylene with predictions from the three models. The experiments were performed for different breathing scenarios that included normal breathing (with no breath holding) as well as paused breathing with different durations of breath holding. However, the simulations were carried out only for the normal breathing scenario at resting inspiratory rates and with the ventilation-to-perfusion ratio set equal to 1. Body weights and lean body weights were considered differently for males and females (as given in ICRP, 1975). While running the Bois model, other parameters remained unchanged across gender. On the other hand, blood flow distributions to various compartments differed across gender in the other two models (as given in Brown et al., 1997).

Although simulations were carried out for a range of tetrachloroethylene exposure concentrations from 0.5 to 9 ppm (only a range has been provided by the authors), the plots in Figures 3-6a and 3-6b show only the simulations for 5 ppm, as there were no substantial differences across this range of exposure concentrations. The agreement with this experimental data is particularly noteworthy, considering that the Bois model was parameterized on the basis of the Monster et al. (1979) data, in which exposures were 5–100 times higher than in the Opdam and Smolders (1986) measurements.

The comparison shows the Reitz and Bois models' predictions to be generally closer to the experimental results. The disagreement of the Rao and Brown model appears to be greatest at the longer time durations: at 40 hrs, it overpredicts by roughly a factor of 1.5, whereas the Reitz and Bois models are in close agreement. Alveolar concentrations in male subjects are generally slightly less than those in females in both simulations and experiment.

Comparisons were also performed for the Altmann et al. (1990) study, in which subjects were exposed to 10 ppm and 50 ppm tetrachloroethylene by inhalation for 4 hrs repeatedly on 4 days. Table 3-5 shows the values corresponding to measurements at the end of exposure on the first day of exposure. Relative to the Reitz model, the Rao and Brown and Bois models appear to compare well with the Altmann et al. (1990) experiment at lower exposure, but they predict nearly twice the experimental levels at higher exposure. The comparison with the Altmann et al. (1990) data may be less conclusive because the measurement was made immediately after exposure, when variation in both experiment and simulation is large.

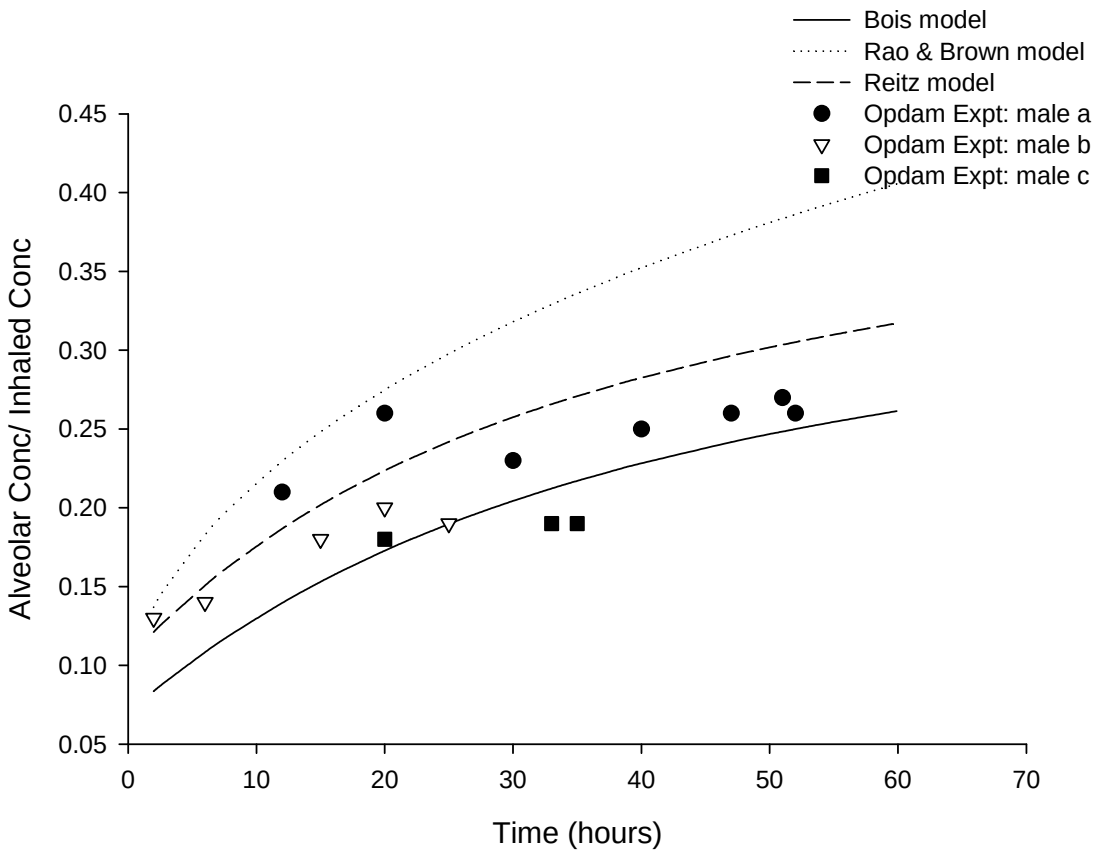

Figure 3-6a. Comparison of model predictions for alveolar concentration as a fraction of inhaled tetrachloroethylene concentration with experimental Opdam and Smolders (1986) data on male human subjects. Some physiological parameters specific for males were used (see text for details). Experiment exposure concentrations ranged from 0.5 to 9 ppm; plots for simulations depict only results for 5 ppm (no significant difference at other exposures in this range). Breathing conditions at rest assumed for the simulations: alveolar ventilation rate of 5.02 L/min and ventilation-to-perfusion ratio of 1). Simulations and experimental data shown here are with no pause in breathing.

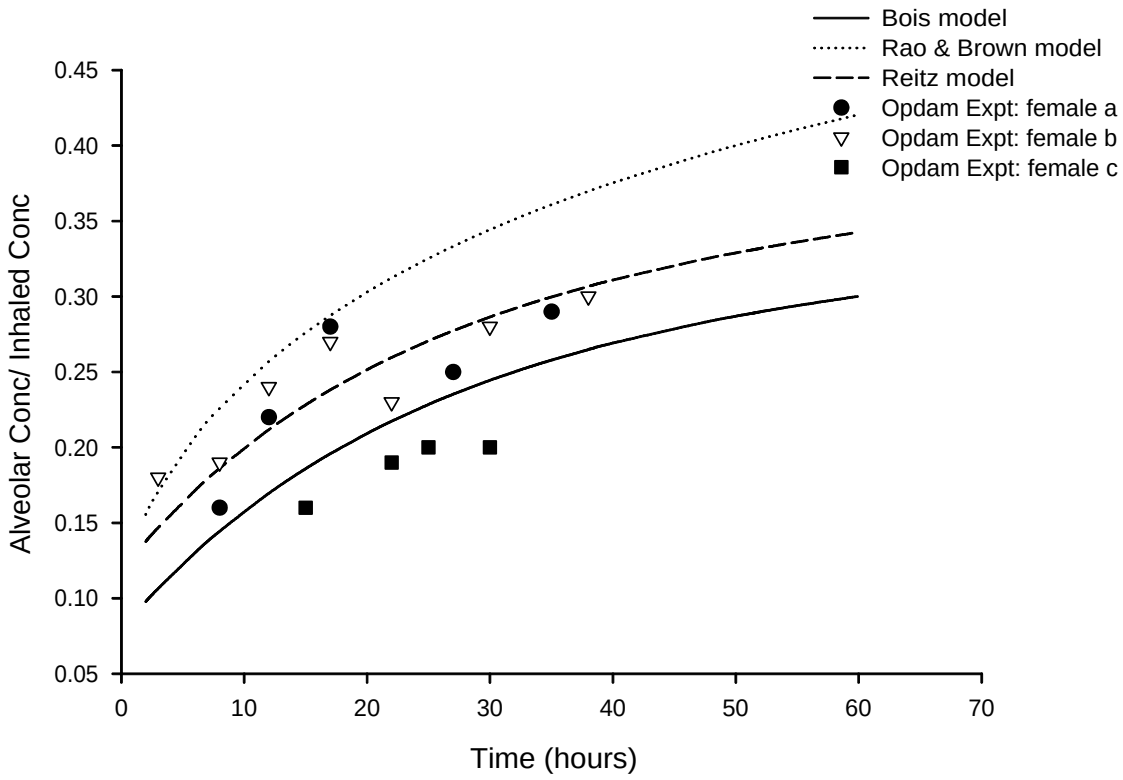

Figure 3-6b. Comparison of model predictions for alveolar concentration as a fraction of inhaled tetrachloroethylene concentration with experimental data on female human subjects. Some physiological parameters specific for females were used (see text for details). Experiment exposure concentrations ranged from 0.5 to 9 ppm; plots for simulations depict only results for 5 ppm (no significant difference at other exposures in this range). Resting breathing conditions (alveolar ventilation rate of 5.02 L/min and a ventilation-to-perfusion ratio of 1.0). Simulations and experimental data shown here are with no pause in breathing.

Source: Opdam and Smolders (1986).

Table 3-5. Comparison of venous blood tetrachloroethylene concentrations: PBPK simulations and Altmann et al. (1990) study[a]

Inhaled exposure concentration (ppm)	Blood concentration (g/L)			
	Altmann	PBPK simulations		
		Bois et al.	Rao and Brown	Reitz
10	333	350	385	262
50	1,106	1,855	1,940	1,332

[a] Values correspond to measurements at the end of the first day of study in the Altmann et al. (1993) experiment.

It is concluded that these four comparisons provide no particular basis for preferring one model over another. The comparisons in Figures 3-4, 3-5, 3-6a, and 3-6b indicate that, with regard to alveolar and blood concentrations, all three models provide reasonably good predictions and are not markedly different from each other.

The three models differ most in their values for the metabolic parameters (see Tables 3-1 and 3-2) and consequently, as shown later, in their predictions of the rate of total metabolite production. The PBPK models presented in this document predict the rate of production of the total amount of metabolites but do not describe their transformation and clearance. It is therefore not possible to compare their predictions on metabolite produced with experimental data without making major assumptions. Such a comparison was attempted with the experimental data of Fernandez et al. (1976) on the amount of TCA excreted in urine.[2] In this experiment, two individuals were exposed to 150 ppm of tetrachloroethylene by inhalation for 8 hrs and followed for 72 hrs. The data indicate that approximately 30 mg of TCA were eliminated through urine in these subjects during the post-exposure period. In order to make a rough comparison, we assumed that urinary excreted TCA constituted the bulk of overall metabolites formed. Simulations using the Rao and Brown and Bois models predict approximately 60 mg and 320 mg (tetrachloroethylene equivalent, determined by multiplying the amount of the metabolite by the ratio of tetrachloroethylene and TCA molecular weights), respectively, of total metabolite produced during the post-exposure period.

[2] Other studies that have reported data on the concentrations of TCA in blood or urine include Stewart et al. (1961, 1970), Monster et al. (1979, 1983), Boettner and Muranko (1969), Ikeda et al. (1972), Essing et al. (1973), Guberan and Fernandez (1974), Volkel et al. (1998), and the New York State Department of Health (NYS DOH, 2000).

In contrast to this large difference, when the two models are applied to the exposure scenario of the Monster et al. (1979) experiment, they predict approximately the same rate of metabolite produced *immediately* following a short 4-hr exposure to 72 ppm tetrachloroethylene.

In addition to the problems in making the comparison, the discrepancies between the models and with experimental data on TCA may point to large uncertainties in the parameters used in these models. Because the accuracy of the models has been evaluated only against blood and breath concentrations of the parent compound, their reliability for predicting the production of overall metabolites is an unknown. The use of all three of these models to provide a range of risk estimates is intended to capture some of this uncertainty.

Furthermore, there are many difficulties associated with estimates of the extent of metabolism in humans based on TCA excretion reported in the experimental studies of Fernandez et al. and Monster et al. Some of the problems encountered are the accurate measurement of the retained dose of tetrachloroethylene from inhalation exposure, the imprecision of the older methodologies using the Fujiwara reaction for metabolite quantification, the possibility of an important contribution for metabolites other than TCA (e.g., oxalic acid, CO_2, TCVC, or as yet unrecognized products) that may be excreted, and the relatively long half-life of certain urinary metabolites, which necessitates extended collection of samples.

The fraction of TCA in urine relative to that in blood or that stored in body organs is not known. Furthermore, TCA is only one component of metabolism. Although it is considered to be the major metabolite (the conclusions from Monster et al. (1979) indicate that it may comprise 60% of the metabolites), some tetrachloroethylene is converted to other compounds, and not all potential metabolites have been adequately evaluated. In addition, TCA, itself, might be further metabolized, reducing the amount of TCA available for urinary excretion.

Gearhart et al. (1993) used a model similar to the one used by Rao and Brown to predict the same urinary TCA data set and included a parameter for urinary excretion of TCA. A comparison of the metabolic parameters is shown in Table 3-1. The ratio V_{max}/K_m is directly proportional to the rate of metabolism at low doses of tetrachloroethylene. The values shown in Table 3-1 indicate that the rate of metabolism predicted by the model of Gearhart et al. (1993), which directly fit the urinary TCA data, is slightly lower than the one used by Rao and Brown. The Reitz and Bois models use values of V_{max}/K_m that are greater than those of Gearhart et al. by an order of magnitude or more.

3.5.5. Physiologically Based Pharmacokinetic (PBPK) Model Comparisons and Interspecies Differences

For an example of tetrachloroethylene tissue concentrations in different species, blood concentrations resulting from a 1 ppm inhalation exposure for a duration of 16 hrs were

simulated. Figure 3-7 indicates blood concentrations in both rats (using the Reitz model) and humans (using the Rao and Brown model). The blood concentrations of the two species differ by a factor of about 2. It will be shown later that the difference in metabolism is significantly greater on a body-weight basis. Evidence of this exists in these plots; in particular, the shape of the decay curve between the two species is different. The blood concentrations are higher in rats up to approximately 20 hrs post exposure; the curves cross at this point in time. In Figure 3-7 the decay appears faster in humans than in rats, which is likely a consequence of steady-state behavior not having been attained.

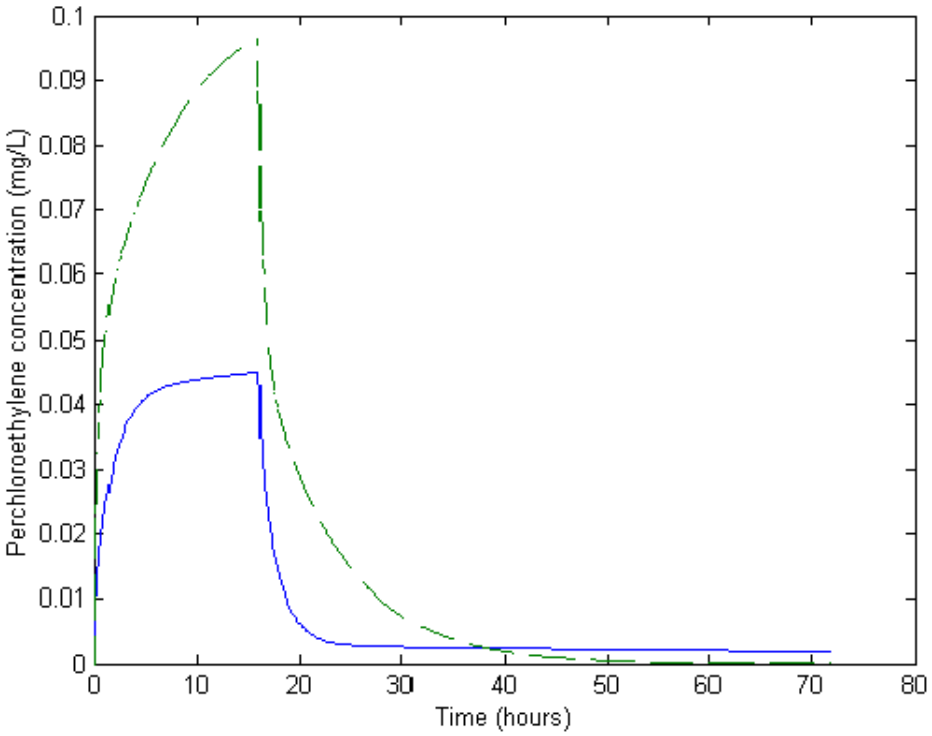

Figure 3-7. Comparison of tetrachloroethylene concentrations in blood in rats and humans. Blood concentrations in humans (solid line) and rats (dashed line) from a 16-hr inhalation exposure to 1 ppm tetrachloroethylene. The PBPK models used were those of Rao and Brown (1993) for humans and Reitz et al. (1996) for rats.

In contrast to Figure 3-7, long-time exposure (12 days at 1 ppm) is simulated in Figure 3-8 so as to ensure that steady state has been attained in both species. The blood concentration shows at least two modes of decay. In the initial phase, the concentrations in the Rao and Brown and Reitz human models decay faster than those in the model for the rat, whereas those in the Bois et al. model decay more slowly. In the second phase, the

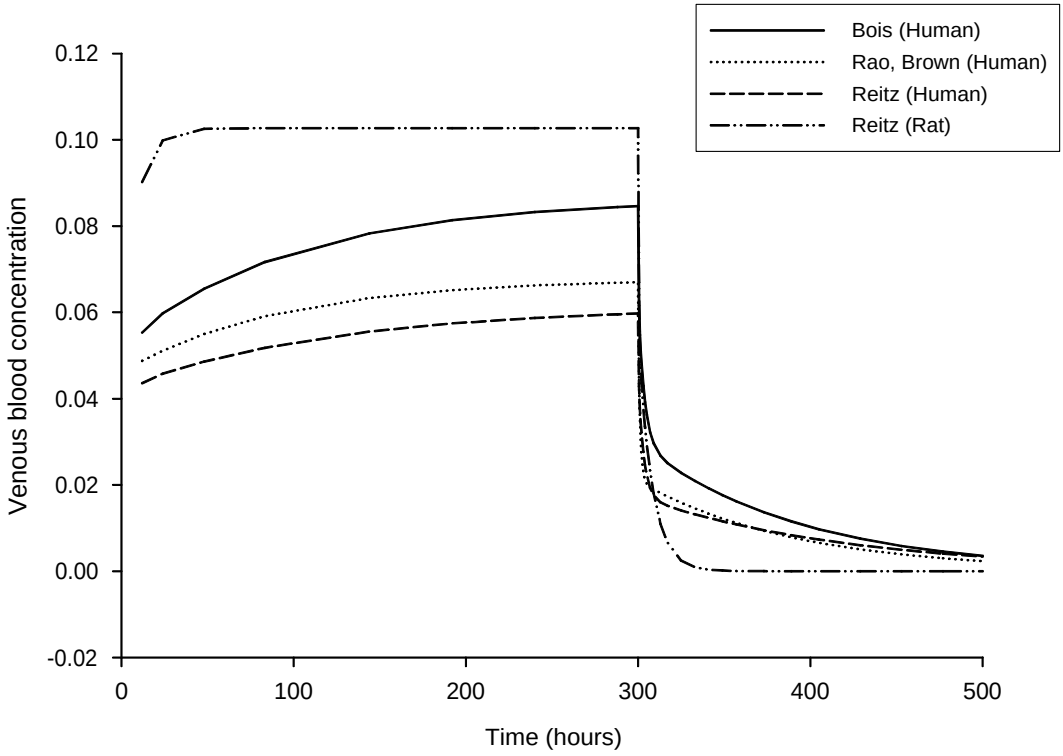

Figure 3-8. Comparison of various model predictions of tetrachloroethylene blood concentration in humans and rats following steady state. Blood concentrations in humans and rats due to 12-day inhalation exposure to 1 ppm tetrachloroethylene. Inspiratory rate is 13.9 L/min, ventilation-to-perfusion ratio is 1.3.

concentrations in all three models for the human decay more slowly than those for the rat. The decay as predicted by the Bois model is much slower than the decay for other models.

Figure 3-9 shows the daily rate of total amount of tetrachloroethylene metabolized following a 6-hr exposure. It illustrates differences in metabolism among the three species (mouse, rat, and human). The human model is that of Rao and Brown; the animal models are those of Reitz. Steady state was not attained—at least not in the rat and human.

The human models differ most in the metabolic parameters V_{max} and K_m (see Table 3-2). The effect of these differences is reflected graphically in Figure 3-10, which shows the rate of metabolism after steady state has been attained as a function of inhaled exposure concentration in units of milligrams per day per kilogram of body weight. At low exposures, the rate of metabolism is nearly equal to the ratio V_{max}/K_m. The values of this ratio in the Rao and Brown and Reitz models are one-tenth and one-half of the value in the Bois model. K_m in the Rao and Brown and Reitz models is greater than in the Bois model by a factor of 35. Therefore,

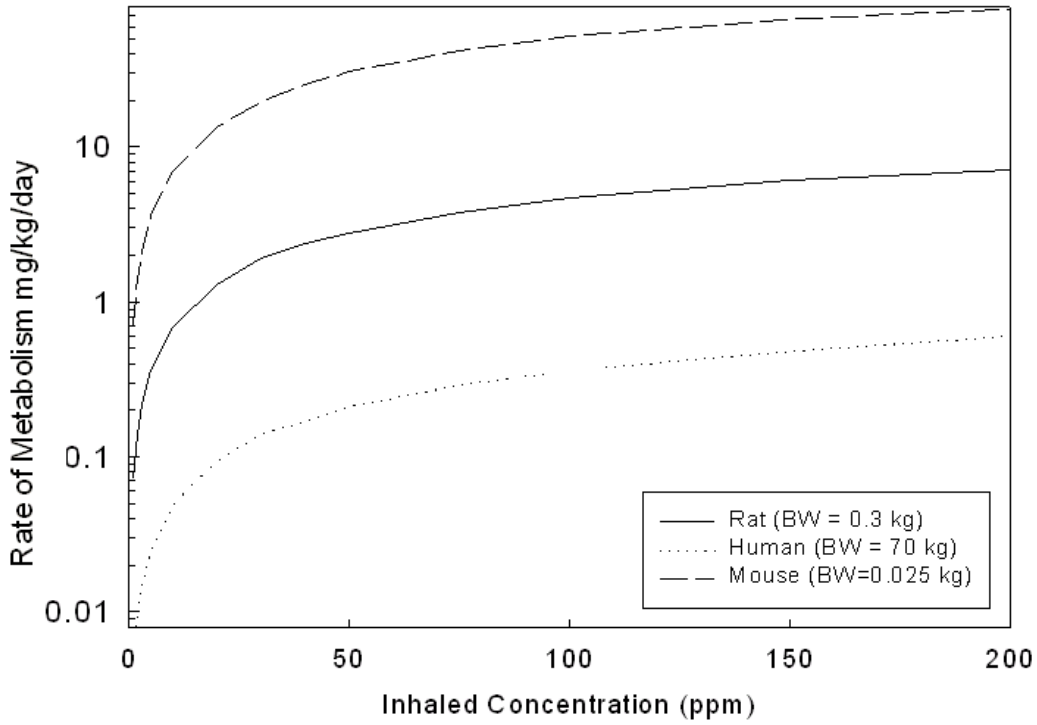

Figure 3-9. Model predictions of total tetrachloroethylene metabolites produced following a 6-hr inhalation exposure in rats, mice, and humans. The PBPK model parameters were from Reitz et al. (1996) for rodents and from Rao and Brown (1993) for humans.

BW = body weight

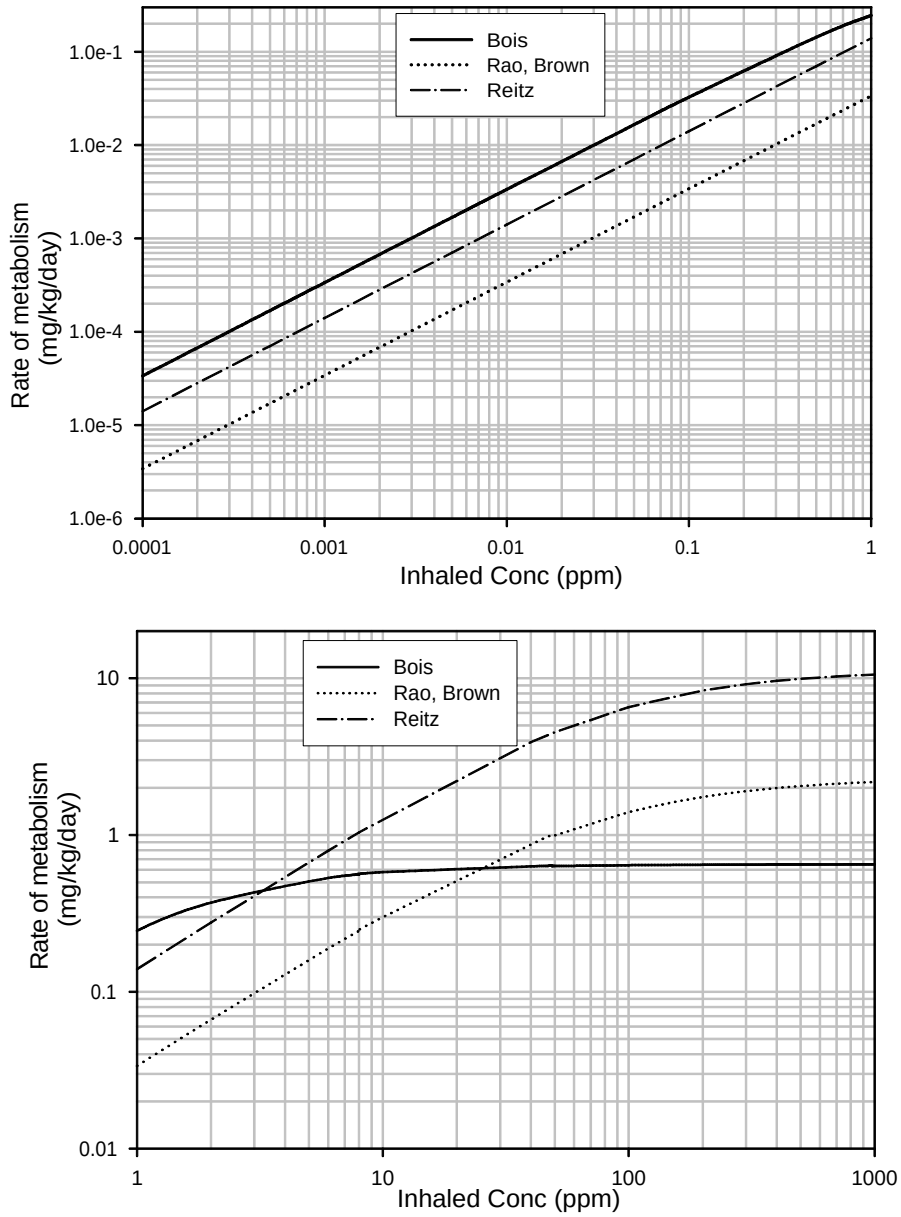

Figure 3-10. Model predictions of rate of total metabolism in humans at steady state. Rate of metabolism in humans, normalized to body weight, with continuous exposure after steady-state conditions have been reached, as predicted by the Bois et al., Rao and Brown, and Reitz models. Inspiratory minute volume is 13.9 L/min, ventilation-to-perfusion ratio is 1.3, and body weight is 70 kg.

saturation occurs at correspondingly higher concentrations in these models (between 1,000 and 2,000 ppm) than in the Bois model (at about 50 ppm). At saturation, the rate of metabolism is lowest in the Bois et al. model, as reflected in the relative values for V_{max}. Ohtsuki et al. (1983) monitored all the trichloro metabolite compounds excreted in the urine of 36 male and 25 female workers exposed to tetrachloroethylene. Their line of regression of the urinary metabolite level versus exposure concentration of tetrachloroethylene indicated saturation of metabolism occurring at roughly 600 ppm of exposure concentration. On the other hand, in an earlier study of 85 male workers, Ikeda et al. (1972) determined this saturation to occur at between 50 and 100 ppm.

In Figures 3-11a and 3-11b, the simulated rate of metabolism is shown as a function of time for two extremes in exposures (0.001 ppm and 50 ppm) for the rat and humans. The exposure time was considered to be 12 days (as in Figure 3-8). At a 50 ppm exposure concentration, the rate of tetrachloroethylene metabolism decreases very slowly with time, post-exposure.

Table 3-4 lists all the parameters used in the models. V_{max} differs considerably between the three models. K_m of the Bois model is the least and is less than the value in the other human models by a factor of 35. There are also other significant differences between the human models. The volume of the rapidly perfused compartment with the associated blood flow and the blood air partition coefficient in the Bois model are considerably different from those in the Rao and Brown and the Reitz models. The perfusion per unit volume of rapidly perfused tissue is much less in the Bois model (equal to 4.2) than in the Reitz (equal to 14) and Rao and Brown (equal to 24) models. The partition coefficient for the slowly perfused compartment in the Rao and Brown model is only about one-third that of the other two human models. The partition coefficient for fat in the Reitz model is considerably higher.

A sensitivity analysis was carried out in order to determine the dominant parameters underlying the differences between the results of the Bois and the Rao and Brown models. The differences in the blood concentrations and in the amount metabolized were found to be largely accounted for by the metabolic parameters (V_{max} and K_m), with the blood/air partition coefficient playing a secondary role. Results were found to be insensitive to the other parameters in the model.

The results of PBPK simulations of oral exposure to tetrachloroethylene are shown in Figures 3-12 and 3-13. In these simulations tetrachloroethylene was orally delivered via drinking water in nine bolus doses spaced 2 hrs apart during an 18-hr time period, followed by 6 hrs of no dosing. Because tetrachloroethylene concentrations and the rate of metabolism were found to be negligible at the end of the 24-hr period, the simulation was terminated after 24 hrs.

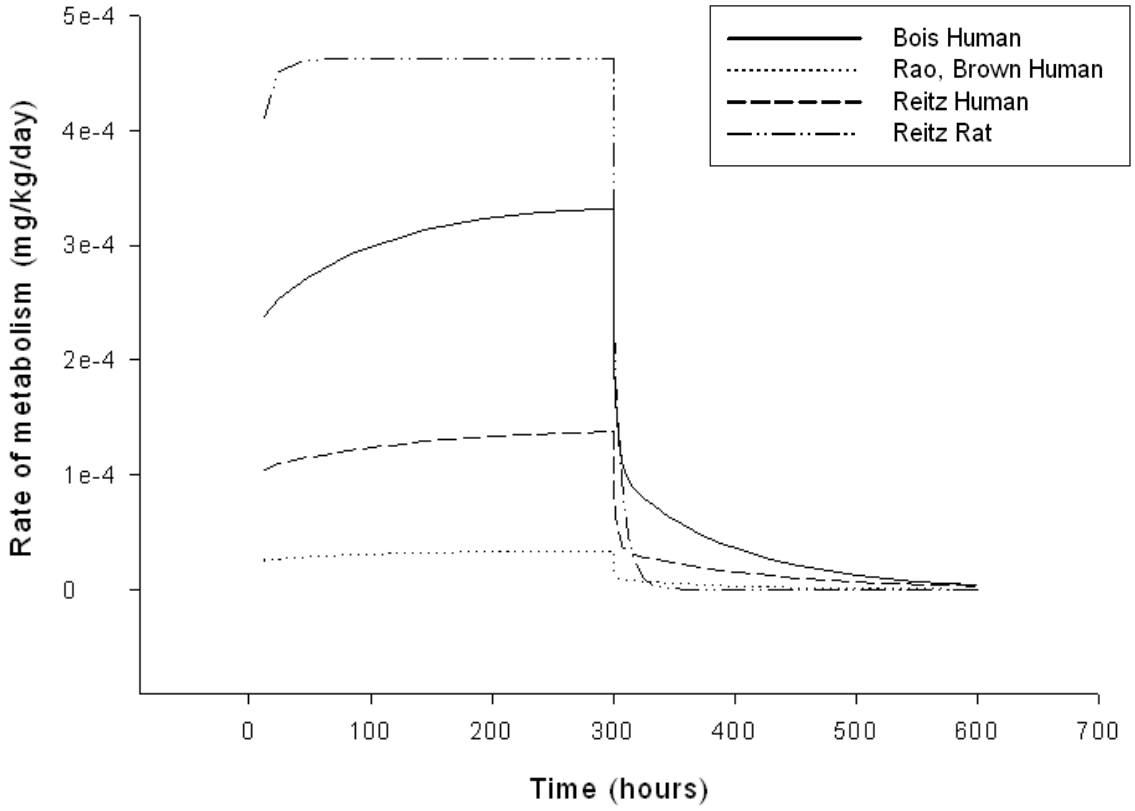

Figure 3-11a. Rate of metabolism in rat and human models: time course for low exposure. Rate normalized by body weight as predicted by the rat model (Reitz model) and various human models (Bois, Rao and Brown, and Reitz) for low-exposure concentration, 0.001 ppm. Human inspiratory minute volume is 13.9 L/min, ventilation-to-perfusion ratio is 1.3; for the rat, these parameters are as given in Table 3-4.

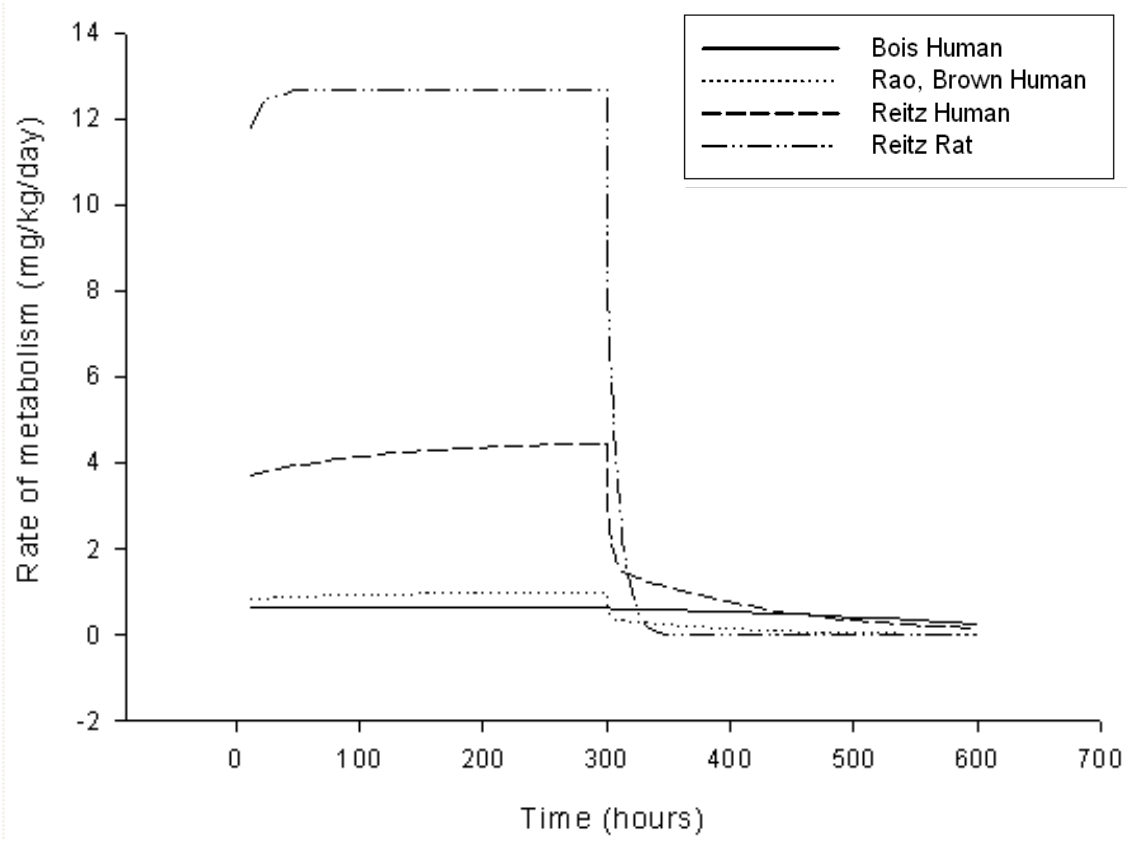

Figure 3-11b. Rate of metabolism in rat and human models: time course for high exposure. Rate normalized by body weight as predicted by the rat model (Reitz model) and various human models (Bois et al., Rao and Brown, and Reitz) for high-exposure concentration, 50 ppm. Human inspiratory minute volume is 13.9 L/min, ventilation-to-perfusion ratio is 1.3; for the rat, these parameters are as given in Table 3-2.

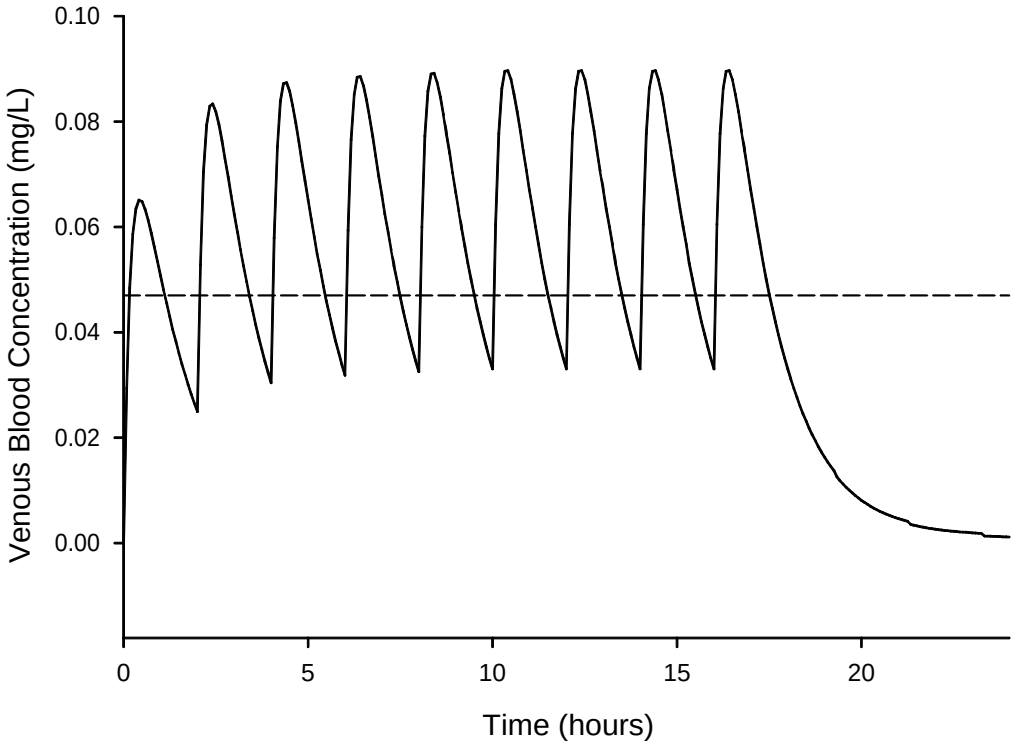

Figure 3-12. Oral ingestion of tetrachloroethylene: blood concentration in humans versus time. Time course of venous blood concentration in humans as predicted by the Rao and Brown model for ingested tetrachloroethylene. A total of 76 mg tetrachloroethylene was delivered orally via drinking water in 9 bolus doses spaced 2 hrs apart during an 18-hr time period, followed by 6 hrs of no dosing. The Bois and Reitz models result in nearly the same blood concentrations at this exposure concentration. The dashed line shows the corresponding steady-state blood concentration due to inhaled tetrachloroethylene of 0.7 ppm exposure concentration that results in the same area under the curve as the above curve integrated over a 24-hr period. The inspiratory rate is 13.9 L/min and the ventilation-to-perfusion ratio is 1.3.

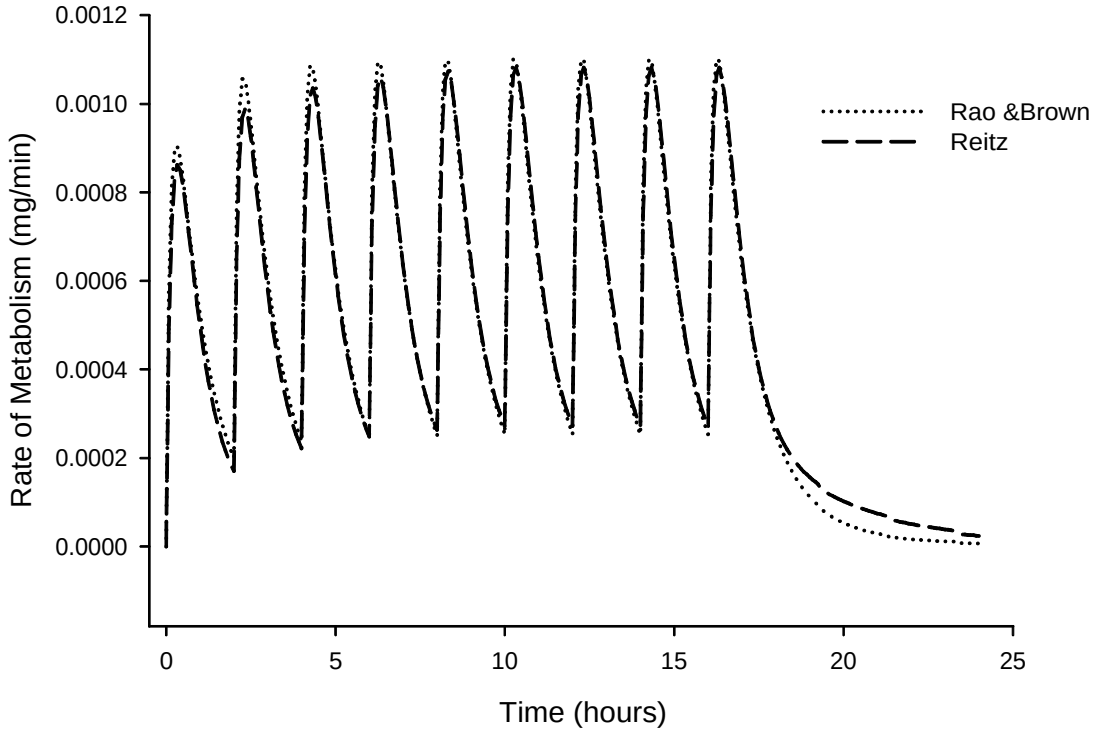

Figure 3-13. Rate of metabolism of tetrachloroethylene in humans: oral exposure. Rate of metabolism (mg/min) versus time of tetrachloroethylene ingested orally as predicted by two PBPK models (Rao and Brown; Reitz). Tetrachloroethylene was delivered orally via drinking water in 9 bolus doses spaced 2 hrs apart during an 18-hr time period, followed by 6 hrs of no dosing. The oral exposures that resulted in production of 0.01 mg/kg/day of overall metabolite were 21, 5.1, and 2.25 mg of total ingested tetrachloroethylene for the Rao and Brown, Reitz, and Bois models, respectively. The Bois model is not shown here to avoid a congested draft.

3.5.6. Metabolic Interactions With Other Chemicals

Fisher et al. (2004) used PBPK modeling and complementary studies in mice to investigate the effect of co-exposures of orally administered carbon tetrachloride (CT) and tetrachloroethylene on metabolic interactions between the two chemicals. CT is known to inhibit its own metabolism (referred to as suicide inhibition). TCA was used as a biomarker to assess the inhibition of the cytochrome P450 system by CT. Oral bolus intubation in the dose range of 1–100 mg/kg of CT was followed by a dose of 100 mg/kg of tetrachloroethylene an hour later. It was concluded that dose additivity could not be used to predict interactions between the compounds in this dose range because the metabolic interactions were found to be highly

nonlinear. The inhibition in metabolic capacity of tetrachloroethylene 2 hrs after administration of CT and 1 hr after administration of tetrachloroethylene was found to be 5, 52, and 90% at CT doses of 1.5, 10, and 19 mg/kg, respectively.

Dobrev et al. (2002) performed gas uptake studies in F344 rats and developed a mixture PBPK model for humans to study interaction effects during co-exposure to mixtures of trichloroethylene (TCE), tetrachloroethylene, and methylchloroform. Corresponding to a 10% increase in TCE blood concentration, the production rates of toxic conjugative metabolites exceeded 17%, pointing to a nonlinear interaction effect due to co-exposure to TCE.

APPENDIX FOR CHAPTER 3: COMPARISONS OF TETRACHLOROETHYLENE METABOLISM WITH TRICHLOROETHYLENE METABOLISM

3.A.1. EXTENT OF METABOLISM

The available data indicate that, overall, tetrachloroethylene is less extensively metabolized than is the closely related chemical, trichloroethylene. The difference is due to the fact that a lower fraction of a tetrachloroethylene dose is metabolized via the major oxidative CYP pathway when compared with an equivalent dose of the trichloroethylene congener (Ohtsuki et al., 1983; Volkel et al., 1998; Lash and Parker, 2001). For example, in balance studies of humans, only about 1–3% of the estimated amounts of tetrachloroethylene inhaled were shown to be metabolized to TCA and other chlorinated metabolites, although these studies fail to account for total dose (see Section 3.3.2 for further discussion). These amounts can be compared to the 40–75% of trichloroethylene shown to be metabolized in various human balance studies similar to the ones conducted for tetrachloroethylene (U.S. EPA, 1985).

Because of its higher lipid solubility, tetrachloroethylene may appear to be less well metabolized than trichloroethylene, at least to a certain degree, simply because it is more slowly metabolized due to fat sequestration. However, the animal data from studies of the two compounds provide results similar to those of the human studies regarding the relative extent of metabolism. For example, using data from laboratory animal studies of tetrachloroethylene (Pegg et al., 1979; Schumann et al., 1980), EPA reported the percent of tetrachloroethylene body burdens excreted as unchanged parent compound following exposure to 10 and 600 ppm for 6 hrs to be 68 and 99%, respectively (U.S. EPA, 1985). By comparison, rats and mice exposed to equivalent 10 and 600 ppm trichloroethylene doses (Stott et al., 1982) metabolized a higher percentage of this compound, with mice metabolizing essentially all of the dose and rats metabolizing 98 and 79% of the low and high doses, respectively.

Saturation of metabolism occurs at a higher dose for trichloroethylene than for tetrachloroethylene; thus, at certain dose levels, the differences in the amounts of the two compounds metabolized is relatively greater than at other dose levels. Tetrachloroethylene appears to be a lower-affinity substrate for CYP enzymes than trichloroethylene (Ohtsuki et al., 1983; Volkel et al., 1998). The K_m value for tetrachloroethylene is certainly higher than the K_m value for trichloroethylene (Lipscomb et al., 1998).

Both tetrachloroethylene and trichloroethylene are liver toxicants and cause liver hepatocellular carcinomas in mice. The liver toxicity, including carcinogenicity, of these compounds is thought to be due to metabolites. It is interesting to note that although

trichloroethylene appears to be more extensively metabolized—due to greater CYP metabolism in the liver—the relative cancer potency for liver tumors is similar for the two compounds.

3.A.2. DIFFERENCES IN CYTOCHROME P450 (CYP) METABOLITES

TCA, DCA, chloral, and TCOH are reported biotransformation products of both tetrachloroethylene and trichloroethylene; however, the relative amounts produced and the precursor intermediates are different for the two compounds. TCA is the major urinary metabolite for tetrachloroethylene and is also an excretion product of trichloroethylene, whereas TCOH is the major trichloroethylene urinary excretion product. The formation of chloral in metabolism of tetrachloroethylene has been called into question, and the measurements of TCOH in urine following tetrachloroethylene exposures have also been challenged.

Regardless, TCOH clearly is not the significant metabolite for tetrachloroethylene that it is for trichloroethylene (Lash and Parker, 2001). The fact that the major urinary metabolite for tetrachloroethylene is TCA (with little, if any, TCOH being formed), whereas the major urinary metabolite for trichloroethylene is TCOH in the form of its glucuronide, clearly indicates qualitative and quantitative differences in precursor intermediates. Very little, if any, TCA produced from tetrachloroethylene metabolism comes through chloral, either directly or indirectly through TCOH (Lash and Parker, 2001). The TCA from tetrachloroethylene comes through trichloroacetyl chloride, possibly via the epoxide. On the other hand, the TCA produced from trichloroethylene metabolism is thought to come through chloral, both directly and through TCOH enterohepatic circulation (Lash et al., 2000).

DCA is a biotransformation product of both tetrachloroethylene and trichloroethylene, although it is believed that a greater portion of DCA coming from tetrachloroethylene metabolism does not arise from CYP metabolism, but rather results from further processing of TCVC, whereas the DCA coming from trichloroethylene metabolism results from CYP oxidation. There are at least three potential routes to DCA in CYP metabolism of trichloroethylene, yet only one likely route—dechlorination of TCA—in the CYP metabolism of tetrachloroethylene. Furthermore, the amount of DCA produced from tetrachloroethylene oxidative metabolism may vary across species.

3.A.3. CYTOCHROME P450 (CYP) ENZYMES

Quantitatively, the liver is by far the predominant site of tetrachloroethylene and trichloroethylene oxidative metabolism; although most other tissues contain the CYPs that could conceivably metabolize these compounds. CYP2E1 has been shown to be important in rodent metabolism of trichloroethylene; however, the chemical-specific data are sparse with regard to

its role in tetrachloroethylene metabolism (Doherty et al., 1996). Still, assuming that CYP2E1 is important to tetrachloroethylene metabolism is not unreasonable.

CYP3A isoenzymes—and especially CYP2B1/2—may be important for tetrachloroethylene. Costa and Ivanetich (1980) showed increased hepatic metabolism following treatment with agents now known to induce these isoenzymes specifically. CYP2B1/2 is probably the most important CYP isoenzyme involved in oxidative metabolism of tetrachloroethylene, at least in the rat, although CYP3As and CYP2E1 are also likely involved.

3.A.4. GLUTATHIONE-DEPENDENT METABOLISM

The GSH-dependent pathway for tetrachloroethylene exists in both rodents and humans, and the pathway is also operative for trichloroethylene in these species (Birner et al., 1996; Volkel et al., 1998). The flux through this pathway is thought to be quantitatively less than that through the P450 pathway. Toxic metabolites can arise from several sources in the pathway; however, for tetrachloroethylene, as well as for trichloroethylene, the GSH pathway is associated with renal toxicity (Anders et al., 1988; Dekant et al., 1989; U.S. EPA, 1991; IARC, 1995; Lash et al., 2000; Lash and Parker, 2001). For both compounds, recovery of urinary mercapturates, the stable end-products of the GSH pathway, comprise 1% or less of the total dose (Lash and Parker, 2001; Dekant et al 1986a), but this does not reflect the total flux through the GSH pathway. In particular, the TCVC metabolite and the corresponding DCVC and their respective N-acetylated forms derived from trichloroethylene might also act as substrates for renal beta lyases and other enzymes: FMO3 and CYP3A (Dekant et al., 1988; reviewed by Anders et al., 1988; Dekant et al., 1989; U.S. EPA, 1991; Lash et al., 2000; Lash and Parker, 2001; see Section 3.2). It should be noted that a higher cysteine S-conjugate-to-mercapturate ratio exists for tetrachloroethylene when compared to trichloroethylene, which could influence the relative bioactivation and nephrotoxicity of these two compounds (Lash and Parker, 2001).

3.A.5. SUMMARY

Tetrachloroethylene is closely related structurally to trichloroethylene and the two chemicals cause similar toxic effects, many of which are attributed to metabolic activation of the parent compounds. Although tetrachloroethylene is not as extensively metabolized as trichloroethylene, there is little difference in potency between the two chemicals. TCA, DCA, chloral, and TCOH are reported P450 biotransformation products of both tetrachloroethylene and trichloroethylene; however, the relative amounts of these metabolites produced, as well as the precursor intermediates in the oxidative pathways, are different for the two compounds. The fact that the two compounds produce different reactive intermediate P450 metabolites is important to

consider. Excretion of urinary mercapturates indicates that, relative to P450 oxidation, tetrachloroethylene is more extensively metabolized via GSH conjugation than is trichloroethylene. However, these urinary excretion products do not reflect the total flux through the GSH pathway since the glutathione and cysteine conjugates of both chemicals have been shown to undergo further processing to products that are highly reactive. Thus, regardless of similarities, both the qualitative and the quantitative differences between tetrachloroethylene and trichloroethylene in metabolite production could have bearing on toxicity and tumor induction, and the relative importance of various mechanisms and different modes of action contributing to their toxic effects, including tumorgenesis, may vary between the two parent compounds. Recognizing similarities and differences is important in attempting to understand how each of these two compounds causes its toxic effects.

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4. HAZARD IDENTIFICATION

4.1. OVERALL APPROACH

This chapter discusses tetrachloroethylene toxicity on an organ-specific basis, with liver, kidney, neurotoxicity, and developmental/reproductive effects as the major emphasis in separate sections. For each of the major organ systems, human effects are presented first, followed by effects in animals and in in vitro systems. Cancer and noncancer toxicity and mode of action (MOA) are also included in the discussions. Of note, site concordance of effect between animals and humans is generally not assumed.

Evidence for each organ system is summarized, but an in-depth discussion or data evaluation is not provided for any individual studies, especially those evaluated and discussed in previous EPA documents and other Agency reports. Several existing publications provide more detailed study descriptions: the World Health Organization-International Programme on Chemical Safety (WHO-IPCS, 2006), the ATSDR's *Toxicologic Profile for Tetrachloroethylene* (ATSDR, 1997) and the International Agency for Research on Cancer (IARC) review the health effect evidence on tetrachloroethylene, trichloroethylene, and their common metabolites; other studies review this evidence on dry cleaner as an occupational title (IARC, 1995); *Tetrachloroethene Ambient Air Criteria Document* (NYS DOH, 1997); and *Public Health Goal for Tetrachloroethylene in Drinking Water* (Cal EPA, 2001). The details for earlier toxicity and carcinogenicity studies may also be found in previous EPA assessments (e.g., U.S. EPA, 1980, 1985a, b, 1986a, 1991a).

4.2. OVERVIEW OF TETRACHLOROETHYLENE METABOLISM

Most tetrachloroethylene toxicity and cancer-causing activity, other than neurotoxicity, is generally attributed to its metabolites. For example, historically, a direct relationship has been demonstrated between the level of hepatic microsomal cytochrome P450, the extent of metabolism of tetrachloroethylene in vivo, and cellular damage (Bonse et al., 1975; Bonse and Henschler, 1976; Moslen et al., 1977; Pegg et al., 1979; Schumann et al., 1980; Buben and O'Flaherty, 1985; U.S. EPA, 1985a, 1991a). In addition, several oxidative (P450) and GSH-derived tetrachloroethylene metabolites have been shown to induce toxic and carcinogenic effects in similar targets when they are administered directly (IARC, 1995; Herren-Freund et al., 1987; Bull et al., 1990; Bull, 2000; Pereira, 1996; DeAngelo et al., 1991, 1999; Daniel, 1963; Carter et al., 2003; Elfarra and Krause, 2007). This metabolism prerequisite for certain toxic effects is true for related halogenated ethylenes and ethanes as well (IARC, 1995; U.S. EPA,

1991a, 2001a; Dekant, 2001). CNS effects are a notable exception in that they are largely attributable to the parent compound.

As detailed in Chapter 3, tetrachloroethylene is metabolized through at least two major pathways. The oxidative P450, or CYP, pathway is quantitatively most important, and it accounts for the greatest amount of observed metabolite in all species at all doses tested (see Section 3.3.3.1). The other is the GSH conjugation pathway, which is associated with renal toxicity and renal carcinogenicity (see Section 3.3.3.2). A significant portion of absorbed dose in human studies (as much as 20–40%) cannot be tracked either as parent compound or metabolites of known pathways, which introduces uncertainty about the identity and the amounts of the metabolites formed in humans (U.S. EPA, 1991a; Bogen and McKone, 1988). TCA, a product of the oxidative pathway, is the major urinary metabolite derived from tetrachloroethylene metabolism. TCA is a mouse liver carcinogen. TCA may contribute in part to findings of liver toxicity and cancer observed in tetrachloroethylene-exposed animals; however, according to some investigators (Clewell et al., 2004, 2005), the amount of TCA produced from tetrachloroethylene in rodent bioassays is insufficient to account in total for observed hepatocarcinogenicity (see Appendix 4A). DCA is another known tetrachloroethylene urinary metabolite that is formed in both the oxidative pathway by dechlorination of TCA and, in organs other than the liver, in the GSH pathway. DCA is known to cause liver cancer in both rats and mice. Whether DCA contributes to tetrachloroethylene-induced toxicity or carcinogenicity in the liver is not known.

Tetrachloroethylene oxide, trichloroacetyl chloride, and chloral/chloral hydrate are proposed reactive intermediates in tetrachloroethylene P450 oxidation. Tetrachloroethylene oxide and trichloroacetyl chloride have the potential to contribute to tetrachloroethylene toxicity and carcinogenicity, particularly in the liver. Detection of TCOH in the urine of tetrachloroethylene-exposed humans and animals would provide evidence for the existence of the chloral hydrate intermediate. However, TCOH—and therefore the evidence that its chloral/chloral hydrate precursor is formed from tetrachloroethylene—is not consistently detected and it might be an artifact of the methodology used in some but not all studies. Chloral hydrate is a liver carcinogen in mice.

The glutathione pathway entails initial glutathione-S-transferase catalyzed conjugation of tetrachloroethylene with GSH to form TCVG. The cellular damage and genotoxic effects of these conjugation products are thought to be from their further metabolism via beta-lyase, FMO3, and/or P450 metabolism to highly reactive toxic products.

All of these metabolites have effects that may contribute to the toxicity and carcinogenicity of tetrachloroethylene, although the role of specific intermediates has not been elucidated. Genotoxicity of the oxidative metabolites TCA, DCA, chloral hydrate,

tetrachloroethylene oxide and the GSH-derived intermediates TCVC, TCVG, and NAcTCVC is discussed in Section 4.3; for TCA and DCA, see Section 4.4.4.3 for peroxisome proliferator-activated receptor alpha (PPAR-α) form activation and Section 4.4.4.4 for hypomethylation. Section 4.10.3 provides a summary of the cancer MOA conclusions for tetrachloroethylene.

4.3. GENOTOXICITY

Tetrachloroethylene has been extensively studied for genotoxic activity in a variety of in vitro assay systems such as bacteria, yeast, and mammalian cells (see reviews by U.S. EPA, 1985c, 1991a; IARC, 1995; ATSDR, 1997). Also, a review of the mutagenicity of trichloroethylene (Moore and Harrington-Brock, 2000) contains a discussion of several of known (TCA, DCA) and proposed (chloral hydrate) tetrachloroethylene metabolites.

The application of mutagenicity data to the question of potential carcinogenicity is based on the premise that genetic alterations are found in all cancers. Mutagenesis is the ability of chemicals to alter the genetic material in a manner that permits changes to be transmitted during cell division. Although most tests for mutagenicity detect changes in DNA or chromosomes, modifications of the epigenome, including proteins associated with DNA or RNA, can also cause transmissible changes. Genetic alterations can occur via a variety of mechanisms including gene mutations, deletions, translocations or amplification; evidence of mutagenesis provides mechanistic support for the inference of potential for carcinogenicity in humans.

The following discussion focuses on the conclusions of the earlier studies and includes details of recent studies that may provide some insight into the potential genotoxicity of tetrachloroethylene. Positive findings were reported in some experiments using technical-grade tetrachloroethylene that contained impurities or used epichlorohydrin or epoxybutane as stabilizers, both of which are clearly mutagenic in a number of biological systems. Purified tetrachloroethylene was negative in the same systems tested without or with mixed-function oxidation activity provided by either rat or hamster liver S9 (Haworth et al., 1983). The results of a large number of in vitro genotoxicity tests in which tetrachloroethylene was the test agent do not clearly support the conclusion that tetrachloroethylene exhibits direct mutagenic activity, although the few studies of conditions that would generate the GSH conjugate were positive (U.S. EPA, 1991a; IARC, 1995; ATSDR, 1997).

An increased level of DNA single-strand breaks (SSB) was seen in liver and kidney tissues but not in the lung tissue of mice 1 hr after single intraperitoneal (i.p.) injections of 4–8 mmol/kg (663–1326 mg/kg) of tetrachloroethylene (Walles, 1986). Potter et al. (1996) found no increases in DNA strand breaks in kidneys of male F344 rats after a single gavage treatment with 1,000 mg/kg tetrachloroethylene. However, differences in species and/or route of exposure preclude direct comparisons of these studies. Muzzullo (1987) found DNA binding of

tetrachloroethylene in mouse liver and rat kidney. Cytosols from several organs were more effective than liver microsomes in enhancing in vitro DNA or protein binding of tetrachloroethylene, and enrichment with GSH enhanced the activity of liver microsomes (Muzzullo, 1987).

Toraason et al. (1999) found no increase in 8-hydroxydeoxyguanosine (8-OHdG) in the urine, the liver, or the kidneys of male F344 rats after a single i.p. injection of tetrachloroethylene at 100, 500, or 1,000 mg/kg (8-OHdG in peripheral lymphocytes was measured only in the 500 mg/kg group). In a subsequent paper, Toraason et al. (2003) reported no increase in 8-OHdG in urine of 18 dry cleaner workers sampled pre- and post-shift work (time-weighted average [TWA] concentration of tetrachloroethylene was 3.8 $\pm$ 5.3 ppm).

Tetrachloroethylene induced damage was observed in the sister chromatid exchange (SCE) assay and in the single-cell gel test in human blood culture treated with up to 5 mM (~830 mg/L) tetrachloroethylene, at which viability was reduced by 40% (Hartmann and Speit, 1995). Tetrachloroethylene exposure increased the frequency of micronuclei in peripheral blood reticulocytes or hepatocytes of ddY mice given single i.p. injections at 1,000 or 2,000 mg/kg tetrachloroethylene given after, but not prior to partial hepatectomy (Murakami and Horikawa, 1995). Tetrachloroethylene-induced micronuclei have also been reported in cultured Chinese hamster kidney cells (Wang et al., 2001) and in human cells (Doherty et al., 1996; White et al., 2001). Micronucleus induction was enhanced by tetrachloroethylene exposure in human lymphoblastoid cells by stable expression of cDNAs encoding either CYP2E1 (hE1 cells) or human CYP1A2, 2A6, 3A4, 2E1 and microsomal epoxide hydrolase (Doherty et al., 1996). In contrast to these findings, neither chromosome aberrations nor SCE were induced in Chinese hamster ovary cells following in vitro exposure to tetrachloroethylene (Galloway et al., 1987).

Tetrachloroethylene when incubated with rat liver GST, GSH, and a rat kidney fraction, exhibited a clear dose-response in the Ames test (Vamvakas et al., 1989b). In addition, it was demonstrated that TCVG was produced from tetrachloroethylene in isolated perfused rat liver and excreted into bile; in the presence of a rat kidney fraction, the collected bile was mutagenic in Salmonella, as was purified TCVG (Vamvakas et al., 1989b). Dreesen (2003) also demonstrated, for TCVG, an unequivocal dose-dependent mutagenic response in the TA 100 strain in the presence of the rat kidney S9-protein fraction; TCVC was mutagenic without metabolic activation in this strain. In a separate study, the tetrachloroethylene metabolite TCVC was also positive in Salmonella (strains TA 98 and TA 100) and inhibition of beta lyase activity blocked the effect (Dekant et al., 1986). A subsequent study indicated that Salmonella also were capable of deacetylating the urinary metabolite NAcTCVC when TA 100 showed a clear positive response without exogenous activation (Vamvakas et al., 1987). Vamvakas et al. (1989a) also

reported concentration-related increases in unscheduled DNA synthesis (UDS) in LLC-PK1 (a porcine kidney cell line) exposed to TCVC, with the effect abolished by a beta lyase inhibitor.

Several identified or putative P450 metabolites of tetrachloroethylene are mutagenic. Tetrachloroethylene-epoxide, a hypothesized intermediate in tetrachloroethylene P450 oxidative metabolism (Henschler et al., 1977a, b), is mutagenic in bacteria (Kline et al., 1982). As reviewed by Moore and Harrington-Brock (2000), the oxidative metabolite TCA, the major urinary excretion product, exhibits little, if any, genotoxic activity. However, in vitro experiments with TCA should be interpreted with caution if steps have not been taken to neutralize pH changes caused by the compound. TCA was positive in genotoxicity studies conducted by Bhunya and Behera (1987), Bhunya and Jena (1996), and Birner et al. (1994) in in vivo mouse and chick test systems. TCA has also been reported to induce DNA SSB in hepatic DNA of mice. A single dose of TCA was administered to Sprague-Dawley rats and B6C3F1 mice by gavage (Nelson and Bull, 1988). The animals were sacrificed 4 hrs later and SSB in liver DNA were analyzed by alkaline unwinding assay. SSB were observed in a dose-dependent manner. The lowest dose of TCA that produced significant SSB in the rats was 0.6 mmol/kg (98 mg/kg). For mice, the lowest dose of TCA that produced significant increases was 0.006 mmol/kg (0.98 mg/kg). Further, in another study by the same authors (Nelson et al., 1989), the incidence of SSB was elevated at 1 hr after a single i.p. dose TCA exposure of 500 mg/kg; the level returned to control levels by 8 hrs. In a second experiment, no increase in SSB in hepatic DNA was observed 24 hrs after 10 days of daily gavage of 500 mg/kg TCA. A later study by Styles et al. (1991), using essentially the same procedures, failed to detect any increase in SSB.

Chang et al. (1992) observed a marginally significant increase in SSB in hepatocyte DNA of mice but not rats at 4 hrs after a single TCA dose of 10 mmol/kg (1,633.9 mg/kg) administered orally. However, the authors considered this finding to be not biologically significant, because SSB were not increased at 1 hr and there were no detectable SSB in isolated hepatocytes exposed to concentrations of TCA as high as 10 mM (~1,650 mg/L). Storer et al. (1996), after evaluating 81 chemicals (carcinogens, noncarcinogens, mutagens, and nonmutagens) for SSB using the alkaline unwinding assay, demonstrated that increased DNA SSB at high doses can be the result of cytotoxicity involving endonucleocytic degradation of DNA.

As reviewed elsewhere (see Salmon et al., 1995; Moore and Harrington-Brock, 2000), chloral hydrate is mutagenic in the standard battery of screening assays. Effects include positive results in bacterial mutation tests for point mutations and in the mouse lymphoma assay for mutagenicity at the Tk locus (e.g., Haworth et al., 1983). In vitro tests showed that chloral hydrate also induced micronuclei and aneuploidy in human peripheral blood lymphocytes or Chinese hamster pulmonary cell lines. Micronuclei were induced in Chinese hamster embryonic

fibroblasts. Several studies demonstrate that chloral hydrate induces aneuploidy (loss or gain of whole chormosomes) in both mitotic and meiotic cells, including yeast (Singh and Sinha, 1976, 1979; Kafer, 1985; Gualandi, 1987; Sora and Agostini-Carbone, 1987), cultured mammalian somatic cells (Degrassi and Tanzarella, 1988), and spermatocytes of mice (Russo et al., 1984; Liang and Pacchierotti, 1988). Chloral hydrate has also been shown to block spindle elongation in insect spermatocytes (Ris, 1949). Chloral hydrate was negative for sex-linked recessive lethal mutations in drosophila (Yoon et al., 1985). It induces SSB in hepatic DNA of mice and rats (Nelson and Bull, 1988) and mitotic gene conversion in yeast (Bronzetti et al., 1984). Schatten and Chakrabarti (1998) showed that chloral hydrate affects centrosome structure, which results in the inability to reform normal microtubule formations and causes abnormal fertilization and mitosis of sea urchin embryos.

The chloroacid metabolite, DCA, is also mutagenic in the standard battery of screening tests (reviewed by Moore and Harrington-Brock, 2000). DCA was positive in bacterial mutation tests, in the in vitro mouse lymphoma assay, the micronucleus induction test, the Big Blue mouse system and other tests (DeMarini et al., 1994; Fuscoe et al., 1996; Nelson and Bull, 1988; Harrington-Brock et al., 1998; Leavitt et al., 1997; Chang et al., 1989; Bignami et al., 1980). Anna et al. (1994) compared mutations in the *ras* gene in liver tumors in mice treated orally with tetrachloroethylene, DCA and trichloroethylene with those in untreated mice. The frequency of mutations at codon 61 of *H-ras* was significantly lower in liver tumors of tetrachloroethylene-exposed mice but not in DCA or trichloroethylene tumors. Thus, the phenotype of tetrachloroethylene-induced mouse tumors appeared to differ from trichloroethylene, DCA or spontaneous occurring tumors. While not sufficient to indicate the MOA, tumor phenotype data regarding H-ras codon 61 suggests that tetrachloroethylene-induced liver tumors differ from those induced by DCA, TCA, or trichloroethylene and those arising spontaneously in the mouse.

In summary, tetrachloroethylene has been shown to induce some genotoxic effects (micronuclei and SCEs following in vitro exposure, DNA binding and SSBs in tumor tissue). Results of in vitro mutagenicity (Ames) or DNA binding assays of tetrachloroethylene have largely been negative except in the few tests of conditions where metabolites of the GSH pathway are generated. The GSH metabolites are clearly mutagenic. TCVC is the most potent bacterial mutagen of the tetrachloroethylene metabolites and induces UDS in a porcine kidney cell line; TCVG and NAcTCVC are also mutagenic in bacteria. The known (DCA) or putative (tetrachloroethylene oxide, chloral hydrate) P450 metabolites also exhibit mutagenicity.

Uncertainties with regard to the genotoxicity characterization include that not all tetrachloroethylene metabolites have been identified, nor have all the known or postulated metabolites been sufficiently tested in the standard genotoxicity screening battery. Of note, bacterial mutation testing protocols typically specify the inclusion of cytotoxic concentrations of

the test article, and the relative potency of the metabolites in vitro may not necessarily inform their relative contribution to the overall mechanistic effects of the parent chemical. This may be especially relevant when evaluating in vitro testing results for tetrachloroethylene, which can undergo inter-organ metabolic processing involving multiple enzyme systems to yield highly reactive species. In addition, such tests do not provide data for all effects that are relevant for carcinogenesis. Thus, other data gaps include incomplete characterization of the metabolites in tests beyond the standard battery of genotoxicity tests including on important genetic and epigenetic endpoints.

Section 4.10.3 addresses the contribution of mutagenicity of tetrachloroethylene and its oxidative and GSH-derived metabolites to the MOA of carcinogeniticy for tetrachloroethylene. Overall, the finding is that the MOA for tetrachloroethylene-induced carcinogenesis is not yet fully characterized, completely tested, or understood. The database for hepatocarcinogenesis is especially limited with regard to chemical-specific studies. It is concluded that the role of genotoxicity in hepatocarcinogenicity, an effect that is thought to be related to products of CYP metabolism, is uncertain (see Section 4.4.4.5). While the complete mechanisms are not yet understood, the weight of evidence, including the known mutagenicity of GSH-derived metabolites produced in the kidney, suggests a mutagenic MOA cannot be ruled out for tetrachloroethylene-induced renal carcinogenesis (see Section 4.5.4.3.3).

4.4. LIVER TOXICITY

4.4.1. Human Effects

A number of hepatotoxic effects, including hepatomegaly, hepatocellular damage, and elevations of several hepatic enzymes and bilirubin degradation byproducts, have been observed after acute high-level exposure to tetrachloroethylene (levels not identified; Meckler and Phelps, 1966; Coler and Rossmiller, 1953; Hake and Stewart, 1977; Saland, 1967; Stewart et al., 1961, as reported in ATSDR, 1997). One case report noted obstructive jaundice and hepatomegaly in an infant exposed orally to tetrachloroethylene (1 mg/dL; Bagnell and Ellenberger, 1977, as reported in ATSDR, 1997).

4.4.1.1. *Liver Damage*

Four cross-sectional studies were available that evaluated the prevalence of liver damage among dry cleaner populations (Lauwerys et al., 1983; Cai et al., 1991; Gennari et al.; 1992; Brodkin et al., 1995). These studies assessed serum concentration of a number of hepatic enzymes in dry cleaner and control populations. Additionally, sonographic changes to hepatic parenchymal tissue were examined in one study (Brodkin et al., 1995). An elevated

concentration of the serum enzyme GGT and mild hepatic changes were notable observations in two studies (Gennari et al., 1992; Brodkin et al., 1995).

Gennari et al. (1992) measured the electrophoretic fractionation patterns of serum GGT isozymes among 141 tetrachloroethylene-exposed dry cleaners and 130 nonexposed controls selected from staff and students from the academic institution of the principal investigators. Both the exposed subjects and the controls had similar lifestyle (smoking, alcohol consumption) and clinical medical histories. The TWA tetrachloroethylene concentration in the dry cleaning facilities was 11.3 ppm. Total GGT was higher in exposed workers (exposed: mean of 12.4 international units per liter [U/L; standard deviation, 6.9 U/L]; controls: 8.8 U/L [4.9 U/L], $p < 0.01$). The GGT-2 isoenzyme component was higher in exposed workers (6.8 U/L [5.7 U/L] in exposed vs. 3.5 U/L [3.3 U/L] in controls, $p < 0.01$) and the GGT-4 component was detectable in exposed workers but not measurable in controls. The authors regarded a GGT-2/GGT-3 ratio of greater than 1 as a sensitive index of the reciprocal behavior of the two isoenzymes. GGT-2 is generally associated with activation of liver microsomal enzymes. GGT-4 is common in liver diseases and indicates hepato-biliary impairment.

This study excluded individuals who presented values for GGT, or other liver enzymes above a normal range, and individuals who had past or current liver disease. None of the workers showed any clinical symptoms of liver disease, and their enzymatic profiles, including GGT, aspartase amino transaminase (AST), alanine amino transaminase, 5'-nucleotidase, and alkaline phosphotase, were within the clinically normal reference limits. Given the study's exclusion criteria, it is not surprising that liver enzyme concentrations were within a normal range. The authors stated that more research is required to develop this GGT fractionation assay into a clinically useful method of measuring liver function. Nevertheless, the study showed that these dry cleaners had markers of tetrachloroethylene oxidative metabolism (GGT-2) and liver impairment (GGT-4).

The study by Brodkin et al. (1995) examined liver function and carried out sonography measurements in a population of 27 dry cleaners and 26 nonexposed laundry workers. Dry cleaners were older and had a longer duration of employment than did laundry workers. The noninvasive imaged penetration of ultrasound into liver tissue can reveal the presence of fat accumulation and fibrous structures. The mean TWA exposure (8 hrs) among all dry cleaners was 15.8 ppm (range: 0.4–83 ppm). The investigators found a higher prevalence of abnormal hepatic sonograms among the dry cleaners (67%) than among laundry workers (38%; $p < 0.05$), the control group. Hepatic parenchymal changes, as assessed by sonography, were graded as mild, moderate, or severe. The prevalence of hepatic parenchymal changes increased both with increasing current concentration and with cumulative exposure ($p < 0.05$). Subjects with serological evidence of active hepatitis infection were excluded from these analyses.

Brodkin et al. (1995) fit logistic regression models to examine possible associations between mild or greater parenchymal changes and tetrachloroethylene exposure. These analyses included adjustment for the effects of ethanol consumption within the past six months, sex, body mass index, age, and serological evidence of active and past hepatitis infection. Subjects with serological evidence of active hepatitis infection were included in the logistic regression analysis due to the ability of the statistical method to account for the effects associated with this factor. These analyses showed subjects exposed during older wet or dry-to-dry transfer processes (average concentration: 19.8 ppm; range: 1.8–83 ppm) was strongly—but imprecisely—associated with mild or greater sonographic changes (odds ratio [OR] = 4.2, 95% confidence interval [CI] = 0.9–20.4) as compared with controls. No association was shown with subacute exposure in new dry-to-dry operations (OR = 0.7, 95% CI = 0.1–5.9). An inverse dose-response association was found with cumulative exposure after adjustment for age due to a strong but imprecise association between tetrachloroethylene exposure and hepatic sonographic changes in younger workers (workers less than 35 years of age, OR = 15; 95% CI = 1.33–170).

Only 21% of the exposed study subjects who had changes graded as mild or greater had increases in any hepatic enzyme (Brodkin et al., 1995). Mean concentrations of GGT, AST, and alanine transferase (ALT) tended to be higher among the dry cleaners as compared with laundry workers; however, the differences were not statistically significant and all mean values were within the normal range of reference values. However, all of the subjects who had elevated ALT concentrations had moderate or severe sonographic changes. Hence, sonographic imaging of the liver appeared to be a more sensitive indicator of toxicity than was measurement of serum hepatic enzymes.

Lauwerys et al. (1983) performed behavioral, renal, hepatic, and pulmonary tests on 22 subjects exposed to tetrachloroethylene in six dry cleaning shops and compared the results with those obtained for 33 subjects nonoccupationally exposed to organic solvents. The mean TWA concentration was 21 ppm. The investigators found no statistically significant differences in mean serum hepatic enzyme concentration between exposed subjects and controls, but they did not describe the statistical methods used to test for differences between the exposed and control groups.

Cai et al. (1991) investigated subjective symptoms, hematology, serum biochemistry, and other clinical signs in 56 dry cleaners exposed to tetrachloroethylene at 20 ppm (as a geometric mean of 8 hr TWA) and compared the results with findings for 69 nonexposed controls from the same factories. Exposure-related increases were observed in the prevalence of subjective symptoms during the workday as well as in the past 3-month period, whereas no significant changes in hematology were seen. There was no effect on liver and kidney function, as measured by enzyme activities, blood urea nitrogen (BUN), and creatinine in the serum.

Table 4-1 presents a summary of the human liver toxicity studies in dry cleaners. Two of the four studies (Brodkin et al., 1995; Gennari et al., 1992) showed clinical signs of liver toxicity, namely, sonographic changes in the liver and higher serum concentrations of liver enzymes indicative of liver injury in the absence of frank toxicity. Subjects in these two studies were exposed to tetrachloroethylene for a longer duration than were subjects in Cai et al. (1991) or Lauwerys et al. (1983), and for this reason these two studies carry greater weight in this analysis. Moreover, the studies by Brodkin et al. (1995) and Gennari et al. (1992) assessed potential liver damage using a different set of markers than those of Cai et al. (1991) or Lauwerys et al. (1983).

Table 4-1. Summary of studies of human liver toxicity

Subjects	Effects	Exposure	Author
27 tetrachloroethylene-exposed dry cleaners 26 nonexposed laundry workers	Sonographic scattering of fat in liver (in vivo) Severity greater with higher cumulative exposure No liver toxicity	Group mean TWA = 15.8 ppm Mean duration of exposure = 12 years	Brodkin et al. (1995)
141 tetrachloroethylene-exposed dry cleaners 130 controls	Elevation of total GGT due to GGT-2 GGT-4 detected in exposed but not in control workers	Mean TWA = 11.3 ppm Mean duration of exposure = 20 years	Gennari et al. (1992)
24 tetrachloroethylene-exposed dry cleaners 33 controls non-occupationally exposed to organic solvents	No effect on serum hepatic enzymes	Mean TWA = 21 ppm Mean duration of exposure = 6 years	Lauwerys et al. (1983)
56 tetrachloroethylene-exposed dry cleaners 69 nonexposed factory controls	Increased subjective symptoms No effects on serum indicators of liver and kidney toxicity	Geometric mean TWA = 20 ppm Mean duration of exposure = 3 years	Cai et al. (1991)

Biological markers of liver effects permit the early identification of adverse effects of xenobiotic exposure. They are an important link between biological markers of exposure and frank liver toxicity, and they offer the most potential for clinical intervention before irreversible

effects have occurred (NRC, 1995). The observations of Brodkin et al. (1995) and Gennari et al. (1992) support the indication that tetrachloroethylene exposure affects liver function; hence, the lowest-observed-adverse-effect level (LOAEL) for liver effects in humans can be established as a range from 12 to 16 ppm (TWA).

4.4.1.2. *Liver Cancer*

Cohort and case-control studies assessing possible association between liver cancer and dry cleaner and laundry workers, or tetrachloroethylene specifically, are identified in Tables 4B-1a, 4B-1b, and 4B-3 (Appendix 4B). An incidence study by Andersen et al. (1999) of dry cleaning and laundry workers in Denmark, Finland, Norway, and Sweden reported the following liver cancer risks: males, standardized indicence ratio (SIR) = 1.3 (95% CI = 0.6–2.3); females, SIR = 1.3 (95% CI = 0.9–1.9); combined, SIR = 1.3 (95% CI = 0.9–1.8). This study included some of the same subjects as the studies by Lynge and Thygesen, who also reported an elevation in liver cancer incidence among Danish female dry cleaners and laundry workers (Lynge and Thygesen, 1990; Lynge, 1994), and the study by Travier et al. (2002) of Swedish dry cleaners, launderers, and pressers.

Risk for primary liver cancer in these analyses was larger than the risk for the liver and biliary tract cancer, which indicates the potential for bias due to disease misclassification in studies that examine liver cancer as a broad category. A nested case-control study (Lynge et al., 1995) suggests that the excess primary liver cancer risk among females observed in Lynge (1994) is attributable to laundry workers rather than to dry cleaners (Table 4B-3 in Appendix 4B). This type of information is not available for primary liver cancer cases in Andersen et al. (1999). Mortality studies are biased due to misclassification of liver cancer on death certificates; and these studies do not report statistically significant elevated risks for liver and biliary tract cancer. Primary liver cancer mortality was not elevated, and observations from case-control studies that assessed generic organic solvents or dry cleaning fluid mixtures did not show a consistent liver response (Wartenberg et al., 2000).

4.4.2. Animal Studies

4.4.2.1. *Liver Toxicity*

Hepatic ffects observed after subchronic or chronic inhalation exposure to tetrachloroethylene include increased liver weight (Kjellstrand et al., 1984; Kyrklund et al., 1990); hypertrophy (Odum et al., 1988); fatty changes (Kylin et al., 1965; Odum et al., 1988); peroxisome proliferation, an increase in the size and numbers of peroxisome organelles (Odum et al., 1988; Goldsworthy and Popp, 1987; Bergamaschi et al., 1992); other histological lesions (Kjellstrand et al., 1984; NTP, 1986a); and necrosis and tumors (NTP, 1986a; JISA, 1993). Liver

toxicity observed in animal studies has been reviewed (see U.S. EPA, 1980, 1985a, b, 1986a, 1991a; IARC, 1995; ATSDR, 1997; NYS DOH, 1997; Cal EPA, 2001).

Species differ in their susceptibility to tetrachloroethylene-induced hepatic toxicity. For example, mice appear to be more sensitve than rats to the adverse liver effects caused by tetrachloroethylene exposure (U.S. EPA, 1985a; NTP, 1986a; Lash and Parker, 2001). In Rowe et al. (1952), guinea pigs exposed to 100 to 2,500 ppm proved to be more susceptible than rabbits, monkeys, and rats to liver toxicity. The lowest reported level for liver effects in laboratory animals is in tetrachloroethylene-exposed NMRI mice at 9 ppm (61 mg/m^3; Kjellstrand et al., 1984). These investigators exposed male and female mice to 9 ppm and higher concentrations of tetrachloroethylene for 30 days and observed changes indicative of adverse health effects including statistically significant increases in liver weight as well as changes in liver morphology. Increases in levels of blood plasma enzyme butyrylcholinesterase (BuChE) were reported at all tetrachloroethylene concentration levels at or above 9 ppm. A recovery period reversed the effects on BuChE, although liver weight was still slightly elevated at 120 days after cessation of tetrachloroethylene exposure for 30 days at 150 ppm.

Chronic lifetime inhalation bioassays of tetrachloroethylene in mice have been conducted by the National Toxicology Program (NTP, 1986a), the Japan Industrial Safety Association (JISA, 1993), and Nagano et al. (1998). In the NTP study, B6C3F1 mice were exposed to 0, 100, and 200 ppm tetrachloroethylene for 104 weeks. In addition to liver tumors in mice of both sexes, the authors reported liver degeneration in 2/49, 8/49, and 14/50 males and in 1/49, 2/50, and 13/50 females. Liver necrosis was seen in some of the mice (1/49, 6/49, and 15/50 males; 3/48, 5/50 and 9/50 females). The authors also observed nuclear inclusions in male mice (2/49, 5/49, and 9/50). No dose-related liver effects were reported in the rats.

In the Japan Industrial Safety Association (JISA, 1993) study (some results reported in Nagano et al., 1998), male and female Crj/BDF1 mice were exposed to 0, 10, 50, and 250 ppm tetrachloroethylene for 104 weeks and sacrificed at 110 weeks. In addition to hepatocellular carcinomas and adenomas in the mice, telangiectasis (vascular lesions formed by dilation of a group of small blood vessels) and focal necrosis occurred in males at 50 ppm and above. Liver degeneration was observed at 250 ppm in both sexes. Liver hemangiosarcomas were also reported in the male mice. The authors described effects in F344/DuCrj rats exposed to 0, 50, 200, 600 ppm for 104 weeks and sacrificed at 110 weeks. Male, but not female, rats had excess incidence of spongiosis hepatitis at 200 ppm and above and hyperplasia at 600 ppm. Liver tumors were not observed in either male or female rats.

Tetrachloroethylene was found to cause liver toxicity in laboratory animals by the oral route in several studies (e.g., Buben and O'Flaherty, 1985; Story et al., 1986; Hayes et al., 1986). The observed effects included increased liver weights, biochemical changes, histological lesions,

necrosis, and polyploidy. Buben and O'Flaherty treated male Swiss-Cox mice with tetrachloroethylene doses of 0, 20, 100, 200, 500, 1,000, 1,500, or 2,000 mg/kg-day for 5 days/week for 6 weeks. These investigators demonstrated that indices of tetrachloroethylene hepatotoxicity (increased liver weight, liver triglyceride accumulation, glucose-6-phosphotase activity, and serum glutamic pyruvic transaminase activity) were highly correlated with the amount of tetrachloroethylene metabolized by the mice. The degree of liver response, as measured by each toxicity parameter when plotted against total urinary metabolites, was linear in all cases. The several dose-related liver effects were reported at doses above the lowest dose of 20 mg/kg-day. Increased liver triglycerides and increased liver-to-body weight ratios were seen in mice receiving 100 mg/kg-day and higher doses. At doses of 500 mg/kg-day and higher, effects in the treated mice also included reduction of DNA content, increased serum levels of liver enzymes, liver degeneration, necrosis, and polyploidy. The LOAEL was 100 mg/kg-day.

Ebrahim et al. (1996) administered 3 g/kg/day tetrachloroethylene in sesame oil to mice for 15 days and observed a significant increase in liver weight and degeneration and necrosis of hepatocytes. These changes occurred simultaneously with a decrease in blood glucose; elevated activities of enzymes hexokinase, aldolase, and phosphoglucoisomerase; and decreased activities of gluconeogenic enzymes. Table 4-2 presents a summary of liver toxicity studies in animals.

4.4.2.2. *Liver Cancer*

In carcinogenicity bioassays, tetrachloroethylene has been shown to cause a statistically significant increase in the incidence of hepatocellular carcinomas in both sexes of B6C3F1 mice following either oral gavage administration or inhalation exposure (NCI, 1977; NTP, 1986a). Both sexes of Crj:BDF1 mice have also been shown to develop an increased incidence of hepatocellular carcinomas when exposed to tetrachloroethylene by inhalation (Nagano et al., 1998; JISA, 1993). The National Cancer Institute (NCI) and NTP bioassays were reviewed previously by EPA (U.S. EPA, 1985a, 1986a, 1991a) and are briefly summarized here. Observations regarding liver cancer from the more recent study (Nagano et al., 1998; JISA, 1993), which confirms the earlier findings of liver tumors in B6C3F1 mice, is also briefly summarized. The tumor incidence data from these studies, with the accompanying tables and figures, are presented in Section 5.3.2.

Several metabolites of tetrachloroethylene have been found to be carcinogenic in mice, and it is thought that the hepatocarcinogenicity of the parent compound is mediated through the action of one or more of its metabolites. Metabolites of tetrachloroethylene, including TCA, DCA, and the putative metabolite chloral hydrate, have been observed to cause liver cancer in mice (Daniel et al., 1992; Rijhsinghani et al., 1986; Herren-Freund et al., 1987; Bull et al., 1990;

Table 4-2. Summary of rodent liver toxicity studies

Subjects	Effects	Exposure	Authors
NMRI mice (both sexes), inhalation	Increase in liver weight Morphological changes Increased plasma butylcholinesterase	9 ppm and above for 4 weeks, inhalation	Kjellstrand et al. (1984)
Swiss-Cox mice (male), gavage	Increased liver/body weight ratio at 100 mg/kg-day Increased triglycerides at 100 mg/kg-day No change at 20 mg/kg-day	0, 20, 100, 200, 500, 1,000, 1,500, 2,000 mg/kg-day for 6 weeks, gavage	Buben and O'Flaherty (1985)
B6C3F1 mice	Liver degeneration and necrosis at 100 ppm and higher in males and at 200 in females	0, 100, 200 ppm for 104 weeks	NTP, (1986a)
Crj/BDF1 mice (both sexes)	Focal necrosis in males at 50 ppm and higher Liver degeneration in males and females at 250 ppm	0, 10, 50, 250 ppm for 110 weeks	JISA (1993)
F344/DuCrj rats (both sexes)	Spongiosis hepatitis in males at 200 ppm and higher Hyperplasia in males at 600 ppm	0, 50, 200, 600 ppm for 110 weeks	JISA (1993)

Richmond et al., 1995; Pereira, 1996; DeAngelo et al., 1991, 1996, 1999; NTP, 2000a, b). In addition, DCA causes liver cancer in rats (DeAngelo et al., 1996; Richmond et al., 1995).

In the mouse gavage study (NCI, 1977), groups of 50 male mice received TWA doses of 536 or 1,072 mg/kg tetrachloroethylene in corn oil by intragastric gavage for 78 weeks (450 or 900 mg/kg for 11 weeks, then 550 or 1,100 mg/kg for 67 weeks). Groups of 50 female mice received TWA doses of 386 or 772 mg/kg of tetrachloroethylene in corn oil by gavage (300 or 600 mg/kg for 11 weeks, then 400 or 800 mg/kg for 67 weeks). Mice were dosed 5 days/week. The tetrachloroethylene used in the study was greater than 99% pure, but impurities were not identified (NCI, 1977; U.S. EPA, 1985a). The test sample was estimated to contain epichlorohydrin concentrations of less than 500 ppm (U.S. EPA, 1985a). It was considered unlikely, however, that the tumor response resulted from this low concentration of epichlorohydrin. Tetrachloroethylene caused statistically significant increases ($p < 0.001$) in the incidences of hepatocellular carcinoma in both sexes of mice in both treatment groups when

compared with untreated controls or vehicle controls. The time to tumor was significantly decreased in treated mice.

The inhalation study (NTP, 1986a) confirmed the finding of hepatocellular carcinoma in B6C3F1 mice. Groups of 50 mice of each sex were exposed to (epichlorohydrin free) tetrachloroethylene concentrations of 0, 100, or 200 ppm, 6 hrs/day, 5 days/week, for 103 weeks. Tetrachloroethylene caused statistically significant dose-related increases in the incidences of hepatocellular carcinoma and in combined hepatocellular adenoma and carcinoma in both sexes.

More recent tetrachloroethylene inhalation studies conducted in Japan using Crj:BDF1 mice resulted in the observation of hepatocellular carcinomas in both sexes (JISA, 1993 [results reported in Nagano et al., 1998]). Groups of 50 male and 50 female mice were exposed to 0, 10, 50, and 250 ppm tetrachloroethylene, 6 hrs/day, 5 days/week, for 104 weeks, and the terminal sacrifice was performed at 110 weeks. Both males and females showed dose-related increased incidences of liver carcinomas and combined liver adenomas and carcinomas. Malignant liver hemangioendotheliomas were also increased in males. Both malignant and combined benign and malignant hemangioendotheliomas in the spleen were increased in males. The investigators also observed Harderian gland adenomas and enlargement of the nucleus in the kidney proximal tubular cells in male mice at the highest dose.

4.4.3. Summary of Liver Effects in Humans and Animals

Two of four studies of occupationally exposed dry cleaners showed indications of liver toxicity, namely sonographic changes of the liver and altered serum concentrations of liver enzymes indicative of liver injury. Frank liver disease was not seen among these workers for a number of possible reasons: individuals with frank liver disease may not have been included in cross-sectional studies because they had left the workforce due to their conditions, the healthy worker effect, and other selection biases. LOAELs in these human studies were between 12 and 16 ppm (TWA).

Primary liver cancer incidence was not consistently elevated across incidence studies and appeared to be associated with laundry work (Andersen et al., 1999; Travier et al., 2002; Lynge and Thygesen, 1990; Lynge et al., 1995). Additionally, elevated risks were also seen for incidence in the combined category of primary liver cancer and cancer of the biliary passages. Primary liver cancer is often misclassified on death certificates; hence, mortality studies that examine mortality from liver and biliary passage cancer are less informative than are studies of incidence. For this reason, greatest weight is placed on the observations of incident studies of primary liver disease. Observations from case-control studies that assess organic solvent generically or dry cleaning fluid mixtures do not show a consistent carcinogenic effect on the

liver (Wartenberg et al., 2000). There are no human studies of drinking water or other oral exposure.

In animals, liver toxicity, manifested by fatty changes, liver enlargement, and enzyme changes in blood, has been observed in rats and mice in several studies. The LOAEL for the inhalation studies, 9 ppm, is from a 30-day-exposure mouse study. A chronic mouse inhalation bioassay showed liver necrotic foci at 50 ppm and higher. In two lifetime inhalation cancer bioassays, increases in liver cancer occurred at 100 ppm and above, and there was a significant dose-response trend in both studies. With oral administration, liver effects have been observed at 100 mg/kg-day, although these were not considered to be irreversible effects. The lowest dose at which liver tumors have appeared is 386 mg/kg-day, administered long term.

4.4.4. Mode of Action for Liver Toxicity

This section summarizes scientific data regarding the MOA for tetrachloroethyene-induced hepatic toxicity and carcinogenicity in mice and its relevance to humans. The MOA for tetrachloroethylene-induced mouse liver cancer is not well understood, and it is highly likely that more than one MOA is operative. The following topics are relevant to the MOA for liver toxicity.

(1) *Tetrachloroethylene metabolites and liver toxicity*. Metabolic activation of tetrachloroethylene is required to produce adverse effects in the liver. TCA is the major urinary excretion product, and it is also a hepatocarcinogen in mice; however, insufficient amounts of TCA are produced from tetrachloroethylene metabolism to quantitatively account for the mouse liver tumor incidences observed in cancer bioassays. In addition, the liver tumor phenotypes with regard to H-*ras* codon 61 mutation do not appear to be similar between TCA, DCA, and tetrachloroethylene. Therefore, it is likely that other tetrachloroethylene metabolites, such as the potentially reactive trichloroacetyl chloride, are contributing to the production of liver tumors. The potential role of GST conjugates of tetrachloroethylene in liver toxicity, although unknown, is presumed to be less than that in the kidney.

(2) *Role of receptor activation*. Data exist to advance the hypothesis that peroxisome proliferators can contribute to liver tumorigenesis in rodents; however, the causal role of PPAR-mediated events in tumorigenesis, and human sensitivity to these effects needs further scientific examination and analysis. Data suggest that tetrachloroethylene is a very weak peroxisome proliferator. The strongest evidence supporting the PPAR MOA for tetrachloroethylene is the data for TCA; however, TCA also has other MOAs, TCA alone does not account quantitatively for tetrachloroethylene induced tumors, and tetrachloroethylene- and TCA-induced tumors are phenotypically distinct (Bull et al., 2002).

(3) *Genotoxic effects*. Tetrachoroethylene has been shown to induce some genotoxic effects (micronuclei and SCEs following in vitro exposure, DNA binding and SSBs in liver). Results of in vitro mutagenicity (Ames) or DNA binding assays of tetrachloroethylene have largely been negative except in the few studies of conditions where metabolites of the GSH pathway are generated. The GSH metabolites are clearly mutagenic. In addition, several known (DCA) and putative (tetrachloroethylene oxide) P450 metabolites exhibit mutagenicity. The mutagenic potential of reactive metabolites of tetrachloroethylene has not been adequately studied. Moreover, the identity of all metabolites is not known.

(4) *Nongenotoxic effects*. Existing data suggest the involvement of events related to tumor induction that are nonspecific to activation of PPAR-α, being common to other nongenotoxic MOAs. Hypomethylation is a common early molecular event in most tumors, and alterations in DNA methylation following exposure to chemicals, both hypomethylation and hypermethylation, may be factors in tetrachloroethylene-induced tumorigenesis. Although tetrachloroethylene-specific data are lacking, its metabolites DCA and TCA are known to induce hypomethylation of DNA and protooncogenes in mouse liver.

4.4.4.1. *Background*

Although hepatocellular tumors are common endpoints in mouse carcinogenicity studies, their biological significance with respect to identifying human hazard has long been a subject of intense controversy and debate (Tomatis et al., 1989; Ward et al., 1979; Nutrition Foundation, 1983; U.S. EPA, 1985c; U.S. EPA, 1986b, 1991a; Popp, 1984; Stevenson et al., 1990). The current controversy in the case of tetrachloroethylene-induced hepatocellular carcinoma in mice involves identifying the operative MOAs and their relevance to human situations.

Hemangiosarcomas, unlike hepatocellular carcinomas, are not a common finding in mouse bioassays (U.S. EPA, 2002, 2000b); in fact, they are considered relatively rare, and their relevance to human health hazard, therefore, is generally accepted. Findings of a positive trend for liver and spleen hemangiosarcomas in the most recent mouse carcinogenicity bioassay of tetrachloroethylene (JISA, 1993; Nagano et al., 1998) in a strain of mice not known to have any type of high background tumor incidence constitute important information about risk of exposure to humans.

The focus of the human relevance of the hepatocellular carcinomas observed in mice has turned to the emerging information on modes of carcinogenic action. Peroxisome proliferation, which is associated with certain rodent liver carcinogens, has gained increasing attention due to its possible relationship to a hypothesized MOA. Studies of tetrachloroethylene and its chloroacid metabolites suggest that the compound is a peroxisome proliferator chemical, albeit a very weak one.

A lack of human relevance for the MOA associated with peroxisome proliferator carcinogens has been proposed (Klaunig et al., 2003; Meek et al., 2003). However, agreement is lacking on the extent to which the MOA hypothesis has been validated or whether the MOA or quantitative differences among species are sufficiently understood to rule out a potential risk of carcinogenicity to humans (Melnick, 2001; U.S. EPA, 2005a). EPA's Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) reviewed a draft EPA proposed science policy report (U.S. EPA, 2003d) and considered the role of PPAR-α agonists in activation of PPAR-α leading to an increase in cell proliferation, a decrease in apoptosis, and eventual clonal expansion of preneoplastic cells leading to liver cancer, and the human relevance of PPAR-α agonist-induced hepatocarcinogenesis. While the majority of the panel concluded that there was sufficient evidence in support of the proposed MOA for PPAR-α agonist-induced rodent hepatocarcinogenesis, some panel members complete disagreed. The majority of the panel agreed that there are relevant data indicating that humans are less sensitive than rodents to the hepatic effects of PPAR-α agonists. However, it was noted that humans are not refractory to the effects of PPAR agonism and many questions remain regarding the specific events PPAR activation entails.

This assessment attempts to evaluate scientific information and review the current issues on MOA hypotheses pertinent to tetrachloroethylene.

4.4.4.2. *Relationship of Metabolism to Potential Mode of Action and Organ Toxicity*

Metabolic activation of tetrachloroethylene is required for adverse effects to occur in the liver. The cancer-causing activity of tetrachloroethylene and other chlorinated ethylenes is generally considered to reside in metabolites rather than in the parent compounds (U.S. EPA, 1991a; Davidson and Beliles, 1991; Lash et al., 2000a; Lash and Parker, 2001; see Section 3.3). Certain metabolites of tetrachloroethylene—specifically, TCA, DCA, and chloral hydrate—have been shown to cause liver tumors in mice (IARC, 1995; Daniel et al., 1992; Rijhsinghani et al., 1986; Herren-Freund et al., 1987; Bull et al., 1990; Pereira, 1996; Odum et al., 1988; DeAngelo et al., 1991, 1999; DeAngelo, 2000; NTP, 2000a, b; Carter et al., 2003), and they may be involved in hepatocarcinogenicity following exposure to the parent compound.

TCA is the metabolite that has received the most attention as being potentially associated with tetrachloroethylene-induced liver tumorigenesis. Although it may play a role in tetrachloroethylene hepatocarcinogenicity, not enough TCA is produced from metabolism to account for all tetrachloroethylene-induced mouse liver tumors (see Appendix 4A) observed in bioassays. Although TCA can be further metabolized to DCA, most DCA originating from tetrachloroethylene is proposed by some investigators to be derived predominantly from the renal beta lyase-mediated cleavage of the TCVC conjugate, where DCA production ultimately results

from dichlorothioketene in the kidney (Volkel et al., 1998). If this is the case, most tetrachloroethylene-derived DCA would not occur in the liver target organ, implying that DCA would not likely be critically involved in tetrachloroethylene-induced liver tumorigenesis.

The potential significance of precursor metabolites—e.g., trichloroacetyl chloride, a major and potentially reactive P450 intermediate—should not be underestimated. The possible roles of key reactive precursor intermediates in causing hepatotoxicity need to be better elucidated, as they may be important to understanding MOA for tetrachloroethylene.

4.4.4.3. *Description of a Hypothesized Mode of Action (MOA): Peroxisome Proliferator-Activated Receptor (PPAR) Mediated Hepatocarcinogenesis*

4.4.4.3.1. ***Background summary.*** Some peroxisome proliferators cause increased incidence of rodent liver tumors. Several investigators (Reddy et al., 1980; Reddy and Lalwai, 1983; Moody et al., 1991; Ashby et al., 1994) have hypothesized a causal relationship between proliferation of peroxisomes and hepatocellular carcinogenicity because peroxisome proliferation in rodent hepatocytes frequently occurs alongside hepatocyte hypertrophy and liver hyperplasia and disproportionate transcriptional increases of peroxisomal enzymes involved in B-oxidation of fatty acids (reviewed by Cattley et al., 1998).

Insight into the possible MOA by which chemicals induce peroxisome proliferation—and possibly cancer—was revealed by the discovery of the PPAR receptor, which was shown to be activated by peroxisome proliferators (Issemann and Green, 1990). Activation of this receptor regulates transcription of the genes that encode the enzymes responsible for biochemical changes, including peroxisomal enzymes responsible for beta-oxidation, liver fatty acid-binding protein (Issemann et al., 1993), certain microsomal P450 (CYP4A, CYP2B, and CYP2C) family enzymes (Heuvel, 1999; Corton et al., 1998; Fan et al., 2003; Simpson et al., 1995, 1996) and other enzymes (Barbier et al., 2003).

The evidence regarding whether peroxisome proliferation induced by tetrachloroethylene or its metabolite TCA is the primary or sole mode of action for carcinogenesis is equivocal at best (Ashby et al., 1994; IARC, 1995a; Goldsworthy and Popp, 1987; Odum et al., 1988; Elcombe, 1985; Elcombe et al., 1985; Goldsworthy and Popp, 1987; DeAngelo et al., 1989; Laughter et al., 2004).

4.4.4.3.2. ***Summary description of postulated mode of action (MOA)—peroxisome proliferation via modification of cell signal pathways through the peroxisome proliferator-activated receptor (PPAR) receptor.*** A recent, in-depth review by Klaunig et al. (2003) summarized the PPAR MOA and supporting data; see also the OPP draft science policy paper and the SAP review (U.S. EPA, 2003d). Klaunig et al. (2003) proposed three events to be

causally related to tumorigenesis: activation of PPAR-α, perturbation of cell proliferation and apoptosis, and selective clonal expansion. The causal role is largely based on evidence that the induction of these events is attenuated in PPAR-α -null mice (or in hepatocytes isolated from such mice) in response to the prototypical agonist WY 14,643 (Lee et al., 1995; Peters et al., 1997). A number of intermediary events are considered associative including: expression of peroxisomal and nonperoxisome genes, peroxisome proliferation, inhibition of gap junction intracellular communication, hepatocyte oxidative stress, as well as Kupffer cell-mediated events.

Historically, the increase in peroxisomal organelles, peroxisomal fatty acid beta-oxidation, and alteration in ratios and production of marker enzymes such as increased acyl-CoA oxidase, observed in rodents treated with the peroxisome proliferator chemicals, was thought to induce oxidative stress in hepatocytes and potentially result in oxidative damage to proteins and DNA, leading to carcinogenesis. Two key factors—oxidative injury and enhanced cell proliferation—were implicated in the rodent hepatocarcinogenicity of peroxisome proliferating agents (Cattley et al., 1998; Klaunig et al., 2003). PPAR-α has been identified as the specific PPAR receptor associated with cell proliferation and hepatocarcinogenesis in mouse liver (Lee et al., 1995; Peters et al., 1997a; Corton et al., 2000). PPAR-α activation has been shown to trigger multiple events, and events other than the proliferation of peroxisomes—or at least manifestations not limited to this phenomenon only—are thought relevant to tumorigenesis.

Peroxisome proliferators have been shown to alter hepatocyte growth and survival by induction of DNA synthesis and suppression followed by enhancement and then depression of apoptosis (cell death; Cattley and Popp, 1989; Roberts et al., 1995; Bursch et al., 1984; Marsman et al., 1992). Cell proliferation is thought to play an important role through specifically enhanced proliferation of normal hepatocytes, resulting in an increase in the frequency of initiated cells or in the selective growth of pretransformed hepatocytes, with subsequent tumorigenesis (Cattley and Popp, 1989; Kraupp-Grasl et al., 1990, 1991; Grasl-Kraupp et al., 1993; Cattley et al., 1991; Marsman et al., 1988; Eacho et al., 1991; Marsman, 1991; Marsman and Popp, 1994). The currently hypothesized MOA for liver carcinogenesis assumes that events such as the increased cell proliferation, inhibition of apoptosis, and clonal expansion of preneoplastic lesions are linked directly to PPAR-α activation.

4.4.4.3.3. ***Identification of potential key events in mode of action (MOA) for liver.*** Certain biochemical and cellular events have been associated with hepatocarcinogenic effects of peroxisome proliferating chemicals. Whether these key events are causally related to liver tumorigenesis remains to be determined. Potential key events include (a) peroxisome proliferation—an increase in the number of peroxisomes and also an increase in their volume density (Meijer and Afzelius, 1989; Ganning et al., 1983; Thangada et al., 1989); (b) certain

disproportionate alterations in levels of peroxisomal enzymes, especially increases in fatty acyl-CoA oxidase levels of 20- to 30-fold, whereas catalase and urate oxidase are increased only 2- to 3-fold, with resulting excess production of hydrogen peroxide, which may affect hepatocytes by oxidative injury; (c) increases in members of the cytochrome P450 CYP4A subfamily; (d) increases in rates of cell proliferation due to increased DNA synthesis and suppression of apoptosis, particularly in basophilic preneoplastic lesions; (e) hepatomegaly; and (f) expression and activation of the α subtype of the PPAR (PPAR-α; Hardwick et al., 1987; Dreyer et al, 1992; Marcus et al., 1993; Tugwood et al., 1992; Zhang et al., 1992; Cattley et al., 1998; Chevalier and Roberts, 1998).

4.4.4.3.3.1. ***Peroxisome proliferator-activated receptor alpha (PPAR-α) activation.*** The hypothesized causal event most associated—and best supported by existing data—with the proposed peroxisome proliferation MOA is the one having true specificity for PPAR-α MOA. This is the activation of the PPAR-α receptor. PPAR-α expression and activation, inducing transcription of selected genes, possibly in concert with altered cell signal transduction initiated by release of cytokines by hepatic macrophages (Kupffer cells), is probably the important process to evaluate as a potential key event to the development of liver hyperplasia and hepatocyte carcinogenesis. The strongest support for a causal relationship between PPAR-α activation and hepatocellular tumorigenesis is found in studies in null mice, i.e., mice lacking PPAR-α, particularly the eleven-month study of WY-14643 (Peters et al., 1997a, b). Such "knockout" mice do not respond to this prototype peroxisome proliferator with increased cell proliferation and decreased apoptosis or with development of other events potentially associated with PPAR-α activation leading to liver cancer. Although a short-term study in null mice has been performed for TCA (Laughter et al., 2004), such a study has not been conducted for tetrachloroethylene. This short-term study of TCA provides data consistent with a relationship between PPAR-α activation and peroxisome proliferation, but provides minimal, if any, support for PPAR-α activation and liver cancer. Although null mouse studies have flaws, some due to deficiencies in the altered mice such as physiological and biological differences in response to stress when compared to wild mice, a well-designed, lifetime tetrachloroethylene carcinogenicity study in null mice could provide valuable information.

4.4.4.3.3.2. ***Alterations in cell replication and death rate.*** Other events could be claimed to be causal for rodent liver tumor induction. One such event is selective clonal expansion. Stauber et al. (1998) and Bull et al. (2004) reported data indicating that TCA acts to induce liver tumors by increasing clonal expansion of a specific group of initiated cells in mouse liver. TCA stimulates growth of colonies of hepatocytes expressing c-Jun phenotype, which is representative of tumors

caused by TCA. TCA also lowers replication rates of normal liver cells. TCA treatment, therefore, results in negative selection, or growth advantage being given to specific characteristic tumor cells over normal cells. Although selective clonal expansion has been associated with PPAR-α MOA, it is not clear that this is the case with the tetrachloroethylene metabolite. In fact, these investigators recently stated that they believe TCA causes cancer independently of peroxisome proliferation (Bull, 2004). The DCA metabolite also selectively stimulates the growth of clones of cells. Clonal expansion is thought to occur with all cancer-causing agents, so it is not limited to PPAR-α MOA. The available data indicate that the MOAs for the two chloroacid metabolites are different.

Another possible causal key event is cell proliferation and apoptosis, although it is not unique to peroxisome proliferator chemicals.

4.4.4.3.3.3. ***Potential key events specific for peroxisome proliferator-activated receptor alpha (PPAR-α).*** Several other events have potential for being key events in the PPAR-α MOA for liver tumors observed in rodents exposed to peroxisome proliferator chemicals. Two events considered specific to PPAR-α activation are the actual proliferation of peroxisome organelles and the expression of peroxisomal genes. These events can be considered biomarkers for peroxisome proliferator chemicals, but a cause-and-effect relationship to liver tumor induction cannot be made. These events are historically linked to the PPAR-α MOA.

4.4.4.3.3.4. ***Other potential key events not limited to proliferator-activated receptor alpha (PPAR-α) mode of action (MOA).*** Yet another key event to consider is an alteration in the expression and activities of nonperoxisomal lipid-metabolizing enzymes that mediate hypolipidemia. PPAR-α agonists shown to cause liver tumors in rodents also induce genes that encode lipid metabolizing enzymes, although these same genes can be altered by other agents.

Alterations in DNA methylation, both hypomethylation and hypermethylation, may be factors in tumorigenesis and occur following exposure to chemicals that also cause peroxisome proliferation (Pereira et al., 2004a, b). Hypomethylation is a common early molecular event in most tumors (Pereira et al., 2004b). DCA and TCA are known to induce hypomethylation of DNA and protooncogenes in mouse liver (Tao et al., 1998, 2000; Pereira et al., 2004a).

Inhibition of gap junction cellular communication has been attributed to certain peroxisome proliferator chemicals (Klaunig et al., 1988; Dybing et al., 1995). This event is thus correlated with the rodent tumorigenesis caused by such chemicals, although similar inhibition of the gap junction cellular communication process also occurs with other nongenotoxic liver carcinogens (Klaunig et al., 2003) and, therefore, cannot be considered specific to peroxisome proliferators and PPAR-α MOA.

Oxidative stress and resulting DNA damage to hepatocytes secondary to that stress have been attributed to peroxisome proliferator chemicals causing liver cancer in rodents, but the exact role is not clear and remains a controversial issue. As discussed above, alterations in the ratios of peroxisomal enzymes by induction of beta-oxidation enzymes results in an imbalance that causes overall increases in hydrogen peroxide leading to oxidative damage.

The nonparenchymal Kupffer cell macrophages may be involved in peroxisome proliferator chemical tumor induction (Klaunig et al., 2003; Rusyn et al., 2000a, 2001). Kupffer cells do not express PPAR-α (Peters et al., 2000). Peroxisome proliferator chemicals activate Kupffer cells directly (Rose et al., 1999; Peters et al., 2000) and independently of PPAR-α activation (Peters et al., 2000). Klaunig et al. (2003) suggest that Kupffer cell mediated events are associated with (i.e., are not causally related to) hepatic tumors induced by the PPAR-α MOA; it is noted that responses of these cells are independent of PPAR-α and are not restricted only to peroxisome proliferator chemicals.

4.4.4.3.4. ***Correlation between proliferator-activated receptor alpha (PPAR-α) activation/peroxisome proliferation and tumor induction.*** Historically, chemicals have been characterized as peroxisome proliferators on the basis of either observations of increases in volume density of peroxisomes or increases in peroxisomal fatty acid beta-oxidation enzyme activity, with characterization by both of these parameters being preferable. Demonstration of induction of the cyanide-insensitive palmitoyl CoA enzyme is viewed as a key biochemical marker acceptable for the detection and quantitation of peroxisome proliferation. Usually, palmitoyl CoA oxidation (PCO) is measured, although palmitoyl CoA oxidase activity can be determined directly where hydrogen peroxide production is measured.

The potential key events described above in Section 4.4.4.3.3 have been correlated with tumorigenesis, although some of these events are not restricted to PPAR-α MOA, and a cause-and-effect relationship is questionable for others. Certain key events are associated with PPAR-α activation and can also be associated with tumorigenesis; however, evidence supporting the link between the receptor activation and tumorigenesis through these key events lacks compelling persuasiveness.

The strongest case currently available for a cause-and-effect link is the results of cancer studies using PPAR-α null mice (see Peters et al., 1997, and Ito et al, 2007). When exposed to the peroxisome proliferator WY-14,643, the null mice do not show increased cell proliferation or decreased apoptosis or evidence of developing hepatocellular carcinogenesis (Peters et al., 1997). The occurrence of events known to be associated with tumorigenesis following exposure to peroxisome proliferator chemicals allows an association to be made between PPAR-α activation and these other events. The events clearly specific to PPAR-α receptor activation—actual

peroxisome proliferation and expression of peroxisomal genes and enzymes—are likely markers for the receptor activation and not cause-and-effect events for carcinogenesis. The other events may be related to tumorigenesis but are not restricted to PPAR-α activation MOA. The TCA metabolite of tetrachloroethylene has been studied in null mice, and although no tumors were observed, this was only a short-term study and findings are only minimally supportive of PPAR-α being related to liver tumorigenesis. Section 4.4.4.3.5 describes the study in greater detail. Tetrachloroethylene has not been studied in such mice. Although some investigators attributed tetrachloroethylene-induced hepatocarcinogenesis to TCA, analysis of the amount of tetrachloroethylene metabolism at the doses administered in the mouse carcinogenicity bioassays demonstrates that not enough TCA is produced to account for the tumor response (see Appendix 4A).

The evidence for peroxisome proliferation by tetrachloroethylene and the chloroacid metabolites is published in Zhou and Waxman (1998), Zanelli et al. (1996), Bruschi and Bull (1993), Nelson et al. (1989), Elcombe (1985), Elcombe et al. (1985), Goldsworthy and Popp (1987), Odum et al. (1988), Channel et al. (1998), DeAngelo et al. (1989), and Daniel et al. (1993).

Studies by Goldsworthy and Popp (1987) indicate that tetrachloroethylene and its metabolite TCA elevate cyanide-insensitive PCO activity in mouse liver, yet only TCA caused increased PCO activity in rat liver. The elevation in PCO activity in mouse liver caused by tetrachloroethylene was not great. In a study conducted by Zanelli et al. (1996), TCA was shown to increase PCO activity in the liver of treated rats of a different strain, so the metabolite does cause the effect, and it may be responsible for the response of the parent compound in mice. Different rat strains were used in the two studies (F344 and Wistar, respectively), and the increase reported by Goldsworthy and Popp was a relatively weak response. Tetrachloroethylene increased both PCO enzyme activity and peroxisome volume density in exposed mice in the study by Goldsworthy and Popp. In the tetrachloroethylene study conducted by Odum et al. (1988), peroxisome proliferation was increased in the livers of mice but not rats. Elcombe (1985) found TCA to cause peroxisome proliferation—as measured by an increase in peroxisomal enzyme activity—in hepatocytes of both rats and mice, both in vivo and in vitro, after short-term exposure. Interestingly, Elcombe reported that the Wistar rat showed a greater peroxisome proliferation response than did mice, as measured by increases in cyanide-insensitive acyl-CoA oxidase activity induction. Clearly, strain and species differences exist.

DeAngelo et al. (1989) demonstrated peroxisome proliferation induction by TCA and by DCA exposures in mice and rat livers, as indicated by increased PCO activity and peroxisomal volume and possibly the observed increased carnitine acetyl transferase activity as well. The investigators examined peroxisome proliferation activity in three strains of rats (Sprague-Dawley,

F344, and Osborne-Mendel) and in four strains of mice (Swiss-Webster, C57BL/6, C3H, and B6C3F1). The conclusion from the DeAngelo et al. (1989) study is that mice are more sensitive than rats with respect to the enhancement of liver peroxisome proliferation by TCA. More recent studies conducted by Waxman and colleagues (Maloney and Waxman, 1999; Zhou and Waxman, 1998) showed induction of peroxisome proliferation in rodents by the tetrachloroethylene metabolites TCA and DCA. DCA and TCA activated the PPAR-α receptor in both mouse and human cells in these studies. Walgren and colleagues (Walgren et al., 2000a, b, 2004) reported expression of PPAR-α in human hepatocytes, activation by TCA and DCA, and peroxisome proliferation by a series of acetates. High concentrations of TCA were used in the studies.

4.4.4.3.5. ***Strength, consistency, specificity of association of the hepatocellular tumor response with key events.*** Whether or not any cause-and-effect relationship exists between peroxisome proliferation per se and cancer-causing activity leading to rodent liver cancer is not clear (Capone, 1994; Cattley et al., 1998; Cattley and Roberts, 2000; Youssef and Badr, 1999). The current majority opinion regards activation of PPAR-α as the important causal key event—the obligatory step—for the MOA of rodent liver carcinogenesis. Even so, not all scientists agree, and not all data support this hypothesis (see Section 4.4.4.1).

The strongest support for a causal relationship between the PPAR-α activation MOA and hepatocellular tumorigenesis using the compound WY-14643 is from studies in null mice by Peters et al. (1997a, b). The existing data show null mice to be refractive to other possible key events—suppression of apoptosis and cell proliferation—and also refractive to tumor formation following 11 months of exposure to this prototype peroxisome proliferator. The null mouse, when challenged by 11 months of exposure to WY-14643, did not respond to a dose that causes 100% tumor response in wild-type mice.

Results from null mouse studies would be convincing data in support of the PPAR-α MOA hypothesis, except for serious shortcomings. For example, the studies are less-than-lifetime studies, or they are in vitro studies conducted in tissues from animals exposed to test compounds. Such studies clearly cannot be considered equal to the standard rodent lifetime bioassays conducted to detect carcinogenic activity, and because of this deficiency, they are not considered adequate for assessing lifetime cancer risk. Also, they cannot be used to demonstrate conclusively that PPAR-α activation is an obligatory step in rodent hepatocellular tumorigenesis simply because some of the key events that could be associated with tumorigenesis are not observed. The complexity of the multitude of effects and the lack of understanding about which of the myriad downstream events result at particular dose levels—mechanisms and steps linking those events to PPAR-α activation—also render the null mouse data somewhat less than adequate for understanding the PPAR-α activation relationship to tumorigenesis. Additionally,

the null mice are innately dissimilar from wild mice—for example, they respond to stress differently (Watanabe et al., 2000; Huss and Kelly, 2005). Their differences include both physiological and biochemical aspects. Some are likely related to PPAR-α-dependent changes in gene expression, but others are not PPAR-α dependent (Valles et al., 2003; Jalouli et al., 2003; Hasmall et al., 2002; Meyer et al, 2003). Because of such inherent differences between null mice and wild mice prior to exposure to test chemicals, the data from studies using these mice should be interpreted with caution.

The most relevant data pertinent to tetrachloroethylene comes from studying TCA. Laughter et al. (2004) attempted to determine whether effects of TCA in the liver associated with carcinogenesis were mediated by PPAR-α. Male wild-type and PPAR-α-null mice were given TCA at 0.25, 0.5, 1, or 2g/L in the drinking water for 7 days. TCA increased liver-to-body weight ratios, but the increases were not significant. The livers from wild-type but not PPAR-α-null mice exposed to 2 g/L TCA exhibited centrilobular hepatocyte hypertrophy. Further, global gene expression was assessed using the mouse Atlas cancer 1.2 array. The induction of CYP4a and acyl-CoA oxidase was examined in the livers of mice after exposure to TCA. In wild-type mice, but not PPAR-α-null mice, CYP4a was induced at 1g TCA/L and above, Aacyl-CoA was induced by TCA at 2g/L, and palmitoyl-CoA oxidase activity was induced at 2 g/L. These data suggest that peroxisome proliferation induced by such compounds could be potentially mediated by PPAR-α (Nakajima et al., 2000). They do not indicate a cause-and-effect relationship between PPAR-α and liver tumorigenesis, however. These studies of TCA were not designed to examine tumor development or show any evidence for a cause-and-effect relationship between receptor activation and tumor development. No comparable study exists for tetrachloroethylene.

If peroxisome proliferation is causally related to the induction of liver cancer, then a detectable quantitative relationship between the two events could be expected. That is, potent peroxisome proliferators should also be potent hepatocarcinogens. However, this does not appear to be the case (Elcombe and Mitchell, 1986; Marsman et al., 1988, 1992; Eacho et al., 1991; U.S. EPA, 199la). A comparison of the tetrachloroethylene chloroacid metabolites indicates that DCA is a more potent hepatocarcinogen than TCA. For example, in a chronic 65-week study of DCA and TCA in male B6 mice, Herren-Freund et al. (1987) found that equal concentrations in drinking water resulted in a nearly threefold higher incidence of liver cancer in DCA-dosed animals than in TCA-dosed animals. DeAngelo et al. (1989), however, reported that TCA was more potent than DCA as a peroxisome proliferator in male B6 mice. Nelson et al. (1989) also reported that TCA produced greater peroxisome proliferation than did DCA in B6 mice dosed for only 10 days. Additionally, evaluation of TCA and DCA indicates that these two metabolites act through different MOAs because they exhibit clearly unparallel dose-response curves.

Compared with oxidative damage, hepatomegaly caused by cell proliferation appears to be better correlated with hepatocarcinogenesis in rodents (Marsman et al., 1988, 1992; Barrass et al., 1993; Cattley et al., 1998). A reversible increase is seen in cell death along with the increased cell proliferation (Bursch et al., 1984; Roberts et al., 1995; Marsman et al., 1992; Cattley et al., 1998). Both TCA and DCA can produce hepatomegaly at carcinogenic doses, although, at least in the case of DCA, the increase is more likely due to cytomegaly (i.e., an increase in cell size), whereas in the case of TCA it is more likely due to an increase in the number of liver cells (Bull, 2000).

Tetrachloroethylene and its major oxidative metabolite, TCA, cause liver tumors in mice, yet do not induce liver tumors in rats (NCI, 1977; NTP, 1986a; DeAngelo et al., 1997). TCA has been demonstrated, however, to produce hepatic peroxisome proliferation in rats as well as in mice (Elcombe, 1985; Zanelli et al., 1996; DeAngelo et al., 1989).

Tetrachloroethylene is not as potent a peroxisome proliferator as are its metabolites. This information is meaningful because some investigators have postulated that although tetrachloroethylene may possess an intrinsic ability to induce peroxisomes, it may be less effective as a peroxisome proliferator and carcinogen in the rat due to a metabolic inability of that species to form sufficient amounts of peroxisome proliferator metabolites, whereas TCA is formed in mice in sufficient amounts from the parent compound bioassay doses to result in a sustained level of peroxisome proliferation. Carcinogenicity bioassay studies in rats disprove that theory because TCA does not cause liver tumors in rats.

The hepatocellular cancer-causing activity of tetrachloroethylene has not been heavily associated with DCA. DCA is thought not to be produced in the liver in sufficient quantity because its only source from tetrachloroethylene oxidative metabolism is the further biotransformation of TCA. TCA, on the other hand, is generally considered to contribute to the mode of carcinogenic action for tetrachloroethylene. Because DCA may be somewhat rapidly further metabolized to other compounds, such a conclusion may be in error.

It is important to note that the peroxisome proliferation effects observed in rodents exposed to tetrachloroethylene are equivocal. In a summary plot of tumor incidence versus peroxisome proliferation from the two studies reporting tetrachloroethylene data, the effects are significant in male mice but not in females (NCI, 1977; NTP, 1986a; Ashby et al., 1994; Goldsworthy and Popp, 1987; Odum et al., 1988). Compared with the effects of potent peroxisome proliferator chemicals, the effects caused by tetrachloroethylene are relatively weak (Ashby et al., 1994).

PPAR-α isolated from mouse liver can be activated by certain tetrachloroethylene chloroacid metabolites. Both the TCA and the DCA metabolites have been shown to activate PPAR-α (Maloney and Waxman, 1999).

4.4.4.3.6. ***Dose-response relationship***. The data for a clear dose-response for increased peroxisome proliferation in mouse liver resulting from tetrachloroethylene exposure are equivocal, especially in female mice (Section 4.4.4.3.5). Some evidence exists for increased tumor response with increased tetrachloroethylene dose in both gavage and inhalation carcinogenicity bioassays, although the dose/concentration levels in those studies were relatively high. Approaching saturation of metabolism blurs the dose-response at these levels, most noticeably in the oral gavage study. There is also some evidence, from studies conducted separately from the cancer bioassays, to support increase in tetrachloroethylene peroxisome proliferation with increase in dose, although these data are not particularly convincing (Ashby et al., 1994).

Likewise, positive dose-responses for cancer-causing activity in lifetime carcinogenicity bioassays and for peroxisome proliferation in short-term studies have been observed in mice following exposure to relatively high doses of TCA (Ashby et al., 1994; Daniel et al., 1993; Goldsworthy and Popp, 1987; Elcombe, 1985; Herren-Freund et al., 1987).

Other potentially adverse effects associated with DCA exposure (e.g., changes in carbohydrate metabolism, as well as other alterations in cell signaling) are expected to occur, in some cases, at lower doses than are required for peroxisome proliferation (Bull, 2000), indicating occurrence of events possibly associated with tumorigenesis at doses below those causing the peroxisome proliferation response.

4.4.4.3.7. ***Temporal association.*** Increases in peroxisome volume density as well as in marker cyanide-insensitive (PCO) oxidation indicative of peroxisome proliferation have been shown to occur following a few days of treatment with tetrachloroethylene, DCA, or TCA (Goldsworthy and Popp, 1987; Elcombe, 1985; Odum et al., 1988; Daniel et al., 1993). On the other hand, following DCA treatment, DNA SSB has been observed prior to peroxisome proliferation (Nelson and Bull, 1988; Nelson et al., 1989), although investigators using a different methodology did not observe the DCA-induced SSB (Chang et al., 1992) after DCA treatment.

4.4.4.3.8. ***Species similarities and differences: human evidence.*** The relevance to humans of rodent hepatocellular carcinomas thought to be induced specifically by peroxisome proliferator chemicals has been questioned. Humans do have a functional PPAR receptor (Sher et al., 1993), which is comparable to PPAR receptors of mice and rats in its affinity for PPAR-α ligands (Klaunig et al., 2003) and it is capable of activating many of the genes regulated in the mouse by PPAR-α (Yu et al., 2001).

PPAR-α, the PPAR subtype considered to be the causal factor for peroxisome proliferation in rodent hepatocytes, has been found in tissue from several species, including mice,

rats, and humans as well as dogs, guinea pigs, hamsters, and nonhuman primates (Yousef and Badr, 1999; Schultz et al., 1999; Lake et al., 1993; Roberts et al., 2000; Reddy et al., 1984; Graham et al., 1994; Kurata et al., 1998). Humans express PPAR-α in liver (Auboeuf et al., 1997), although reportedly to a lesser extent than do rats and mice (Klaunig et al., 2003). PPAR-α mRNA in human liver samples have been reported by some investigators to be one order of magnitude lower than those observed in mice (Palmer et al., 1998; Tugwood et al., 1996).

Only a few human liver samples have been examined for quantification of PPAR-α transcription factors (Klaunig et al., 2003), and one study by Walgren et al. (2000a) reported that one of six human samples was equivalent to mice in expression of PPAR-α protein. Although the number of human liver samples examined is limited, evidence exists for mutations in PPAR-α (Flavell et al., 2000; Sapone et al., 2000; Vohl et al., 2000; Yamakawa-Kobayashi et al., 2002), which could contribute to the large variations in PPAR-α levels (Walgren et al., 2000a). Interindividual variability (Tugwood et al., 1996), along with the inducibility of PPAR-α expression by chemicals and other factors (Sterchele et al., 1996), indicate the likelihood of a susceptible subpopulation (Heuvel, 1999).

Although some studies have shown no increase in DNA synthesis in primary human hepatocytes following treatment with several peroxisome proliferator chemicals, other evidence indicates that humans may indeed be responsive to adverse effects of peroxisome proliferators. For example, investigations of human hepatocytes following treatment with certain fibrate chemotherapeutic agents found dose-dependent induction of acyl-CoA oxidase activity and, in one case, increased peroxisome density (Cimini et al., 2000; Perrone et al., 1998). Increases of liver peroxisomes have been reported in human patients taking the hypolipidemic therapeutic agents clofibrate and ciprofibrate (Hanefeld et al., 1983; Bentley et al., 1993; Hinton et al., 1986). The increases in volume density of peroxisomes (23–30%) are comparable to or greater than those observed in rodents exposed to tetrachloroethylene. Also, both humans and rodents respond to peroxisome proliferators with reduction of serum lipids, indicating similar capabilities for modification of gene expression. Epidemiologic evidence of cancer from exposure to peroxisome proliferator chemicals is limited to only a few studies in patients taking fibrate drugs, and is inconclusive (Newman and Hulley, 1996; Melnick, 2001).

Taking into account kinetic and dynamic factors, the proposed animal MOA is plausible in humans. If peroxisome proliferation is involved in a mode of carcinogenic action for tetrachloroethylene, the cancer-causing activity cannot be dismissed for humans, especially since PPAR-α has been identified in human liver, and both TCA and DCA metabolites similarly activate human as well as mouse PPAR-α, even if to different degrees. Chemical-specific data regarding the ability of the major tetrachloroethylene metabolite TCA to activate PPAR-α in

humans as well as in mice indicate cross-species relevance (Maloney and Waxman, 1999), although there exists a quantitative difference between these two species in hepatic PPAR-α activation (Walgren et al., 2000a, b). Site concordance is not a requirement for extrapolation of tumorigenesis in animal models to the human situation, however, and PPAR-α is found in several different organs. It is highly expressed in cells having active fatty acid capacity, such as hepatocytes in liver, and also in renal proximal tubule cells, cardiomyocytes, and enterocytes. Some PPAR-α agonists cause tumors in rodents at sites other than the liver.

PPAR-α is proposed to be involved in causing Leydig cell tumors. The human relevance of PPAR-α induction of Leydig cell tumors is not so controversial. The understanding of the science is not good enough to explain why humans have a functional PPAR-α capable of gene expression modulation in a manner similar to that of rodents but does not respond similarly. PPAR-α in humans may be capable of modulating lipid homeostasis through alteration of expression of other, different, genes in the liver and genes in other target organs that express higher levels of PPAR-α. Similar events occurring in various tissues and organs could lead to carcinogenesis in those tissues and organs; therefore, target organs could differ among species. The liver carcinogenesis in mice could be a red flag for tumorigenesis at some other site in humans. This is the case for other chemicals, such as arsenic.

A different PPAR receptor also involved in lipid homeostasis may be involved in human liver cancer, because cross-talk is known to occur among these receptors. Glinghammar et al. (2003) found that PPAR delta (PPAR-δ) receptor activation in human hepatocellular carcinoma cells induced COX2 expression, a factor associated with carcinogenesis, and increased cellular proliferation. Such results suggest a potential role for PPAR-δ in human hepatocellular carcinoma induction. PPAR-α also can mediate mRNA alterations associated with prostaglandin synthesis in rodents, i.e., COX2 expression (Peters and Vanden Heuvel, 2002). PPAR gamma has been shown to induce COX2 expression as well. Because cross-talk is known to occur among the PPAR receptors, the potential exists for cross-talk to be involved in receptor activation related to carcinogenicity.

4.4.4.3.9. ***Biological plausibility and coherence of the database.*** Tetrachloroethylene induces liver cancer in treated mice, but it has not been shown to cause liver cancer in treated rats; thus, an inconsistency exists between rodent species. Epidemiologic evidence is insufficient for determining whether tetrachloroethylene causes liver cancer in humans (see Section 4.3.1.2).

Inadequate amounts of TCA are produced from tetrachloroethylene metabolism to account totally for the liver tumor induction observed in bioassays (Appendix 4A). DCA is less likely to contribute to tetrachloroethylene hepatocarcinogenesis because relatively small amounts would be produced in the liver target organ from metabolism of tetrachloroethylene. Limited

data on tumor phenotype indicate that tetrachloroethylene tumors differ phenotypically from TCA tumors or DCA tumors (Maronpot et al., 1995; Anna et al., 1994).

An important distinction needs to be drawn between biomarkers that are causally related to carcinogenic activity and those that are merely correlative. There is much confusion and debate over the reliability of hepatic peroxisome proliferation as a marker for hepatocarcinogenesis. As reviewed in Section 4.4.4.1, some scientists maintain that there is a causal relationship between peroxisome proliferation and hepatocarcinogenesis, whereas others have questioned the validity of this relationship and suggest that the degree of peroxisome proliferation correlates poorly with relative hepatocarcinogenic effectiveness and potency. Although many of the responses generally observed in the overall peroxisome proliferation phenomena are often manifested in tumorigenesis, causality is uncertain, and in the specific case of tetrachloroethylene, the data are especially limited.

Several recent studies have expanded the scientific understanding of the PPAR-α mode of action (see Caldwell et al., 2008). First, Yang et al. (2007) demonstrated that PPAR-α activation in hepatocytes induces peroxisome proliferation but not liver tumors. The approach entailed targeting expression of PPAR-α to hepatocytes by placing the VP16 PPAR-α transgene gene under control of the liver enriched activator protein (LAP) promoter. LAP-VP16 PPAR-α transgenic mice showed a number of PPAR-α-mediated effects: decreased serum triglycerides and free fatty acids, peroxisome proliferation, enhanced hepatocyte proliferation, and induction of cell-cycle and PPAR-α target genes. However, compared with wild-type mice exposed to Wy-14,643, the extent of hepatomegaly was reduced and no hypertrophy or eosinophilic cytoplasms was seen in LAP-VP16 PPAR-α mice. Also in contrast with wild-type mice exposed to Wy-14,643, no evidence of non-parenchymal cell proliferation was observed in the LAP-VP16 PPAR-α transgenic mice. Moreover, at one year of age no evidence of preneoplastic hepatic lesions or hepatocellular neoplasia was observed in LAP-VP16 PPAR-α transgenic mice. As noted by the authors, PPAR-α activation only in mouse hepatocytes is sufficient to induce peroxisome proliferation and hepatocyte proliferation but "…is not sufficient to induce liver tumors."

Secondly, Ito et al. (2007) found that di (2-ethylhexyl)phthalate (DEHP), a proposed robust example of PPAR-α agonism-induced hepatocarcinogenesis, yields liver tumors in a 2-year study in PPAR-α knock-out mice. This study demonstrates the limitations, cited by the FIFRA SAP, of drawing conclusions from the one-year bioassay of high doses of WY-14,643 referenced above (e.g., Peters, 1997). It supports the view that knock-out mouse bioassays should be carefully characterized and conducted for 2 years to assess whether PPAR-α activation is indeed necessary for induction of liver cancer.

In summary, limited evidence supports the hypothesis that tetrachloroethylene tumor induction could be related to PPAR-α activation, but critical review of the scientific literature reveals significant data gaps regarding the relationship between the PPAR-α activation and neoplasia induced by tetrachloroethylene. If PPAR-α does play a role in tetrachloroethylene-induced tumorigenesis, available information suggests relevance to humans cannot be ruled out.

4.4.4.4. *Effects That Could Be Related to Other Potential Modes of Action*

4.4.4.4.1. ***Mutagenicity and genotoxic effects.*** The available evidence is inconclusive regarding mutagenicity of tetrachloroethylene or its metabolites and hepatocarcinogenesis (see Section 4.3). Tetrachloroethylene induces SSB and DNA binding in liver tissue, and existing data implicate a potential role for genotoxic effects of certain metabolites, such as DCA and the proposed intermediate chloral hydrate; the epoxide tetrachloroethylene oxide is a bacterial mutagen. Interestingly, and the phenotype and frequency of tumors produced by DCA and tetrachloroethylene tumors differ (Bull, 2000; Anna et al., 1994; Maronpot et al., 1995; Moore and Harrington-Brock, 2000; also see Section 4.4.4.2). The mutagenic potential of several metabolites has not been studied. Not all of the P450 metabolites, including the unstable, potentially reactive intermediates, such as trichloroacetyl chloride, for example, have been sufficiently tested in the standard genotoxicity screening battery.

4.4.4.4.2. ***Immunosuppressive effects.*** Although tetrachloroethylene-specific data are lacking, it is possible that inhibition of the natural immune surveillance could be related to hepatocarcinogenic properties of tetrachloroethylene (see also Section 4.8.3). Immune suppression could play a role in the induction of cancer as many immunosuppressive agents are human carcinogens (Tomatis et al., 1989). Exposure to organic solvents has been generally associated with autoimmune diseases such as scleroderma (Nietert et al., 1998). A strong association has been reported between exposure to solvents structurally similar to tetrachloroethylene and systemic sclerosis in patients who have autoantibodies (Nietert et al., 1998).

Binding of reactive compounds to cellular macromolecules has been proposed as an important step in the pathogenesis of several diseases, both for cancer (Hinson and Roberts, 1992) and for chemically induced autoimmune disease (Uetrecht et al., 1988). Reactive metabolites of tetrachloroethylene have been shown to bind irreversibly to cellular macromolecules in vitro (e.g., Costa and Ivanetich, 1980) and in vivo (Pegg et al., 1979; Schumann et al., 1980). Binding occurs proportionally to the amount metabolized, and metabolism is proportional to toxicity (e.g., Buben and O'Flaherty, 1985). Several published studies have demonstrated formation of trichloroacylated protein adducts, for example, in liver

and kidney of rats (Birner et al., 1994) and in plasma of rats and humans (Pahler et al., 1999) following exposures to tetrachloroethylene. Another example is the detection of trichloroacetylated protein adducts formed in mice treated with tetrachloroethylene (Green et al., 2001). Further studies designed to identify the adducted proteins may help to elucidate an MOA for tetrachloroethylene-induced autoimmune response, which, in turn, may be related to cancer-causing activity.

4.4.4.4.3. ***Effects on the insulin receptor/glucose metabolism.*** Tumorigenesis is associated with changes in enzymes involved in carbohydrate metabolism (Ahn et al., 1992) and tumor cells depend uniquely on glucose as an energy source. Transformation of many cell types is associated with an increase in glucose metabolism including increases in important glycolytic enzyme activities (Baggetto, 1992), and often these enzyme activities correlate with malignancy (Weber and Lea, 1966; Harap, 1975). Ebrahim et al. (1996) observed tetrachloroethylene-induced alterations in glycolytic and gluconeogenic enzymes in liver and kidney of mice treated with the chemical. Administration of 2-deoxy-D-glucose and vitamin E controlled the changes in glycolytic and gluconeogenic enzymes induced by tetrachloroethylene.

4.4.4.4.4. ***Alteration in DNA methylation.*** No tetrachloroethylene-specific data are available regarding a role of alteration in DNA methylation in tumorigenesis. Such changes are reported to be a common early molecular event in most tumors (Zingg and Jones, 1997; Baylin et al., 1998). Alterations in DNA methylation following exposure to chemicals, both hypomethylation and hypermethylation, may be factors in tetrachloroethylene-induced tumorigenesis. Although tetrachloroethylene-specific data are lacking, its metabolites DCA and TCA are known to induce hypomethylation of DNA and protooncogenes in mouse liver.

4.4.4.4.5. ***Alterations in cell replication and death rate.*** Although modification of cell replication and death rates may be important to tetrachloroethylene liver tumorigenesis, no tetrachloroethylene-specific data are available.

4.4.4.4.6. ***Cytotoxicity and compensatory hyperplasia.*** Cytotoxicity and reparative hyperplasia are not marked findings resulting from tetrachloroethylene exposures capable of causing liver cancer in mice.

4.4.4.4.7. ***Hepatomegaly/cytomegaly.*** Increase in liver size is highly correlated with liver tumorigenesis in mice. Treatment with tetrachloroethylene can lead to increased liver weight. In carcinogenicity studies, hepatomegaly occurred following exposures in the dose ranges that

cause liver tumors and at experimental exposures well below the carcinogenicity bioassay dose levels. It is not clear exactly how the phenomenon is related to tumorigenesis in the case of tetrachloroethylene.

4.4.4.5. *Summary and Conclusions*

At the present time, the specific mechanisms or MOA for tetrachloroethylene-induced hepatocarcinogenesis in mice are not known. The cancer-causing activity of tetrachloroethylene is thought to be metabolism-dependent, however the specific contribution of particular metabolites has not been elucidated. TCA induces liver tumors with different phenotype and is not produced in sufficient amounts to account quantitatively for the liver tumor response observed with tetrachloroethylene. Not all metabolites have been identified or characterized, but several known metabolites including those derived from P450 as well as GSH pathways are clearly mutagenic in the standard battery of tests. Tetrachloroethylene is mutagenic in bacterial assays in the presence of GST and GSH whereas the standard S9 fraction has typically yielded negative results. Tetrachloroethylene at higher concentrations also induces SSBs and DNA binding in liver tissue. The metabolite DCA is the most potent mutagen of the P450-derived metabolites, exhibiting mutagenic activity in a number of assays. A putative P450 derived metabolite, 1,1,2,2-tetrachloroethylene oxide, is also mutagenic; the mutagenicity of this epoxide would be predicted from structure-activity relationships. Given the demonstrated mutagenicity of several tetrachloroethylene metabolites, it is expected that mutagenicity contributes to the MOA for tetrachloroethylene carcinogenesis, although the specific metabolic species or mechanistic effects are not known.

Chemical-specific data for PPAR-α activation are limited but suggest that this is not the primary MOA for hepatocarcinogenesis. The existing data for tetrachloroethylene show minimal peroxisome proliferator activity, and no chemical-specific data correlate peroxisome proliferation with tumor induction for tetrachloroethylene. As noted above, TCA produces tumors of a different phenotype than tetrachloroethylene and TCA is not produced in sufficient amounts to account quantitatively for the tetrachloroethylene liver tumor response. Moreover, several recent studies have expanded the scientific understanding of the PPAR-α mode of action (see Caldwell et al., 2008) including the demonstration that PPAR-α activation in hepatocytes induces peroxisome proliferation but not liver tumors (Yang et al., 2007). In particular, peroxisome proliferation and hepatocyte proliferation, but not liver tumors, were observed with PPAR-α activation in mouse hepatocytes. Furthermore, Ito et al. (2007) found that DEHP, a proposed robust example of PPAR-α agonism-induced hepatocarcinogenesis, yields liver tumors in a 2-year study in PPAR-α knock-out mice.

In summary, the MOA for tetrachloroethylene-induced liver toxicity and tumorigenesis is not understood. Data are lacking particularly for tetrachloroethylene P450 intermediates that could be involved in mutagenicity and carcinogenicity of the parent compound. Among the data gaps is the incomplete characterization of the metabolites in tests beyond the standard battery of genotoxicity tests, including on important genetic and epigenetic endpoints.

4.5. KIDNEY TOXICITY

4.5.1. Human Studies

4.5.1.1. *Kidney Toxicity in Humans*

High concentrations of inhaled tetrachloroethylene given acutely as an anaesthetic are associated with symptoms of renal dysfunction, including proteinuria and hematuria (Hake and Stewart, 1977, ATSDR, 1997). Controlled inhalation exposure to tetrachloroethylene at levels of 0, 20, 100, or 150 ppm for up to 11 weeks did not affect a number of urine parameters or BUN (a measure of kidney function) in 12 healthy individuals (Stewart et al., 1977, as reported in ATSDR, 1997). Whether renal effects would occur from these acute exposure levels in a larger, more diverse population than the one studied by Stewart et al. (1977) is not known.

The evidence for kidney effects from chronic inhalation of tetrachloroethylene is limited because many of the available reports do not include information on even a minimal core battery of tests for kidney function. The ATSDR (Amler et al., 1998; Lybarger et al., 1999) recommends a core battery of kidney function tests that includes serum creatinine, urinalysis with microscopic examination of urine sediment, albumin, retinol binding protein (RBP), N-acetyl-β-D-glucosaminidase (NAG), alanine aminopeptidase (AAP), osmolality, and urine creatinine (Lybarger et al., 1999). These indicators evaluate a range of toxicity, from effects on general kidney function to effects on specific segments of the nephron. For example, the overall integrity of the nephron can be evaluated from the urinalysis, and albumin is an indicator of the integrity of the glomerulus; three indicators—RBP, NAG, and AAP—assess damage to the proximal tubules (Lybarger et al., 1999). The proximal tubules house beta lyase enzymes and are hypothesized to be a target of tetrachloroethylene toxicity due to the bioactivation of reactive metabolites produced from the further metabolism of TCVC (see Section 4.2). For this reason, this analysis places greater weight on urinary indicators of proximal tubule function.

The epidemiologic studies are suggestive of subtle damage to the renal tubules. Five studies (Trevisan et al., 2000; Verplanke et al., 1999; Mutti et al., 1992; Solet and Robins, 1991; Lauwerys et al., 1983) have examined the three core indicators of tubule function—RBP, NAG, or AAP—in urine of dry cleaners. Three studies measured RBP, with two of the studies reporting a statistically significant elevated prevalence of abnormal values among study participants (Mutti et al., 1992) or a statistically significant elevated geometric mean

concentration of RBP (Verplanke et al., 1999) for tetrachloroethylene-exposed workers as compared with controls. The mean concentration of RBP for exposed subjects (75.4 μg/g creatinine) in the Verplanke et al. (1999) study is within a normal range,[1] indicating the absence of concurrent tubule toxicity.

As a comparison, Nomiyama et al. (1992) suggest a critical level of RBG of 200 μg/g creatinine as indicative of cadmium-induced kidney toxicity. Exposure levels were to a median of 15 ppm (range: limit of detection to 85 ppm) in Mutti et al. (1992) and 1.2 ppm (range: 0.3–6.5 ppm) in Verplanke et al. (1999). Lauwerys et al. (1983), the only other study to assess RBP, did not observe any differences in the geometric mean concentration of RBP between dry cleaners with mean tetrachloroethylene exposure of 21 ppm and their controls; however, this study contained fewer exposed subjects with a shorter duration of exposure than did that of Mutti et al. (1992).

The four studies that measured urinary excretion of NAG (Solet and Robins, 1991; Mutti et al., 1992; Verplanke et al., 1999; Trevisan et al., 2000) and the one study that measured AAP (Verplanke et al., 1999) did not observe any differences between exposed subjects and controls. These findings are not surprising; NAG is not a sensitive and specific marker of tubular dysfunction (Lybarger et al., 1999). Mean exposures were 14 ppm in Solet and Robins (1991) and 9 ppm in Trevisan et al. (2000); both studies assessed exposure from personal monitoring of exhaled breath.

The above findings are further supported by the observation of elevated urinary excretion of other proteins that are also indicators of damage to the proximal tubules: $beta_2$-microglobulin, intestinal alkaline phosphatase (IAP), tissue non-specific alkaline phosphatase (TNAP), lysozyme, $beta_2$-glucuronidase, and glutamine synthetase. Both IAP and TNAP are indicators of proximal tubule brush border integrity (Price et al., 1996), whereas lysozyme and $beta_2$-microglobulin indicate a failure of the tubule to reabsorb protein (Lybarger et al., 1999; Bernard and Lauwerys, 1995; Kok et al., 1998). Glutamine synthetase is a mitochondrial enzyme located in the proximal tubules and has been recently suggested as a marker of tubular damage in rats exposed to 1,3-hexachlorobutadiene (Trevisan et al., 1999).

Mutti et al. (1992) observed an elevated prevalence of abnormal values for $beta_2$-microglobulin and brush border antigens, a higher geometric mean concentration of brush border antigens in urine, and a higher concentration of TNAP in urine among 50 exposed dry cleaners as compared with 50 blood donors matched by sex and age with the exposed subjects. Furthermore, markers of renal damage were highly predictive of exposure status in discriminant

[1] Lapsley et al. (1998) found a median and an upper 98% confidence limit of 67 and 143 μg/g creatinine, respectively, in a survey of 70 adults, and this range closely matches the findings of Topping et al. (1986), who observed a mean and a 98% upper limit of 64 and 185 μg/g creatine in 118 subjects.

analysis. Beta$_2$-microglobulin, however, was not elevated among exposed subjects as compared with controls in the other two studies that examined this protein (Lauwerys et al., 1983; Vyskocil et al., 1990), although the mean concentration of beta$_2$-microglobulin appeared higher in subjects studied by Vyskocil et al. than the mean concentration in controls. Both these studies contained fewer numbers of exposed subjects than did the study by Mutti et al. (1992), and reduced power as a consequence of fewer subjects may be a reason for the null observations. Further, tetrachloroethylene exposure appears to affect reabsorption in the renal tubules. Two studies that assessed lysozyme or beta-glucuronidase observed a statistically significant elevated mean concentration of these proteins among dry cleaners as compared with controls (Franchini et al., 1983; Vyskocil et al., 1990).

It is not clear whether tetrachloroethylene exposure engenders an effect on other parts of the kidney. The study by Mutti et al. (1992) is suggestive of damage to the glomerulus; however, the lack of an elevated excretion of albumin, an indicator of glomerular function (Lybarger et al., 1999), in the study by Verplanke et al. (1999) argues for further assessment of possible glomerular effects.

Taken together, the epidemiologic studies support an inference of subtle effects on the renal proximal tubules. Effects are seen in populations of both males and females, and potential differences in susceptibility due to sex-related differences in rates of metabolism (see Section 4.2) cannot be determined from the available evidence. Median exposure levels in the studies that observed alterations in renal enzymes were 9 ppm (Trevisan et al., 2000), 10 ppm (Franchini et al., 1983), and 15 ppm (Mutti et al., 1992), representing LOAELs for these studies. Only the study by Trevisan et al. (2000) observed an exposure-response relationship, a correlation between urinary tetrachloroethylene and the concentration of glutatmine synthatase ($p < 0.001$). None of the other studies reported exposure-response relationships, which is a limitation on the inference of an association between tetrachloroethylene and renal damage. However, as pointed out by Mutti et al. (1992), this is a common finding among solvent-exposed populations, and inadequate definition of the dose metric most likely contributes to the null finding. Table 4-3 summarizes the human kidney toxicity studies.

4.5.1.2. *Kidney Cancer*

The evidence supporting a hypothesis of an association between tetrachloroethylene exposure and kidney cancer consists of the observation of elevated risks in the larger case-control studies (Asal et al., 1988; Aschengrau et al., 1993; Dosemeci et al., 1999; Mandel et al., 1995; McCredie and Stewart, 1993; Mellemgaard et al., 1994; Schlehofer et al., 1995; Partanen

Table 4-3. Summary of human kidney toxicity marker studies in dry cleaners

Author	Number of subjects	Estimated mean exposure concentration[a] (ppm)/duration (yrs)	RBP	NAG	AAP	Beta2-micro-globulin	Albumin	Total protein	Lyso-zyme	TNAP	Beta-glucuro-nidase	Gluta-mine synthe-tase
Mutti et al. (1992)	50 dry cleaners and 50 matched controls	15/10 PM	D	NS		D	D	NS		NS		
Verplanke et al. (1999)	82 exposed and 19 nonexposed dry cleaners	1.2 (TWA)/ 5.1 PM	D	NS	NS			NS				
Solet and Robins (1991)	192 exposed dry cleaners, no control group	14/11.6 PM		NS			NS	NS				
Lauwreys et al. (1983)	24 exposed dry cleaners and 33 unexposed controls	21/6.4 PM	NS			NS						
Vyskocil et al. (1990)	16 exposed dry cleaners and 13 nonoccupationally unexposed controls	23 (TWA)/9 U				NS	NS		D			
Franchini et al. (1983)	57 exposed dry cleaners and (a) 81 unexposed and (b) 80 unexposed factory workers	10 (TWA)2/14 PM					NS	NS	D		D	
Trevisan et al. (1999)	40 dry cleaners and 45 laundry workers	8.8/15 PM, B, U										Dose-response observed

[a] TWA tetrachloroethylene concentration developed using the relationship of between excretion of TCA in urine and breathing-zone, TWA concentration of Ikeda and Otsuji (1972).

B = Biological monitoring of blood
D = Detected with statistically significant elevation with respect to controls
IA = Indoor air monitoring
NS = Not statistically significant
PM = Personal monitoring of breath
U = Biological monitoring of urine for trichloroacetic acid (U-TCA)

et al., 1991; Pesch et al., 2000a). The studies by Aschengrau et al. (1993), Partanen et al. (1991), and Pesch et al. (2000a) are of high quality because they had good exposure information, controlled adequately for confounding, and used histologic confirmation of outcomes. For these reasons, observations in these two case-control studies carry greater weight than observations in the other case-control studies identified in Table 4B-4 (Appendix 4B). The remaining studies included large numbers of cases self-reported to determine exposure. These types of reports are more subject to misclassification errors.

In many of the case-control studies there are concerns about selection bias, blinding of investigators or interviewers, and, particularly, exposure characterization (Wartenberg et al., 2000). Three studies (Pesch et al., 2000a; Dosemeci et al., 1999; Schlehofer et al., 1995) present risks for tetrachloroethylene exposure explicitly. The studies by Pesch et al. (2000a) and Dosemeci et al. (1999) both suggest that there may be gender differences in renal cell carcinoma risk with occupational exposure to tetrachloroethylene; in both studies the risks were higher in males than in females. Exposure-response gradients were not observed in any of the three studies.

Cohort studies of kidney cancer incidence among dry cleaners and laundry workers in Sweden and Denmark (Andersen et al., 1999; Travier et al., 2002; Lynge and Thygesen, 1990; McLaughlin et al., 1987) did not observe excess risks of kidney cancer (see Table 4B-1a and Appendix 4B)—an inconsistency with the case-control studies. Few kidney cancer deaths were observed in cohort studies assessing mortality among dry cleaners (Ruder et al., 2001; Blair et al., 2003; see Table 4B-1b and Appendix 4B). The highest risks (not statistically significant) were reported for tetrachloroethylene-exposed subjects (Ruder et al., 2001) and for subjects identified with higher levels of exposure as compared with subjects with little or no exposure (Blair et al., 2003). There are too few cases of kidney cancer in the tetrachloroethylene subcohorts (degreaser studies) to assess any relationship with tetrachloroethylene (see Table 4B-2 and Appendix 4B).

4.5.2. Animal Studies

4.5.2.1. *Kidney Toxicity in Animals*

Tetrachloroethylene causes renal toxicity across several species, including rats, mice, rabbits, dogs, guinea pigs, and humans (for reviews, see U.S. EPA, 1985a, ATSDR, 1997; NYS DOH, 1997; Cal EPA, 2001).

Adverse effects on the kidney have been observed in studies of animals exposed to high concentrations of tetrachloroethylene by inhalation, oral intake, and i.p. injection. These effects include hyperplasia and increased kidney-to-body weight ratios, hyaline droplet formation, glomerular "nephrosis," karyomegaly (enlarged nuclei), cast formation, and other lesions or indicators of renal toxicity. The effects occurred following very high doses or chronic, relatively

high-doses of tetrachloroethylene exposures. The LOAEL for renal toxicity reported in the scientific literature is 100 ppm (678 mg/m^3) for inhalation exposure in mice (NTP, 1986a).

Oral administration of tetrachloroethylene in sesame oil (3 g/kg/day for 15 days) to mice caused an increase in kidney weight as well as increases in glomerular nephrosis and degeneration (Ebrahim et al., 1996). A lifetime animal carcinogenicity study in which tetrachloroethylene was administered to rats and mice by oral gavage in corn oil for 78 weeks resulted in clear evidence of kidney toxicity in both species (NCI, 1977). The TWA doses (mg/kg-day) used in the bioassay were 471 and 941 for male rats, 474 and 949 for female rats, 536 and 1,072 for male mice, and 386 and 772 for female mice. Nephropathy was observed in almost all of the test animals.

Hayes et al. (1986) reported renal effects in rats exposed to 400 mg/kg-day tetrachloroethylene in drinking water for 90 days. In a study by Jonker et al. (1996), tetrachloroethylene nephrotoxicity was observed in female Wistar rats administered the chemical in corn oil by oral gavage for 32 days. Nephrotoxic effects were noted at 2,400 mg/kg.

4.5.2.2. *Kidney Cancer in Animals*

In the studies conducted by NTP (1986a), groups of 50 male and 50 female F344/N rats were exposed for 6 hrs/day, 5 days/week, for 103 weeks by inhalation to atmospheres containing 0, 200, or 400 ppm tetrachloroethylene. Tubule cell hyperplasia was observed in male rats (control, 0/49; low dose, 3/49; high dose, 5/50) and in one high-dose female rat. Renal tubule adenomas and adenocarcinomas were observed in male rats (control, 1/49; low dose, 3/49; high dose, 4/50).

The spontaneous incidence rate for renal tubule tumors in F344/N rats, the strain used in the NTP bioassay, as well as for other rat strains reported by NTP was less than 1%, making the appearance of tubule neoplasms in 8% of the treated animals in the NTP study (low-dose and high-dose groups combined) convincing evidence of a treatment-related effect (Goodman et al., 1979; Solleveld et al., 1984; U.S. EPA, 1986a, 1991a). Also notable is the fact that no malignant renal tubule neoplasms had ever been observed in any control rats examined by NTP—including chamber controls from the performing laboratory and the untreated controls and vehicle controls from gavage studies—whereas two of the tumors observed in high-dose animals in the NTP study were carcinomas. The probability of two rare carcinomas appearing by chance in a group of 50 animals has been calculated to be less than 0.001 (U.S. EPA, 1987a, 1991a; NTP, 1986a).

In addition, when statistically compared with historical control incidences of renal tubule tumors, a significant dose-related positive trend exists, and tumor incidences in both low-dose and high-dose groups are significantly elevated. Standard statistical analyses of tumor incidence data did not reveal a significant increase in kidney tumors, and the tumor incidence is not

statistically significant when compared with concurrent controls; however, when the incidences of tubule cell hyperplasia and neoplasms and tumor severity are all considered, a dose-response relationship is apparent.

A slight increase in renal tumors was observed in other studies in male Sprague-Dawley (SD) rats receiving tetrachloroethylene by gavage or by inhalation (Maltoni and Cotti, 1986; Rampy et al., 1978; cited in U.S. EPA, 1991a), consistent with the findings reported in the NTP studies. However, in the rat chronic bioassay reported by JISA (1993), there was no increase in the incidence of kidney tubular cell adenoma or carcinoma in excess of that in the concurrent or historical control animals (see Tables 5-7 and 5-8) at administered concentrations of 50, 200, and 600 ppm.

The findings of rare kidney tumors in some cancer bioassays constitute suggestive evidence that tetrachloroethylene can induce kidney cancer in humans. The findings of rare kidney tumors in tetrachloroethylene bioassays in laboratory animals have been reviewed in EPA assessment documents on tetrachloroethylene (U.S. EPA, 1985a, 1986a, 1991a) and by an EPA risk assessment forum technical panel (U.S. EPA, 1991b) reporting on the association of alpha-2μ-globulin with renal lesions in the male rat. The closely related tetrachloroethylene congener trichloroethylene also induces low increased incidences of rare renal tumors in rats (U.S. EPA, 2001a) and NTP has found low incidences of tubule neoplasms in rats dosed with other chlorinated ethanes and ethylenes (NTP, 1983, 1988, 1986a, b, 1987, 1989, 1990b).

4.5.3. Summary of Kidney Effects in Humans and Animals

Taken together, the epidemiologic studies support an inference of subtle effects on the renal proximal tubules from inhalation exposure in tetrachloroethylene. The elevated urinary RBP levels seen in two studies (Mutti et al., 1992; Verplanke et al., 1999) provide some evidence for effects to the proximal tubules from tetrachloroethylene exposure. Exposures in the two studies that observed renal toxicity were 1.2 ppm and 15 ppm (means), representing an observational LOAEL for human kidney effects. None of the reviewed studies reported exposure-response relationships, and this is an important limitation of the available data. However, as pointed out by Mutti et al. (1992), this is a common finding among solvent-exposed populations, and inadequate definition of the dose metric most likely contributes to the absence of exposure-response relationships. No human studies investigating drinking water or other oral exposures on kidney toxicity have been published.

Positive associations between kidney cancer (renal cell carcinoma) and exposure to dry cleaning and laundry workers or to tetrachloroethylene specifically were observed in several well-conducted studies (Mandel et al., 1995; McCredie and Stewart, 1993; Pesch et al., 2000a; Schlehofer et al., 1995).

Adverse effects on the kidney have been observed in studies of animals exposed to high concentrations of tetrachloroethylene by inhalation, oral gavage, and i.p. injection. These effects include hyperplasia and increased kidney-to-body weight ratios, hyaline droplet formation, glomerular "nephrosis," karyomegaly, enlarged nuclei, cast formation, and other lesions or indicators of renal toxicity. Increased incidences of relatively rare renal tumors have been observed in multiple studies of male rats. The renal effects occurred following very high (or chronic, relatively high) doses of tetrachloroethylene exposures. The LOAEL for renal toxicity reported in the scientific literature is 100 ppm (678 mg/m^3) for inhalation exposure in mice (NTP, 1986a).

4.5.4. Mode of Action for Kidney Toxicity and Carcinogenicity

4.5.4.1. *Background*

The data support the conclusion that the chronic administration of tetrachloroethylene produces nephrotoxicity in both sexes of mice and rats and an increased incidence of proliferative lesions of the kidney tubules in male rats. The renal tumors observed in male rats exposed to tetrachloroethylene are of a rare type and include carcinomas. However, the use of these data to infer risk of carcinogenesis to humans has been a focus of scientific debate. Of particular consequence in this debate are two issues: the possibility that quantitative species differences in conjugative metabolism of tetrachloroethylene may greatly reduce the potential risk of human hazard and the possibility that the induction of renal tubule tumors by tetrachloroethylene may be unique to male rats and, therefore, is inappropriate for deducing potential human health hazard.

There are multiple hypothesized MOAs for kidney toxicity induced with tetrachloroethylene exposure, including mutagenicity, alpha-2μ-globulin accumulation, and cytotoxicity unrelated to alpha-2μ-globulin. When clearly demonstrated to develop from the sequence of events induced by alpha-2μ-globulin accumulation, kidney tumors in male rats caused by exposure to a test chemical, are generally considered to be species and sex specific and not relevant for assessing human hazard. Limited data from studies of tetrachloroethylene indicating hyaline droplet formation provide some evidence for the alpha-2μ-globulin MOA. The phenomenon occurs only at very high doses of tetrachloroethylene, however, above the doses used in cancer bioassays in which tumors were observed. There is also data supporting other MOAs for tetrachloroethylene-induced renal tumors, particularly findings of mutagenicity, and also cytotoxicity not associated with alpha-2μ-globulin accumulation. This mutagenicity and cytotoxicity are attributed to the further biotransformation of glutathione and cysteine conjugates of tetrachloroethylene to reactive chemical intermediates. Humans are known to conjugate tetrachloroethylene with glutathione and excrete the mercapturate end product,

therefore, exhibiting evidence for operation of the same metabolic pathway. Quantitative measurements of urinary excretionmetabolites from this pathway do not provide data for estimations of the amount of chemical coverted to toxic intermediates; therefore, relative amounts of tetrachloroethylene processed by enzymes activating the conjugates to toxic products in rats versus humans are not known.

4.5.4.2. *Summary Description of a Postulated Mode of Action—alpha-2μ-globulin Accumulation*

A variety of organic compounds have been shown to cause sex- and species-specific lesions in the renal tubules of male rats in the form of what is known as "hyaline droplet nephropathy" (NTP, 1983, 1986a, b, 1987, 1990a; U.S. EPA 1991b; Alden, 1985; MacNaughton and Uddin, 1984; Alden and Repta, 1984; Phillips et al., 1987). These chemicals have been associated with interference of normal renal proximal tubule reabsorption of protein from the glomerular filtrate, resulting in accumulation of alpha-2μ-globulin in phagolysosomes of renal proximal tubule cells (U.S. EPA, 1991a, b). This accumulation is believed to be the reason for an excessive number of hyaline droplets (Stonard et al., 1986; Olson et al., 1987) and associated nephropathy. The sequence of functional changes in the epithelial cells of proximal tubules, with subsequent tubule necrosis and compensatory cell proliferation, is hypothesized to culminate in the renal tubule tumors observed in the male rats exposed to these compounds in bioassays (UAREP, 1983; Alden et al., 1984; Halder et al., 1984; Swenberg et al., 1989; U.S. EPA, 1991b).

Alpha-2μ-globulin is considered unique to the male rat and is the major component of its urinary protein load. Alpha-2μ-globulin is synthesized in the liver under hormonal control, but it has not been detected in the liver of female rats or in other species (Roy et al., 1975; U.S. EPA, 1991b), although homologous proteins do exist in other species, including humans (Flower et al., 1993).

The renal tubule tumors associated with alpha-2μ nephropathy appear to be the end product in the following histopathological sequence of functional changes in the epithelial cells of proximal tubules:

1. Excessive accumulation due to increased number and size of hyaline droplets in the P2 segment of renal proximal tubule cells, representing lysosomal overload, leads to tubule cell degeneration, cell loss, and regenerative cellular proliferation. The excessive hyaline droplet accumulation occurs in male rats only, and the accumulating protein is identified as alpha-2μ-globulin.

2. Cell debris in the form of granular casts accumulates at the corticomedullary junction, with associated dilation of the affected tubule segment and, more distally, mineralization of tubules within the renal medulla.

3. The chronic progressive nephropathy characteristically found in aging rats is exacerbated as a consequence of the induced nephrotoxicity.

4. Renal tubule hyperplasia and neoplasia develop subsequently. The increased cellular proliferation is thought to cause development of renal cell tumors due to increases in DNA damage in replicating cells.

This proposed MOA for kidney tumorigenesis seems plausible to many scientists and may provide an adequate explanation of the specific susceptibility of the male rat to renal tubule tumor induction by certain chemicals. However, data gaps still exist, and the mechanism of cellular damage in alpha-2μ-globulin nephropathy is not known (Melnick et al., 1997). Several chemical compounds have been shown to cause the acute renal nephropathy associated with alpha-2μ-globulin accumulation, but not to cause tumors (Melnick et al., 1997; Swenberg and Lehman-McKeeman, 1999) even though a critical level of regenerative cellular proliferation may have to be attained for renal tumorigenesis to occur (Swenberg and Lehman-McKeeman, 1999).

EPA has developed specific criteria for use in evaluating the likelihood of a chemical's inducing renal tumors through the hypothesized alpha-2μ-globulin MOA (U.S. EPA, 1991b). Although EPA downgrades the finding of kidney cancer in male rats as being unimportant to the human situation if it can be shown that the criteria for alpha-2μ-globulin are clearly met, the proposed MOA, although reasonable, is still hypothetical, and other reasonable alternative hypotheses have been proposed. As described and discussed below, in the case of tetrachloroethylene, evidence for alpha-2μ-globulin accumulation exists only at doses above cancer-causing doses, and other alternative MOAs are well supported.

4.5.4.2.1. ***Human relevance of alpha-2μ-globulin nephropathy.*** The U.S. EPA has specific guidance (U.S. EPA, 1991b) for evaluating chemically induced male rat renal tumors to determine the use of the data for human risk assessment. It is interesting to note, however, that controversy still exists within the scientific community regarding this mode of carcinogenic action and its relevance to human health risk assessment (Lash et al., 2000b; Ashby, 1996; de la Iglesia et al., 1997; Dietrich, 1997; Huff, 1995, 1996; Melnick et al., 1997; Melnick, 2001, 2002).

4.5.4.2.2. ***Identification of tetrachloroethylene-specific key events in support of the alpha-2μ hypothesis.*** Goldsworthy et al. (1988) observed increases in alpha-2μ-hyaline droplets in exposed male but not female F344 rats following 10 days of gavage with 1,000 mg/kg tetrachloroethylene. This finding was correlated with both protein droplet nephropathy (crystalloid accumulation) and increases in cellular proliferation. The cell replication was enhanced in the male rats specifically in damaged P2 segments, suggesting a link between the

alpha-2μ-globulin accumulation and kidney tumors. These investigators reported similar findings for pentachloroethane in the same study, but at a dose of 150 mg/kg for 10 days. Trichloroethylene has a similar structure but did not cause any alpha-2μ accumulation or increase in protein droplets, nor did it stimulate cellular proliferation in either male or female rats in this study when a dose of 1,000 mg/kg was administered for 10 days. Bergamaschi et al. (1992) also demonstrated alpha-2μ-accumulation in P2 segments of rat proximal tubule cells resulting from a daily exposure of rats to 500 mg/kg tetrachloroethylene in corn oil for 4 weeks.

In short-term, high-dose studies, Green et al. (1990) found that the oral administration of from 1,000 to 1,500 mg/kg of tetrachloroethylene daily for up to 42 days caused an accumulation of alpha-2μ-globulin in the proximal tubules of male rats. The animals were sacrificed within 24 hrs of the last dose of tetrachloroethylene. The effect was accompanied by evidence of nephrotoxicity, with the formation of granular tubular casts and evidence of tubular cell regeneration. These effects were not observed in female rats or in mice. Inhalation exposure to 1,000 ppm tetrachloroethylene for 10 days resulted in the formation of hyaline droplets in the kidneys of male rats, but granular casts and tubule cell regeneration were not observed, although the time period may have been too short to allow progression to this stage. These results show that very high doses of tetrachloroethylene are capable of precipitating hyaline droplet nephropathy in male rats. The results also show that male rats are more sensitive to the effect than are female rats or mice of either sex. It is possible, therefore, that alpha-2μ-globulin accumulation may indeed play a role in the tumorigenesis observed in male rats exposed to tetrachloroethylene. EPA has listed tetrachloroethylene as an alpha-2μ-accumulator (U.S. EPA, 1991b) and in the same report specifically identified trichloroethylene as not being an alpha-2μ-accumulator.

It is interesting to note that tetrachloroethylene-induced alpha-2μ-globulin accumulation is probably more likely to be caused by the parent molecule rather than by its metabolites, because its occurrence is related more to the charge and lipid solubility of the inducing agent than to specific interactions with reactive chemical species (Lash and Parker, 2001).

4.5.4.2.3. ***Points relevant to biological plausibility and coherence of this mode of action (MOA) for tetrachloroethylene-induced kidney tumors.*** The following points show that factors other than the specific protein droplet nephropathy may have as much—or more—of a significant role in explaining renal tumor formation resulting from tetrachloroethylene exposure, although some contributions of alpha-2μ-globulin accumulation cannot be entirely ruled out.

The alpha-2μ-globulin response reported following exposure to tetrachloroethylene is relatively mild, and the fact that renal tumors have been observed at doses lower than the ones shown to cause the alpha-2μ-globulin response is inconsistent with this phenomenon being

responsible for tumorigenesis. Although the alpha-2μ-globulin response occurs in male rats exposed to tetrachloroethylene, it has been observed following only high doses. Green et al. (1990) tested lower inhaled tetrachloroethylene doses in rats—up to 400 ppm for 6 hrs/day for 28 days, with the animals being sacrificed within 18 hrs of termination of the final exposure—but found no evidence of hyaline droplet formation; however, there may have been time for recovery prior to sacrifice.

It is noteworthy that the 400 ppm concentration was the same exposure level used for the high-dose rats in the NTP inhalation carcinogenicity bioassay (NTP, 1986a). In the NTP study, the 400 ppm concentration caused a high incidence of nontumor nephropathy and resulted in the formation of kidney tubule adenomas and adenocarcinomas. The renal pathology of rats in the NTP study was reported to be different from the specific alpha-2μ-globulin nephropathy, but the age of the rats as well as the length of time that elapsed between final exposure and sacrifice may explain some of the differences. However, mineralization in the inner medulla and papilla of the kidney—a characteristic trait of alpha-2μ-globulin nephropathy—was not seen.

Green et al. (1990) proposed the possibility that longer-term exposure to the 400 ppm concentration of tetrachloroethylene is required for the hyaline droplet accumulation in the kidney of rats. Alpha-2μ-globulin accumulation can be demonstrated, however, after only short-term exposures (even a single administration) to several agents, such as d-limonene, decalin, unleaded gasoline, and trimethylpentane (Charbonneau et al., 1987; NTP, 1988). Lack of hyaline droplet formation, increase in alpha-2μ-globulin, or signs of the characteristic renal nephropathy at the high dose level of the NTP inhalation study (NTP, 1986a) may indicate a threshold effect and thus diminish the likelihood that the renal tumors associated with exposure to tetrachloroethylene are induced through this mechanism (Green et al., 1990). Pharmacokinetic differences between oral and inhalation exposure may contribute to the observed discrepancies in some of the results.

NTP did not report the presence of hyaline droplets in rats that had been exposed to either 200 or 400 ppm tetrachloroethylene for up to 2 years. These doses were associated with the production of renal tubule neoplasms in male rats. However, the fact that NTP did not report the presence of hyaline droplets in the 14-day, 90-day, or 2-year studies is not definitive, because the NTP protocol at that time was not designed specifically to detect hyaline droplets or alpha-2μ-globulin accumulation in the kidney (NTP, 1990a). Thus, the procedures followed at the time of the study were not necessarily conducive to detecting hyaline droplets. For example, in the chronic study of tetrachloroethylene, at least 1 week elapsed between the final tetrachloroethylene exposure and the scheduled sacrifice of the surviving animals. It is possible that had hyaline droplets been present, they could have regressed. Also, the nephropathy observed at the end of a 2-year bioassay could be difficult to distinguish from the old-age

nephropathy that occurs in these rats. Other investigators (Goldsworthy et al., 1988; Green et al., 1990) observed hyaline droplets containing alpha-2μ-globulin following very high doses of tetrachloroethylene administered to male rats.

On the other hand, the renal pathology reported in the NTP bioassay is not entirely consistent with the results generally found for chemicals where there is alpha-2μ-globulin accumulation (NTP, 1986a; letter from Scot Eustis, National Toxicology Program, to William Farland, Director, Office of Health and Environmental Assessment, EPA, 1988). For example, there was no mineralization in the inner medulla and papilla of the kidney, a frequent finding in bioassays of chemicals that induce alpha-2μ-globulin accumulation (e.g., for pentachloroethane, the incidence of renal papillar mineralization was 8% in controls, 59% in the low-dose group, and 58% in the high-dose group). In addition, it is important to note that some aspects of toxic tubular nephropathy were also observed in female rats and male mice exposed to tetrachloroethylene, clearly contrary to sex and species specificity.

Thus, chronically induced tetrachloroethylene nonneoplastic kidney lesions exhibit neither species nor sex specificity. Unlike with other chemicals that induce alpha-2μ-globulin accumulation and have been tested by NTP in chronic carcinogenicity bioassays, renal lesions occurring in animals exposed to tetrachloroethylene were not limited to the male rat. Although the female rat did not develop any renal tubule tumors, the incidence of karyomegaly was significantly elevated in the female rat as well as in the male rat; 1 of 50 female rats exposed at the high dose developed tubule cell hyperplasia.

In the mouse, "nephrosis" was observed at increased incidences in dosed females, casts were observed at increased incidences in dosed males and high-dose females, and karyomegaly of the tubular cells was observed at increased incidences in both sexes of treated mice. The severity of the renal lesions was dose related, and one low-dose male had a renal tubular cell adenocarcinoma.

In the NCI gavage study of tetrachloroethylene (NCI, 1977), toxic nephropathy, which was not detected in the control animals, occurred in both male and female Osborne-Mendel rats administered tetrachloroethylene. Tetrachloroethylene also clearly caused nephropathy in both sexes of mice in the study. Unfortunately, animal survival in the rat study was not adequate to support any conclusions about tetrachloroethylene carcinogenicity.

Other chlorinated ethanes and ethylenes also produce nephrotoxicity and renal tubule tumors in laboratory animals. Hexachloroethane causes accumulation of hyaline droplets and renal tubule tumors in male rats (NTP, 1989). On the other hand, trichloroethylene, which was also tested by NTP, induces kidney tumors in male rats and also possibly in female rats, but it does not cause an accumulation of hyaline droplets or an increase in levels of alpha-2μ-globulin (Goldsworthy et al., 1988). Consequently, kidney tumors induced by this compound are not

considered to be associated with alpha-2μ-globulin accumulation (U.S. EPA, 1991b, 2001a). Tetrachloroethylene is related in structure to trichloroethylene, and both chemicals have been shown to be metabolized in the kidney to cytosolic and mutagenic compounds.

4.5.4.3. *Other Modes of Action for Tetrachloroethylene-Induced Renal Tumors in Rats*

4.5.4.3.1. ***Genotoxicity.*** The glutathione conjugation of tetrachloroethylene in the kidney, discussed in Chapter 3, leads sequentially to S(1,2,2-trichlorovinyl)glutathione and S(1,2,2-trichlorovinyl)cysteine—TCVG and TCVC. TCVC can be further processed by beta-lyase to yield an unstable thiol, 1,2,2-trichlorovinylthiol, that may give rise to a highly reactive thioketene, a chemical species that can form covalent adducts with cellular nucleophiles including DNA. Additionally, sulfoxidation of both TCVC and its N-acetylated product via FMO3 or P450s occurs, resulting in reactive metabolites (Ripp et al, 1997, 1999; Werner et al., 1996). While most of these intermediates have not been characterized for mutagenic potential, TCVG, TCVC, and NAcTCVC are clearly mutagenic in Salmonella tests. In addition, tetrachloroethylene exhibited mutagenicity in Salmonella in the few studies of conditions that could generate GSH-derived metabolites and, following in vivo exposures, induces SSB and DNA binding in kidney. See Section 4.3 for more details of genotoxicity.

4.5.4.3.2. ***Peroxisome proliferation.*** Peroxisome proliferation has been linked to tumorigenesis in rodents; however, the mechanisms involved have not been clearly elucidated (see Section 4.3). The PPARs, a class of nuclear receptors, are believed to be transcriptionally activated to mediate the effects of peroxisome proliferators (Issemann et al., 1993; Desvergne and Wahli, 1995). Although most of the focus on peroxisome proliferation and PPAR receptor activation, and their relationship to tumor development, has been on the liver (see Section 4.3.4 for a more detailed discussion), the phenomenon can also occur in other tissues. In fact, peroxisomes were first noted in rodent renal tubule epithelial cells, and peroxisome proliferation in these cells in response to peroxisome proliferating agents is not unusual (reviewed by Stott, 1988; Klaunig et al., 2003). Data exist to support increased peroxisome proliferation in rodent kidney following exposure to tetrachloroethylene (Goldsworthy and Popp, 1987; Zanelli et al., 1996).

Goldsworthy and Popp (1987) investigated the ability of tetrachloroethylene to induce peroxisome proliferation in both liver and kidney of rats and mice using increases in cyanide-insensitive PCO activity as a marker enzyme. Tetrachloroethylene caused elevations in enzyme activity in mouse kidney as well as liver but not in rat kidney. It seems somewhat unlikely that any peroxisome proliferation observed following tetrachloroethylene exposure would be associated with renal tumors if one considers the magnitude of the measurable peroxisome proliferation effect across species. Renal tumors occur in rats, but greater peroxisome

proliferation is observed in the kidneys of mice (Goldsworthy and Popp, 1987). Modification of cell signaling pathways that control rates of cell division and apoptosis, for example, may occur through activation of PPAR receptors. The occurrence of peroxisome proliferation per se may be only a marker for PPAR receptor activation, the key event having the most support for being causally related to other tumor types. The dissimilarity in the peroxisome response observed in different tissues may be related to variability in the levels of PPAR receptors in these tissues.

It is important to note that, relatively speaking, chlorinated ethylenes, particularly tetrachloroethylene, and their chloroacid metabolites are not very potent peroxisome proliferators compared to many other compounds that are known to cause the phenomenon. PPAR receptors belong to the superfamily of proteins that control almost every metabolic and developmental event in mammals, and PPAR receptor activation is known to result in numerous biochemical, physiological, and molecular events. Therefore, the possibility of PPAR receptor activation being related to tetrachloroethylene-induced kidney tumorigenesis in rats is plausible, especially since peroxisome proliferation, the phenomenon that could be considered a biomarker for PPAR activation, has been shown to occur in the kidneys of mice following exposure to tetrachloroethylene. Moreover, a causal link between PPAR activation and kidney tumorigenesis has yet to be established for tetrachloroethylene or other PPAR agonists.

4.5.4.3.3. ***Cytotoxicity/sustained chronic nephrotoxicity not associated with alpha-2μ-globulin nephropathy.*** The kidney is a major target organ for tetrachloroethylene-induced toxicity through the reactive metabolites of TCVC. Tetrachloroethylene has been reported to produce nephrotoxicity across species, although its relative potency is not extremely high. Other chlorinated ethanes and ethylenes also induce nephrotoxicity, although the toxicity manifests itself differently with specific chemicals. The observed effects vary across species and between sexes and may include tubular cell cytomegaly, karyomegaly and pleomorphism, tubular cell dilation, or the formation of granular casts. There may be a link between renal toxicity and tumorigenesis, and sustained kidney damage may be a risk factor for tumorigenesis. It is reasonable, therefore, to suspect that renal tubule neoplasia observed in tetrachloroethylene-exposed male rats may be influenced by cytotoxicity and subsequent cellular regeneration.

It has been suggested that renal neoplasms induced by tetrachloroethylene may be secondary to renal cytotoxicity and subsequent cellular proliferation without regard to alpha-2μ-accumulation. Thus, sustained chronic nephrotoxicity, independent of alpha-2μ-globulin accumulation and its resulting neuropathic cascade of events, may be a possible MOA for tetrachloroethylene carcinogenesis. If this is the case, renal tubule neoplasia observed to occur in male rats would not be expected to be a species- or sex-specific response because the nontumor lesions appear in both sexes of both rodent species tested. In support of this expectation, the

renal lesions occurring in animals exposed to tetrachloroethylene are not limited to the male rat. Signs of tetrachloroethylene-induced kidney damage appeared in both rats and mice during the early phases of the NTP inhalation study, for example, indicating that animals of both species surviving to the scheduled termination of the study had long-standing nephrotoxicity. Although the female rats did not develop any renal tubule tumors, the incidence of karyomegaly was significantly elevated in females as well as in males, and 1 of 50 female rats exposed at the high dose developed tubule cell hyperplasia.

In the NTP study of the mouse, "nephrosis" was observed at increased incidences in dosed females, casts were observed at increased incidences in dosed males and high-dose females, and karyomegaly of the tubule cells was observed at increased incidences in both sexes of treated mice. The severity of the renal lesions was dose related, and one low-dose male had a renal tubule cell adenocarcinoma. In the NCI gavage study of B6C3F1 mice and Osborne-Mendel rats exposed to tetrachloroethylene, toxic nephropathy was not detected in control animals but did occur in both male and female rats as well as in mice. On the other hand, findings using in vitro models studied by Lash et al. (2002) suggest a marked sex difference between male and female rats in the severity of acute renal toxicity caused by both tetrachloroethylene and its TCVG metabolite. Tetrachloroethylene and TCVG also produced signs of toxicity in mitochondria; i.e., mitochondrial dysfunction, such as inhibition of state 3 respiration by specific inhibition of several sulfhydryl-containing enzymes in both sexes of mice (Lash et al., 2000, 2001, 2002).

Mechanistic studies of tetrachloroethylene nephrotoxicity are relatively sparse. More and better data are available for trichloroethylene. Most studies performed to elucidate information related to understanding tetrachloroethylene renal toxicity have concentrated on the GSH pathway metabolites rather than on the parent chemical; this is because much available data for both tetrachloroethylene and trichloroethylene suggest that it is flux through this pathway that generates reactive chemical species responsible for nephrotoxicity. Vamvakas et al. (1989c, d) have shown the tetrachloroethylene conjugate metabolites TCVG and TCVC to cause dose-related cytotoxicity in renal cell preparations and prevention of this toxicity by beta lyase enzyme inhibitor. Renal beta lyases are known to cleave TCVC to yield an unstable thiol, 1,2,2-trichlorovinylthiol, that may give rise to a highly reactive thioketene, a chemical species that can form covalent adducts with cellular nucleophiles. Additionally, sulfoxidation of both TCVC and its N-acetylated product occurs, resulting in toxic metabolites (Ripp et al, 1997, 1999; Werner et al., 1996). Contribution to overall toxicity is unknown, however, and it is interesting to note that human CYP3A4 catalyzed sulfoxidation of the N-acetyl metabolite of a structurally related chemical, HCBD, at rates comparable to those of rat CYP3A1 (Werner et al., 1995), indicating a relative value of the human-to-rat rate constant of 1 (Lash and Parker, 2001).

4.5.4.3.4. ***Immunotoxicity/immunosuppression.*** Although specific data about tetrachloroethylene are lacking, immune suppression could contribute to the induction of kidney tumors caused by tetrachloroethylene exposure. Many immunosuppressive therapeutic agents are human carcinogens (see Tomatis et al., 1989), although they are usually associated almost exclusively with lymphoma. Organic solvent exposure in general is associated with autoimmune disease such as schleroderma and other autoimmune responses (Nietert et al., 1998). Several published studies have demonstrated formation of trichloroacylated protein adducts, for example, in liver and kidney of rats (Birner et al., 1994) and in plasma of rats and humans following exposures to tetrachloroethylene (Pahler et al., 1999). Another recent example is the detection of trichloroacetylated protein adducts formed in mice treated with tetrachloroethylene (Green et al., 2001).

4.5.4.4. ***Summary***

The kidney is a target organ in mammalian species for tetrachloroethylene and other related chlorinated ethanes and ethylenes, and tetrachloroethylene causes kidney cancer in male rats. It is likely that several mechanisms contribute to tetrachloroethylene-induced kidney toxicity, including cancer, and that the relative importance of specific MOAs varies from high-dose to low-dose exposure. Peroxisome proliferation, alpha-2μ-globulin nephropathy, mutagenicity, and cytotoxicity not associated with alpha-2μ-globulin accumulation are MOAs that have been investigated. The pathogenesis of immunosuppression is another potential MOA that may also be related to tumorigenesis.

Tetrachloroethylene-induced renal toxicity is likely associated with its metabolites rather than with the parent compound, except for toxicity associated with alpha-2μ-globulin accumulation, which is more likely due to tetrachloroethylene itself (Lash and Parker, 2001).

The GSH conjugate and reactive metabolites generated from further processing of TCVC or its acetylated metabolite NAcTCVC by beta lyase, FMO3/P450 and/or CYP3A, are the most likely tetrachloroethylene metabolites to induce renal toxicity and tumorigenicity, as opposed to the metabolites resulting from oxidative CYP processing. These conjugate metabolites are known to be mutagenic, and they are known to occur in rats, mice, and humans.

Due to tetrachloroethylene's nephrotoxic effects, it has been suggested that the low-level renal tumor production observed in exposed rats is secondary to sustained cytotoxicity and necrosis leading to activation of repair processes and cellular regeneration. However, "nephrotoxicity" occurs in both sexes of rats and mice whereas cell replication and tumorigenesis occurs in male but not in female rats In addition, tetrachloroethylene induces kidney tumors at lower doses than those required to cause alpha-2μ-globulin accumulation,

raising serious doubt that alpha-2μ-globulin plays a key role—especially any major role—in the rat kidney tumor formation.

Because tetrachloroethylene has been shown to induce peroxisome proliferation, an indicator of PPAR activation, the possibility exists that certain responses resulting from activation of PPAR receptors might be involved in cancer-causing activity leading to tetrachloroethylene-induced renal tumors. However, there is no evidence causally linking PPAR-α activation to kidney tumorigenesis for tetrachloroethylene or other compounds.

The weight of evidence suggests that for tetrachloroethylene the further processing of conjugative metabolites by beta lyase, FMO3 and/or CYP3A leads to reactive and mutagenic metabolites responsible for nephrotoxicity and carcinogenicity. The glutathione conjugation of tetrachloroethylene in the kidney, discussed in Chapter 3, leads sequentially to S(1,2,2-trichlorovinyl)glutathione and S(1,2,2-trichlorovinyl)cysteine—TCVG and TCVC. TCVC can be further processed by beta-lyase to yield an unstable thiol, 1,2,2-trichlorovinylthiol, that may give rise to a highly reactive thioketene, a chemical species that can form covalent adducts with cellular nucleophiles including DNA. TCVC can also undergo FMO3 or P450 oxidation to reactive intermediates; additionally, sulfoxidation of both TCVC and its N-acetylated product occurs, resulting in reactive metabolites (Ripp et al, 1997, 1999; Werner et al., 1996). While most of these intermediates have not been characterized for mutagenic potential, TCVG, TCVC, and NAcTCVC are clearly mutagenic in Salmonella tests. In addition, tetrachloroethylene exhibited mutagenicity in Salmonella in the few studies of conditions that could generate GSH-derived metabolites. Tetrachloroethylene, following in vivo exposures, also binds to kidney DNA and induces SSB in kidney.

In summary, the complete mechanisms of tetrachloroethylene-induced renal carcinogenesis are not yet understood. Given the known mutagenicity of the GSH-derived tetrachloroethylene metabolites that are formed in the kidney, and the observed in vitro mutagenicity of tetrachloroethylene under conditions that would generate these metabolites, a mutagenic MOA contributing to the development of the kidney tumors clearly cannot be ruled out.

4.6. NEUROTOXICITY

4.6.1. Human Studies

A wide range of effects on neurologic function are well-documented for both acute and chronic exposure to tetrachloroethylene. Acute controlled inhalation exposures of 100 ppm and higher have induced symptoms consistent with depression of the CNS and included dizziness and drowsiness (ATSDR, 1997). Changes in electroencephalograms (EEGs) have also been noted with controlled inhalation exposures at this level (Stewart et al., 1977). Acute exposure to

lower levels of tetrachloroethylene has induced alterations in neurobehavioral function. For example, Altmann et al. (1990, 1992) reported increases in the latency of visually evoked potentials (VEPs) and significant performance deficit for vigilance and eye-hand coordination in subjects with inhalation exposure to 50 ppm for 4 hrs/day for 4 days as compared with that seen among subjects exposed to 10 ppm (the control group in these reports). These observations indicate visual system dysfunction, including delayed neuronal processing time, is related to tetrachloroethylene exposure. ATSDR (1997) developed an acute exposure minimal risk level from this study and considered 10 ppm as the no-observed-adverse-effect level (NOAEL).

Studies by Stewart et al. (1997) and Hake and Stewart (1977), funded primarily by the National Institutes of Occupational Safety and Health (NIOSH), are third-party studies and are considered by EPA to be protective of human subjects. EPA and other federal agencies subscribe fully to principles articulated in EPA's Protection of Human Subjects Rule ("the Common Rule"), 40 CFR Part 26. EPA recently issued an interim policy on accepting human test data stating that the Agency will continue to accept third-party test data on a case-by-case basis (U.S. EPA, 2005d). A description of the studies by Altmann et al. (1990, 1992) is also included because ATSDR used these studies to develop an acute minimal risk level (MRL; ATSDR, 1977). EPA also considers these studies to be third-party. No information is provided in the published papers regarding the procedures the study investigators adopted for informed consent or protection of human subjects, and staff of the National Center for Environmental Assessment (NCEA) contacted study investigators requesting this information (e-mail communication from Robert McGaughy, U.S. EPA, to L. Altmann, Heinrich-Heine University, Dusseldorf, Germany, October 8, 2003).

Epidemiologic studies of workers or residents with chronic exposure to tetrachloroethylene show that the nervous system is a target, with decrements reported in several nervous system domains (Altmann et al., 1995; Schreiber et al., 2002; Seeber, 1989; Ferroni et al., 1992; Cavalleri et al., 1994; Gobba et al., 1998; Spinatonda et al., 1997; Echeverria et al. 1994, 1995). Table 4-4 presents select details of available chronic studies evaluating neurological function. Furthermore, neurotoxic effects from chronic-duration exposure to tetrachloroethylene are similar to neurotoxic effects reported for other solvents (Arlien-Sorborg, 1982). Case reports and case studies also show the nervous system as a target of organic solvent exposures such as tetrachloroethylene (Seppalainen and Antti-Poika, 1983; Antti-Poika, 1982a, b; Onofrj et al., 1998). Electrophysiological abnormalities in tetrachloroethylene- and other organic solvent-exposed patients, with diagnosed chronic solvents intoxication, persisted post-exposure (Seppalainen and Antti-Poika, 1983; Antti-Poika, 1982b).

Table 4-4. Summary of human neurotoxicology studies

Subjects	Exposure	Effects	Author(s)
24 dry cleaners exposed to tetrachloroethylene, 33 subjects nonoccupationally exposed to other solvents	Mean TWA = 21 ppm, PM Mean duration of exposure = 6 years	Statistically significant differences for simple reaction time and critical flicker fusion	Lauwerys et al. (1983)
101 dry cleaners (both sexes), 84 nonexposed controls	Low-exposure group: 12 ppm TWA, IA, PM, 11.8 years High-exposure group: 53 ppm TWA IA, PM, 10.6 years	Decrease in information processing speed (perceptual threshold, choice reaction time), visual scanning (cancellation dZ test), visuo-spatial function No fine motor function deficits Neurological signs	Seeber (1989)
56 tetrachloroethylene-exposed dry cleaning workers, 69 non-exposed factory controls	Geometric mean TWA = 20 ppm, PM Mean duration of exposure = 3 years	Statistically significant increase in the prevalence of several CNS symptoms such as nasal irritation and dizziness	Cai et al. (1991)
64 dry cleaners exposed to tetrachloroethylene, 120 controls	Mean duration of employment not reported Geometric mean = 15 ppm (males), 11 ppm; (females), PM	No statistically significant differences between exposed and controls in color vision loss	Nakatsuka et al. (1992)
Dry cleaners, 60 females and 30 nonsolvent-exposed controls	15 ppm IA, 10.1 years	Impaired performance on three tests (simple reaction time, vigilance, stress) No fine motor function deficit No effects on digit symbol test	Ferroni et al. (1992)
22 dry cleaners, 13 ironers, 35 controls	Mean TWA = 6 ppm (7 ppm, dry cleaning workers; 5 ppm, ironers), PM, 8.8 years (exposed subjects)	CCI statistically significantly elevated among dry cleaners with a statistically significant exposure (TWA)-response relationship. No effect on CCI in ironers	Cavalleri et al. (1994)

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Table 4-4. Summary of human neurotoxicology studies (continuted)

Subjects	Exposure	Effects	Author(s)
45 dry cleaners matched to 69 laundry workers, 59 pressers or counter clerks	<0.2 ppm, 3 ppm, 9 ppm, PM, 2.6 to 11 years	Statistically significant association between chronic lifetime tetrachloroethylene exposure and reduced test performance on three cognitive tests: switching, pattern memory, and pattern recognition.	Echeverria et al. (1994)
65 dry cleaners, no unexposed group	11 ppm, 23 ppm, 41 ppm, PM, 1.2 to 14.6 years	Statistically significant differences between "high" and "low" lifetime exposure groups in three tests of visuo-spatial function No effect on digit span test	Echeverria et al. (1995)
Residents near dry cleaning facilities, 14 exposed and 23 age- and gender-matched nonexposed controls	0.7 ppm (mean), IA (7-day monitoring period), B, 10.6 years	Increase in simple reaction time Decrements in continuous performance and visuo-spatial function No fine motor function deficits	Altmann, et al. (1995)
24 solvent-exposed workers and 24 controls	Past use of solvent mixture, including tetrachloro-ethylene (10%) for at least 3 years, solvent exposure for 6 years average	Color vision impairment ($p < 0.05$) among exposed subjects as compared with controls	Muttray et al. (1997)
35 dry cleaners, 39 age- and education-matched controls	8 ppm TWA, IA, grab sample Mean duration of employment not reported	Increase in vocal reaction time to visual stimuli Concentration-response relationship	Spinatonda et al. (1997)

Table 4-4. Summary of human neurotoxicology studies (continuted)

Subjects	Exposure	Effects	Author(s)
33 dry cleaners and ironers, self control (follow-up of Cavalleri et al., 1994, subjects)	Group A, 4 ppm, PM Group B, 0.7 ppm, PM	Worse CCI in subjects who experienced a higher mean TWA exposure than that reported in Cavalleri et al. No impairment in CCI in subjects who experienced a lower mean TWA exposure than that reported in Cavalleri et al.	Gobba et al. (1998)
30 patients with solvent-induced encephalopathy	Several solvents, including tetrachloroethylene, 20 years	No peripheral neuropathy	Albers et al. (1999)
Apartment residents living above dry cleaning facilities: 17 exposed and 17 age- and gender-matched unexposed controls	0.4 ppm (mean, monitoring taken before closure of dry cleaners) IA, PM, B, U Duration of residence, 6 years (mean)	No effects on visual acuity or color discrimination (comparison of group means) Lower (worse) scores on tests of visual contrast sensitivity	Schreiber et al. (2002)
Employees of a day care facility located in the same building as a dry-cleaning business, 9 exposed and 9 age- and gender-matched unexposed controls	0.32 ppm (mean, monitoring taken before closure of dry cleaners) IA No information on duration of employment	No effects on visual acuity color discrimination (comparison of group means) Lower (worse) scores on tests of visual contrast sensitivity	Schreiber et al. (2002)
14 dry cleaners exposed to tetrachloroethylene, 27 non-exposed (Control 1) and 27 support staff of Universiti Kebangsaan Malaysia (Control 2)	No exposure information presented in paper other than tetrachloroethylene was used for dry cleaning	No effect on color vision using Ishihara plates. 43% and 93% of dry cleaners compared to no controls had errors on the color vision D-15 test and FM 100 Hue test, respectively. Dry cleaners had more errors on FM 100 Hue than control group 2 ($p < 0.05$) but not control group 1.	Sharanjeet-Kaur et al. (2004)

Table 4-4. Summary of human neurotoxicology studies (continuted)

Subjects	Exposure	Effects	Author(s)
65 households (67 adults and 68 children) in residential buildings with co-located dry cleaners, 61 households (61 adults and 71 children) in residential buildings without dry cleaners	Geometric mean = 5 ppb (0.005 ppm), IA Duration of residence, 10 years (mean)	Association ($p < 0.05$) between tetrachloroethylene concentrations in indoor air and in blood and performance on test of visual contract sensitivity in children. No association observed in adults. Color vision impairment ($p < 0.05$) among children but not adult exposed subjects as compared with controls	NYS DOH (2005a), McDermott et al. (2005)
4-year follow-up of 13 former students, 13 children matched to exposed children on age, gender, and daycare experience	Exposure had ceased 4 years earlier.	No difference in visual function (VCS, color vision) or neurobehavioral function between exposed children and controls.	NYS DOH (2005c)
88,820 births from 1964–1976 identified from the Jerusalem Perinatal Study and linked to Israel's national Psychiatric Registry for hospitalization with a schizophrnia-related diagnosis as of 1-1-98.	Occupation of mother and father on birth certificate as dry cleaner.	Four cases were identified in 144 offspring of dry cleaners. Unadjusted relative risk (RR) of 3.4 (95% CI = 1.3–9.2) for schizophrenia in the offspring of dry cleaners using proportional hazard modeling. Control for a number of potentially confounding variables did not lead to appreciable different RR estimates.	Perrin et al. (2007)

B = Biological monitoring of blood
IA = Indoor air
PM = Personal monitoring of breath
U = Biological monitoring of urine for trichloroacetic acid
VCS = visual contrast sensitivity

Additionally, other reports (Laslo-Baker et al., 2004; Till et al. 2001a, b, 2005) suggest a vulnerability of the fetus to organic solvent exposures, including tetrachloroethylene exposure. Deficits in neurobehavioral parameters and in visual system functioning in young children of mothers exposed during pregnancy were observed in these reports. These reports are not fully discussed in this section. Case series help identify target organ toxicity and can support generating hypotheses; however, the lack of information in an unexposed population in these types of studies limits the ability to infer observations reported among cases to other populations.

Most human data on tetrachloroethylene exposure and nervous system effects—from cross-sectional or prevalence studies—is of dry cleaner and laundry workers and assessment of neurobehavioral effects; one report on neurobehavioral effects is of residents living in close proximity to a dry cleaning establishment. Three studies assessed the visual system; two reports of the same population are of dry cleaner and laundry workers, and one report is of two populations living or working in a building co-located with a dry cleaning establishment. Few studies are available on neurologic diseases such as Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease and organic solvents (IOM, 2002), and none of these reports uniquely assess tetrachloroethylene.

Tetrachloroethylene concentrations reported in the dry cleaning and laundry worker studies ranged from an 8-hr TWA mean of 6 ppm for dry cleaner and laundry workers in Cavalleri et al. (1994) to an 8-hr TWA of 41 ppm for operators of wet-transfer dry cleaning machine in Echeverria et al. (1995). Tetrachloroethylene concentrations reported for exposed residents were 0.4 ppm (mean) to residents living in an apartment building containing an operating dry cleaning business (Schreiber et al., 2002) and 0.7 ppm (mean) in indoor air of residents in close proximity to a dry cleaning business (Altmann et al., 1995).

4.6.1.1. *Environmental Chamber Studies*

4.6.1.1.1. ***Stewart, R.O., E.D. Baretta, H.C. Dodd and T.R. Torkelson. 1970. Experimental human exposure to tetrachloroethylene. Arch. Environ. Health. 20:225–229.*** In a study by Stewart et al. (1970), 12 healthy adults were exposed to 100 ppm for 7 hrs; eye and nose irritation was reported by 60% of the subjects, a slight frontal headache by 26%, slight light-headedness by 26%, feeling slightly sleepy by 40%, and difficulty in speaking by 25%. Some of these complaints were made during the first 2 hrs. Of five healthy men exposed to 100 ppm for 7 hrs/day on 5 consecutive days, one reported a mild frontal headache during each exposure and two consistently reported mild eye and throat irritation. Other symptoms were not reported, and individual responses during exposures to 0 ppm were not assessed. Subjects reported that their ability to detect the odor of tetrachloroethylene decreased during the course of daily and weekly exposure.

Three tests of equilibrium (a modified Romberg test,[2] where an individual stands on one foot with eyes closed and arms at side; a heel-to-toe test; and a finger-to-nose test) were performed every 60 minutes during each day of exposure. After 6 hrs, neurobehavioral tests of motor function (the Crawford manual dexterity and Flanagan coordination tests), cognitive function (arithmetic test), and motor/cognitive function (inspection test) were also performed. Three of the subjects had increased difficulty in maintaining their equilibrium when tested within the first 3 hrs of exposure (i.e., performance on the Romberg equilibrium test was impaired). The three subjects were able to perform the test normally when given a second chance. Performance on the other tests was not impaired. An additional subject, exposed during the third day of testing, showed a slight deterioration in his Romberg test and complained of slight dizziness and slight impairment of his intellectual faculties after 1 hr of exposure. No improvement in his Romberg test occurred during the next hour, and he was removed from the test chamber. The subject performed the test normally when retested 30 minutes later.

The investigators concluded that there were CNS effects in some subjects exposed to 100 ppm and that there exists a large range of individual susceptibility to tetrachloroethylene. The latter conclusion was based on the observations that only 3 of 17 subjects reported light-headedness and 4 of 17 subjects had an abnormal Romberg test.

4.6.1.1.2. ***Hake, C.L., and R.D. Stewart. 1977. Human exposure to tetrachloroethylene: inhalation and skin contact. Environ. Health Perspect. 21:231–238.*** As part of a 6-week study, four healthy men were exposed 7.5 hrs/day to 0 ppm (2 days in week one, 1 day in week three, and 2 days in week six), 21 ppm (4 consecutive days in week three), 100 ppm (5 consecutive days in week two), and a TWA of 100 ppm (5 consecutive days in week four) when exposure levels were more than 53, 100, or 155 ppm (5 consecutive days during week five). In addition, four healthy women were exposed to 100 ppm for 7.5 hrs/day on 5 consecutive days and to 0 ppm on 2 days. A fifth woman became sick with influenza during the study and was exposed to 100 ppm on only 2 days.

All subjects were cautioned to either abstain from the use of alcohol and drugs (or to limit their use to very moderate amounts) and they were asked not to drink coffee until 1 hr after the end of each exposure period. The subjects were told that they would be exposed to various concentration of tetrachloroethylene, but they were not told their sequence of exposures (a single-blind protocol). All subjects were sedentary during exposure except that men exercised on a bicycle ergometer for 6 minutes at 1 and 5 hrs of exposure.

[2] The Romberg test measures CNS depression.

Reports of symptoms (e.g., headache) varied among individuals but, overall, complaints during exposures were similar to those during exposures to 0 ppm of tetrachloroethylene. All subjects were able to detect the odor of tetrachloroethylene at all levels of exposure immediately upon entering the chamber; thereafter, they varied in their ability to detect odors. Some subjects retained the ability to detect odors during the entire experimental period, particularly at 155 ppm. A few other subjects detected no odor after the first hour of the first day.

The evaluation of EEG recordings made during exposure suggested altered patterns indicative of cortical depression in three of four men and four of five women exposed to 100 ppm (constant or TWA). In five subjects, altered EEG recordings occurred during hours 4 to 7 of exposure; another subject had altered recordings within 10 minutes of exposure, which gradually returned to normal during continued exposure, and the seventh subject showed changes between 30 minutes and 6–7 hrs of exposure. Recordings of VEPs in response to bright flashes of light (i.e., neurophysiological measurements of the electrical signals generated by the visual system in response to visual stimuli) and equilibrium tests (Romberg and heel-to-toe) were normal in men and women.

The performance of men on neurobehavioral tests of cognitive function (arithmetic), motor function (alertness), motor/cognitive function (inspection), and time estimation were not significantly affected by any exposure. The performances of men on a second test of motor function (Flanagan coordination) were significantly decreased ($p < 0.05$) on 1 of 3 days during each of 2 weeks of exposure to 100 ppm and on 2 of 3 days during the week of exposure to 155 ppm, but the investigators concluded that only the results at 155 ppm were related to tetrachloroethylene. In women, alertness (the only neurobehavioral endpoint evaluated) was not affected by exposure to tetrachloroethylene.

The study authors concluded that (1) there is considerable interindividual variation in response to tetrachloroethylene vapors, (2) EEG analysis indicates preliminary signs at narcosis in most subjects exposed to 100 ppm for 7.5 hrs, (3) impairment of coordination may occur in subjects exposed to 155 ppm for 7.5 hrs, and (4) the effects are likely due to tetrachloroethylene itself, given its slow metabolism in humans. They also reported that their data suggested that a threshold limit value of 100 ppm contains no margin of safety for susceptible subjects—both subjectively and neurologically—to the vapors of tetrachloroethylene, a surprising finding, given the study's sample size.

4.6.1.1.3. ***Hake, C.L., R.D. Stewart, A. Wu, J. Kalbfleisch, P.E. Newton, S. K. Marlow and M. Vucicevic-Salama. 1977. Effects of perchloroethylene/drug interaction on behavior and neurological function. DHEW (NIOSH) Publ. No. 77-191.*** Stewart et al. (1977) conducted a complex study with 12 healthy adults (6 men and 6 women) on the behavioral effects of inhaled

tetrachloroethylene and oral doses of alcohol or Valium. Individuals were typically exposed for 6.5 hrs to 0 ppm on Monday or Tuesday, 100 ppm on Wednesday and Friday, and 25 ppm on Thursday during each of the 11 weeks of exposure and were given a placebo capsule, alcohol, Valium, or nothing during each period. Numerous neurological tests were performed throughout each exposure, and all testing was done in a double-blind mode (neither the testers nor the subjects were told the exposure level).

Exposure to 25 or 100 ppm of tetrachloroethylene for 6.5 hrs did not increase the overall prevalence of reported symptoms (e.g., headache) or alter the subjects' mood. There were exposure-related increases in the strength and persistence of the tetrachloroethylene odor perceived by the subjects. Exposure did not significantly reduce performance on two equilibrium tests (Romberg and heel-to-toe) and two neurobehavioral tests of motor function (Michigan eye-hand coordination test and rotary pursuit test). At 100 ppm (but not 25 ppm) there was a significant decrease ($p < 0.05$) in scores on a third test of motor function (Flanagan coordination test) on some days of exposure. Statistical analyses performed by the investigators revealed no effect of tetrachloroethylene exposure alone on EEGs and no significant interactive effects between tetrachloroethylene and either alcohol or Valium.

The study authors reported that exposure to 100 ppm tetrachloroethylene did not have a significant consistent effect on performance, although it did have a significant but inconsistent detrimental effect on the performance of the Flanagan coordination test (given during the 3rd and 4th hrs of exposure).

4.6.1.1.4. ***Altmann, L., A. Bottgor and H. Wiegand. 1990. Neurophysiological and psychophysical measurements reveal effects of acute low-level organic solvent exposure in humans. Int. Arch. Occup. Environ. 3:493–499. Altmann, L., H. Wiegand, A. Bottger, F. Eistwmelor and G. Winneke. 1992. Neurobehavioral and neurophysiological outcomes of acute repeated perchloroethylene exposure. Appl. Psych. 41:269–279.*** This study, conducted in Germany, reports intentional inhalation exposure of human subjects to tetrachloroethylene for the purpose of measuring potentially adverse health outcomes. No information is provided about ethical principles espoused by the U.S. government for exposure to human subjects. Therefore, the principal author was contacted by EPA staff (e-mail communication from R. McGaughy, EPA to L. Altmann, October 8, 2003). Information was requested regarding procedures that were used to select the subjects and inform them about the nature of the exposure, institutional procedures that were taken to review the design of the study, and ethical standards and guidelines that the institution was operating under at the time of the study. No response had been received by EPA staff as of October 19, 2004. Although the report is not of crucial importance in the

evaluation of chronic neurotoxic effects of tetrachloroethylene, the scientific and ethical issues associated with intentional dosing of human subjects is of importance to EPA.

Altmann et al. (1990, 1992) used neurophysiological and neurobehavioral techniques to evaluate the neurological effects of tetrachloroethylene on healthy adults exposed to 10 ppm or 50 ppm for 4 hrs on 4 consecutive days. All subjects denied prior occupational exposure to solvents and drug use at the time of the study. Visual acuity of all subjects was normal or corrected to normal. The study was a single-blind study (subjects were not told their level of exposure), and subjects were randomly assigned to either group. Sixteen subjects were exposed to 10 ppm and 12 subjects were exposed to 50 ppm, but neurophysiological measurements were made on only 22 subjects (12 at the low level and 10 at the high level). There was no unexposed control group.

Three neurophysiological measurements were taken on the day before exposure started and on each of the four exposure days: (1) VEPs in response to black-and-white checkerboard patterns were measured; the VEPs of some subjects (exact number not reported) were also measured on the day after exposure ceased; (2) a visual contrast sensitivity (VCS) test (a test of the central spatial vision that determines the minimum contrast necessary for an individual to see patterns of various sizes) was given to five subjects (three from the low-exposure group and two from the high-exposure group); (3) recordings of brainstem auditory-evoked potentials (neurophysiological measurements of the electrical signals generated by the hearing system in response to auditory stimuli) were made to evaluate peripheral hearing loss. All measurements were started 2 hrs after a subject entered the chamber and were completed within 1 hr.

A German version of the Neurobehavioral Evaluation System was used to assess motor, motor/cognitive, and cognitive function of subjects. The battery included nine tests (finger tapping, eye-hand coordination, simple reaction time, continuous performance, symbol digit, visual retention, pattern recognition, digit span, and paired associates). A vocabulary test and a test of emotional state (moods) were also given. Each subject was assessed with a complete battery of tests during the pre-exposure baseline assessment and at the end of the study. Subsets of the battery covering motor function and mood were given repeatedly at the beginning and end of each 4-hr exposure period.

Tetrachloroethylene was not detected in blood samples collected before the start of the first exposure period. The detection limit was less than 0.0005 mg/L. Mean tetrachloroethylene blood levels increased slightly over the 4-day period. Among subjects exposed to 10 ppm, mean blood levels were 0.33, 0.36, 0.4, and 0.38 mg/L at the end of days one, two, three, and four of exposure, respectively. Among subjects exposed to 50 ppm, mean blood levels were 1.1, 1.2, 1.4, and 1.5 mg/L at the end of days one, two, three, and four of exposure, respectively.

On the first day of testing, a faint solvent odor was reported by 33% and 29% of the subjects exposed to 10 ppm and 50 ppm, respectively. On the fourth day, these incidences changed to 17% and 36%, respectively. The VEP latencies of subjects during the 3rd hr of exposure to 50 ppm on days one, two, three, and four of exposure were significantly longer ($p < 0.05$) from those measured on the control day, and the differences became progressively longer on successive exposure days. One set of VEP latencies on the day after the end of the exposure period remained longer than the control day values (statistical significance not reported). VEP latencies in subjects with exposure to 10 ppm were not statistically significantly longer than those recorded on the control day. There were significant differences ($p < 0.05$) between the VEP latencies of subjects exposed to 10 ppm and those exposed to 50 ppm.

Data on contrast sensitivity indicated greater effects at 50 ppm than at 10 ppm; effects were most pronounced on the last day of exposure. However, statistical analysis was not reported, and the data are limited by the small number of subjects. There were no indications of peripheral hearing loss at either exposure level.

Neurobehavioral tests results were reported for only those tests given repeatedly on 4 consecutive days (finger tapping, eye-hand coordination test, simple reaction time, continuous performance, and moods). There were significant post-exposure performance deficits (p 0.05) among subjects exposed to 50 ppm when compared with the group exposed to 10 ppm in tests of motor/cognitive function (continuous performance test for vigilance) and motor function (eye-hand coordination), and a near-significant difference ($p = 0.09$) on a test of motor function (simple reaction time). In all cases, the degree of improvement shown by the subjects exposed to 50 ppm was less than that shown by the subjects exposed to 10 ppm. There were no exposure-related effects on the finger-tapping or moods test.

The study authors concluded that visual function in healthy, young, adult males is mildly affected by tetrachloroethylene exposures to 50 ppm maintained for 4 hrs on each of 4 days (Altmann et al., 1990), and they stated that the impaired performance on tests of motor/cognitive and motor function suggests that 50 ppm cannot be considered a NOAEL for neurobehavioral end-points indicative of CNS depression (Altmann et al., 1992).

4.6.1.2. *Chronic Exposure Studies*

4.6.1.2.1. ***Lauwerys, R., J. Herbrand, J.P. Buchet, A. Bernard and J. Gaussin. 1983. Health surveillance of workers exposed to tetrachloroethylene in dry-cleaning shops. Int. Arch. Occup. Environ. Health. 52:69–77.*** Lauwerys et al. (1983) studied 26[3] workers (24 women and 2 men) occupationally exposed to tetrachloroethylene in six dry cleaning shops in Belgium for a mean of

[3] Abstract of paper reports 22 subjects were exposed to perchloroethylene.

6.4 years. The authors evaluated potential effects on the CNS, the kidney, the liver, and the lungs in these workers and in controls (31 women and 2 men) working in a chocolate factory (20) or an occupational health service (13) who did not report occupational exposure to organic solvents. No information is provided in the paper on the methods used to identify subjects or their reasons for participating in the study. Several characteristics of the two groups were similar (sex ratio, mean age [32.9 vs. 34.5 years], and level of education). However, 13 of the 26 dry cleaning workers—but only 9 of the 33 controls—were smokers. Neurobehavioral tests of motor function (simple and choice reaction time), sensory function (critical flicker fusion), and cognitive function (sustained attention test) were given twice to each worker, once before work and once after work. Both groups were tested in the middle of the work week. Individuals also were questioned about chronic symptoms related to nervous system disturbances. Blood samples were collected both before and after work. Urine samples for kidney function tests were collected after work.

The mean tetrachloroethylene air concentration (8-hr TWA) was 21 ppm and the range of TWA values was 9 to 38 ppm, using results from active sampling of personal air. The mean tetrachloroethylene blood level (30 minutes after the end of work) was 1.2 mg/L (range of means from the shops was 0.6 to 2.4 mg/L). There was no significant connection between air concentrations and blood levels. Trichloroacetic acid, a metabolite of tetrachloroethylene, was not detected (LOD not identified in published paper) in urine specimens from exposed subjects.

Seventeen of 22 symptoms related to nervous system disturbances were reported by study investigators as being more prevalent among the workers than among unexposed controls. Although statistical testing was conducted, the study investigators did not describe statistical methods or tests in the published paper. The investigators reported no statistically significant differences in prevalences for most symptoms and no relationships with duration of exposure. Lauwerys et al. (1983) reported more complaints in the exposed group than in control workers, particularly memory loss (7/26 exposed vs. 3/33controls) and difficulty falling asleep (11/26 exposed vs. 6/33 controls). EPA analysis of the data found the latter complaint to be statistically significant using Fisher's exact test ($p = 0.04$). The mean cycles/second, the score of the critical flicker fusion test (a test of sensory function) was significantly increased (better performance) in the exposed group than in controls when given both before work and after work. The dry cleaning workers did not differ from controls on the other three neurobehavioral tests. The prevalence of abnormal scores (those beyond the 5^{th} or 95^{th} percentile of the control group) did not vary significantly between the two groups.

4.6.1.2.2. ***Seeber, A. 1989. Neurobehavioral toxicity of long-term exposure to tetrachloroethylene. Neurotoxicol. Teratol. 11:579–583.***[4] Seeber (1989) evaluated the neurobehavioral effects of tetrachloroethylene on 101 German dry cleaning workers (machine operators, ironers, touch-up workers, counter attendants, and other employees) who were employed in coin-operated or while-you-wait shops, all affiliated with one organization. The workers were separated into a low-exposure group (50 women, 7 men) and a high-exposure group (39 women, 5 men). A third group of 84 sales personnel (64 women, 20 men) from several department stores and receptionists from large hotels served as unexposed controls. No information was provided on the methods used to identify subjects or their reasons for participating in the study, although the authors reported that 29 service technicians were excluded from the study because of either discontinuous exposure conditions with peak concentrations or long periods of no exposure. Predominant characteristics of both groups included primarily standing work, contact with customers, and moderate physical exercise. The mean ages of the low-exposure, high-exposure, and control groups were 38.2, 38.4, and 31.8 years, respectively.

Details on air monitoring methods were sparsely reported, but mean tetrachloroethylene concentrations (8-hr TWA) for the low- and high-exposure groups were 12 ($\pm$8) ppm and 53 ($\pm$17) ppm, respectively, using results from active sampling of room air and passive sampling of personal air. The mean duration of occupational exposure for the low- and high-exposure groups was 11.8 and 10.6 years, respectively.

A number of tests of neuropsychological functioning were administered, including standardized tests of symptoms and personality; tests of sensorimotor function, including finger tapping and aiming; and the Mira and Santa Ana dexterity tests, which are published standardized tests. Threshold of perceptual speed was assessed by recognition of stimuli flashed briefly on a screen; whether this procedure used a standardized instrument was not noted. Choice reaction time was also determined using "nine light and tone stimuli." It is not clear whether the auditory and visual stimuli occurred together or whether some trials consisted of an auditory stimulus and others a visual stimulus. Details of the timing of the stimulus presentation were not provided. One of the response variables, "delayed reactions," was not defined. The typical dependent variable measured in this task—response reaction time—apparently was not measured; only the number of correct reactions was reported. Subtests of the Wechsler Intelligence Test (digit span, digit symbol, and cancellations) were used, as was recognition of words, faces, and digits. The instrument used and the scoring of the last three tests were not

[4] Dr. Seeber provided additional information on this study in written correspondence to the New York State Department of Health dated January 19 and May 20, 1996. This information appears in NYS DOH (1997).

described. Intelligence was assessed using the logical thinking subtest of the German Performance Test System.

Each subject was examined during a 1.5-day stay at a clinic located at a large institute for occupational medicine. Each subject came to the clinic in the evening hours, stayed overnight, and started the examination and testing process the next morning. The clinic examined numerous people daily, and the dry cleaners and the control group took up only a small part of the daily routine of the clinic staff. Neurobehavioral tests were given by two specialized clinic staff who did not question the subjects about exposure status. However, clinic psychologists (six at the time of the study) did inquire about the exposure and living conditions of the subjects. Because the dry cleaner groups and the control group differed in gender ratios, age, and scores on the intelligence test, stratified analysis was used to statistically control the influence of these confounding factors on test scores. As discussed in the section that describes Altmann et al. (1995), the use of dichotomous or categorical variables may not fully control for confounding effects of these factors on the endpoint. The groups also differed in alcohol consumption, so a separate analysis was used to examine the role of alcohol on effects associated with tetrachloroethylene.

Performance of both the low-exposure and high-exposure groups differed significantly ($p < 0.01$) from that of the unexposed control group on the threshold of perceptual speed and "delayed responses" on a choice reaction time task, both of which are measures of information processing speed ($p = 0.08$ and 0.03 for low exposure and high exposure, respectively). Both exposed groups also had worse scores ($p < 0.01$) on two tests of attention (digit reproduction and digit symbol) and on visual scanning (cancellations). Group mean scores for digit reproduction and digit symbol did not appear to increase from the low-exposure to the high-exposure group. The low-exposure group also showed significantly higher scores than did the control group on questionnaires, on neurological signs ($p < 0.01$), and emotional liability ($p < 0.05$). Scores of the high-exposure group for these measures appeared higher than those for the control group; however, the scores did not show a statistically significant difference. There were no differences between groups on the other tests. Controlling for group differences in alcohol consumption did not alter any test results.

4.6.1.2.3. ***Cai, S.X., M.Y. Huang, Z. Chen, Y.T. Liu, C. Jin, T. Watanabe, H. Nakatsuka, K. Seiji, O. Inoue and M. Ikeda. 1991. Subjective symptom increase among dry-cleaning workers exposed to tetrachloroethylene vapor. Ind. Health. 29:111–121.*** Cai et al. (1991) evaluated the CNS effects of tetrachloroethylene exposure among 56 dry cleaning workers (27 women and 29 men) from three shops in China. The control group (37 women and 32 men) were of similar mean age (34 years vs. 35 years for dry cleaning workers), but the male dry cleaning workers

were 4 years younger than the male controls and the women were 4.9 years older than the female controls. The controls were recruited from the same factories as the dry cleaning workers but from workshops without known solvent exposures. No information is provided in the paper on the methods used to identify subjects or their reasons for participating in the study. Further, no information was provided on test procedures or the questionnaire used to assess subjective symptoms. The geometric mean tetrachloroethylene air concentration (8-hr TWA) was 20 ppm and the range of TWA values was 4 to 97 ppm, using results from passive sampling of personal air. The mean duration of occupational (tetrachloroethylene) exposure was 3 years.

The prevalence of symptoms of tetrachloroethylene exposure was significantly higher among the dry cleaning workers (men, women, and men and women combined; $p < 0.001$) than among the unexposed controls. Five symptoms (dizziness, drunken feeling, floating sensation, a heavy feeling in the head, and facial flushes) in men and women (combined) were significantly more prevalent in the dry cleaning workers than in the controls ($p < 0.001$). Nasal irritation and unusual smell were also reported significantly more often by the dry cleaning workers than by controls ($p < 0.05$). Similar findings were reported when the workers were asked about the symptoms they had noticed during the 3 months before the study. The investigators found exposure-related increases in the prevalence of subjective symptoms among dry cleaning workers exposed to 21 ppm (8-hr TWA).

4.6.1.2.4. ***Nakatsuka, H., T. Watanabe, Y. Takeuchi, N. Hisanaga, E. Shibata, H. Suzuki, M.Y. Huang, Z. Chen, Q.S. Qu and M. Ikeda. 1992. Absence of blue-yellow color vision loss among workers exposed to toluene or tetrachloroethylene, mostly at levels below occupational exposure limits. Int. Arch. Occup. Environ. Health. 64:113–117.*** Nakatsuka et al. (1992) evaluated the effects of tetrachloroethylene exposure on the color vision of 64 dry cleaning workers (34 women and 30 men) in China. The workers were from the same shops studied by Cai et al. (1991). Control workers (72 women and 48 men) were recruited from the clerical sections of dry cleaning shops and from other factories (paint production plants or plants producing tetrachloroethylene from trichloroethylene). No information is provided in the paper on the methods used to identify subjects or their reasons for participating in the study. The mean ages of the dry cleaning workers (34 years for men, 35 years for women) were lower than those of the controls (34 years for men, 33 years for women). A screening color test (Lanthony's new color test) and a test used for confirmation of red-green vision loss were carried out by ophthalmologists or occupational health doctors in charge of the factories under one of two lighting conditions (natural sunlight or a daylight fluorescent light). The published report does not identify what procedure was used on which test; illumination is a critical component in administering color vision tests to subjects (Geller and Hudnell, 1997).

The geometric mean air concentrations of tetrachloroethylene (averaging time not reported) were 16 and 11 ppm for the men and women, respectively, using results from passive sampling of personal air. The overall geometric mean was 13 ppm.

There was no significant difference in the performance of the dry cleaning workers and unexposed controls on Lanthony's new color test. The study authors reported that the percentages of dry cleaning workers who correctly separated colored caps from monochromatic caps were not significantly different from the percentages in the corresponding control group. A statistical analysis of these data reported in public comments of the Halogenated Solvents Industry Alliance to EPA (HSIA, 2004) on the Neurotoxicity of Tetrachloroethylene Discussion Paper (U.S. EPA, 2003b) showed—using a chi-square test for differences in proportions—that tetrachloroethylene-exposed women were more likely to have normal color vision as compared with unexposed women. An EPA analysis of male workers did not show any differences, either better color vision or worse color vision, in exposed males compared with unexposed male controls. Nakatsuka et al. concluded, overall, that they found no distinct case of color vision loss among the dry cleaning workers.

4.6.1.2.5. ***Ferroni, C., L. Selis, A. Mutti, D. Folli, E. Bergamaschi and I. Franchini. 1992. Neurobehavioral and neuroendocrine effects of occupational exposure to perchloroethylene. Neurotoxicol. 13:243–247.***[5] Ferroni et al. (1992) evaluated neuroendocrine and neurobehavioral effects of tetrachloroethylene exposure among 60 female dry cleaners and 30 unexposed female controls who were comparable in age (mean ages 39.7 and 37.6 years, respectively) and vocabulary level. Each dry cleaning shop in a small town outside of Parma, Italy was visited. The workers were invited to participate in the study, which was part of a preventive health program implemented by the local health office and professional associations of small businesses. There were no refusals. Controls were selected from the workers at a hospital who cleaned clothes using a water-based process. Their jobs were essentially the same as those of the dry cleaners, but they were not exposed to any organic solvents. Both groups filled out a questionnaire on their health status, medication (including oral contraceptives), lifestyle, and current and past jobs. Both groups met the following criteria: no history of metabolic disorders, no history of psychiatric disorders, and low level of daily alcohol intake. The two groups were similar in height, weight, body mass index, smoking habits, and use of medication, but alcohol intake was about 5% higher ($p < 0.03$) in the control group than in the dry cleaner group.

[5] Dr. Mutti provided details on the selection process of exposed and control subjects and also clarified reported results to Dr. Ken Bodgen, New York State Department of Health, in written correspondence dated July 29 and September 5, 1995 (see NYS DOH, 1997).

Workers and controls were given five neurobehavioral tests (part of the Swedish Performance Evaluation System, "adapted" Italian version: finger tapping with both dominant hand and nondominant hand, simple reaction time, digit symbol test, shape comparison-vigilance, and shape comparison-response to stress). All subjects were examined in the morning before their work shift in the same room by the same examiners (NYS DOH, 1997). The tests were part of a computer-based battery, and the same machines and software were used to administer the tests and score the results. The same sequence of tests and protocols were used for all subjects. Although the examiners were not blind to the status of the subjects (dry cleaner or control), they were blind to the worker's exposure level (NYS DOH, 1997). Serum prolactin levels were measured in all subjects; blood samples were collected during the working day during summer and winter. Prolactin secretion by the pituitary is under control by hypothalamic dopamine; dopamine is also important to neurotransmitter systems.

One proposed alternative for assessment of nervous system toxicity is the study of biochemical signals in peripheral tissues as biomarkers of nervous system function (Manzo et al., 1996). Samples from dry cleaners and controls were alternated and analyzed in the same experimental runs. For women, only those samples obtained during the proliferative phase of the menstrual cycle were used for comparison between groups (41 dry cleaners and 23 controls).

Workplace air samples were randomly collected throughout the work week during summer and winter to account for variability related to either the work cycle or seasonal environmental fluctuations. The median tetrachloroethylene air concentration (4-hr TWA) was 15 ppm and the range of TWA values was 1 to 67 ppm. The subjects' range of tetrachloroethylene blood levels was 0.012 to 0.864 mg/L (median = 0.145 mg/L; incorrectly expressed in Ferroni et al., 1992, as 12,864 and 145 mg/L [NYS DOH, 1997]). The mean duration of occupational exposure was 10 years.

The dry cleaners showed significantly reduced performance when compared with the unexposed matched controls in three tests (simple reaction time, $p < 0.0001$; vigilance, $p < 0.005$; and stress, $p < 0.005$), as reported by Ferroni et al. (1992). Performance on the finger-tapping test (both hands) and digit symbol test was not affected (NYS DOH, 1997). Additionally, the mean serum level of prolactin was significantly higher in the workers than in the matched controls ($p < 0.001$). None of the three measures of exposure (duration of exposure and air or blood concentration of tetrachloroethylene) was significantly associated with decreased test scores or increased serum prolactin levels among the dry cleaners.

The study authors concluded that tetrachloroethylene exposure in dry cleaning shops may impair performance and affect pituitary function but that the cross-sectional design prevented distinguishing acute effects from chronic effects. Ferroni et al. (1992) also reported that the most likely bias of cross-sectional studies is a spontaneous selection of the sample (i.e., workers who

believe exposure is making them sick or workers who actually become sick may quit work prematurely and not be included in the study) and, as a result, the actual risk was likely to be underestimated rather than overestimated, although no data are presented in the paper with which to evaluate this statement. On the other hand, the exposed and unexposed study population of women was tested during the proliferation phase of menstruation, which may better capture changes in prolactin secretion, but also may potentially confound findings if there are individual differences in severity of menstruation and in the timing of test session relative to the day of menstruation (U.S. EPA, 2004).

4.6.1.2.6. ***Cavalleri, A., F. Gobba, M. Paltrinieri, G. Fantuzzi, E. Righi and C.L. Aggazzoti. 1994. Perchloroethylene exposure can induce colour vision loss. Neuroscience Lett. 179:162–166.[6] Gobba, F., E. Righi, G. Fantuzzi, G. Predieri, L. Cavazzuti and G. Aggozzotti. 1998. Two-year evaluation of perchloroethylene-induced color-vision loss. Arch. Environ. Health 53:196–198.*** Cavalleri et al. (1994) reported on a control-matched, cross-sectional, observational, occupational study that evaluated the effects of tetrachloroethylene exposure on the color vision of dry cleaners. The investigators compiled a list of all the dry cleaning shops in the municipality of Modena, Italy, (110 shops employing 189 workers) and randomly selected 60 dry cleaners from 28 premises for recruitment into the study (Aggazzotti et al., 1994a). Only full-time workers (n = 52) were asked to participate, and two declined. No information is provided in the paper on a subject's motivation for participating or not participating in the study. All 50 workers provided, via questionnaires, information on work history, health status, occupational and hobby use of solvents, drinking and smoking habits, and drug use. Thirty-five of the 50 dry cleaners (33 women, 2 men) met the inclusion criteria; others were excluded for hypertension, smoking more than 30 cigarettes a day, alcohol consumption exceeding 50 g of alcohol a day, oculo-visual pathology, or working less than 1 year. Another worker was excluded because a matched control could not be found.

The controls were factory workers who were not occupationally exposed to solvents or other neurotoxic chemicals; they were selected and recruited into the study using the same methods that were used for dry cleaners. The controls (n = 35) were from factories in the Modena area and met the same inclusion criteria as the dry cleaners. They were matched to dry cleaners by gender, age ($\pm$3 years), alcohol consumption ($\pm$10 g/day), and cigarette use ($\pm$5 cigarettes a day). The mean age of both groups (35 years) and the percentages of each group that were smokers (43%) or alcohol drinkers (71%) were comparable.

[6] Dr. Cavalleri provided additional information on this study in written correspondence to the New York State Department of Health dated October 8, 1996 (see NYS DOH, 1997).

All subjects appeared healthy and met minimal status of visual acuity. None of the subjects reported hobby exposure to solvents or other substances toxic to the eye. There were no known systematic differences between exposed and control groups or between machine operators and ironers.

Color vision was assessed using Lanthony's D-15 desaturated panel test, in which subjects are asked to put a series of small round "caps" in order by color. The types of errors made can distinguish specific types of color vision deficiency; e.g., red-green color confusion errors (blindness) is a common condition in males, mostly but not entirely of congenital origin, whereas blue-yellow color confusion errors are very rarely due to congenital conditions and therefore are considered as a hallmark of an acquired condition. Impairments in color vision, beginning as blue-yellow confusion errors, have been reported in numerous populations exposed to organic solvents (Mergler and Blain, 1987; Mergler et al., 1987, 1988a, b, 1991; Campagna et al., 1995, 1996). Test scores are based on the ability of each subject to arrange a set of 15 caps colored with desaturated colors according to a definite chromatic sequence, with each mistake increasing the score above a perfect score of 1.00. A formula (the Color Confusion Index [CCI]) is used to calculate total errors.

Exposed and control subjects were tested in a random order (NYS DOH, 1997). All subjects were tested at the same time of day (in the morning, before work) under the same lighting conditions by the same investigator. With respect to exposed subjects, the investigator was unaware of both the exposure levels and the job (operator or ironer) of each dry cleaner.

For all dry cleaners, the mean tetrachloroethylene air concentration (8-hr TWA) was 6 ppm and the range of TWA values was 0.4–31 ppm, using results from passive sampling of personal air. For operators ($n = 22$), the mean air concentration 8-hr TWA was 7 ppm and the range of TWA values was 0.4–31 ppm. For ironers ($n = 13$), mean air concentration (8-hr TWA) was 5 ppm and the range of TWA values was 0.5–11 ppm. The mean duration of occupational exposure was 8.8 years. Tetrachloroethylene concentrations were also measured in alveolar air for a subset of these dry cleaners, with a high correlation observed between tetrachloroethylene concentration in alveolar air and the 8-hr TWA levels in ambient air ($r = 0.8$, $p < 0.001$; Aggazzotti et al., 1994a).

Only three dry cleaning workers, as opposed to 13 controls, scored a perfect test score ($p < 0.01$). Mistakes were made mainly in the blue-yellow range. Overall, the workers showed poorer performance on the test as compared to controls, and they had a significantly higher mean CCI using a Student's t-test ($p = 0.03$). The effect was statistically significant among operators but not among ironers. Study investigators also evaluated whether CCI values were normally distributed, which is important if using a Student's t-test, but they did not present any information about the result of the Kolmogorov-Smirnov test. The observation for ironers may

reflect a lower statistical power in this group due to fewer subjects (13 ironers vs. 22 operators). There also was a statistically significant positive correlation ($p < 0.01$) between TWA air concentrations and the CCI ($r = 0.52$), which remained after multivariate analysis considered previous tetrachloroethylene exposure duration, age, number of cigarettes a day, and daily intake of alcohol as covariates.

The effect on color vision may not be rapidly reversible; preliminary data showed that the scores of some workers did not improve when retested after 4 weeks of vacation (NYS DOH, 1997). Moreover, some of these workers showed poorer performance on this test in the follow-up study by Gobba et al. (1998), described below, suggesting color vision impairment as a chronic effect. The CCI values were not associated with two other measures of tetrachloroethylene exposure (mean duration and an integrated index of exposure, yearly TWA level). The study authors suggested that this may reflect the difficulty in controlling for the interactive effects of age and exposure and accurately evaluating exposure.

Gobba et al. (1998) reexamined color vision after a period of 2 years in 33 of the 35 dry cleaners and ironers examined by Cavalleri et al. (1994). Two subjects had retired during the 2-year period between examinations. These investigators used the Lanthony D-15 test, the test used by Cavalleri et al. (1994) to assess color vision, and performance was compared with the subject's score from the initial survey (self-control). Tetrachloroethylene concentration in the occupational setting was determined in the breathing zone using personal passive samplers. Monitoring was carried out during the afternoon shift, as Cavalleri et al. (1994) did not show any differences between morning and afternoon samples. Gobba et al. (1998) found that tetrachloroethylene concentration had increased during the 2-year period for 19 subjects, identified as Group A, (geometric mean, from 1.67 ppm at the first survey to 4.35 ppm at the second survey) and had decreased for 14 subjects, identified as Group B (geometric mean, from 2.95 ppm to 0.66 ppm). For the 33 workers overall, tetrachloroethylene concentration did not change over the 2-year period (geometric mean, from 2.4 ppm to 1.94 ppm at the second survey, $p > 0.05$).

Color vision deteriorated between the two surveys for the entire group, a reflection of the color vision loss among Group A subjects, whose exposure had increased by the second survey. As found in the first survey, color vision was impaired primarily in the blue-yellow range of color, with few subjects presenting a red-green deficit. Color vision performance for the entire group was related significantly to age ($r = 0.45$) and tetrachloroethylene concentration ($r = 0.39$). The mean CCI score for Group A subjects showed a statistically significant difference between the two surveys ($p < 0.05$). Analysis of variance methods that controlled for an effect of age further supported the finding of color vision deterioration among these subjects. For Group B subjects, who experienced lower exposure concentrations by the second survey, the CCI score

did not change from that of the initial survey. The findings in Groups A and B were also supported using analysis of variance methods that adjusted for age, alcohol consumption, or cigarette smoking between the subgroups.

4.6.1.2.7. ***Echeverria, D., N. Hyer, J.A. Bitner, H. Checkoway, G. Toutonghi and N. Ronhovde. 1994. Behavioral effects of low level exposure to perchloroethylene (PCE) among dry cleaners. In: Battelle Centers for Public Health Research and Evaluation. A behavioral investigation of occupational exposure to solvents: perchloroethylene among dry cleaners and styrene among reinforced fiberglass laminations. Final Report SSRC-10OM4/040. Seattle, WA: pp. 6.1–6.37.*** Echeverria et al. (1994) reported a study designed to evaluate a hypothesis in a previous study (Echeverria et al., 1995)[7] of frontal/limbic system effects[8] as the site underlying tetrachloroethylene pathology, where degradation in behavior may be the earliest indicator of acute, subchronic, or chronic neurotoxicity. The study was conducted in the Seattle/Tacoma, Washington area from 1989 through 1993, when the area's dry cleaning industry was switching from wet-transfer to dry-to-dry machines. Initially, 320 dry cleaning shops and laundries were sent introductory letters requesting permission to allow their employees to participate in the study. Of the 181 owners who responded, 39 agreed to participate. Of the owners who did not agree to participate, 22% expressed no specific reason, 19% cited time constraints, 17% feared legislative reprisal from federal agencies, 17% did not speak English, 15% were unavailable or never contacted, and 10% cited various other reasons.

Recruitment ended when a total of 45 operators were enrolled in the study (total $n = 173$). Each operator was matched with a less-exposed person from the same shop. The subjects included laundry workers ($n = 69$), pressers or counter clerks ($n = 59$), and operators or former operators ($n = 45$). The mean ages of the groups were 42.5, 34.2, and 46.2 years, respectively. Women comprised 63% of the study population (109/173). The subjects, who were paid volunteers, were eligible if they spoke English, had no history of diabetes or CNS disorders, and had worked for more than one year in the trade. The final sample excluded three subjects for limited knowledge of English and reading skills and six subjects for not wearing glasses or missing covariate information such as vocabulary performance on the test.

[7] Although published a year after this study, the study by Echeverria et al. (1995), discussed in Section 4.6.1.2.8, was conducted in 1986, 3 years before this study.

[8] Echeverria et al. (1994) hypothesized that exposure to solvents particularly affected attention, executive function, visuospatial skill, short-term memory, and mood, leaving motor, language-based skills, and long-term memory intact. The frontal system has been associated with executive function, such as that measured by Switching Attention, Trailmaking A and B, and the Wisconsin Card Sort. Tests associated with the limbic system include mood, as measured by the Profile of Mood States, short-term memory as measured by digit span, visual reproductions, and pattern memory.

An index of chronic exposure and measures of subchronic and acute exposure were developed for each subject. The chronic exposure index was based on a detailed work history, including consideration of the type of dry cleaning machine, job title, percentage of time at each job title, estimated air levels associated with each job title, and employment duration. The measures of subchronic and acute current exposure were based on mean 8-hr TWA air concentrations measured on the day of neurobehavioral testing. Mean chronic indices were zero for the never-exposed group of laundry workers, 68 for the dry cleaning workers with low exposure (pressers/clerks), and 1,150 for the dry cleaning workers with high exposure (operators). Mean exposures (8-hr TWA, using results from passive sampling of personal air) for workers placed in these chronic exposure categories were <0.2 ppm (laundry workers), 3 ppm (pressers/clerks), and 9 ppm (operators). Dry cleaning workers placed in the chronic exposure categories of low and high had been employed in their current job for 2.6 and 11 years, respectively.

The subjects also were placed in acute and subchronic exposure categories of <1 ppm (laundry workers and some dry cleaning workers, e.g., clerks), low (mainly pressers), and high (operators), with corresponding current tetrachloroethylene 8-hr mean concentrations of 0.5, 3, and 20 ppm. Dry cleaning workers placed in the low and high categories had been employed in their current job for 5 and 9 years, respectively. Because of the changes in dry cleaning practices over the course of the study, many subjects who were placed in the high chronic-exposure category, which was based on detailed work history, were frequently found in the low acute- or subchronic-exposure group, which was based on air concentrations on the day of testing.

The test battery included tests of cognitive function, including visuospatial ability, motor skills, mood, CNS symptoms, and basic verbal and arithmetic skills. The chronic and subchronic assessment was based on tests given during the morning of each subject's day off and on pre-shift scores. Additional tests considered to have an acute component were given 1 hr before work on the first day of the work week and again at the end of the day, allowing acute effects to be examined by using pre-shift performance to predict post-shift performance and then comparing predicted with observed performances. On their day off, the subjects were tested at home. At the work site, subjects were tested in a minivan. Each subject signed a consent form, provided a breath sample at each test session, and completed a questionnaire covering transient factors that could affect performance (e.g., headache). This was followed by questionnaires on medical history, medication, drug and alcohol use, occupational and nonoccupational exposure to chemicals, symptoms, and mood. The subject was then administered the neurobehavioral tests.

Multivariate analysis was used to evaluate the relationship between exposure indices and levels and performance on neurobehavioral tests after adjusting for the variables of age, gender, race, vocabulary level (surrogate for education and test-taking), and alcohol consumption. After

adjustment for those variables that were significant confounders, associations between increased indices of chronic (lifetime) exposure and reduced test performance were found in three tests of cognitive function: switching ($p = 0.1$), pattern memory ($p = 0.03$), and pattern recognition ($p = 0.09$). The magnitude of change attributable to tetrachloroethylene was a 3% loss in function for the latency of pattern memory and an 11% loss in function for the correct number in visual reproductions; losses in function that are well within pre-clinical values. Subjective measures of mood and symptoms were not significantly associated with exposure. Dry cleaning workers scored lower (but not significantly) on all but one of the remaining tests (the digit span test).

Analysis of the association between test scores and measures of subchronic exposure (8-hr TWA tetrachloroethylene concentrations on the day of testing) confirmed the findings of the chronic analysis: reduced scores on tests of switching ($p = 0.1$) and pattern recognition ($p = 0.04$) as exposure increased. Analysis of effects of acute exposures showed no relationship between workday exposure at any level and post-work performance on nine neurobehavioral tests.

Echeverria et al. (1994) detected deficits in visuospatial function (reduced performance in tests of pattern memory and pattern recognition) in the dry cleaning workers categorized as having high lifetime chronic exposure and whose current exposure level was 9 ppm, 8-hr TWA. However, the exposure level of 9 ppm was not considered representative of past chronic exposure levels because the industry in the study area was switching from wet-transfer to dry-to-dry machines during the study. The investigators attributed the reduced performance to prior exposures that were about two to four times higher 3 to 5 years previously, and they hypothesized that a few years of reduced exposure may not be long enough to eliminate the residual effects on visuospatial skills caused by the exposures associated with wet-transfer machines.

4.6.1.2.8. ***Echeverria, D., R.F. White and C. Sampaio. 1995. A behavioral evaluation of PCE exposure in patients and dry cleaners: a possible relationship between clinical and preclinical effects. J. Occup. Environ. Med. 37:667–680.*** Echeverria et al. (1995) assessed neurobehavioral effects and mood disturbances in four patients diagnosed with tetrachloroethylene encephalopathy. Subject 1 was exposed chronically over a 1-year period when the interior woodwork of her home was mistakenly treated with tetrachloroethylene. The three other cases were occupationally exposed. Subject 2 was exposed during two separate periods: first, for 3 years in a dry cleaning establishment and, second, for 7 years cleaning parts. Subject 3 was exposed for 16 years as a dry cleaning worker. Subject 4 was also exposed as a dry cleaning worker, but her duration of employment was not reported. Subjects 2, 3, and 4 were working

with tetrachloroethylene when first tested. Air monitoring data were not available; however, occupational health physicians diagnosed each case with tetrachloroethylene encephalopathy on the basis of symptoms, neurophysiological assessment, and their own examinations.

A large battery of standard neurobehavioral tests was given to each subject. For most tests, impairment was inferred clinically when a subject's score was greater than one standard error of measurement below expectation, which is less restrictive than the criterion (more than two standard deviations below mean) commonly used in neurobehavioral testing to separate normal from abnormal scores (Lezak, 1995). Test results for the four subjects most consistently indicated complaints of fatigue and confusion, accompanied by cognitive deficits on tests assessing memory and motor, visuospatial, and executive function. Repeated testing of subjects 3 and 4 indicated post-exposure improvement on neurobehavioral tests of all the affected functional domains, although performance on some of the more difficult tests in each domain remained impaired. These results suggest an association between CNS effects and tetrachloroethylene exposure, but a conclusion of a causal relationship is precluded by the lack of data on the duration and severity of the tetrachloroethylene exposure.

The investigators also assessed the performance of 66 dry cleaning workers on neurobehavioral tests designed to detect the same impairments noted in the clinical cases. The testing was conducted in 1986. The owners of 125 shops in Detroit, Michigan were contacted, and 23 agreed to allow their workers to participate in the study. Within each shop, operators were matched on education and age ($\pm$5 years) with a lower-exposure subject.

The subjects (35 men and 30 women) were grouped into three categories of chronic tetrachloroethylene exposure (low, moderate, and high), based on type of shop (wet-transfer or dry-to-dry), job title (counter clerk, presser, or operator), and years of employment. All the operators were placed in the high-exposure category. There was no unexposed control group. Dry cleaning workers placed in the chronic exposure categories of low, moderate, and high had been employed at their main job for 2.1, 3.9, and 14.6 years, respectively. Their mean age was 40.9, 40.6, and 43 years. The three groups were also characterized by estimates of current exposure (low, medium, and high), which corresponded to mean tetrachloroethylene air concentrations (8-hr TWA) of 11, 23, and 41 ppm, respectively, for counter clerks, pressers, and operators in the more common wet-transfer shops (17 of 23 shops). Estimated air concentrations for counter clerks, pressers, and operators in the dry-to-dry shops were 0.5, 10, and 11 ppm. The estimates were based on a relationship between breath and air concentrations derived from a larger independent study (Solet et al., 1990). The study authors noted that the estimates were comparable to those found in other surveys of dry cleaning facilities in the United States.

All subjects were tested in groups of two in the afternoon after work on the first or second day of their work week. The tests were conducted in a minivan. Each subject provided a

breath sample and completed a medical, symptom, work history, and hobby questionnaire. The subjects were administered six neurobehavioral tests, a test of verbal skills, and questionnaires on emotional states (moods) and CNS symptoms. The neurobehavioral test battery consisted of one test of motor/cognitive function (symbol digit) and five tests of cognitive function (digit span, trailmaking A and B, visual reproduction, pattern memory, and pattern recognition), including three tests of an individual's ability to process and remember visuospatial stimuli (the latter three tests).

Multivariate analysis was used to evaluate the relationship between a chronic index of lifetime exposure and performance on neurobehavioral tests, after adjusting for the confounding variables of current exposure, a 3-year index of exposure, age, education, verbal skill, alcohol consumption, hours of sleep, fatigue, mood, symptoms, medication, and secondary exposures to neurotoxicants. After adjustment for factors affecting performance, the scores of the dry cleaning workers with high chronic exposure were statistically significantly lower ($p < 0.01$) than those of the workers with low chronic exposure in three tests of visual function: visual reproduction, pattern memory, and pattern recognition. Adjusted scores were reduced from 6 to 15%; the two most sensitive tests were those that measured short-term memory of visual designs. These impairments of visually mediated function were consistent with the impairment of visuospatial functions observed in the four patients previously studied by Echeverria et al. who were diagnosed with tetrachloroethylene encephalopathy. Other effects seen in the patients (mood changes and decreased cognitive function in nonvisual tests) were not found in the dry cleaning workers with high lifetime exposures. Among complaints by the dry cleaning workers, only the number of complaints of dizziness from standing up rapidly and "solvent-induced dizziness" over the previous 3 months was significantly elevated ($p<0.04$) in the high-exposure group.

The study authors concluded that effects on visuospatial function were consistently found in subjects employed as operators for an average of 14.6 years and exposed to an estimated tetrachloroethylene 8-hr TWA air concentration of 41 ppm, suggesting a vulnerability of visually mediated functions with tetrachloroethylene exposure. This conclusion was based on the impaired performance of the high-exposure group when compared with a group of dry cleaning workers with low lifetime exposure, including 16/22 workers who were probably clerks in wet-transfer shops where the mean current exposure level was 12 ppm. This exposure level is substantially above background ambient levels, and whether the performance of the low-exposure group was impaired when compared with that of a group without occupational exposure (i.e., an unexposed control group) is not known. The lack of an unexposed control group limits the ability of the study to fully characterize the magnitude of the effects on visuospatial ability and to detect exposure-related symptoms or effects on tests of nonvisual

cognitive ability. It also limits the extrapolation of the results to other populations exposed to tetrachloroethylene.

4.6.1.2.9. ***Altmann, L., H.F. Neuhann, U. Kramer, J. Witten and E. Jermann. 1995. Neurobehavioral and neurophysiological outcomes of chronic low-level tetrachloroethylene exposure measured in neighborhoods of dry cleaning shops. Environ. Res. 69:83–89.***

Altmann et al. (1995) used neurophysiological and neurobehavioral techniques to assess the effects of long-term exposures to tetrachloroethylene. A total of 19 tetrachloroethylene-exposed subjects (residents of Mulheim, Germany) were chosen from a population of 92 subjects living in neighborhoods close to dry cleaning facilities. Three criteria were used to select subjects: a tetrachloroethylene blood level above 0.002 mg/L, a period of living above or next to a dry cleaning facility for at least 1 year, and no occupational exposure to organic solvents. The mean age of the exposed subjects was 39.2 years (range: 27–58 years) and the mean duration of living near a dry cleaning facility was 10.6 years (range: 1–30 years). The daily activity pattern of the exposed subjects was not reported. A total of 30 controls were selected from volunteers; their mean age was 37.2 years (range: 24–63 years). One or two controls, matched for age ($\pm$1 year, but 3 years in one case and 6 years in another case) and gender, were chosen for each exposed subject. The control subjects were recruited mainly from the staff of a public health office or an institute for environmental hygiene, and none reported a history of solvent exposure. No information is provided in the paper on the motivation for exposed and control subject to participate in the study. Voluntary consent was obtained from all subjects prior to the initiation of testing.

All subjects were given medical examinations. Five exposed (26%) and seven control subjects (23%) were excluded for various medical reasons, including impaired vision, diseases with potential neuropathy, hypertension, and joint impairment. The reasons for exclusion were similar in both groups. All subjects met standards for visual acuity and vibration perception. The final exposed group was composed of 5 men and 9 women and the control group was composed of 9 men and 14 women. The two groups did not differ with regard to consumption of alcoholic beverages, regular medication, smoking, or body mass index, but they did differ in degree of education, which was used as an indicator of social status.

The effect of tetrachloroethylene exposure on the neurophysiological and neurobehavioral measurements was evaluated using both univariate and covariate linear regression. The multivariate regression analysis accounted for age, gender, and education as covariates. Degree of education was defined as "low," "medium," or "high" level.

VEPs in response to black-and-white checkerboard patterns were recorded for all individuals. Vibration perception using a tuning fork—a crude measure of peripheral

neuropathy—was assessed at the ankle. Five tests included in the Neurobehavioral Evaluation System developed in the United States and adapted for testing on a German population were used: (1) finger-tapping speed with the index finger of both the dominant and the nondominant hand; (2) hand-eye coordination using a joy stick to follow a sine wave on a computer screen; (3) a continuous performance test for assessment of vigilance, which requires a response to a specific stimulus appearing on the computer screen and failure to respond to other stimuli; (4) simple reaction time, which requires the fastest possible response to a simple visual stimulus (measured twice); and (5) visual memory on the Benton visual retention test, which requires a match of a previously displayed stimulus out of several choices after a short delay interval. All of these tests are commonly used to assess occupationally exposed adults, and the software for testing and analysis is available for purchase. All testing was completed in a single 3-hr session; testing times were selected randomly for both exposed or control subjects.

Blood samples were taken once in the examination room immediately before testing (all subjects) and, if possible, once when the exposed subjects were at home. The mean blood level for exposed subjects, based on samples collected in the examination room, was 0.0178 mg/L (standard deviation, 0.469 mg/L). For seven of the nine exposed subjects, blood concentrations in samples collected at home were higher than those in samples collected in the examination room. None of the blood concentrations in the control group exceeded the detection limit of 0.0005 mg/L. For the exposed subjects (data from 13 apartments), indoor air sampling indicated that the mean (7-day TWA) air concentration was 0.7 ppm (standard deviation, 1 ppm) and the median was 0.2 ppm. For the control group, the mean and median values were 0.0005 ppm (standard deviation, 0.0005 ppm) and 0.0003 ppm. There was a good correlation between home indoor air concentrations and blood levels of tetrachloroethylene in the exposed subjects ($r = 0.81$). The correlation was much lower when the examination room blood samples were used ($r = 0.24$).

After adjusting for covariates and possible confounders of age, gender, and education, there were statistically significant group differences between the adjusted mean scores of exposed and control subjects on three neurobehavioral tests (simple reaction time, $p < 0.05$ for the first test and $p < 0.01$ for the second test; continuous performance, $p < 0.05$; and visual memory, $p < 0.05$). In all cases, the exposed subjects had slower response times or more errors than did the unexposed controls. No statistically significant differences were observed between the performance of the exposed and control groups on the finger-tapping or hand-eye coordination tests, which are measures of fine motor function; on VEP, which may be less sensitive than direct measurement of visual function; or on vibration perception at the ankle using a tuning fork.

The relationship between indoor tetrachloroethylene concentration and individual performance was not reported, so it was not possible to evaluate concentration-response relationships. The small numbers of study subjects in this study compared to the occupational studies is a limitation; however, this study appeared to have sufficient power to detect associations with tetrachloroethylene exposure. Additionally, exposed and control groups did not differ with regard to consumption of alcoholic beverages, regular medication, smoking, or body mass index, but they did differ in degree of education. Statistical analysis of the data took into account effects from important covariates such as age, gender, and education. Univariate analyses showed that age, gender, and education were not predictors of deficits in tests of continuous performance, visual memory, and the second reaction time, although education and gender were predictors of deficits in the first reaction time.

The use of three categories for education in the multivariate regression analyses may not fully account for all effects from these covariates (U.S. EPA, 2004), although it is not possible to evaluate whether residual confounding from these covariates may explain observations on the neurobehavioral tests. Furthermore, because the responses in the exposed group for the tests highlighted above (simple reaction time, continuous performance, visual retention) were statistically significantly different from those of the control group, whether or not the covariates were considered, an approximate estimate of the impact of the tetrachloroethylene exposures can be derived by comparing the reported response levels for the two groups. The degree of change from control was approximately 15–20% for this subset of tests.

4.6.1.2.10. ***Spinatonda, G., R. Colombo, E.M. Capodaglio, M. Imbriani, C. Pasetti, G. Minuco and P. Pinelli. 1997. [Study on speech production processes: application for a group of subjects chronically exposed to organic solvents (part II).] Med. Lav. 19:85–88.*** Spinatonda et al. (1997) assessed the effect of tetrachloroethylene exposure on vocal reaction times among 35 dry cleaners and 39 unexposed controls. Controls were matched to exposed individuals for age (mean age of 35 years for both groups) and education. The published paper did not identify the population from which exposed and controls were drawn, the inclusion criteria for exposed subjects and controls—and hence, whether potential study subjects may have been excluded—and duration of exposure in a tetrachloroethylene-exposed job.

Exposure was assessed by a "grab sample" and not as a weighted average (as often reported in other occupational studies reviewed in this section). Exposure monitoring indicated a median concentration of tetrachloroethylene of 8 ppm (range: 2–136 ppm). An index of cumulative exposure to tetrachloroethylene was also developed for each exposed subject by multiplying the tetrachloroethylene concentration by the number of years worked.

Latency to and duration of vocal response to the stimulus (reading) were measured in each subject after the presentation of a sequence of words on a computer screen. For each condition, subjects were asked to say the word immediately or following delays of 0.1 or 0.5 seconds. The test was performed using a random sequence of concrete or meaningless disyllabic words. These tests were carried out at the place of employment for dry cleaners and in a clinical setting for controls, indicating that the investigators were not blinded as to a subject's exposure status. Testing conditions may have differed between exposed group and controls.

Compared with the control group, the exposed group had statistically significant longer mean reaction times and/or vocalization durations under all response conditions (immediate or delayed response) with either real or meaningless words. Furthermore, statistically significant positive correlations were observed between cumulative tetrachloroethylene exposure and immediate reading and delayed reading tasks $r = 0.69$ and $r = 0.73$, respectively). No information on alcohol consumption or other potential differences between exposed subjects and controls was reported, precluding an analysis of how these factors may have affected the observed association between tetrachloroethylene and reaction time.

4.6.1.2.11. ***Schreiber, J.S., H.K. Hudnell, A.M. Geller, D.E. House, K.M. Aldous, M.E. Force, K.W. Langguth, E.J. Prohonic and J.C. Parker. 2002. Apartment residents' and day care workers' exposure to tetrachloroethylene (perc) and deficits in visual contrast sensitivity. Environ. Health Perspect. 110:655–664.*** Schreiber et al. (2002) reported the findings from investigations using visual tests to assess neurologic function in two populations: apartment residents and day care employees who had potential environmental tetrachloroethylene exposure due to close proximity to dry cleaning facilities.[9] Residential exposure to tetrachloroethylene can result in nearly continuous exposure (NYS OAG, 2004a) and is distinct from the pattern of tetrachloroethylene exposure experienced by the occupational populations described in the preceding paragraphs. Objectives of the residential and day care investigations were to characterize tetrachloroethylene exposure and to screen for subclinical neurological effects using a battery of visual function tests. All participants—or their guardians in the case of the residential study—signed voluntary consent forms prior to study commencement.

For the residential study, the exposed group consisted of 17 tetrachloroethylene-exposed subjects (11 adults between the ages of 20 and 50, 2 adults over the age of 60, and 4 children)

[9] The apartment residents lived in two separate buildings in New York City that each contained a dry cleaning business. The residential study served as a pilot for a larger study that is investigating visual effects among tetrachloroethylene-exposed residents. The day care study was part of an investigation of staff and children carried out by the NYS DOH and the Centers for Disease Control and Prevention. The day care facility, located in Albany, New York, was in a building that also housed a business that did dry cleaning. Visual testing for both studies was carried out by the same investigator using the same testing apparatus.

from six families residing for an average of 5.8 years (6 years median) in two apartment buildings in New York City. Preliminary monitoring of these buildings indicated tetrachloroethylene concentrations were elevated compared to eight other buildings also monitored by the NYS DOH. These eight buildings were identified by NYS DOH from discussions with the New York City Department of Health and from a review of files on dry cleaning facilities (NYS OAG, 2004b).

Study subjects were identified through several methods: (1) both families in the first building (Buildling A) had been referred to the NYS DOH for information about participating in the study by Consumer Union/Hunter College researchers, (2) one family in the second building (Buildling B) had previously contacted NYS DOH about exposure concerns and desired to participate in a study, and (3) three other families in Building B were recruited by a participating family (NYS OAG, 2004b). Exposed residents were an affluent, English-speaking, Caucasan population living near New York City's Central Park (telephone communication from K. Hudnell, EPA, to D. Rice, EPA, February 2003). Exposed participants were generally unaware of the tetrachloroethylene exposure, although some study participants did observe tetrachloroethylene-like odors prior to the study period.

Control subjects were recruited from among NYS DOH Albany, New York employees and their families. They were considered representative of the general population not living near dry cleaning facilities. All controls were Caucasan, except for one Asian individual, and were age- and sex-matched to exposed apartment residents. In some cases, more than one control participant was matched to an exposed subject, and an average of the multiple control visual function test scores was used for comparison to that of an exposed subject. Mean age was 34.5 years for exposed apartment residents and 33.2 years for control subjects.

Nine adult staff (all females) of a day care facility agreed to participate in the day care study. Controls were age- and gender-matched acquaintances of the exposed participants, local retail shop employees, NYS DOH employees, or staff from other local day care centers with no known tetrachloroethylene exposure. All subjects in the exposed and control groups were Caucasan (telephone communication from K. Hudnell, EPA, to D. Rice, EPA, February 2003). Mean age was 27.7 years for control participants and 27.2 years for day care staff; mean duration of employment for exposed subjects was 4 years at the center.

Information on sociodemographics; lifestyle factors such as exposure to direct or passive smoke, alcohol consumption, and exercise; medical history; and neurotoxicant exposure in addition to the visual tests was obtained by questionnaire from both study populations and their controls. Exposed participants had no known exposure to other neurotoxicants, ongoing illness, current use of neuroactive drugs, or a medical history indicative of neurologic dysfunction, and both exposed participants and controls reported low or moderate alcohol consumption that did

not differ between either exposed group and their controls. Moreover, the profile of moods test scores of all residential exposed subjects were within normal limits. The investigators also administered visual tests of acuity, contrast sensitivity, and color discrimination to exposed subjects and their referents. The investigators were not blinded as to a subject's status as either exposed or nonexposed.

The assessment of tetrachloroethylene exposure of residents consisted of tetrachloroethylene concentrations in indoor air and personal air samples, exhaled breath, and blood, which were collected at the time of visual testing. Testing was performed during a period of active dry cleaning for four of the families and one month after closure of the facility for the remaining two families in the residential study. Additionally, adult residents provided urine samples, which were analyzed for tetrachloroethylene as well as for three products of its metabolism: TCA, trichloroethanol, and the urinary acetyl metabolite. Breast milk samples were provided from two exposed breastfeeding mothers.

Ambient concentrations of tetrachloroethylene were available for all study participants for an earlier time frame (from 1 to 3 months before the date of visual testing), when active dry cleaning was occurring in both apartment buildings. These measurements were used by NYS DOH to identify study sites. Concentrations of airborne tetrachloroethylene levels in apartment rooms were higher in these samples than in the monitoring data obtained at the time of the visual testing. Median concentrations in these samples, which were taken during the day during active periods of dry cleaning, were 0.21 ppm (mean = 0.36 ppm; range: 0.1–0.9 ppm). Airborne tetrachloroethylene concentrations had decreased in samples collected at the time of visual testing; median tetrachloroethylene concentration was 0.09 ppm (mean = 0.18 ppm; range: 0.01–0.78 ppm). Tetrachloroethylene levels in blood correlated well with levels in room air, personal air, and breath.

Atmospheric monitoring of the day care facility before closure of the dry cleaning business showed airborne concentrations of tetrachloroethylene ranging from 0.27 to 0.35 ppm, with median and mean concentrations of 0.32 ppm. Samples obtained at the time of visual testing, five weeks after removal of the dry cleaning machines, approached background concentrations (range: 0.0012–0.0081 ppm).

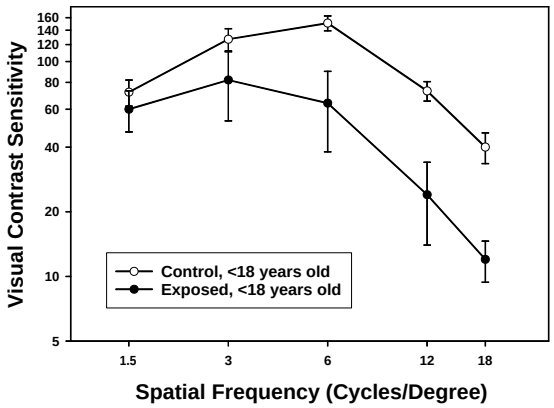
Visual function testing consisted of near visual acuity, near VCS, and color vision. All study participants who wore corrective lenses for reading wore their lenses when taking the vision tests. The visual acuity test measured the ability to discriminate high- frequency (i.e., small) images at high contrast; e.g., reading successively smaller black-on-white letters as part of an examination for corrective lenses. This measure typically is dependent on the optics of the eye (and corrective lenses when needed) and is insensitive to subclinical deficits in neurologic function. In neither assessment did the groups differ in visual acuity.

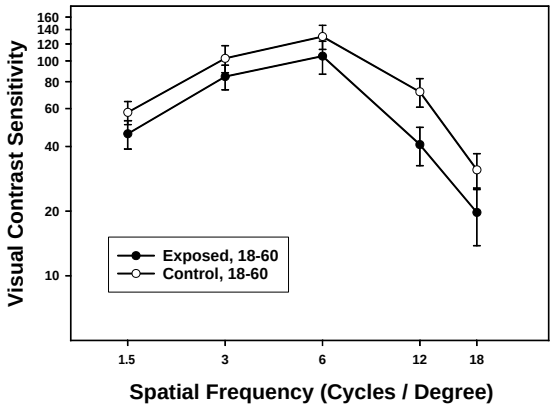
The contrast sensitivity test is sensitive to subclinical deficits in neurologic function in the visual pathways. The test measured the least amount of luminance difference between dark and light bars that was needed to detect the bar pattern. Luminance varied between the bars in sine-wave fashion, and each test pattern represented one size of bars or spatial frequency. The bar patterns were presented at five different spatial frequencies, thereby breaking spatial visual function into its essential components. The least amount of luminance contrast needed to detect each bar size was measured. The contrast sensitivity data are presented in Figure 4-1. A strength of this study is that the test of contrast sensitivity employed a forced-choice procedure, providing better reliability and consistency than other approaches.

Multivariate analysis of variance was used to analyze the VCS data. Group mean scores for VCS across spatial frequencies were statistically significantly lower in exposed residents than in controls and in day care employees as compared with controls, indicating poorer visual function in the exposed groups. An exposure-response analysis did not show an association between poorer performance and increasing tetrachloroethylene concentration. Among apartment residents, mean scores of VCS in all four children and in both older adults (60 years of age) were lower than the 12^{th} percentile score of all control subjects. (The 12^{th} percentile represents the two control subjects with the poorest performance out of the 17 total data points.) In contrast, 5 of the 11 adults aged less than 60 years scored below the 12^{th} percentile. It is unknown whether the difference between groups would have been statistically significant on the basis of the adults under 60 years alone. However, there was a statistically significant lower group mean VCS score across all spatial frequencies when day care employees were compared with the control group (data not shown).

In the residential study, exposed subjects were retested twice after the initial assessment, 6 to 10 months and 17 to 21 months after closure of the dry cleaning facility. Performance appeared to worsen over successive evaluations, although statistical comparisons were not performed (NYS DOH, 2000). Control subjects from the initial testing were not retested, preventing a comparison with observations from exposed subjects.

Color vision was also assessed in both the residential and the day care groups. Subjects were asked to put a series of small round "caps" in order by color. The types of errors made could distinguish specific types of color vision deficiency: e.g., red-green color blindness, which is common in males, or blue-yellow color blindness, which is associated with solvent exposure (Mergler and Blain, 1987; Mergler et al., 1987, 1988a, b, 1991; Campagna et al., 1995, 1996). Group differences in the CCI were assessed using two-tailed Student's t-tests for matched-pair analyses. CCI scores of exposed groups did not show statistically significant impairment as compared with referents, although the performance of the exposed groups, particularly the residential group, appeared worse than that of control.





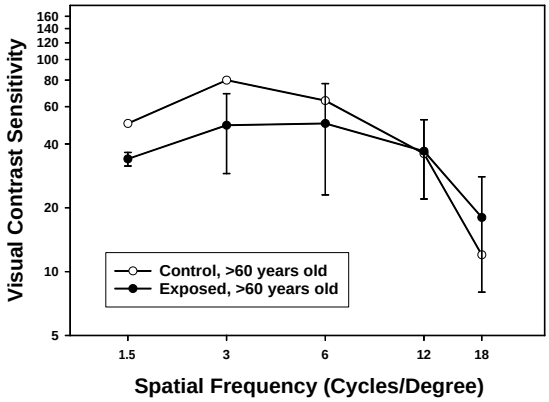


Figure 4-1. Visual contrast sensitivity functions for control and exposed children (top), adults that were identified as having impaired function (i.e., 5 of the total 11) and their matched controls (middle), and the control and exposed individuals over 60 years of age. The x-axis represents the frequency of the stimulus bars, with finer bars toward the right. The y-axis represents the inverse of the contrast at which the subject could no longer distinguish the orientation of the bars (threshold). For any frequency, a higher contrast sensitivity threshold represents better visual function. It is apparent that the group of children is relatively more impaired than the impaired group adults.

Observations in the study paper have been questioned, particularly on selection bias in the residential investigation as an explanation of observed VCS deficits (HSIA, 2004). Although motivation for study participation is not known, the New York State Office of Attorney General (NYS OAG, 2004b) noted that test results were provided to individual study subjects, which probably encouraged participation; however, the principal investigator does not believe selection bias was a factor for study participation. Some information in the study paper may also be used to judge the potential for selection bias. The study authors noted that the profile of moods test scores of all exposed residential subjects were within normal limits, with no cases of clinical depression or other neuropsychiatric conditions. Hence, it does not appear that exposed residents had major psychological impairments. Additionally, bias may be introduced through the use of controls living in Albany for comparison with exposed residents living in New York City. Information on covariates is lacking, and the impact of these covariates on VCS function cannot be adequately assessed.

Some general information is available to evaluate potential confounding due to education, occupation, and residential location. Factors such as education, socioeconomic status, and smoking do not affect the VCS test (NYS OAG, 2004b; Hudnell et al., 2001; Mergler et al., 1991; Frenette et al., 1991; U.S. EPA, 2004). Occupation is highly correlated with socioeconomic status (Deonandan et al., 2000) and is not likely to confound the VCS test. Moreover, urban-rural differences between exposed and control subjects are not thought to strongly bias findings. For example, Kaufman et al. (1988) did not show that urban or rural residence was related to performance on specific subtests of the Wechsler Adult Intelligence Scale, although associations were seen with other variables such as sex, age, and education, variables that are similar or matched for exposed and referent subjects in the current study.

Public comments to EPA (NYS DOH, 2004) discuss that two of the four children in the residential study had medically verified diagnoses of learning disabilities or developmental delays; however, no information was provided in these public comments about these conditions in referent children. Without comparable information on control children, it is difficult to draw any conclusions about whether these conditions may or may not have also contributed to the VCS deficits observed in residential subjects.

Finally, as with all other studies discussed in this section, unmeasured differences or residual confounding between exposed and referent groups may possibly explain observations; however, in the absence of information, it is not possible to evaluate the unmeasured variables.

4.6.1.2.12. ***Sharanjeet-Kaur, Mursyid A, Kamaruddin A, Ariffin A. 2004. Effect of petroleum derivatives and solvents on colour perception. Clin Exp Optom 87:339-343.*** Fourteen healthy subjects of ages 24–53 years working in 3 dry cleaning facilities using tetrachloroethylene are

included in a study assessing color vision. This study was part of a larger study assessing color vision in two other occupationally-exposed populations, 39 workers in a factory producing polyethylene resins plastic storage containers and 40 workers manufacturing polystyrene plastic bags. The published study is poorly reported, lacks many details, and adopts post-hoc statistical testing. The paper reports neither how facilities were identified nor recruitment methods for study subjects. Furthermore, the paper does not present any information on tetrachloroethylene concentrations or on tetrachloroethylene biomarkers, making it difficult to judge the degree of exposure to tetrachloroethylene. Control selection criteria are not identified in the published paper other than 27 healthy subjects (mean age 27 $\pm$ 4 years) composed Control Group 1 and 2 healthy subjects (mean age 33 $\pm$ 4 years) who were support staff of Universiti Kebangsaan Malaysia. Dry cleaning workers differed from controls on several variables: work duration, hours worker per day, cigarette smoking, mean age (compared to Control Group 1), and race. Also, no information is presented on possible difference between dry cleaners and controls on socio-economic status (SES). Voluntary consent was provided by all subjects.

Visual testing was carried out at the factory or dry cleaner, for exposed subjects, and at the Optometry Clinic in the Universiti Kebangsaan Malaysia for control subjects. Given these testing conditions, a subject's exposure status was known, i.e., no blinding. Visual acuity was measured at distance using the Snellen chart and at near using a reading chart. Subjects were excluded with poor visual acuity or with systemic, ocular, or neurological diseases; the number of excluded subjects is not identified in the published paper. Color vision was assessed binocularly using Ishihara plate, D-15 test, and Farnsworth Munsell 100 Hue test under a light box at illumination of 1,000 lux. Subjects wore the best corrective lens and testing was carried out at a distance of 35 to 40 cm.

The number of subjects with abnormal scores, using criteria of Vingrys and King-Smith (1988), is presented but not group-mean color confusion index scores. None of the controls or dry cleaners had color vision errors with the Ishihara plates. In contrast, 6 dry cleaners (43%) and 13 subjects (93%) compared to no controls were identified with errors on the D-15 test and FM 100 Hue test, respectively. Statistical testing of differences is lacking. Total error scores for the FM 100 Hue test differed between dry cleaners and control group 2 ($p < 0.05$) but not with control group 1. It is difficult to interpret these findings due to the lack of exposure information on potential tetrachloroethylene exposure other than job title, and differences between dry cleaners and controls regarding test conditions, SES, and smoking.

4.6.1.2.13. ***New York Department of Health (NYS DOH). 2005a. Improving human risk assessment for tetrachloroethylene by using biomarkers and neurobehavioral testing. Final Technical Report to US EPA Star Grant #R827446. Accessed 5 December 2006,***

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/977/report/0. NYS DOH (2005a) examines the effect of tetrachloroethylene exposure on visual function in two populations, residents living in a building co-located with a dry cleaning establishment and among employees and former students of a day care establishment exposed 4 years previously during a period when the day care was co-located near a dry cleaner. The first study, the New York City Perc Project, did not include subjects studied by Schreiber et al. (2002) and employed different methods for testing visual contrast sensitivity and color vision. NYS DOH (2005a) enlisted 65 households in 24 residential buildings with dry cleaners using tetrachloroethylene on-site and 61 households in 36 buildings without dry cleaners located in the study area in Manhattan, New York City. Health outcome and tetrachloroethylene concentrations as measured from indoor air monitoring and in exposed subject's breath and blood were obtained over the period from 2001 to 2003. The full report of the residential study has not received public peer review nor has it been published as a literature paper although McDermott et al. (2005) presents exposure monitoring findings from the dry cleaner households.

The second project, the Pumpkin Patch Day Care Center (PPDCC) Follow-up Evaluation, is a 5-year follow-up of visual function among some employees and neurobehavioral function among children in a day care center that had been previously co-located in a building with a dry cleaning establishment. The PPDCC Follow-up Study also included first-time visual function tests of former students. NYS DOH together with the U.S. Centers for Disease Control and Prevention carried out the initial evaluation of PPDCC staff and students in 1998 (NYS DOH, 2005b). Funding to NYS DOH for the residential study and the PPDCC Follow-up study was provided though U.S. EPA STAR Grant #827446010 (NYS DOH, 2005a, 2005c).

4.6.1.2.13.1. ***New York City Perc Project*****.** The objectives of the New York City Perc Project are as follows: 1) to document tetrachloroethylene exposures in buildings where dry cleaners were present; 2) to evaluate whether living in a building with a dry cleaner was associated with CNS effects; 3) to evaluate the relationship(s) between measures of tetrachloroethylene exposure and CNS effects; and, 4) to assess whether children were disproportionately exposed to and/or affected by tetrachloroethylene compared to adults. Study design and protocols were approved by Institutional Review Boards at the NYS DOH and other collaborating institutes (Mr. Sinai Medical Center and U.S. CDC).

Subjects were identified in buildings from eleven zip code areas surrounding Central Park, New York City, contiguous with one another, but different in demographic and socioeconomic characteristics. Eligible households for participating in this study include at least one adult (20–55 years old) and one child (5–14 years old), so as to assess whether residential tetrachloroethylene exposure would disproportionately affect children. Initial monitoring

indicated few residences in dry cleaner buildings with elevated indoor air concentration of tetrachloroethylene above the current NYS DOH residential air guideline of 0.015 ppm (0.1 mg/m^3). The study area was broadened to include buildings subject of a resident complaint and to include buildings in additional zip codes, primarily characterized by lower SES or higher percentage of minority residents. This decision was made after the finding of elevated tetrachloroethylene levels in households in dry cleaner buildings in one zip code area, a low income, minority area, compared to other zip codes area. Mail and telephone contact were the primary methods of recruiting subjects, with door-to-door recruitment by a not-for-profit child advocacy organization (Northern Manhattan Perinatal Partnership, Inc.) providing assistance with recruiting bilingual subjects primarily living in 3 of the 11 zip code areas. Of the 1,261 dry cleaner and 1,252 reference households contacted, 132 dry cleaner households and 175 reference households included age-eligible adult-child pairs and a total of 65 dry cleaner (67 adults, 68 children) and 61 reference households (61 adults, 71 children) participated in the study. All participants or their guardians signed voluntary consent forms prior to study commencement.

Tetrachloroethylene indoor air concentrations in dry cleaner buildings had decreased since 1997, the period of the pilot study (Schreiber et al., 2002), and ranged up to around 0.77 ppm (5 mg/m^3) with a geometric mean of 0.005 ppm (0.035 mg/m^3). Monitoring was carried out using passive monitoring badges. In comparison, tetrachloroethylene concentrations in building without dry cleaners ranged up to 0.014 ppm (0.09 mg/m^3) with a geometric mean of 0.0004 ppm (0.003 mg/m^3). Both breath and blood tetrachloroethylene levels were significantly ($p < 0.05$) correlated with indoor air concentration for adult and for child subjects of dry cleaner buildings. Levels of detection (LODS) were 5 μg/m^3 air and 0.048 mg/ml blood. Air, breath, and blood tetrachloroethylene concentrations were inversely correlated with income and were higher among minority compared to non-minority subjects.

NYS DOH staff visited participants in their residences to collect 24-hr indoor air samples, breath samples, and to give adult participants a questionnaire seeking information on residential, occupational, and medical history for themselves and their children. Opthalmologic examinations were scheduled at the same time for the Mt Sinai School of Medicine Department of Opthalmology research clinic. Participants received financial compensation after completing the home visit ($50.00) and ophthalmology clinic visit ($50.00).

No differences between exposure groups were observed for participants recruited using the mail and telephone method, although this was not so for participants recruited using door-to-door methods. For these individuals, language and adult age differed significantly between exposed and non-exposed groups with more English speaking households participating in the non-exposed group and non-exposed adults were slightly older than exposed adults. Overall, differences between adult residents of reference buildings or buildings with dry cleaners in SES

characteristics, residence duration, education level, age smoking or alcohol use are not apparent. Differences between child residents in gender or residence duration are not apparent, but the highest exposure group is about a year younger and has about one less year of education than children in the other exposure groups.

Ophthalmologic examinations and visual function tests were given to study participants at the Mt. Sinai Medical School of Medicine. The final report does not describe whether examiners were or were not blinded as to a subject's exposure status (NYS DOH, 2005a). The examination included determination of past ocular and medical history; measurement of visual acuity, pupil size, extrocular motility, and intraocular pressure; and anterior and posterior segment exams. Subjects with abnormalities or taking medications that could influence VCS and/or color vision were excluded from further testing. Furthermore, visual functional tests for some children were excluded from the statistical analysis because of their young age or because they were identified by their parents as learning disabled or having attention deficit hyperactivity disorder. VCS was determined using the Functional Acuity Contrast Test (FACT) distance chart placed 10 feet from the participant under light conditions of 68–240 cd/m2. These testing conditions differ from those employed by Schreiber et al. (2002) in their residential study where visual test was carried out assessing near contrast sensitivity.

Adults and children demonstrated a ceiling effect with VCS performance, i.e., a maximum score at 1.5, 3, 6, 12, and 18 cycles per degree (cpd) is achieved by some study participants. VCS scores among adults were not correlated with any SES factor or personal characteristics (smoking, alcohol use, education level, duration of residence). Among all children, poorer VCS at 1.5, 3, and 6 cpd were significantly correlated with speaking primarily Spanish at home.

NYS DOH examined possible association between VCS and tetrachloroethylene exposure by, (1) comparing the percent of exposed subjects with maximum VCS score (no errors) to referents, (2) comparing mean differences in VCS scores between adult and child subjects living in the same residence across exposure categories, and (3) using logistic regression to assess the effect of tetrachloroethylene in indoor air, blood or breathe on the achievment of maximum VCS score at 6 and 12 cpd. Analyses examining relationships between tetrachloroethylene and visual function were conducted with the referent exposure group (background exposure, living in a building without a dry cleaner), <0.015 ppm (<100 $\mu g/m^3$), and >0.015 ppm (>100 $\mu g/m^3$).

Several analyses suggest a susceptibility of exposed subjects, particularly among children, to tetrachloroethylene on VCS performance at higher spatial frequencies. A decreasing trend ($p < 0.05$) was observed between increasing residential tetrachloroethylene exposure and the proportion of adults achieving the maximum contrast sensitivity score at 6 cpd and in the

proportion of children achieving the maximum contrast sensitivity score at 6 and 12 cpd; i.e., a lower proportion of participants with a maximum VCS score in the highest exposure category compared to referents living in a building without a dry cleaner. Stratified analyses suggested a lower percentage of low income and minority children with maximum VCS scores at a given cpd than higher income and non-minority children, but sample sizes in the highest exposure group, especially in higher income, non-minority groups, limit reliability of this observation. Race did not appear to confound the association in adults between VCS at 6 cpd and tetrachloroethylene.

VCS scores in children at a given cpd were generally higher (better contrast vision) than the VCS score of an adult living in the same apartment. Using differences between adult-child pairs in each exposure grouping to assess possible tetrachloroethylene effects, the advantage of children over adults appeared much smaller in the >0.015 ppm (>100 $\mu g/m^3$) category at 12 cpd (mean difference of 10.9) compared to referents (mean difference of 21.6) but was not statistically significant from the mean difference in the referent population ($p = 0.16$).

Results from logistic regression analyses further support susceptibility of children but not adults. Whereas adult VCS at 6 or 12 cpd was not significantly influenced by any measure of tetrachloroethylene exposure, VCS performance at 12 cpd among children was significantly influenced ($p < 0.05$) by tetrachloroethylene concentrations in either indoor air or in blood; that is, a lower percentage of children achieved a maximum VCS score with higher tetrachloroethylene exposure. Analyses of tetrachloroethylene breath concentrations and VCS performance at 12 cpd in children appeared to support the findings with indoor air and blood, but were of borderline statistical significance. Logistic regression models examining VCS findings in either children or adults are not adjusted for potentially confounding factors such as SES, education, smoking, alcohol use, age (for children) and gender (for children); these variables were correlated with one another as well as with tetrachloroethylene, but not with VCS performance.

Color vision was assessed biocularly using both the Farnsworth D15 and Lanthony's Desaturated 15 Hue Test. Both tests were administered under light conditions specified by the manufacturer. The number of errors for each eye was recorded by noting instances of inversions involving a single cap (minor error) and instances of inversions involving two or more caps (major errors). Total Color Distance Scores (TCDS) were determined and a CCI was calculated for each participant according to Geller (2001) and Bowman (1982).

Analyses were carried out using the proportion of subjects with no errors, comparing quantitative differences in CCI, and logistic regression modeling to assess associations between tetrachloroethylene exposure measures and occurrence of any major errors. A comparison of differences in CCI and major error between children and adults residing in the same household was used to assess the possible vulnerability of children. A high proportion of adult and child

participants scored perfectly on both the Farnsworth and Lanthony color vision tests. Lower annual household income, being a member of a minotiry group, speaking primarily Spanish at home, and fewer years of education were all significantly associated with increased CCI on both color vision tests.

Tetrachloroethylene measures of exposure were unrelated to color vision performance among adults; however, similar to VCS performance, children appear as a susceptible population. There were no differences between exposure groups for either adults or children in the percent of subject with major effort on both color vision tests. A comparison of mean CCI between exposure groups showed that children in the highest exposure category performed worse (mean CCI of 1.3, range 1.0–1.9) than children in the low exposure category (mean CCI of 1.1, range 1.0–1.7) and to referent children (mean CCI of 1.2, range 1.0–2.0) on the Lanthony test; the test for trend for the three exposure groups was statistically significant ($p < 0.05$). Performance (mean CCI) on the less sensitive Farnsworth test was not associated with tetrachloroethylene exposure in either adults or children. Moreover, for children, tetrachloroethylene in breath was significantly associated ($p < 0.05$) with making one or more major errors on the Lanthony color vision test in logistic regression analyses that adjusted for the effects of age and gender. Logistic regression analyses examining color vision and other tetrachloroethylene measures such as indoor tetrachloroethylene concentration or breath concentration were not discussed in NYS DOH (2005a.). Last, the higher mean difference in CCI between children and adults in the highest exposure category, >0.015 ppm (>100 $\mu g/m^3$), and referents was statistically significant. Children in the high exposure group were a year younger than in other exposure groups; age was correlated with CCI and with tetrachloroethylene exposure in this study. The highly correlated variables and the few numbers of children in the high exposure group limits analysis of age effects on the association between breath tetrachloroethylene concentration and CCI.

In summary, this study adopts a different approach than Schreiber et al. (2002) to assess vision, using far vision methods as opposed to the near vision methods of Schreiber et al. (2002). For both contrast vision and color vision, a number of analyses in NYS DOH (2005a) are suggestive of vulnerability among children. The association with vision effects in children and exposure to >0.015 ppm (>100 $\mu g/m^3$) tetrachloroethylene support findings from the earlier pilot study (Schreiber et al., 2002). Exposure to >0.015 ppm (>100 $\mu g/m^3$) tetrachloroethylene was highly correlated with race and children's age, and the sample sizes in the highest exposure group, especially in higher income, non-minority groups, makes it difficult to fully examine possible effects of income, race, and age on vision. However, association of tetrachloroethylene exposure >0.015 ppm (>100 $\mu g/m^3$) with visual deficits suggests a susceptibility of the population studied.

4.6.1.2.13.2. ***Pumpkin Patch Day Care Center follow-up evaluation*****.** The objective of the PPDCC Follow-up Evaluation was to assess neurobehavioral function in former students of PPDCC after a 5 year post exposure period and to carry out first-time testing of visual function of the former students. Additionally, visual function testing was carried out on five staff exposed to tetrachloroethylene 5 years previously. The NYS DOH final report to EPA (NYS DOH, 2005c) provides a full description of testing in children but not adults. The discussion of visual tests on former PPDCC is contained in NYS DOH (2005b). The initial testing in 1998 of vision in PPDCC staff and of neurobehavior in children is contained in NYS DOH (2005b).

Children eligible for testing in the current evaluation were enrolled in the New York State Volatile Organic Chemical (VOC) Registry and had attended PPDCC. There were 115 children who met this criteria. Of this group, 27 children with the highest number of hours spent at PPDCC were asked through letters or by phone to participate; 17 children completed vision testing and 13 children completed some or all of the neurobehavioral assessment. Referents were children who attended other day care centers and who were about the same age as PPDCC participants. No information is provided on methods employed for referent participation. Exposed and referent subjects were matched on daycare experience, age, and gender. Overall, 17 PPDCC and 13 comparison children completed vision testing and 13 PPDCC and 13 comparison children completed neuropsychological testing. Of these subjects, 13 matched pairs completed vision test; but only 8 matched pairs completed the neurobehavioral test. No information is provided in the NYS DOH final report on how many of the 13 former PPDCC students were part of the PPDCC student group who underwent neurological testing 5 years previously one month after the close of the dry cleaner facility.

Neurobehavioral evaluations consisted of a battery of tests that assess general intellectual function, attention/information processing speed, visuospatial ability, reasoning and logical analysis, memory, motor functions, and sensory-perceptual functions. Tests were administered in fixed order on two different days. All children completed the same tests with the exception of the Halstead-Reitan Neuropsychological Batteries. Children age eight or younger were administered the Reitan-Indiana Neuropsychological Test Battery and the Halstead-Reitan Neuropsychological Test Battery for Old Children was administered to children age nine or older. Children also performed portions of the computerized Neurobehavioral Evaluation System-2 (NES-2) which assessed perceptual-motor skills, attention, visual memory, and mood. A parent or guardian completed the Child Behavioral Checklist and a background history questionnaire. All neurobehavioral evaluations were conducted at the office of Albany Psychological Associated, P.C., in Albany, NY.

Independent samples t-tests were performed on the scores from the Wechsler Intelligence Scale for Children, Children's Memory Scale, the Halstead-Reitan Neuropsychological Test

Battery for Old Children, and Reitan-Indiana Neuropsychological Test Battery. Age was significantly correlated with performance on the Purdue Pegboard and many subtests in the NES-2 and analysis of covariance was completed on subtests from the NES-2 and Purdue Pegboard with age as a covariate. Each child's performance level on the neurobehavioral tests was determined by comparing his/her test score to normative information for the specific test or battery. For NES-2, performance of the referent children, children who attended other day care centers in Albany and who were about the same age as PPDCC participants, was used as the normative basis, with scores 2 S.D. below the mean of the same age and gender from the normative data being classified as impaired.

Neurobehavioral function of the 13 PPDCC children evaluated in this follow-up study did not differ from that of the 13 referent children. PPDCC children performed better than referent children on several tests but performance was within normative ranges. These results are not surprising. Neuropsychological or behavioral testing was conducted in October 1998 by the auspices of the U.S. Centers for Disease Control (U.S. CDC) on children then of ages four and five. No consistent differences in neurological function were found between 18 children who then attended the day care center and 18 age- and gender-matched control children who did not attend the day care center, although a statistically significant association was found between duration of attendance at PPDCC and poorer performance on the Purdue pegboard test with the dominant hand (NYS DOH, 2005b).

Visual function testing consisted of visual acuity, far VCS, and color vision. Visual contrast sensitivity was determined monocularly using the Functional Acuity Contrast Test distance chart placed 10 feet from the participant under light conditions specified by the manufacturer. Scores for each eye were recorded on a graph showing a normal range (90% confidence interval) of VCS at each spatial frequency. Color vision was assessed using both the Farnsworth D15 and Lanthony's Desaturated 15 Hue Test under light conditions specified by the manufacturer. For both tests, for each eye, participants were shown a rectangular box containing 16 color caps arranged in chromatic order. The test administrator removed 15 caps, leaving the first as a stand, and randomized them in front of the participant. Participants were asked to place the cap which most closely matched the stand in hue in the box next to the stand, and to continue the process until all colored caps were in the box. When the participant was done, the order of cap placement was recorded and diagramed on templates accompanying the tests. Both color vision and contrast sensitivity tests were performed monocularly. Ophthalmologic examinations and visual function testing was performed by Cornea Consultants of Albany, NY. Examiners were not blinded but were not told whether participants were associated with the PPDCC.

VCS results for all 13 matched pairs of children were analyzed using the Wilcoxon matched-pairs signed-ranks test. Two pairs of PPDCC and comparison children were six years

old during vision testing; all other children were aged seven or more years. So as to examine the effect of age on visual function, analyses were conducted using the 13 child pairs and excluding two pairs who were ≤6 years old. PPDCC children performed better on the VCS test compared to referent children.

Color vision results were evaluated in several ways. Statistical analyses were performed for matched pairs (n = 13) only. Proportions of pairs of children with discordant clinical judgements and with discordant numbers of major errors were assessed using McNemar's Exact Test for Correlated Proportions. Furthermore, difference in CCI between matched PPDCC and referent children were assessed using Wilcoxon Matched-Pairs signed-rank test. As for VCS results, analyses were conducted using the all child pairs and excluding pairs who were ≤6 years old. No significant difference in proportions of children with abnormal color vision or with children making major errors between PPDCC and comparison children for either of the color vision tests were found. Similarly, PPDCC and referent children were not significantly different on CCI for either color vision test.

4.6.1.2.14. ***Perrin, MC; Opler, MG; Harlap, S; Harkavy-Friedman, J; Kleinhau,s K; Nahon, D; Fennig, S; Susser, ES; Malaspina, D. 2007. Tetrachloroethylene exposure and risk of schizophrenia: Offspring of dry cleaners in a populaton birth cohort, preliminary findings. Schizophr Res. 90(1-3):251-254.*** Perrin et al. (2007) evaluates the time to a diagnosis of schizophrenia among a cohort of 88,829 births born between 1964–1976 in the Jerusalum Perintal Project, a population-based cohort. Births in this cohort were linked to the database of Israel's Psychiatric Case Registry (PCR), with cases identified using a broad definition of schizophrenia-related disorders as recorded as hospital discharge codes. Diagnoses for individuals with psychosis were validated and the date of onset was identified as the date of first psychiatric admission. Of the 88,829 births, 136 offspring were born to parents identified as having a job title of dry cleaner on the birth certificate; 120 offspring whose fathers but not mothers were dry cleaners, 20 whose mothers but not fathers were dry cleaners; and 4 with both parents as dry cleaners; 4 of the 136 births had a later diagnosis of schizophrenia. The relative risk (crude) between schizophrenia and parental employment in dry cleaning was 3.9 (95% CI = 1.3–9.2) using proportional hazard methods. The investigators noted risk estimates did not greatly change when fitting proportional hazard models that adjusted for a number of potentially confounding variables; although adjusted relative risk (RR) estimates are not reported in the paper. Variables considered as possible confounders were parents' age, father's social class, duration of marriage, rural residence, religion, ethnic origin, parental immigration status, offspring's birth order, sex, birth weight and month of birth. Family history of mental illness was not included as a covariate; rates of schizophrenia are higher among relatives of patients

than in the general population (Mueser and McGurk, 2004). The magnitude of this possible bias on the association between parental occupational employment as a dry cleaner and schizophrenia in offspring can not be judged given the information provided in the paper.

4.6.1.3. *Summary of Neuropsychological Effects in Low- and Moderate-Exposure Studies*

It is important to compare outcomes across studies in order to determine whether it is possible to identify a pattern of neuropsychological deficits produced by tetrachloroethylene. Table 4-5 is presented as an aid for this comparison. Primarily these studies have assessed neurobehavioral and, to a limited extent, neurophysiological effects of tetrachloroethylene exposure using a number of statistical methods of varying sensitivity, from simple methods that are more susceptible to multiple comparison errors to regression analyses that control for potentially confounding effects. A clinical neurological examination that includes the Romberg test, tests of body balance, and neuroradiological examination has not been widely incorporated into the tetrachloroethylene epidemiologic studies. Neurophysiological tests such as EEGs, nerve conduction tests, and evoked potentials (EPs) have seen limited use for assessing neurotoxicologic effects in tetrachloroethylene-exposed populations. Although statistically significant alterations in VEPs were reported by Altmann et al. (1990, 1992) with 4-hr acute exposure at 10 ppm, they were not altered in residents exposed chronically to a median of around 1 ppm tetrachloroethylene (Altmann et al., 1995).

Acute and chronic exposures are of different patterns, short-term peak exposure versus longer duration exposure, and, therefore, may result in a different pattern of toxicity. Furthermore, studies assessing peripheral neuropathy and tetrachloroethylene uniquely were not found, and studies reporting tetrachloroethylene exposure as one of a number of solvent exposures (Albers et al., 1999; Antti-Poika, 1982a, b) are not informative, as discussed in Section 4.6.1.

Several occupational studies of dry cleaner and laundry workers and the residential study by Altmann et al. (1995) share a common set of tests from a neurobehavioral battery. Tests in this battery have been widely administered to occupational populations in different settings with a reasonably high degree of reliability (Anger et al., 2000). Moreover, these tests have been used in clinical or experimental research to assess normal nervous system functioning, and they measure a range of sensory and cognitive function. Studies that used a test battery include Ferroni et al. (1992), Seeber (1989), Echeverria et al. (1994, 1995), and Altmann et al. (1995). Both the Seeber and the Echeverria et al. studies involved more subjects than did the studies by Ferroni et al. and Altmann et al. and statistical analyses, such as in Altmann et al., controlled for a number of potentially confounding factors. The Ferroni et al. study was not well-reported and was methodologically poorer than the other studies.

Table 4-5. Summary of neuropsychological effects of tetrachloroethylene in humans

Neurological effects	Study, number of subjects, exposure level (ppm)										
	Cavalleri et al. (1994)	Echeverria et al. (1994, 1995)[a]		Ferroni et al. (1992)	Seeber (1989)	Spinatonda et al. (1997)	Altmann et al. (1995)	Schreiber et al. (2002)		Lauwerys et al. (1983)	Nakatsuka et al. (1992)
								Residents	Daycare		
	70	173	65	90	185	74	37	34	18	59	184
	7	<0.2 3 9	11 23 41	15	12 53	8	0.7	0.4	0.3	21	15
Contrast sensitivity (spatial vision)								+	+		
CCI (color vision)	+/– [b]							trend +			– [c]
VEP (vision)							–				
Fine motor function				–	–		–				
Simple RT (attention, motor)				+			+			--	
Continuous performance (vigilance)				+			+				
Visuospatial function		+, +, + pattern recognition, reproduc-tion, memory	+, +, + pattern recogni-tion, reproduc-tion, memory		+ digit reproduc-tion		+ Benton				

Table 4-5. Summary of neuropsychological effects of tetrachloroethylene in humans (continued)

Neurological effects	Study, number of subjects, exposure level (ppm)										
	Cavalleri et al. (1994)	Echeverria et al. (1994, 1995)[a]		Ferroni et al. (1992)	Seeber (1989)	Spinatonda et al. (1997)	Altmann et al. (1995)	Schreiber et al. (2002)		Lauwerys et al. (1983)	Nakatsuka et al. (1992)
								Residents	Daycare		
	70	173	65	90	185	74	37	34	18	59	184
	7	<0.2 3 9	11 23 41	15	12 53	8	0.7	0.4	0.3	21	15
Information processing speed			–	–	+ perceptual threshold + "delayed reaction" on choice reaction time	+ vocal reproduction					
Digit span, digit symbol			–, – (both)	–	+/-, +						
Cancellation (visual scanning)					+						
Trailmaking (executive function)											

[a] Field study, no unexposed controls.
[b] Positive in dry cleaners, negative in ironing workers with lower exposure.
[c] Using less sensitive test instrument and data analysis procedure than the other studies.

RT = Reaction time
\+ = Impaired performance in exposed group
– = No effect of tetrachloroethylene

Cognitive domains affected by tetrachloroethylene include visuospatial function, attention, vigilance, and speed of information processing (choice reaction time; Table 4-5). Effects on visuospatial function are of particular interest, given the finding in the four studies that examined this domain and similar reports for other solvents (Morrow et al., 1990; Daniel et al., 1999). Echeverria et al. (1995) found effects on tests of pattern memory, visual reproduction, and pattern recognition in the absence of effects on attention (digit symbol and digit span) or executive function (Trailmaking A and B). Further, Echeverria and colleagues (1994) confirmed these findings in an independent sample of dry cleaners in their follow-up study (U.S. EPA, 2004).

Seeber (1989) also reported impaired visuospatial recognition in both exposure groups, and Altmann et al. (1995) observed deficits on a test of visuospatial function in residents with much lower exposure concentrations than those of the two occupational studies. These studies are considered to provide strong weight, given the numbers of subjects and their use of appropriate statistical methods, including adjustment for potentially confounding factors. Additionally, they considered potential bias and confounding more carefully than did other studies in this review.

Altmann et al. (1995) and Ferroni et al. (1992) assessed vigilance using a continuous performance procedure in which the subject faces a screen that presents one of several different stimuli at random intervals. The subject must make a response to a specified stimulus and not to the others. This test measures sustained attention and is correlated with performance on tests of executive function. Both studies found deficits as a result of tetrachloroethylene exposure on this task. Seeber (1989) found effects on two tests of attention (cancellation d2 and digit symbol) that are subsets of the Weschler IQ tests and were designed to be sensitive to performance within the normal range. These investigators also found positive effects on a visual scanning test that is usually used to assess laterality of brain damage but has also proved sensitive to toxicant (lead) exposure (Bellinger et al., 1994). In contrast, Echeverria et al. (1995) and Ferroni et al. (1992, as described in NYS DOH, 1997) did not find effects on digit span, which is given as a test of attention and memory, or digit symbol, despite higher levels of exposure than in Seeber (1989).

Two of these studies—an occupational study with relatively higher exposure (Ferroni et al., 1992) and the Altmann et al. (1995) residential study—also assessed simple reaction time, a task that uses a motor response and demands a relatively modest amount of attention; results were positive in both studies. Speed of information processing was assessed in two studies, Seeber (1989) and Spinatonda et al. (1997). Seeber used two tasks: recognition and choice reaction time. Effects were observed in both groups on a task requiring recognition of briefly presented stimuli. In a choice reaction time task, effects were borderline in the lower-exposure

group and negative in the higher-dose group, with no exposure-response relationship. Spinatonda et al. (1997) found effects on response to vocal and visual stimuli. A third study, Lauwerys et al. (1983), reported better performance on simple and choice reaction times.

Of the occupational studies, greatest weight is placed on the Seeber (1989) observations due to the larger number of study subjects and to their consideration in the statistical analysis of potentially confounding factors. Ferroni et al. (1992), Spinatonda et al. (1997), and Lauwerys et al. (1983) all reported limited information in their published papers, particularly regarding potential confounding and bias, and because of this, they have greater inherent uncertainties than does Seeber (1989).

Tetrachloroethylene exposure has not been reported to affect fine motor tests. Seeber (1989), Ferroni et al. (1992), and Altmann et al. (1995) each assessed fine motor control using various instruments and all three found no significant decrements in fine motor performance.

Deficits in blue-yellow color vision, a well established effect of solvents, were observed in the high-exposure group (mean tetrachloroethylene concentration of 7 ppm) but not the low-exposure group (mean tetrachloroethylene concentration of 5 ppm) in Cavalleri et al. (1994) and in Muttray et al. (1997)—a study carrying lesser weight than that of Cavalleri et al.—of workers previously exposed to a mixture of solvents that contained tetrachloroethylene. Overall, the findings of the Cavalleri et al. study and its follow-up study (Gobba et al., 1998) are in agreement with previous reports on other solvents (Geller and Hudnell, 1997; Mergler et al., 1996; Mergler and Blain, 1987): the blue-yellow range of color vision was primarily affected in the dry cleaners, with only a few workers showing an effect on red-green perception.

The absence of a color vision effect in Nakatsuka et al. (1992), who used confirmatory methods to augment their screening method of Lanthony's new color test, may not be inconsistent with the findings of Cavalleri et al. (1994) and Gobba et al. (1998). There are uncertainties regarding testing lighting conditions in Nakatsuka et al. (1992)—an important determinant of a subject's response (Geller and Hudnell, 1997)—and the fewer subjects in this study than in Cavalleri et al. (1994). A pilot study of residents living above dry cleaners with mean tetrachloroethylene exposure during active dry cleaning of 0.4 ppm (Schreiber et al., 2002) also reported a trend of decreasing color vision, although this finding was not statistically significant. The follow-up study of NYS DOH (2005a), reported to U.S. EPA as a final grant report, is further suggestive of tetrachloroethylene effects on color vision, particularly in children compared to their parents. Tetrachloroethylene exposure concentrations had decreased since Schreiber et al. (2002), making it it difficult to find higher-exposed subjects. Higher tetrachloroethylene exposure, that is, exposure at or over 0.1 ppm, was highly correlated with SES and belonging to a minor population. This study is not able to adjust for these possible

confounders given the sample size. Studies of a larger number of residents with similar exposure concentrations are needed to draw more definitive conclusions.

Only Schreiber et al. (2002) and NYS DOH (2005a) assessed spatial vision, an effect reported for exposure to other solvents (Bowler et al., 1991; Broadwell et al., 1995; Campagna et al., 1995; Donoghue et al., 1995; Frenette et al., 1991; Hudnell et al., 1996a, b; Mergler et al., 1991). VCS deficits in subjects with normal visual acuity were observed at low exposure concentrations in residential populations that were subject to very different dose-rates than those incurred by occupational workers (U.S. EPA, 2004). This finding is based on few subjects in this study and is noteworthy for this reason, even in light of questions regarding potential biases. Potential bias and confounding could be introduced, in part, from a lack of blinding of testers, differences in motivation between exposed and referent subjects for participating in the study, and individual differences in exposed and control populations (U.S. EPA, 2004). As discussed in Section 4.6.1.2.11, occupation has not been found to strongly affect contrast sensitivity (Hudnell et al., 2001), nor does motivation (U.S. EPA, 2004).

Peer consultation comments on EPA's earlier draft "Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper" (U.S. EPA, 2003b) noted that the deficit in contrast sensitivity could reflect a sensitivity of the visual system to tetrachloroethylene, or it may be that this test was simply carried out by a superior test method (U.S. EPA, 2004). Furthermore, the peer consultants also suggested that contrast sensitivity loss may reflect impaired function throughout the brain, because contrast sensitivity is affected by retinal, optic nerve, or central brain dysfunction (U.S. EPA, 2004). Nonetheless, drawing strong conclusions from a single study is difficult, particularly in light of the paucity of data on this test in occupational populations with higher exposure concentrations and in animal studies. The finding of poorer performance among children with exposure to >0.1 ppm tetrachloroethylene compared to adults in NYS DOH, (2005a) adds some support for observations in Schreiber et al. (2002).

Mental disease of neurologic origin has not been well studied with respect to environmental factors. Perrin et al. (2007), who reports an association between schizophrenia and parental exposure in dry cleaning, is the only such study. Other studies are needed to understand the role of parental tetrachloroethylene exposure in the development of mental disease in children.

The epidemiologic studies all have limitations. The body of evidence is characterized generally by a lack of studies that adopt sensitive tests of important functions affected by solvents. The exceptions are the studies employing vision assessment and the Echeverria et al. (1994, 1995) studies. In most studies, investigators were not blinded to subject status, a potential source of bias, particularly in situations in which the investigator interacted directly with the subject during testing. All studies used a cross-sectional design, which is weaker than a

longitudinal design for a number of reasons, including a greater potential for selection bias and exposure misclassification (the latter of which would bias the results toward the null). One possible source of selection bias is motivational differences between exposed and control populations. The designs of the Schreiber et al. (2002) and Ferroni et al. (1992) studies may have introduced such unwanted bias, although motivation has been found to more strongly influence performance on color vision but not contrast sensitivity tests (U.S. EPA, 2004). Several studies provided insufficient details on the population from which controls were selected (Seeber, 1989; Spinatonda et al., 1997; Ferroni et al., 1992), or the details provided raise concerns regarding the appropriateness of the control group (Seeber, 1989; Spinatonda et al., 1997; Schreiber et al., 2002 [residents only]).

For some of the occupational studies, the descriptions of behavioral testing procedures or results were insufficient or ambiguous (Ferroni et al., 1992; Seeber, 1989; Nakatsuka et al., 1992; Spinatonda et al., 1997). A number of studies had either insufficient control for possible influences of education (Cavalleri et al., 1994; Gobba et al., 1998; Schreiber et al. (2002) [day care study]) or provided insufficient detail on the study populations (Schreiber et al., 2002 [residents]; Nakatsuka et al., 1992; Ferroni et al., 1992; Spinatonda et al., 1997). Further, adjustment for education in the statistical analysis may not have been fully adequate due to the use of categorical variables (Seeber, 1989; Altmann et al., 1995). Additionally, Spinatonda et al. (1997) did not provide detailed information on variables other than age of subjects, precluding a determination of whether subjects may or may not have been comparable.

Alcohol by itself cannot explain the observed deficits in neurobehavioral functions, because either study designs excluded subjects who were moderate to heavy drinkers, or statistical analyses of the epidemiologic observations controlled for this covariate. However, effects from the interaction between tetrachloroethylene exposure and alcohol consumption were not well investigated in these studies. Valic et al. (1997) showed greater decrements in color vision among subjects with exposures to both tetrachloroethylene and ethanol when compared with individuals with solvent exposure only to the solvents or to neither substance.

Many studies did not include exposure monitoring of individual subjects, and the statistical analyses compare groups using t-tests or chi-square tests, with the result of a greater dependency on the performance in the control group. Dose response analyses are statistically more powerful. However, despite the use of t-tests or chi-square tests, deficits in neurobehavioral or neurophysiological functions were reported in these studies. A number of statistical comparisons were made in these studies, increasing the possibility of a type I, or false positive, error. The issue of multiple comparisons is always present in risk assessment and evaluation activities; however, unfortunately, reducing the type I error increases the type II, or false negative, error for those associations that are not null. As mentioned above, inferences

about associations between exposure and effects are best drawn from a body of evidence where consistency across studies of different designs, populations, and statistical methods may be obtained; value and richness can be found when consistency emerges from the diversity and despite the flaws.

These studies do have important strengths. They describe susceptibility to tetrachloroethylene toxicity in humans, providing evidence to augment findings from animal toxicity testing. Further, the majority of studies, with the exception of Schreiber et al. (2002), exceeded a smallest cell size of 40 exposed subjects that is generally considered sufficient to detect preclinical effects in a group that range from 3 to18, or 20%, from normal function. Several studies (Altmann et al., 1995; Schreiber et al., 2002; Echeverria et al., 1995) employed multiple measures of exposure (indoor air monitoring, personal monitoring, and in some cases, biological monitoring), with a high degree of correlation between tetrachloroethylene concentration as assessed from indoor air monitoring or personal monitoring and biological metrics such as blood tetrachloroethylene concentration, suggesting indoor air concentration as a reasonable exposure metric.

Several independent lines of evidence can be found in the occupational and residential studies to support an inference of a broad range of cognitive and behavioral deficits following tetrachloroethylene exposure (U.S. EPA, 2004). First, adverse effects on visuospatial function are reported in three studies (Seeber, 1989; Altmann et al., 1995; Echeverria et al., 1995), with Echeverria et al. (1994) as a confirmatory study of Echeverria et al. (1995). The results across these three studies appear reasonably consistent, despite substantial differences in study design.

A second line of evidence can be found in both the occupational (Nakatsuka et al., 1992; Cavalleri et al., 1994) and residential studies (Schreiber et al., 2002), both of which evaluated performance on the Lanthony color vision test. Cavalleri et al. (1994) reported a decrement in color vision in the high exposure group, but not the low exposure group, and a significant dose-response relationship between CCI value and tetrachloroethylene concentration. The lack of an association between color vision and tetrachloroethylene exposure in Nakatsuka et al. (1992) may not be inconsistent, given significant weaknesses in this study (U.S. EPA, 2004). Performance of the residents and day care workers who worked in buildings with a co-located dry cleaner appeared worse (particularly that of residents) than the performance of controls, although CCI scores were not statistically significantly different from referents (Schreiber et al., 2002). Last, VCS deficits were observed in these residents and day care workers. These subjects received exposures of lower dose rates, but a different and, for residents, a more prolonged daily exposure duration than typical occupational exposures occurred. Overall, the evidence reveals a high degree of consistency in visually mediated function.

Effects on spatial vision are well-known consequences of solvent exposure in industrial workers (Bowler et al., 1991; Broadwell et al., 1995; Campagna et al., 1995; Donoghue et al., 1995; Frenette et al., 1991; Hudnell et al., 1996a; Mergler et al., 1991). Other organic solvents, as well as alcohol, induce effects on memory and color vision (Altmann et al., 1995; Mergler et al., 1991; Hudnell et al., 1996a, b). By analogy, the observations on other solvents also support an inference of neurobehavioral deficits following exposure to tetrachloroethylene.

In conclusion, the weight of evidence across the available studies of humans exposed to tetrachloroethylene—and by analogy to other organic solvents—indicates that chronic exposure to tetrachloroethylene may be associated with adverse decrements in nervous system function.

4.6.2. Animal Studies

4.6.2.1. *Inhalation Studies*

Mattsson et al. (1998) studied the effects of acute exposure to tetrachloroethylene for 13 weeks observing flash-evoked potentials (FEPs), somatosensory-evoked potentials (SEPs), EEGs, and rectal temperature in F344 rats. During the acute (pilot) study, male rats were exposed to 0 or 800 ppm tetrachloroethylene for 6 hrs/day for 4 days and tested before and after exposure on the 4th day. Changes in FEP, SEP, and EEG components were observed after acute exposure. In the subchronic study, the above evoked potentials and caudal nerve conduction velocity were determined in male and female rats exposed to 0, 50, 200, or 800 ppm for 6 hrs/day for 13 weeks. Testing was performed during the week following cessation of exposure. Changes in FEP were observed at the highest dose (800 ppm). Several measures of the evoked potential were affected, at 50 ppm but not at higher doses. Other measures were not affected, and no dose response was observed. The finding of an overall greater effect following short-term (4-day) exposure as compared with longer-term exposure is similar to the findings of Moser et al. (1995) on a number of measures of a neurotoxicity battery.

The effects of exposure to 90–3,600 ppm tetrachloroethylene for 1 hr on motor activity were examined in male MRI mice (Kjellstrand et al., 1985). A strong odor (cologne) was used as the control condition. Total activity was monitored during the dark period during exposure and for several hours thereafter. All doses produced increased activity during exposure; activity decreased over several hours after cessation of exposure. Although apparently no statistical analyses were performed, it is clear from the figures that the lowest dose produced an average performance that was well outside the boundary of the 95% CIs of the cologne-treated controls and was dose-dependent. Tetrachloroethylene induced motor activity at concentrations lower than those of any of the other organic solvents tested (methylene chloride, toluene, trichloroethylene, 1,1,1-trichloromethane).

De Ceaurriz et al. (1983) exposed male Swiss OF1 mice to 596, 649, 684, or 820 ppm tetrachloroethylene for 4 hrs. Immediately following exposure, subjects were immersed in a cylinder filled with water and the duration of immobility was observed for 3 minutes. The term "behavioral despair" has been coined for this initial immobility, and the length of immobility is shortened by antidepressant administration. Tetrachloroethylene exposure also shortened the period of immobility, with a no-observed-effect level (NOEL) of 596 ppm.

Nelson et al. (1980) of NIOSH, investigated developmental neurotoxicity in SD rats by exposing pregnant dams to tetrachloroethylene at concentrations of 100 ppm or 900 ppm during both early pregnancy (gestation days 7 to 13) or late pregnancy (gestation days 14 to 20). The investigators made morphological examinations of the fetuses and performed behavioral testing and neurochemical analysis of the offspring. There were no alterations in any of the measured parameters in the 100 ppm groups. At 900 ppm there were no skeletal abnormalities, but the weight gain of the offspring as compared with controls was depressed about 20% at weeks 3–5. Developmental delay was observed in both the early and late pregnancy groups. Offspring of the early pregnancy-exposed group performed poorly on an ascent test and on a rotorod test, whereas those in the late pregnancy group underperformed on the ascent test only at postnatal day 14. However, later in development (days 21 and 25), their performance was higher than that of the controls on the rotorod test. These pups were markedly more active in the open field test at days 31 and 32.

There were no effects on running in an activity wheel on days 32 or 33 or avoidance conditioning on day 34 and operant conditioning on days 40 to 46. Neurochemical analyses of whole brain (minus cerebellum) tissue in 21-day-old offspring revealed significant reductions in acetylcholine levels at both exposure periods, whereas dopamine levels were reduced among those exposed on gestation days 7–13. Unfortunately, none of the statistics for the 100 ppm treatments was presented. The authors observed that more behavioral changes occurred in offspring exposed during late pregnancy than in those exposed during early pregnancy.

Szakmáry et al. (1997) exposed CFY rats to tetrachloroethylene via inhalation throughout gestation (i.e., gestation days 1–-20) for 8 hrs/day at concentrations of 0, 1,500, or 4,500 mg/m^3 tetrachloroethylene. The primary focus of the study was prenatal developmental evaluations (see Section 4.7.2). However a cohort of rats (15 litters/group) was allowed to deliver, and the offspring (standardized to 8 pups/litter) were maintained on study until postnatal day 100 and evaluated for growth, development and neurotoxic effects. The report did not specify whether the animals were exposed to tetrachlorotehylene after birth. Pre-weaning observations included weekly body weights, developmental landmarks (pinna detachment, incisor eruption, and eye opening), and functional assessments (forward movement, surface righting reflex, grasping ability, swimming ontogeny, rotating activity, auditory startle reflex, and examination of

stereoscopic vision). After weaning, exploratory activity in an open field, motor activity in an activity wheel, and development of muscle strength were assessed. The study authors reported that adverse findings included a decreased survival index (details were not provided), a minimal decrease of exploratory activity and muscular strength in treated offspring (presumably at both exposure levels) which normalized by postnatal day 51, and significantly increased motor activity on postnatal day 100 of females exposed to 4,500 mg/m^3. Litter was evaluated as the statistical unit of measure for all outcomes. There is no clear indication of group means for postnatal measures reported. The lack of experimental detail in the postnatal evaluation part of this study reduces the overall confidence in the findings. There was no evaluation of postnatal histopathology of the nervous system reported or cognitive testing during the post weaning period or during adulthood.

Wang et al. (1993) exposed male SD rats to 300 ppm tetrachloroethylene continuously for 4 weeks or 600 ppm for 4 or 12 weeks. Exposure to 600 ppm at either duration resulted in reduced brain weight gain, decreased regional brain weight, and decreased DNA in frontal cortex and brain stem but not hippocampus. Four specific proteins (S-100 [an astroglial protein], glial fibrallary acidic protein, neurone specific enolase, and neurofilament 68 kD polypeptide) were decreased at 4 and/or 12 weeks exposure to 600 ppm; 300 ppm had no effect on any endpoint.

The effects of exposure to 200 ppm tetrachloroethylene 6 hrs/day for 4 days in male SD rats were examined on a number of endpoints (Savolainen et al., 1977a, b). Rats were killed on the 5th day following a further 0–6 hrs of exposure. Tetrachloroethylene levels were highest in fat, followed by liver, cerebrum, cerebellum, lung, and blood. Tissue levels increased in all tissues over the 6 hrs of exposure. Brain RNA content decreased, and brain nonspecific cholinesterase was increased on the 5th day, although no statistical comparisons were performed. Locomotion in an open field was increased immediately following the end of exposure on the 4th day, with no difference 17 hrs after exposure, although no statistical comparisons were made. Brain protein, GSH, and acid proteinase were unaffected.

A series of experiments were performed on the effects of tetrachloroethylene on brain lipid patterns. Exposure to 320 ppm for 90 days (Kyrklund et al., 1990) or 30 days (Kyrklund et al., 1988) in male SD rats resulted in changes in the fatty acid composition of cerebral cortex, which persisted after a 30-day recovery period (Kyrklund et al., 1990). Similar results were observed in cerebral cortex and hippocampus after exposure to 320 ppm in the Mongolian gerbil (sex unspecified) in the presence of reduced brain weight (Kyrklund et al., 1987). Exposure of male Mongolian gerbils to 120 ppm for 12 months also resulted in decreases in long-chain, linolenic acid-derived fatty acids in cerebral cortex and hippocampus (Kyrklund et al., 1984).

The effect of tetrachloroethylene on neurotransmitter levels in the brain was explored in male SD rats exposed continuously to 200, 400, or 800 ppm for a month (Honma et al., 1980a, b).

The 800 ppm dose produced a decrease in ACh in striatum, and there was a dose-related increase in a peak containing glutamine, threonine, and serine in whole brain preparations. GABA, NE, 5-HT, and other amino acids were not affected.

In a study from the same laboratory (Rosengren et al., 1986), Mongolian gerbils of both sexes were exposed to 60 or 300 ppm tetrachloroethylene for 3 months, followed by a 4-month solvent-free period. Changes in both S-100 and DNA concentrations in various brain regions were observed at the higher concentration, and decreased DNA in frontal cortex was observed after exposure to 60 ppm. The higher concentration also produced decreased brain but not body weight. The results at 60 ppm were replicated in a follow-up study (Karlsson et al., 1987).

In a related study (Briving et al., 1986), Mongolian gerbils were exposed for 12 months to tetrachloroethylene at 120 ppm. At the end of exposure, out of a total of 8 amino acids assayed, taurine was significantly decreased in the two brain regions assessed (hippocampus and cerebellum), and glutamine was elevated in hippocampus. γ-Aminobutyric acid (GABA) levels were unaffected, as was uptake of GABA and glutamate.

Kyrklund and Haglid (1991) exposed pregnant guinea pigs to airborne tetrachloroethylene continuously from day 33 through day 65 of gestation. The exposure was continuous at 160 ppm except for 4 days at the beginning and end of the exposure period, when it was reduced to 80 ppm. In the control group there were three dams with litter sizes of four, three and two pups, and in the exposed group there were three dams with litter sizes of two each. The pup body weights differed between litters. In the data analysis, three pups in the control group were eliminated and the six pups in the treatment and control groups were assumed to be independent, which is an invalid assumption. According to the authors' analysis, the offspring had a slightly altered brain fatty acid composition, with a statistically significant reduced stearic acid content in the tetrachloroethylene treatment group, which is consistent with the authors' earlier findings in rats. This conclusion might have been different if the investigators had grouped litters rather than pups as independent groups. The results suggest that tetrachloroethylene could have reduced the litter size, but a much larger study would be necessary to establish reduced litter size as an effect of tetrachloroethylene.

Caucasan male and female NMRI mice were exposed to 9, 37, 75, or 150 ppm continuously for 30 days, to 150 ppm for one of several exposure periods ranging from 5 to 30 days, or to 150 ppm tetrachloroethylene for 30 days with various recovery periods (Kjellstrand et al., 1984). Other groups were exposed intermittently on several dosing and exposure regimens that resulted in a TWA of 150 ppm for 30 days. Plasma BuChE levels, organ weights, liver morphology, and motor activity were assessed. BuChE was elevated after continuous exposure to 37 ppm or greater. Liver weight was increased at all doses following continuous exposure, and body weight decreased at 37 ppm or above. Motor activity results following continuous

exposure were not reported. BuChE and liver weight were both elevated at a TWA of 150 ppm for 30 days, regardless of the length of the exposure pulse. This was true even for an hour's exposure (at 3,600 ppm) as well as at the lowest concentration (225 ppm). All concentrations of intermittent exposure increased motor activity. A recovery period reversed the effects on BuChE, whereas liver weight was still slightly elevated at 150 days after cessation of exposure. Changes in liver morphology were detected following exposure to 9 ppm for 30 days and reversed after cessation of exposure.

Tinston (1994) performed a multi-generation study of the effects on rats exposed to airborne concentrations of tetrachloroethylene. The details of the study are discussed in Section 4.7.2. The investigators observed several developmental effects. Of interest here were the signs of CNS depression (decreased activity and reduced response to sound) observed for the first 2 weeks in both adult generations and when the exposure was resumed on day 6 postpartum in the F1 generation (adults and pups). These effects disappeared about 2 hrs after cessation of the daily exposure. Other overt signs of tetrachloroethylene poisoning among the adults included irregular breathing and piloerection at both 300 and 1,000 ppm. These changes stopped concurrently with cessation of exposure or shortly thereafter.

4.6.2.1.1. ***Summary of animal inhalation neurotoxicity studies*.** In order to compare the animal inhalation neurotoxicity studies with each other and to evaluate whether there is any relationship across studies between the LOAEL of the administered dose and the duration of treatment, the data were summarized (Table 4-6). In order to estimate the lowest concentration at which a given effect occurs, the experiment showing that effect must have both a LOAEL (the lowest concentration at which the effect occurred) and a NOAEL (the next lower concentration where the effect did not occur). The experiments that meet this criteria are Mattsson et al. (1998), De Ceaurriz et al. (1983), Wang et al. (1993), Honma et al. (1980 a, b), and Kjellstrand et al. (1984).

The total duration of exposure in these experiments is plotted in Figure 4-2 as a function of the LOAEL concentration in order to discover whether there is a systematic trend in this relationship. The plot shows that there is no systematic trend. It also shows that the LOAEL varies over a 22-fold range: from 37 ppm for 30 days for increased brain butyl cholinesterase in mice observed by Kjellstrand et al. (1984) to 800 ppm for 13 weeks for alteration in the flash-evoked potential in rats observed by Mattsson et al. (1998). Table 4-6 shows other observations at comparatively low concentrations: decreased DNA in gerbils by Rosengren et al. (1986) and Karlsson et al. (1987) at 60 ppm and increased motor activity in mice at 90 ppm, observed by Kjellstrand et al. (1985). The LOAEL for these studies as a group is therefore in the range of 37 to 90 ppm, and the effects at these levels are changes in neurotransmitter levels and increased

Table 4-6. Summary of animal inhalation neurotoxicology studies

Subjects	Effect	NOAEL/ LOAEL[a] (ppm)	Authors
F344 rats Pilot study: male 10/dose Follow-up study: males and females 12/sex dose	Changes in FEP, SEP, EEG Increased amplitude and latency in late component of FEP	0, 800, 4 days, 6 hr/d 50, 200, 800, 13 wks, 6 hr/d, 5 d/wk	Mattsson et al. (1998)
NMRI mice, males	Increase in motor activity	90, 3,600 1 hr	Kjellstrand et al. (1985)
Swiss OF1 mice, males 10/dose	Decrease in duration of immobility	596, 649, 684, 820 4 hrs	De Ceaurriz et al. (1983)
SD rats pregnant females 13–21 litters/dose males and female Offspring assessed	Decreased weight gain Behavioral changes, more extensive for late pregnancy exposure Decreased brain acetylcholine	0, 100, 900 on GD 7–13 or on GD 14–20 7 hr/d	Nelson et al. (1980)
CFY rats pregnant females 15 litters/dose male and female offspring assessed	Transient decreases in muscular strength and exploratory behavior. Latent increases in motor activity in females at 100 days postnatally	0, 1,500 or 4,500 mg/m^3 GD 1-20 for 8 hrs/day	Szakmáry et al. (1997)
SD rats, males 8/dose	Reduced brain weight, DNA, protein	300, 600, 4 or 12 wks continuous (24 hr/d)	Wang et al. (1993)
SD rats, males 10/dose	Decrease in brain RNA, increase in brain cholinesterase and increase motor activity	200, 4 days	Savolainen et al. (1977a, b)
SD rats, males 5–6/dose	Change in fatty acid composition of cerebral cortex	320, 12 wks continuous (24 hr/d), 30-day washout period 320, 4 wks continuous (24 hr/d)	Kyrklund et al. (1990, 1988)
SD rats, males 5–6/dose	Neurotransmitter changes, brain regions	200, 400, 800, 4 wks continuous (24 hr/d)	Honma et al. (1980a, b)
Mongolian gerbils males and females 6/sex/dose	Decrease in DNA, frontal cortex Decrease in brain weight	60, 300, 12 wks, continuous (24 hr/d) 16-week washout period	Rosengren et al. (1986)

Table 4-6. Summary of animal inhalation neurotoxicology studies (continued)

Subjects	Effect	NOAEL/LOAEL (ppm)	Authors
Mongolian gerbils gender unspecified	Decrease in DNA, frontal cortex Decrease in brain weight	60, 12 wks, continuous (24 hr/d)	Karlsson et al. (1987)
Mongolian gerbils males and females 8/sex/dose	Taurine, glutamine changes in brain regions	120, 12 mos continuous (24 hr/d)	Briving et al. (1986)
Mongolian gerbils gender unspecified 6/dose	Decrease in brain weight, change in fatty acids	320, 12 wks continuous (24 hr/d)	Kyrklund et al. (1987)
Mongolian gerbils males 6/dose	Decreased brain long-chain fatty acids	120, 52 wks continuous (24 hr/d)	Kyrklund et al. (1984)
Guinea pigs pregnant females 3/litters/dose males and female Offspring assessed	Decrease in brain stearic acid in offspring after in utero exposure[b]	Maximum exposure 160, GD 33 to 65 continuous (24 hr/d)	Kyrklund and Hagid (1991)
NMRI mice, males and females 3–8/sex dose	Increase in butyl cholinesterase	9[c], 37, 75, 150 4 wks continuous (24 hr/d)	Kjellstrand et al. (1984)
Males and females 10/sex dose	Increased motor activity	150, 4 wks intermittent- (1, 2, 4, 8, or 16 hr/d)	Kjellstrand et al. (1984)
SD rats, multigeneration study 28 litters/dose	CNS depression in first 2 wks of F1 and F2 generations, which ceased 2 hrs after daily exposures	0, 100, 300, 1,000 6 hr/d, 5 d/wk, except during mating, 6 hr/d-7 d/wk	Tinston (1994)

[a] Experimental/observational NOAEL is underlined, LOAEL is double-underlined.
[b] Questionable findings because litter was not used as the unit of measure in analysis.
[c] LOAEL for changes in liver weight.

FEP = Flash-evoked potential
GD = Gestational day
SEP = Somatosensory-evoked potential

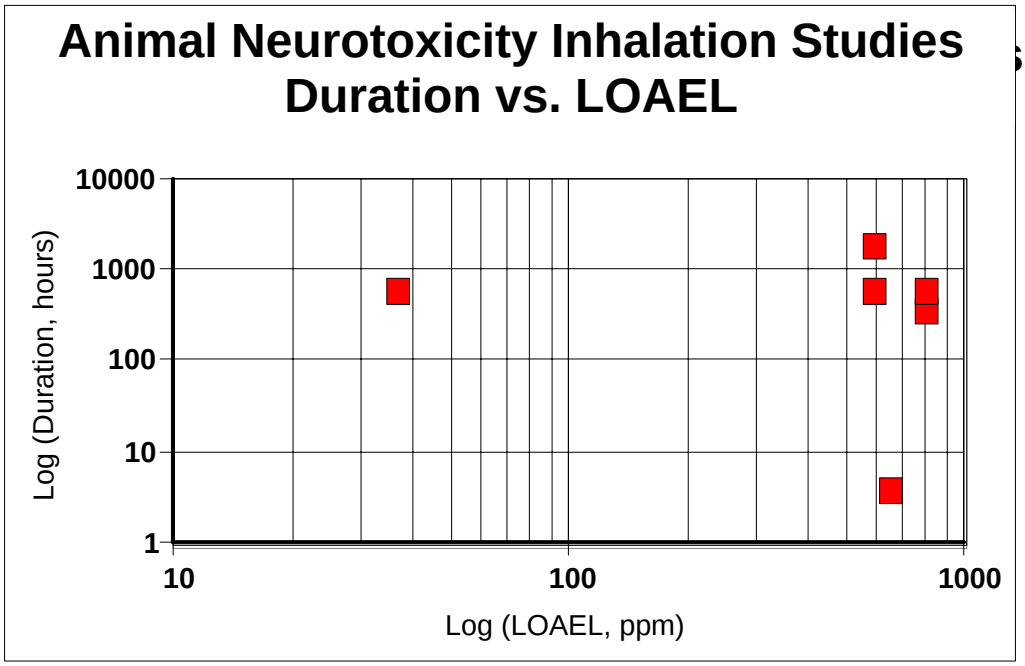


Figure 4-2. Summary of the relationship between LOAEL concentrations (ppm) and treatment duration (hours).

LOAEL = Lowest-observed-adverse-effect level

motor activity. Changes in fatty acid composition were observed at somewhat higher concentrations (320 ppm).

4.6.2.2. *Oral and Intraperitoneal Studies*

A study in male SD rats assessed the acute or short-term effects of tetrachloroethylene by gavage on several screening tests (Chen et al., 2002). A single dose of 500 mg/kg to adult rats produced changes on three different tests of pain threshold, locomotor activity, and seizure susceptibility threshold following pentylenetetrazol infusion, whereas 50 mg/kg had statistically significant effects on seizure threshold only. In the short-term study, young 45–50 gram rats were dosed 5 days/week for 8 weeks with 5 or 50 mg/kg. Behavioral testing began 3 days after the last dose. Locomotion was affected only at the high dose, whereas both doses produced effects on the other four endpoints. The 8-week exposure resulted in retarded weight gain in both treated groups, which was about 10% at the end of the dosing period.

The interpretation of these results is problematic. The tests were all observational in nature, requiring scoring by the observer. The study by Chen et al. (2002) does not state whether

the observer(s) was blind to the treatment group of the animals, a condition that is essential for such tests to be valid. In fact, because there were differences in weight between control and treated rats, it would probably be easy to distinguish treated from control animals simply by looking at them. Further, the paper does not state whether all animals were tested by the same person for each task or, if not, whether there was any indication of inter-observer correlation. The potential effect of the difference in weight between the control and the treated groups on these measures is also unknown. Given that the difference between the control and the treated groups in response latency to painful stimuli is tenths or hundredths of a second with no dose response, these issues are of serious concern.

Various behavioral endpoints were assessed in 8-week-old ICR male mice at the beginning of an experiment by Umezu et al. (1997). Righting reflex was affected after single-dose i.p. administration of tetrachloroethylene at 4,000 but not at 2,000 mg/kg or less, and ability to balance on a wooden rod was decreased at 2,000 but not at 1,000 mg/kg or less. Response rate on a fixed-ratio 20 (FR20) schedule—which requires 20 responses for each reinforcement—was affected at 2,000 but not at 1,000 mg/kg or less 30 minutes after administration. In a procedure in which a thirsty mouse was shocked every 20th lick of a water spout, mice dosed with 500 mg/kg but not with higher or lower doses received an increased number of shocks. In an FR20-FR20 punishment schedule, responding in the punishment condition was increased at 1,000 but not at 500 mg/kg or less. A puzzling aspect of the study is the mention in the methods section of "breeding animals," with no further explanation. If the investigators bred their own mice, there is no indication of how pups were assigned to treatment groups.

Moser et al. (1995) examined the effects of a number of potentially neurotoxic agents, including tetrachloroethylene, on a neurotoxicity screening battery in adult female F344 rats following either a single gavage dose (acute exposure) or repeated gavage doses over 14 days (subacute exposure). For the acute study, subjects were tested 4 and 24 hrs following exposure. After acute exposure, a LOAEL of 150 mg/kg was identified for increased reactivity to being handled 4 hrs after dosing, with increased lacrimation, decreased motor activity, abnormal gate, decreased response to an auditory stimulus,
decreased righting ability, and increased landing foot-splay at higher doses at 4 and/or 24 hrs post-dosing. A NOAEL was not identified. In the subacute study, no endpoint was significantly different from those of controls at doses of 50–1,500 mg/kg. This presumably represents behavioral adaptation following repeated exposure to tetrachloroethylene.

Locomotor activity was monitored in NMRI mice gavaged with 5 or 320 mg/kg tetrachloroethylene for 7 days beginning at 10 days of age (Fredriksson et al., 1993). Twelve male pups from three or four litters were assigned to each treatment group. This study design does not conform to traditional developmental toxicity testing guidelines. Locomotion, rearing,

and total activity (vibration of the cage) were measured for 60 minutes at 17 and 60 days of age. A stastically significant increase in locomotor activity of treated mice in both dose groups was observed, and rearing behavior decreased as compared with controls for all three measures at 60 days of age but not at 17 days of age in which testing followed shortly after the last dose.

The persistent effects of subacute developmental exposures in this study raises some concerns. Some caution in interpreting the results of the effect of tetrachloroethylene exposure is warranted for two reasons: (1) the results at 320 mg/kg were no different than at 5 mg/kg, indicating no clear dose-response relationship between exposure and this effect, and (2) litter mates were used as independent observations in the statistical analysis. This procedure can increase the apparent α and result in an erroneous statistical result. For example, Holson and Pearce (1992) demonstrated that for body weight, using three or four littermates as independent observations, as in the above study, resulted in the nominal α increasing from 0.05 to a range of 0.23 to 0.38. Similar litter effects have been demonstrated for behavioral data (Buelke-Sam et al., 1985).

Fredriksson et al. completed a study that parametrically compared the effects of postnatal dosing and resulting alternations in motor activity using both litter as the unit of measure and their own within-litter randomization (Ericksson et al., 2005). Their results were similar in both the magnitude of effect across dose groups and in the variability within each dose group for both experimental designs. The authors' key assertion for using this randomization within a small number of litters rather than the traditional litter as the unit of measure is that it reduces the overall number of animals needed to be generated to statistically determine an effect of chemical exposure.

Locomotor activity was assessed in 6-week-old male Wister rats following i.p. doses of 100, 500, or 1,000 mg/kg tetrachloroethylene for 3 consecutive days, with activity being monitored for at least 1 week following cessation of administration (Motohashi et al., 1993). Animals were monitored 24 hrs/day, and locomotor activity (measured as change in electrical capacitance of a circuit beneath the floor of the cage) was analyzed by time-series analysis and spectral analysis. All doses of tetrachloroethylene changed circadian rhythm in a dose-dependent manner, with the increased activity at the start of the dark period delayed by tetrachloroethylene exposure. Recovery took 3–5 days after cessation of exposure.

Operant performance on a fixed-ratio 40 schedule of reinforcement was assessed in adult male SD rats gavaged with 160 or 480 mg/kg tetrachloroethylene immediately before testing (Warren et al., 1996). The lower dose produced no effect on response rate over the 90-minute session, whereas the higher dose produced a transient rate decrease in three of six animals (with recovery after 20 to 40 minutes) and induced a complete cessation of response in two of the six animals. Tetrachloroethylene concentrations increased rapidly after administration in blood,

brain, fat, liver, and muscle. For the duration of the 90-minute period of testing, blood tetrachloroethylene levels were approximately linearly related to the administered dose, but brain tetrachloroethylene levels were similar for both dose groups. This study did not evaluate the persistent effects of exposure to tetrachloroethylene on cognitive performance.

Table 4-7 presents a summary of the oral neurotoxicity animal studies. For the six oral neurotoxicity studies in rodents reviewed here, only one (Fredriksson et al., 1993) describes effects lasting more than 1 week. In that study the effect (increased motor activity) was the same at 5 and 320 mg/kg, and the results do not represent a clear dose-response relationship across two orders of magnitude of administered doses. The lowest LOAEL occurring in the four remaining studies is 100 mg/kg for delayed onset of circadian activity in rats (Motohashi et al., 1993). This LOAEL is based on an i.p.-administered dose describing transient neurological effects and is not comparable to inhalation or ingestion LOAELs without pharmacokinetic modeling of an appropriate dose metric. No information is available for irreversible neurological effects via the oral route because no studies have evaluated the potential for neurotoxicity following chronic oral exposure.

4.6.3. Summary of Neurotoxic Effects in Humans and Animals

Taken together, the animal and epidemiologic evidence is supportive of an association between neurobehavioral deficits and tetrachloroethylene exposure. The pattern of effects on the visual system in humans may be consistent with decrements in visually mediated dysfunction, as suggested by Echeverria et al. (1995). The test for VCS in humans is sensitive to neurological dysfunction associated with many diseases affecting the nervous system (NYS DOH, 2000). Moreover, VCS deficits as well as color discrimination deficits are commonly present prior to detectable pathology in the retina or optic nerve head, making this one of the earliest signs of disease (Regan, 1989). Additionally, other organic solvents, as well as alcohol, induce effects on memory and color vision (Altmann et al., 1995; Mergler et al., 1991; Hudnell et al., 1996a, b). The consistency of these observations suggests construct validity for organic solvents as a class because of their effects on visually mediated function. Hence, these observations, by analogy, add support to an inference of tetrachloroethylene-induced neurobehavioral effects.

Studies of occupational (Seeber, 1989; Echeverria 1994, 1995) and residential (Altmann et al., 1995) exposures indicate that cognitive performance in humans exposed to tetrachloroethylene is affected with effects on choice reaction times, visual-spatial information processing, and other measures of cognitive performance.

The three epidemiological studies on dry cleaners chronically exposed to tetrachloroethylene showed decrements in color vision at 7 ppm (Cavalleri et al., 1994, with a follow-up of these workers [Gobba et al., 1998] showing greater loss in color discrimination in

Table 4-7. Summary of oral neurotoxicity animal studies

Subjects	Effect	NOAEL/LOAEL[a] (mg/kg)	Author
SD rats, male	Pain threshold, pain susceptibility, weight gain decrement Interpretation is unclear	5, 50 mg/kg daily for 8 weeks	Chen et al. (2002)
SD rats, male	Operant responses stopped immediately after 480 mg/kg dose, then 2/3 of animals recovered by 40 minutes. Brain tetrachloroethylene concentrations were the same at both doses	Gavage single dose at 0, 160, 480 mg/kg	Warren et al. (1996)
ICR mice, male 8–10/dose	NOAEL/LOAEL: Righting reflex, 2,000/4,000 Balance, 1,000/2,000 Operant responses, 1,000/2,000 Punishment, 500/1,000	Single intraperitoneal doses at 0, 500, 1,000, 2,000, 4,000 mg/kg	Umezu et al. (1997)
F344 rats, female	Increased reactivity, decreased motor activity, decreased righting ability, increased landing foot splay, abnormal gait after one dose No effect after repeated doses	Single doses: 150 mg/kg is LOAEL Repeated dosing for 14 days: 1,500 mg/kg is NOAEL	Moser et al. (1995)
NMRI male mice, post-natal exposure 12 pups/ dose (derived from 3 litters)	Increased locomotion and decreased rearing at day 60 in both dose groups No effect immediately after treatment	Gavage treatment 5, 320 mg/kg daily for postnatal days 10–16	Fredriksson et al. (1993)
Wistar rats, male n/dose?	Transient delay in circadian activity, dose-related	Intraperitoneal doses 0, 100, 500, 1,000 mg/kg-day for 3 days	Motohashi et al. (1993)

[a] Experimental/observational NOAEL is underlined, LOAEL is double-underlined.

n/dose = Number of animals per dose not clearly defined

those who were subsequently exposed to a higher concentration and smaller loss in those exposed for lower concentrations), longer reaction times to visual stimuli at 8 ppm (Spinatonda et al., 1997) and decrements in cognitive function at 12 ppm (Seeber, 1989).

Two studies of tetrachloroethylene exposure in residences near a dry cleaning facility (Altmann et al., 1995) and a day care facility (Schreiber et al., 2002) found decrements in several neurological parameters at lower exposures than did the studies of occupational exposures cited above. This indicates that CNS effects can occur at a lower concentration than inferred from occupational studies of dry cleaners, which have been of exposures several-fold higher than those in these residential studies. LOAELs in the human studies of CNS effects ranged from 0.7 ppm to 41 ppm.

The lack of exposure-response relationships is a limitation; however, this lack may be a reflection of poorer characterization of exposure in these studies. Exposure-response relationships are based only on an estimate of current exposure; historical exposure to tetrachloroethylene is lacking and may be more important to an analysis of exposure response. Moreover, most analyses use a concentration x time [C × t]) dose metric. Another metric, such as peak concentration, may be more relevant of an exposure-response relationship.

Alcohol by itself cannot account for the observed deficits in neurobehavioral functions, because statistical analyses of the epidemiologic observations accounted for this covariant. However, effects from the interaction between tetrachloroethylene exposure and alcohol consumption was not well investigated in these studies. Valic et al. (1997) showed greater decrements in color vision among subject with both exposures as compared with individuals with solvent exposure only or with neither exposure.

No epidemiological studies investigating drinking water or other oral exposures to tetrachloroethylene have explored the potential for neurotoxicity.

The research in animal models (rodents) on the effects of tetrachloroethylene on functional endpoints consists almost exclusively of screening studies (functional observation battery, motor activity) or effects on sensory system function, as assessed by evoked potentials. Effects on motor activity and motor function have been observed with some consistency following either adult or developmental exposure. Changes in VEPs were also reported following acute (4-day) and subchronic (13-week) exposure. In addition, changes in brain DNA, RNA, or protein levels and lipid composition were altered following inhalation, with changes observed in cerebellum, hippocampus, and frontal cortex. The replication of these changes in biochemical parameters and effects in brain weight in both rats and gerbils is pathognomonic.

Changes in neurotransmitters systems (Honma et al., 1980 a, b, Briving et al., 1986) and circadian rhythm (Motohashi et al., 1993) in animal studies are consistent with neuroendocrine alterations observed in humans (Ferroni et al., 1992). Operant tasks that test cognitive

performance have demonstrated performance deficits in rats and mice following acute tetrachloroethylene oral (Warren et al., 1996) and i.p. (Umezu et al., 1997) exposures. These findings in animal studies are consistent with observed effects on cognition and memory in humans. However, no studies to date have evaluated the persistent effects of tetrachloroethylene exposure on cognitive performance deficits in animal models. This is a clear data need that could help resolve the dose-response relationship in cognitive performance observed in both human occupational and residential studies. The neurophysiological findings in animal studies, albeit at high doses (800 ppm), are consistent with the physiological dysfunction observed in visually mediated functions in humans. In addition, the persistent changes in neurotransmitter levels, regional DNA content, and brain weight in animal studies is consistent with neurological effects in humans. Therefore, effects observed in human and rodent models exhibit a reasonable degree of congruence.

The inhalation LOAEL for neurotoxic effects in humans is 0.2–41 ppm. For animals it is 37–90 ppm, with no apparent correlation between the LOAEL of administered concentration and duration of treatment (see Section 4.6.2.1). Information for oral effects in humans is missing, and the only animal data applicable to an oral exposure are from gavage administration of tetrachloroethylene. No information on long-term neurological effects in animals via the oral route is available.

4.6.4. Mode of Action for Neurotoxic Effects

The MOA for the neurotoxic effects of tetrachloroethylene is unknown; however, at present, the best surrogate for the dose metric for neurotoxicity is blood tetrachloroethylene. There may be multiple mechanisms or MOAs, which may differ for adult and developmental exposure. The acute effects of tetrachloroethylene share much in common with those of other solvents such as toluene, volatile anesthetics, and alcohols. There is emerging evidence that such agents act on the ligand-gated ion channel superfamily in vitro (Shafer et al., 2005), particularly on the inhibitory amino acids NMDA, nicotinic, and GABA receptors in vivo (Bale et al., 2005). Volatile anesthetics and alcohol both interact with the glycine receptor (Yamakura et al., 1999; Wick et al., 1998; Mihic, 1999). Affinities depend on specific subunits of the receptor and are correlated with behavioral effects on tests such as loss of righting ability. Similarly, ethanol and volatile anesthetics enhance $GABA_A$ receptor function (Mihic, 1999). Chronic effects of these agents may also be dependent on the $GABA_A$ system (Grobin et al., 1998).

Other receptors, such as the dopaminergic/N–methyl-D-aspartate (NMDA) receptor, may also be involved in the mediation of the effects of these agents, as may the glutamate kainate or α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA) receptors (Harris et al., 1995; Cruz et al., 1998). The solvents 1,1,1-trichloromethane and trichloroethylene enhanced

neurotransmitter-activated currents at α 1β1 $GABA_A$ and α 1 glycine receptors (Beckstead et al., 2000). It seems reasonable to speculate that tetrachloroethylene would also have as part of its MOA modulation of these systems, although specificity for different receptor subunits would undoubtedly differ from those for other nervous system depressants. Consistent with this hypothesis, glutamine levels were elevated in hippocampus following tetrachloroethylene administration (Briving et al., 1986), although GABA levels and uptake were unchanged.

Tetrachloroethylene also affects the fatty acid composition of the brain following 30- or 90-day exposure and persists for at least 30 days after cessation of exposure (Kyrklund et al., 1984, 1987, 1988, 1990). It has long been known that the potency of an anesthetic is proportional to its lipid solubility, from which it was inferred that anesthetics act on the lipid bilayer of the plasma membrane. However, this observation has not led to elucidation of the mechanism of action of anesthetic agents or solvents. It is nonetheless interesting that tetrachloroethylene produces changes in fatty acid composition of the brain.

Tetrachloroethylene effects on the nervous system are not explained simply by any direct dosing studies with metabolites. In particular, the pattern of neurotoxicity observed with DCA is qualitatively different. DCA has been found to produce hind-limb paralysis, altered gait, muscle weakness, and pathology in the spinal cord (Moser et al., 1999). In the one study that examined the effects of chronic exposures to tetrachloroethylene on the histology and function of the nervous system (Mattsson et al., 1998) there was no observed neuropathology or functional deficits (i.e., hind-limb paralysis, altered gait, or muscle weakness). This discrepancy between tetrachloroethylene and DCA could be explained by differences in target tissue dose available from metabolized tetrachloroethylene and more direct exposure to DCA.

4.7. DEVELOPMENTAL/REPRODUCTIVE STUDIES

4.7.1. Human Studies

Adverse effects on reproduction and development assessed by epidemiologic investigation include effects on fecundity (defined as reproductive potential and measured by time to pregnancy); effects on sperm; the risk of adverse pregnancy outcomes such as spontaneous abortion, stillbirth, congenital malformation, or low birth weight; and effects on postnatal development (which in this evaluation includes the occurrence of childhood cancer). Several of the adverse pregnancy outcome studies evaluated exposure during a critical window, the first trimester of the pregnancy. In general, the epidemiologic studies evaluating effects on reproduction and the developing fetus do not present quantitative information on level of exposure to tetrachloroethylene. When information is available (identified in the discussion below), an assertion of exposure to tetrachloroethylene in most cases was derived from self-reported information provided by study subjects in mailed questionnaires or interviews. More

rarely, biological measures of exposure, such as tetrachloroethylene in blood or in urine, were available for a subset of these subjects.

A number of studies found elevated risks of spontaneous abortions among women employed as dry cleaners (Doyle et al., 1997; Windham et al., 1991; Olsen et al., 1990; Lindbohm et al., 1990; Kyyrönen et al., 1989; Bosco et al., 1987). Two reports of the same study population do not note associations between spontaneous abortions and dry cleaning and laundry employment (McDonald et al., 1986, 1987). These reports are not inconsistent with the remaining body of literature due to possible bias. Approximately 25% of the women hospitalized for a spontaneous abortion were not interviewed, and this may have introduced a bias into the study if a decision to participate was related to solvent exposure. The studies assessing spontaneous abortions among the wives of men exposed to tetrachloroethylene (Taskinen et al., 1989; Eskenazi et al., 1991a) were not remarkable due to the few numbers of exposed cases.

The study by Doyle et al. (1997) is the largest: 3,517 pregnant women who were currently or previously employed in dry cleaning or laundry shops. The authors analyzed the data by applying several different approaches and taking into account a number of important covariates, which is a strength of this study. The findings were all suggestive of an increased risk of spontaneous abortions among pregnancies reported by women who were employed as dry cleaners at any time during pregnancy or three months before conception as compared with unexposed pregnancies. In fact, lower 95% CIs for many of these approaches were above a relative risk of 1. Adding support was the observation that risk for pregnancies in dry cleaning operators was larger than risks observed for pregnancies reported by women working in jobs in laundry or nonoperator dry cleaning, suggesting the presence in this study of an exposure-response association.

Doyle et al. (1997) is considered to carry greater weight than the other studies discussed below due to its use of a pregnancy as the unit of analysis. Analyses that do not adjust for previous pregnancy loss, such as those presented in McDonald et al. (1987), could lead to biased estimates because a previous spontaneous abortion is a risk factor for a spontaneous abortion with the current pregnancy. The study design of Doyle et al. (1997) minimizes the potential for this type of bias because a woman with repeated pregnancy losses may be counted in both exposed and unexposed categories, depending on her exposure status at the time of the pregnancy.

Three other studies (Olsen et al., 1990; Bosco et al., 1987; Windham et al., 1991) examined the association between spontaneous abortions and occupational exposure to tetrachloroethylene. Olsen et al. (1990) presented findings from a four-country Nordic study of spontaneous abortion, low birth weight, and congenital anomalies and observed a relative risk of

2.9 (95% CI = 1.0–8.4; eight exposed cases) for all data sets between spontaneous abortion and "high exposure" to tetrachloroethylene during the first trimester of pregnancy, primarily due to the large risk seen among subjects from Finland (OR = 4.5, 95% CI = 1.1–18.5, six exposed cases). These analyses were based on 3,279 pregnancies among women dry cleaners and laundry workers linked to national registers of birth and reproductive failures. Biological monitoring data were available for some of the subjects from Finland; blood tetrachloroethylene concentration ranged from 0.1 μmol/L to 2.6 μmol/L for cases (n = 4) and from 0.3 μmol/L to 3.6 μmol/L for controls (n = 3; Kyyrönen et al., 1989). Unfortunately, the number of subjects in Kyyrönen et al. (1989) who were also included in Olsen et al. (1990) is not known.

The result for Finnish workers reported by Olsen et al. (1990) is of a similar magnitude as that reported for essentially the same study subjects by two other Finnish investigators (Kyyrönen et al., 1989; Lindbohm et al., 1990). Overall, greater weight is placed on the Olsen et al. (1990) findings due to the investigators' more systematic approach for evaluating an exposure-effect association.

Bosco et al. (1987) observed a 4-fold higher history of prior spontaneous abortions among women working in dry cleaning shops than among these same women when they were not employed outside their homes. These findings were based on a small number of subjects and were not statistically significant. Mean urinary TCA levels among women employed in dry cleaning shops was 5 μg/L, compared to 1.4 μg/L for women employed in shops that operated only as an ironing service. Windham et al. (1991) reported a statistically significant elevated risk of spontaneous abortions (OR = 4.7, 95% CI = 1.1–21.1) in tetrachloroethylene-exposed women in analyses that adjusted for age, race, education, prior fetal loss, smoking, and number of hours worked. This analysis was based on seven women identified with tetrachloroethylene exposure, of which four were identified as having exposure to trichloroethylene, for which the odds ratio was also elevated (OR = 3.1, 95% CI = 0.9–10.4). Both trichloroethylene and tetrachloroethylene share a number of common or like metabolites, although human metabolism via the P450 oxidative pathway is more extensive for trichloroethylene than for tetrachloroethylene.

There is more limited evidence for reduced fecundity and effects on sperm with exposure to tetrachloroethylene, but it is suggested in several studies (Rachootin and Olsen, 1983; Eskenazi et al., 1991a, b; Sallmén et al., 1995). Sallmén et al. (1995) observed a lower probability of achieving a clinically recognized pregnancy among women employed in dry cleaning shops (incidence density ratio [IDR] = 0.44, 95% CI = 0.22–0.86, 11 women) in analyses that adjusted for a number of other covariates. Furthermore, tetrachloroethylene exposure was associated with a decreased probability of pregnancy, although an exposure-response pattern was not apparent (low exposure to tetrachloroethylene, IDR = 0.63, 95% CI =

0.34–1.17, based on 13 women; high exposure to tetrachloroethylene, IDR = 0.69, 95% CI = 0.31–1.52, based on 7 women). Exposure was defined as frequency of tetrachloroethylene use, with no attention paid to level of exposure. Hence, exposure misclassification may partially explain the lack of an exposure-response relationship. Sallmén et al. (1995) examined these exposures as part of a larger evaluation of general organic solvent exposure for which a statistically significant association was noted (high level exposure, IDR = 0.41, 95% CI = 0.27–0.62).

Eskenazi et al. (1991a, b) reported a statistically significant reduced probability of pregnancy among highly exposed individuals. Eskenazi et al. (1991a) noted a lower per-cycle pregnancy rate among wives of men who received higher-level exposure to tetrachloroethylene (RR = 0.94, 95% CI = 0.85–1.04) as compared with wives of men who received lower-level exposure. The potential contribution of tetrachloroethylene exposure on time to conception was small compared to the contribution observed from Hispanic ethnicity and smoking, which were found to be stronger and statistically significant predictors of time to conception.

Eskenazi et al. (1991b) also found subtle spermatogenic effects among dry cleaners when compared with laundry workers. These effects were characterized as a greater proportion of round sperm and a lower proportion of narrow sperm. Furthermore, tetrachloroethylene level was a statistically significant predictor of decreased number of narrow sperm and of increased numbers of spermatids with amplitude of lateral head displacement, a measure of the unsteady movement of the sperm head about its average path of motion. Mean tetrachloroethylene exposure was 1.2 ppm for dry cleaners and 0.01 ppm for laundry workers. More traditional measures of semen quality, such as number of sperm, concentration, volume, average percentage of motile sperm or average percentage of abnormally shaped sperm, as well as the prevalence of study subjects identified as azospermic or oligospermic, did not differ between these two job titles. Moreover, it did not appear that the effects on semen parameters or the per-cycle pregnancy rate had a large impact on fertility rates. Partners of these male dry cleaners did not have fewer pregnancies as compared with a national standard (Eskenazi et al., 1991a).

The findings from these studies are consistent with those observed by Rachootin and Olsen (1983), whose study subjects were couples seeking treatment for infertility. These investigators noted that employment as a dry cleaner was associated with hormonal disturbances and delayed conception in analyses that took into account the woman's age, education, residence, and parity. Exposure to tetrachloroethylene was inferred but not documented.

Few epidemiologic studies exist that evaluate other developmental toxicity endpoints such as decreased birth weight, intrauterine growth restriction (IUGR; also known as small for gestation age [SGA]), and congenital anomalies. Many of the analyses were included in the reports of spontaneous abortions discussed above. Overall, no associations were noted in several

studies that assessed maternal or paternal exposure to tetrachloroethylene and increased incidences of stillbirths, congenital anomalies, or decreased birth weight (Olsen et al., 1990; Kyyrönen et al., 1989; Taskinen et al., 1989; Windham et al., 1991). These findings may be a reflection of the small numbers of exposed cases, or they may be attributable to exposure misclassification (biological marker data were available for only a few study subjects) or disease misclassification (which could be introduced from the grouping of several different outcomes into one category).

The case-control study by Windham et al. (1991) observed a strong but imprecise association between IUGR and exposure to tetrachloroethylene (OR = 12.5, 95% CI not given in the published paper and too few data for NCEA staff to calculate). This observation was based on only one exposed case who also had exposure to trichloroethylene; an RR greater than 1 was also observed for trichloroethylene exposure (OR = 4.2).

Studies of populations serviced by drinking water containing several contaminants, including tetrachloroethylene and trichloroethylene, report elevated risks such as adverse pregnancy or postnatal outcomes attributed to living in a residence receiving contaminated water effects (Lagakos et al., 1986; Bove et al., 1995; ATSDR, 1998). Lagakos et al. examined the relationship between several birth outcomes that were identified from questionnaires given to a sample of residents from Woburn, MA. This study was part of a larger study evaluating the association between childhood leukemia among residents of this town and living in a residence receiving drinking water from two wells contaminated with trichloroethylene, tetrachloroethylene, and chloroform. The levels of these contaminants in the wells at the time they were closed were 267 ppb (µg/L) trichloroethylene, 21 ppb (µg/L) tetrachloroethylene, and 12 ppb (µg/L) chloroform. The investigators observed statistically significant associations between a residence receiving contaminated water and three outcomes: perinatal deaths since 1970, eye and ear anomalies, and CNS/oral cleft anomalies.

An analysis by Shawn and Robins (1986) of events among residents of East Woburn, the location of the contaminated wells, noted a statistically significant exposure-response trend only for perinatal deaths. This analysis was presented in comments by the study authors to the study by Lagakos et al. (1986) and was carried out to evaluate recall bias in that study, which these authors concluded did not exist.

A case-control study of leukemia cases among children in Woburn, MA (MA DPH, 1997) noted a large risk between maternal exposure (e.g., living during the first trimester of pregnancy in a residence that received contaminated water) and leukemia (OR_{adj} = 8.3, 90% CI = 0.7–94.7, 10 exposed case). Risks increased significantly ($p < 0.05$) with increasing exposure (never exposed, least exposed, most exposed). This study is more fully discussed in the section reviewing the epidemiologic evidence on cancer effects.

In a prevalence study, Bove et al. (1995) assessed the relationship between a number of birth outcomes, as identified from the birth certificate or from the New Jersey Birth Defects Registry, and residence in 75 towns for which monitoring data were available. Concentrations of trihalomethanes, trichloroethylene, and tetrachloroethylene, along with a wide range of other solvents, were identified from the monitoring data. Bove et al. (1992, 1995) observed risks above 1.5 between residence in a town with >10 ppb tetrachloroethylene detected in drinking water and oral cleft defects (OR = 3.5, 90% CI = 1.3–8.78, four exposed cases). No associations were reported for other birth outcomes such as CNS defects, neural tube defects, low birth weight, and small for gestational age and tetrachloroethylene exposure.

This analysis lacks information on risk factors for an individual. To address this limitation, the investigators conducted a case-control study of oral cleft defects (Bove et al., 1992), where findings did not support the observations in the ecological study. The association between tetrachloroethylene in water (>5 ppb) and oral clefts was not elevated (OR_{adj} = 0.4, 95% CI = 0–4.3, four exposed cases) in the case-control analyses. Given the better design of the case-control study and its ability to include information on individual study participants, this study carries a greater weight than does the 1995 ecological study in the overall evaluation of the relationship between tetrachloroethylene exposure and developmental effects.

An analysis by ATSDR (1998; results published by Sonnenfeld et al., 2001) examined birth weight and gestational age among births of residents living in base family housing at Camp Lejeune, NC. The residences received drinking water contaminated by solvents, including trichloroethylene, tetrachloroethylene, and/or benzene. A large number of births (n = 6,117) between 1968 and 1985 were identified from birth records and were classified as exposed to tetrachloroethylene, i.e., the mother had resided at any time of pregnancy in the base housing, specifically Tarawa Terrace, which had received tetrachloroethylene-contaminated drinking water. Although quantitative information on exposure is limited in this study (water samples were collected on only three different occasions between 1982 and 1985), it is thought that the well providing water to Tarawa Terrace was contaminated with tetrachloroethylene for as long as 30 years (ATSDR, 1998; Sonnenfeld et al., 2001). The highest concentrations of contaminants measured in tap water from Tarawa Terrace were 215 ppb (µg/L) tetrachloroethylene, 8 ppb (µg/L) trichloroethylene (single sample), and 12 ppb (µg/L) 1,2,-dichloroethylene (single sample). No information on level of tetrachloroethylene exposure was available prior to 1982.

The frequency of a birth of an SGA[10] infant was slightly increased among women who were identified as tetrachloroethylene exposed (10.2%) as compared with those who were not (9%; OR = 1.2, 90% CI = 1.0–1.3, 622 exposed births). The investigators also observed a smaller mean birth weight of exposed infants as compared with infants of mothers who lived in unexposed housing—a difference of 24 g, which was not considered to be of biological significance. Two susceptible groups were identified from this analysis: mothers 35 years or older and mothers with previous fetal deaths. For older mothers, the adjusted difference in mean birth weight between tetrachloroethylene-exposed and unexposed births was 205 g (90% CI = 78–333), with a risk (OR) of 4 (95% CI = 1.6–10.2, 11 exposed births) between exposure and birth of an SGA infant. For mothers with prior fetal losses, exposure to tetrachloroethylene in drinking water was associated with a 60% higher risk for an infant that was identified as SGA (95% CI = 1.2–2.1, 147 exposed births).

Inferences regarding developmental and reproductive effects from tetrachloroethylene are limited due to small risks of low precision, the lack of a direct measure of tetrachloroethylene in many studies, the small numbers of exposed cases, and possible biases such as recall or misclassification bias. The epidemiologic evidence is strongest for spontaneous abortions among exposed women. A number of studies have reported an elevated risk of spontaneous abortions and maternal exposure to tetrachloroethylene, primarily exposure received through employment as a dry cleaner (Doyle et al., 1997; Windham et al., 1991; Olsen et al., 1990; Lindbohm et al., 1990; Kyyrönen et al., 1989; Bosco et al., 1987). The epidemiologic evidence for infertility is further suggestive of an association with tetrachloroethylene exposure (Rachootin and Olsen, 1983; Eskenazi et al., 1991a, b; Sallmén et al., 1995). Any conclusions of effects on birth weight, IUGR (SGA), or congenital anomalies and tetrachloroethylene exposure cannot be drawn from the available occupational studies, although drinking water studies of exposures to multiple chemicals, including tetrachloroethylene, provide some limited evidence. There is very little information about what exposure pattern (concentration and duration) is associated with these effects. Table 4-8 summarizes these studies.

4.7.2. Animal Studies

Evaluation of the developmental and reproductive effects of tetrachloroethylene exposure in animal models is based on several studies of in utero exposures to maternal animals during specific periods of pregnancy. These studies include embryo explant in rats, multigeneration

[10] SGA is measured by comparing birth weight at specific gestational ages with a gestational-age-specific-birth-weight distribution. Live births of infants weights less than the 10th percentile are classified as SGA. Three standards were examined by Sonnenfeld et al. (2001), and the standard of Williams et al. (1982) provided the best fit to the data.

Table 4-8. Developmental/reproductive studies in humans

Subjects	Effect	Exposure	Authors
3,517 pregnancies in women employees in dry cleaners and laundries	Spontaneous abortions	Elevated risks by several definitions of "exposed." No tetrachloroethylene measurements	Doyle et al. (1997)
3,279 pregnancies among dry cleaners in four Nordic countries. Occupational records were linked to national registries of birth and reproductive failures.	Spontaneous abortions elevated among "high-exposure" workers in all countries, primarily attributed to excesses in Finland	Blood levels of TCA not elevated in some of these workers	Olsen et al. (1990)
53 women dry cleaners and ironers whose pregnancy outcomes were compared with outcomes during time as a housewife.	Spontaneous abortions fourfold higher than among nonexposed housewives but not statistically significantly different	TCA in dry cleaners was 5 µg/L; TCA in ironers was 1.4 µg/L	Bosco et al. (1987)
Case-control study of spontaneous abortions (1,361 cases, controls with live births)	Spontaneous abortion excess in 7 women, 4 of whom were also exposed to trichloroethylene		Windham et al. (1991)
20 women in dry cleaning shops, 90 unexposed control women	Lower probability of clinically recognized pregnancy	No dose-response with tetrachloroethylene exposure	Sallmen et al. (1995)
Wives of 17 men exposed to tetrachloroethylene dry cleaners, wives of 32 male laundry workers	Lower per-cycle pregnancy rate but no decrease in fertility rate	High vs. low tetrachloroethylene exposure categories	Eskenazi et al. (1991a)
34 male dry cleaners, 48 unexposed male laundry workers	Subtle sperm changes but no change in clinical sperm quality criteria	Mean exposure was 1.2 ppm	Eskenazi et al. (1991b)
Case-control study of infertility and delayed conception: 1,069 infertile case couples; 4,305 fertile control couples	Delayed conception and hormonal disturbances: OR = 3 for exposure to dry-cleaning chemicals (95% CI = 1.2–7.4)	Tetrachloroethylene exposure inferred	Rachootin, and Olsen (1983)

Table 4-8. Developmental/reproductive studies in humans (continued)

Subjects	Effect	Exposure	Authors
4,396 pregnancies among residents of Woburn, MA.	Statistically significant positive association between access to contaminated water and (a) perinatal deaths since 1970 and (b) eye/ear birth anomalies No association between water access and (a) incidence of spontaneous abortion (b) low birth rate (c) perinatal deaths before 1970 (d) musculoskeletal birth anomalies (e) cardiovascular birth anomalies	Tetrachloroethylene: 21 µg/L Trichloroethylene: 267 µg/L Chloroform: 12 µg/L	Lagakos et al. (1986)
80,938 live births and 594 fetal deaths among residents in 75 New Jersey towns	Oral cleft defects, OR = 3.5, 95% CI = 1.3–8.8) based on four exposed cases	>10 µg/L in drinking water	Bove et al. (1995)
Case-control study of selected birth outcomes in New Jersey; 49 cases of oral cleft defects, 138 controls	The association between tetrachloroethylene in water (>5 ppb) and oral cleft defects was not elevated.		Bove et al. (1992)
11,798 births among women living in United States Marine Corp base housing	Excess of age (SGA) births in women >35 years of age among mothers with prior fetal losses	Tetrachloroethylene: <215 µg/L	ATSDR, (1998), Sonnenfeld et al. (2001)

reproduction in rats, and an in vitro oocyte fertilization assay following in vivo exposure of adult female rats.

In an inhalation developmental toxicity study (Schwetz et al., 1975), Sprague-Dawley rats and Swiss-Webster mice were exposed to airborne tetrachloroethylene at 300 ppm 7 hrs/day on days 6–15 of gestation. Following laparohysterectomy on gestation days 21 or 18 (for rats and mice, respectively), fetuses were weighed and measured, examined for external abnormalities, and processed for the evaluation of either soft tissue or skeletal abnormalities. Three other organic solvents were also tested with the same protocol; the concentration of all agents was chosen to be approximately twice their threshold limit values. Although the study

authors concluded that there was no significant maternal, fetal, or embryo toxicity for any of the solvents tested, the maternal and fetal data demonstrated a number of statistically significant differences from control values following gestational exposures to tetrachloroethylene in rats and mice. In the rats, exposures to tetrachloroethylene produced slight but statistically significant maternal toxicity (4–5% reductions in mean maternal body weight gains) and embryotoxicity (increased resorptions; 9% in treated vs. 4% in controls). In the mice, maternal toxicity consisted of a significant 21% increase in mean relative liver weight as compared with controls. The mean fetal weight in mice was significantly (9%) less than in the concurrent control, and the percent of litters with delayed ossification of the skull bones, delayed ossification of the sternebra, and subcutaneous edema were significantly increased.

Szakmáry et al. (1997) exposed CFY rats to tetrachloroethylene via inhalation throughout gestation (i.e., gestation days 1–20) for 8 hrs/day at concentrations of 1,500, 4,500, or 8,500 mg/m^3. In the same study, the study authors exposed C57Bl mice via inhalation on gestation days 7–15 (i.e., during the period of organogenesis) to a concentration of 1,500 mg/m^3 and New Zealand white rabbits during organogenesis (gestation days 7–20) to a concentration of 4,500 mg/m^3. Maternal animals were killed approximately 1 day prior to expected delivery; a gross necropsy was conducted, organ weights were recorded, blood was taken by aorta puncture for hematology and clinical chemistry evaluations, ovarian corpora lutea were counted, and uterine contents were examined (number and position of living, dead, or resorbed fetuses; and fetal and placental observations and weights). The numbers of litters available for evaluation were as follows: 20 control and 21 or 22 per treated group in the rat, 77 control and 10 treated in the mice, and 10 control and 16 treated in the rabbit. One-half of the fetuses from each litter were evaluated for visceral abnormalities, and the other half were evaluated for skeletal development. The study authors reported that the organs of five dams and five embryos from each group were also evaluated by routine histological methods. To evaluate the concentration of tetrachloroethylene in maternal and fetal blood and in amniotic fluid, another subset of rats (number not specified) was studied. (For the 1,500 and 8,500 mg/m^3 exposure levels, maternal blood concentrations of tetrachloroethylene were 17.8+8.9 and 86.2+13.0 µL/mL, respectively. Concentrations in the fetal blood were 66% and 30% of maternal blood concentrations, and amniotic fluid concentrations were 33% and 20% of maternal blood concentrations.) In the rat, at 4,500 and 8,500 mg/m^3, maternal body weight gain during gestation was significantly decreased (37 and 40%, respectively), relative maternal liver mass was significantly increased (10 and 6%, respectively), and serum aspartate amino transferase activity was increased (data not provided) as compared to controls. Percent pre-implantation loss was significantly increased from controls by 133 and 117% at these exposure levels, while percent post-implantation loss was increased non-significantly from controls by 80% in each group. Also, at 4,500 and

8,500 mg/m^3, fetal weight was significantly decreased in 98.5 and 100% of all fetuses, the number of fetuses with skeletal retardation was significantly increased in 98.5% and 100% of fetuses, and the percent of fetuses with malformations was both significantly increased to 6.4% and 15.7% as compared to the control incidence of 2.0%. Although the study authors judged the 1,500 mg/m^3 exposure level to be the NOAEL for the rat study, it is noted that there were concentration-dependent non-significant decreases in maternal body weight gain (13% lower than control), and increases in pre- and post-implantation loss (49% and 38% greater than control, respectively). The percent of weight-retarded fetuses increased to 3.4 times the control incidence, and the incidences of fetuses with skeletal retardation (48% increased) or total malformations increased by 2.3 times the control incidence observed at the low-exposure level of 1,500 mg/m^3. Therefore, these findings are judged to be adverse consequences of treatment. The attribution of these findings to treatment, and the designation of 1,500 mg/m^3 as the study LOAEL is consistent with the adverse developmental findings of Schwetz et al. (1975). In mice (1,500 mg/m^3) and rabbits (4,500 mg/m^3), relative liver mass was significantly increased; decreased maternal body weight gain was also observed in the rabbits. In the mice, a significantly increased number of fetuses with visceral malformations (details not specified) was observed, while in the rabbits, two (of 16) does aborted, total resorption of four litters was reported, and the percent of post-implantation loss was significantly increased. The percent of rabbit fetuses with malformations (details not provided in the report) was also increased, although not significantly.

An additional cohort of rats from the Szakmáry et al. study (15 litters/group at exposure levels of 1,500 or 4,500 mg/m^3 tetrachloroethylene) was allowed to deliver, and the offspring (standardized to 8 pups/litter) were maintained on study to postnatal day 100. It was not clearly specified in the report whether the daily inhalation exposures continued throughout the postnatal period. Pre-weaning observations included weekly body weights, developmental landmarks (pinna detachment, incisor eruption, and eye opening), and functional assessments (forward movement, surface righting reflex, grasping ability, swimming ontogeny, rotating activity, auditory startle reflex, and examination of stereoscopic vision). After weaning, exploratory activity in an open field, motor activity in an activity wheel, and development of muscle strength were assessed. The study authors reported that adverse findings included a decreased survival index (details not provided), a minimal decrease of exploratory activity and muscular strength in treated offspring (presumably at both exposure levels) that normalized by postnatal day 51, and significantly increased motor activity on postnatal day 100 of females exposed to 4,500 mg/m^3 of tetrachloroethylene.

Nelson et al. (1980) investigated developmental neurotoxicity in Sprague-Dawley rats by exposing pregnant dams to tetrachloroethylene at concentrations of 100 ppm and 900 ppm during

either early pregnancy (gestation days 7 to 13) or late pregnancy (gestation days 14 to 20). They performed morphological examinations of the fetuses (gross, visceral, and skeletal) and behavioral testing and neurochemical analyses of the offspring.

There were no alterations in any of the measured parameters in the 100 ppm groups. At 900 ppm there were no skeletal abnormalities, but the weight gain of the offspring as compared with controls was depressed about 20% at postnatal weeks 3–5. Developmental delay was observed in both the groups exposed in early and in late pregnancy. Offspring of the early pregnancy-exposed group performed poorly on an ascent test and on a rotorod test, whereas those in the late pregnancy group underperformed on the ascent test at only postnatal day 14. However, later in development (days 21 and 25) their performance was higher than that of the controls on the rotorod test. These pups were markedly more active in the open field test at days 31 and 32. Activity wheel testing on days 32 and 33 did not reveal statistically significant changes. Avoidance conditioning on day 34 and operant conditioning on days 40–46 failed to suggest effects. Neurochemical analyses of whole brain (minus cerebellum) tissue in 21-day-old offspring revealed significant reductions in acetylcholine levels at both exposure periods, whereas dopamine levels were reduced among those exposed on gestation days 7–13.

All of the described effects in the 900 ppm group were statistically significant as compared with controls. Unfortunately, none of the statistics for the 100 ppm treatments were presented. The authors observed that more behavioral changes occurred in offspring exposed during late pregnancy than in those exposed during early pregnancy.

Beliles et al. (1980) described an experiment in which male rats and mice were exposed via inhalation to tetrachloroethylene concentrations of 100 and 500 ppm for 7 hrs/day for 5 days. Sperm head abnormalities and abnormal sperm were evaluated at 1, 4, and 10 weeks after the last dose. Rats were unaffected. At 4 weeks but not at 1 or 10 weeks after exposure there was a significant increase ($p < 0.05$) in the percentage of mice with abnormal sperm heads (19.7%) for animals inhaling 500 ppm. For the 100 ppm and control groups the percentages were 10.3% and 6% (not statistically significant at the $p < 0.05$ level), respectively. A positive control group administered triethylene melanime was adversely affected (11.1%). The authors suggested that the temporal appearance of the abnormal sperm heads indicated that the spermatocyte and/or spermatogonia were the stages most sensitive to the effects of inhaled tetrachloroethylene. In this study the NOAEL was 100 ppm and the LOAEL was 500 ppm.

Hardin et al. (1981; see also Beliles et al., 1980) found no developmental toxicity among the fetuses from Sprague-Dawley rats or New Zealand White rabbits inhaling 500 ppm of tetrachloroethylene for 7 hrs/day, 5 days/week. Tetrachloroethylene was administered with and without three-week pregestation exposures and with both full-term and terminal two-thirds-term exposure.

In a developmental toxicity study, Carney et al. (2006) investigated the effects of whole-body inhalation exposures to pregnant Sprague-Dawley rats at nominal concentrations of 0, 75, 250, or 600 ppm (actual chamber concentrations of 0, 65, 249, or 600 ppm) tetrachloroethylene for 6 hrs/day, 7 days/week on gestation days (GD) 6–19. This study was conducted under Good Laboratory Practice (GLP) regulations according to current EPA and OECD regulatory testing guidelines. Maternal toxicity consisted of slight but statistically significant decreases in body weight gain during the first 3 days of exposure to 600 ppm, establishing a no-adverse-effect concentration of 249 ppm for dams. A slight, statistically significant decrease in gravid uterine weight at 600 ppm correlated with significant reductions in mean fetal body weight (9.4%) and placental weight (15.8%) at GD 20 cesarean section. At $\geq$249 ppm, mean fetal and placental weights were significantly decreased by 4.3% and 12.3% from control, respectively. A significant increase in the incidence of incomplete ossification of the thoracic vertebral centra at this exposure level was consistent with fetal growth retardation. No treatment-related alterations in fetal growth or development were noted at 65 ppm. Therefore the LOAEL for this study is 249 ppm.

Saillenfait et al. (1995), using a rat whole embryo (day 10) culture system, found tetrachloroethylene-induced embryo toxicity, including mortality, malformations, and delayed growth and differentiation. No adverse effect was produced at the 2.5 mM concentration, but concentration-related trends of increasing toxicity occurred from 3.5 mM through 15 mM. Statistical tests for a concentration-related trend were not reported. The investigators found that trichloroethylene produced similar effects, with potency somewhat less than that of tetrachloroethylene. They also found that TCA and DCA caused a variety of abnormalities in this culture system.

In a developmental toxicity screening study, timed-pregnant F344 rats were treated by gavage with tetrachloroethylene doses of 900 or 1,200 mg/kg-day in corn oil vehicle on gestation days 6–19 (Narotsky and Kavlock, 1995). There were 17 dams in each of the tetrachloroethylene-treated groups and 21 in the control groups. The dams were allowed to deliver, and their litters were examined on postnatal days 1, 3, and 6. At 1,200 mg/kg no live pups were delivered on day 22 of gestation. At 900 mg/kg-day there was maternal ataxia, and weight gain was markedly less than in the controls. The number of pups per litter was reduced ($p < 0.01$) as compared with the controls at day 22 of gestation. On postnatal day 6 the number of pups per litter was reduced ($p < 0.001$) as compared with the controls. The investigators noted that full-litter resorptions were not observed with other chemicals they tested in the presence of maternal toxicity. An increase in micro/anophthalmia was found in the offspring. There was no evaluation for skeletal changes, and not all available pups were examined for soft tissue changes.

Because of the high dose levels and limited evaluation of the soft tissue changes, the malformations described are of limited impact.

A multigeneration study of the effects on rats of exposure to airborne concentrations of tetrachloroethylene was performed by Tinston (1994). Although this study has not been published, it was submitted to EPA (Office of Prevention, Pesticides, and Toxic Substances and to the IRIS Office as a result of the data call-in for the IRIS update). It was conducted under good laboratory practice standards and received frequent quality assurance audits. In this study, weanling male and female (Alpk:APfSD) rats (F0) were exposed to airborne tetrachloroethylene concentrations of 0, 100, 300, or 1,000 ppm 6 hrs/day, 5 days/week, for 11 weeks prior to mating and then for 6 hrs/day during mating and through day 20 of gestation. There were no exposures from day 21 of gestation through day 5 postpartum. One litter was produced in the first generation (F1A). The first-generation dams and their litters were exposed to tetrachloroethylene from postnatal day 6 through 29, at which time parental animals for the second generation were selected. The second-generation parents (F1) were then exposed 5 days/week during the 11-week pre-mating period. In the second generation, three litters were produced: F2A, F2B, and F2C. The F2A dams and litters were exposed from days 6 to 29 (control and 100 ppm) or days 7 to 29 (300 ppm). The 1,000 ppm exposure for the F1 dams stopped after the F2A littering.

F2B litters were generated by mating the F1 parental males and females in the control, 300, and 1,000 ppm groups; the dams and F2B litters were not exposed to tetrachloroethylene during lactation. An F2C litter was produced by mating F1 males exposed to 1,000 ppm with unexposed females. These females and the F2C litters were killed on postnatal day 5 and discarded without further examination. Overall, the F0 males were exposed for 19 weeks and the F1 males were exposed up to 35 weeks. Postmortem evaluation in adults and selected weanlings included organ weight and histopathology examination of liver, kidney, and reproductive organs; sperm measures were not assessed.

Table 4-9 summarizes the results of the Tinston study. Signs of CNS depression (decreased activity and reduced response to sound) were observed at 1,000 ppm for the first 2 weeks in both adult generations and again when the exposure was resumed on day 6 postpartum in the F1 generation (adults and pups). Other signs of overt tetrachloroethylene toxicity in the adults included irregular breathing and piloerection at both 1,000 and 300 ppm and salivation and tip-toe gait (in one F1 female) at 1,000 ppm. These changes stopped with the cessation of exposure or within approximately 30 minutes thereafter.

There were a number of changes relative to controls that were of minor biological significance. One change was transient statistically significant reductions of mean body weights

Table 4-9. Exposure concentrations (ppm) at which effects occurred in a two-generation study

Parameter	Generation					
	F0	F1A	F1	F2A	F2B	F2C[a]
Clinical signs (piloerection, irregular breathing)	1,000, 300		1,000, 300			
Behavioral effects (decreased activity; reduced response to sound)	1,000	1,000	1,000			
Transient decreased body weight gains	1,000, 300		1,000, 300			
Decreased mean testes weight		1,000	1,000			
Increased liver and kidney weights	1,000		1,000			
Renal histopathology	1,000		1,000			
Decreased pups born alive (%)		1,000[b]		1,000[c]	1,000[c]	
Decreased mean % pup survival days 1–5		1,000		1,000[c]		1,000[c]
Decreased mean % pup survival days 5–22		1,000[b]		1,000[b]		NA
Decreased mean male pup weight day 1		1,000[c]		1,000[c]	1,000[c]	
Decreased mean female pup weight day 1		1,000[c]		1,000[b]	1,000[c]	
Decreased mean male pup weight day 29		1,000[b], 300[b], 100[b,d]				NA
Decreased mean female pup weight day 29		1,000[b], 300[b], 100[d]				NA

[a] Not exposed after delivery.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] trend $p < 0.05$.

NA = Not applicable (pups terminated on day 5 postnatal)

Source: Adapted from Tinston (1994).

(originating from treated males and nontreated females) suggests the absence of male-mediated effects on reproductive outcome. Nevertheless, the alterations in testes weight cannot be discounted as a possible effect of treatment.

In females, dystocia was noted in one F0 dam at 100 ppm, two F1 dams at 300 ppm, and a total of four dams (two each F0 and F1) at 1,000 ppm; these dams were terminated without completion of delivery. From the data for surviving dams and litters, it can be assumed that the difficulties in parturition were not associated with or attributable to alterations in mean gestation length or increased mean pup or litter weights. In fact, mean pup body weights showed a statistically significant decrease throughout the lactation period at 300 and 1,000 ppm for F1A litters and in early lactation for F2A and F2B litters. Additionally, mean F1A male pup body weight was significantly decreased (5% less than controls; $p < 0.05$) at 100 ppm on postnatal day 29. These postnatal day 29 mean body weight deficits in all treated groups were observed in the animals selected as parents of the second generation, but by the second week of the F1 pre-mating period, mean body weights were similar to those of controls for both 100 and 300 ppm animals.

Mean litter size was decreased at 1,000 ppm for F2A and F2B litters. Statistically significant decreases in the number of live pups on postnatal day 1 (25% and 37% lower than controls for F2A and F2B, respectively) are suggestive of either an adverse effect on fertilization or on in utero survival. Early postnatal survival (i.e., on postnatal day 1 and between postnatal days 1 and 5) was also compromised in F2A and F2B pups at 1,000 ppm, with mean litter sizes decreasing to 48% and 53% of those of controls, respectively. The number of dead pups and litters with dead pups was also increased, although not significantly, at 300 ppm for F2A litters. Clinical observations data for 1,000 ppm litters reported an increased incidence of F2A and F2B pups that were found dead, were killed in extremis or were missing and presumed dead. The apparent increase in adverse survival findings at 300 and 1,000 ppm in the second generation as compared with the first generation could not be definitively attributed to any particular aspect of study design or conduct (e.g., differences in the duration of treatment), although it is noted that, unlike the second generation (F1) parental animals, the first generation (F0) rats were not exposed to tetrachloroethylene during preconception and in utero development.

A deficiency of the Tinston study is that the pregnant rats were not exposed from gestation day 21 through lactation day 6 or 7, and the exposure at the 1,000 ppm treatment level stopped for the F1 dams at the littering of the F2B pups. The F2B pups were not exposed postnatally. A summary of the doses at which the effects were observed in the study is presented in Table 4-9.

In a study designed to examine the fertilizability of rat oocytes, female rats were exposed to inhaled tetrachloroethylene at 12,000 mg/m^3 (2 hrs/day, 5 days/week) for 2 weeks (Berger and

Horner, 2003). The percentage of extracted oocytes that were fertilized in vitro was reduced for tetrachloroethylene-treated females as compared with controls.

4.7.2.1. *Summary of Animal Studies*

Table 4-10 summarizes the findings of the animal studies described in this section. The data show that inhalation of tetrachloroethylene by pregnant mice and rats during various periods of gestation resulted in fetal growth retardation and mortality in several studies and in delayed behavioral changes in the three studies that measured these effects (Szakmaćy et al 1997; Nelson et al. 1980; Tinston, 1994). Single studies have shown changes in brain acetyl choline and dopamine, altered brain fatty acid composition, and altered sperm morphology. These effects occurred at doses higher than 300 to 1,000 ppm in various studies.

The overall NOAEL for the animal developmental/reproductive inhalation studies is 100 ppm, based on Tinston (1994). The overall LOAEL is 300 ppm, based on Tinston (1994) and Schwetz et al. (1975), in which increased mortality and decreased body weight of the offspring were observed. All of these studies used the inhalation route of exposure.

4.7.3. Summary of Human and Animal Developmental/Reproductive Studies

Inferences regarding developmental and reproductive effects from tetrachloroethylene exposure in humans are limited due to small risks of low precision, the lack of a direct measure of tetrachloroethylene in many studies, the small numbers of exposed cases, and possible biases, particularly in studies where the information on birth outcome or exposure is obtained by questionnaire or inferred by residence. The epidemiologic evidence is strongest for spontaneous abortions among exposed women. A number of studies have reported an elevated risk of spontaneous abortion and maternal exposure to tetrachloroethylene, primarily exposure received through employment as a dry cleaner (Doyle et al., 1997; Windham et al., 1991; Olsen et al., 1990; Lindbohm et al., 1990; Kyyrönen et al., 1989; Bosco et al., 1987).

The epidemiologic evidence for infertility is further suggestive of an association with tetrachloroethylene exposure (Rachootin and Olsen, 1983; Eskenazi et al., 1991a, b; Sallmén et al., 1995). Strong conclusions about effects on birth weight—IUGR (SGA)—or congenital anomalies and tetrachloroethylene exposure cannot be drawn from the available occupational studies, although drinking water studies of exposures to multiple chemicals, including tetrachloroethylene, provide some limited evidence. There is very little information about what exposure pattern (concentration and duration) is associated with these effects.

Inhalation of tetrachloroethylene by pregnant mice and rats during various fractions of the gestation period has resulted in fetal growth retardation and mortality in several studies and

Table 4-10. Summary of animal developmental/reproductive studies fortetrachloroethylene, in chronological order

Subjects	Effects	Concentration	Authors
SW Mice	Maternal toxicity, decreased fetal weight, delayed ossification, 9% decrease in birth weight	300 ppm on gestation days 6–15	Schwetz et al. (1975)
SD Rats	Maternal toxicity, increased resorptions (fetal death)	300 ppm on days 6–15	Schwetz et al. (1975)
CFY Rats	Maternal toxicity (decreased body weight gain, increased liver weight and serum enzymes); increased pre- and post-implantation loss, skeletal retardation, and total malformations; decreased fetal weight	1,500, 4,500, 8,500 mg/m^3 on gestation days 1–20 LOAEL = 1,500 mg/m^3	Szakmáry et al. (1997)
CFY Rats	Decreased postnatal survival, minimal transient decreases in exploratory activity and muscular strength, and increased motor activity in females on postnatal day 100	1,500, 4,500 mg/m^3 on gestation days 1–20 (and perhaps postnatally to PND 100) LOAEL = 1,500 mg/m^3	Szakmáry et al. (1997)
C57Bl Mice	Maternal toxicity (increased liver weight); visceral malformations	1,500 mg/m^3 on gestation days 7–15 LOAEL = 1,500 mg/m^3	Szakmáry et al. (1997)
NZW Rabbits	Maternal toxicity (decreased body weight gain, increased liver weight); abortions, total litter resorptions, increased postimplantation loss, malformations	4,500 mg/m^3 on gestation days 7–20 LOAEL = 4,500 mg/m^3	Szakmáry et al. (1997)
SD Rats	Decreased weight gain, behavioral changes (more extensive for late pregnancy exposure), decreased brain acetylcholine	0, 100, 900 ppm on days 7–13 or on days 14–20 NOAEL = 100 ppm LOAEL = 900 ppm	Nelson et al. (1980)
Mice	Abnormal sperm heads at 500 ppm but not at 100 ppm, spermatogonia or spermatocyte stage affected	0, 100, 500 ppm for 5 days LOAEL = 500 ppm	Beliles et al. (1980)

Table 4-10. Summary of animal developmental/reproductive studies fortetrachloroethylene, in chronological order (continued)

Subjects	Effects	Concentration	Authors
Rats. rabbits	No developmental toxicity	Exposures throughout gestation NOAEL = 500 ppm	Hardin et al. (1981)
SD rats	Maternal toxicity (decreased body weight gain; decreased gravid uterine weight); fetal body weight and placental weight decrements, increased delays in thoracic vertebral ossification	0, 75, 250 or 600 ppm (actual concentrations: 0, 66, 249, 600 ppm), 6 hr/day, 7 days/week, on gestation days 0–19 Maternal LOAEL = 600 ppm Fetal LOAEL = 250 ppm	Carney et al. (2006)
Rat embryo in culture	Mortality, malformations, delayed growth and differentiation	No effect at 2.5 mM, effects at 3.5 mM and higher	Saillenfait et al. (1995)
F344 rats	100% mortality at 1,200 mg/kg-day, increased mortality and micro/anophthalmia at 900 mg/kg-day; soft tissues not examined	Gavage, 900, 1,200 mg/kg-day on gestation days 6–19	Narotsky and Kavlock (1995)
SD rats, two-generation study	Increased death of F1A and F2A and F2B pups, decreased body weight	0, 100, 300, 1,000 ppm NOAEL = 100 ppm for body weight reduction	Tinston (1994)
Rats	Reduced fertilizability of extracted oocytes	12,000 mg/m^3, 2 hrs/day, 5 days/week for 2 weeks	Berger and Horner (2003)

in delayed behavioral changes in the two studies that measured these effects. Single studies have shown changes in brain acetyl choline and dopamine, altered brain fatty acid composition, and altered sperm morphology. These effects occurred at doses higher than 300 to 1,000 ppm in various studies.

The overall NOAEL for the animal developmental/reproductive inhalation studies is 100 ppm, based on Tinston (1994). The overall LOAEL is 300 ppm, based on Tinston (1994) and Schwetz et al. (1975), in which increased mortality and decreased body weight of the offspring were observed. All studies used the inhalation route of exposure except for one gavage study (Fredriksson et al., 1993), which showed behavioral toxicity.

The finding of spontaneous abortions in several human studies of dry cleaners is supported by the occurrence of reduced birth weight and mortality in several animal studies. The finding of low birth weight in the Camp Lejeune studies by ATSDR is supported by reduced birth weight in five animal studies (Schwetz et al., 1975; Szakmaćy et al., 1997; Nelson et al., 1980; Carney et al., 2006; and in the F1 generation but not the F2 generation of Tinston (1994). There are no human observations of behavioral changes to compare with the animal evidence of CNS effects. The subtle nonadverse effects on sperm seen in humans correspond to one report of abnormal sperm in mice. The LOAEL for developmental/reproductive effects in animals is 300 ppm, but no quantitative measures are available for human effects, although other dry cleaner studies cited in previous sections have 8-hr TWA exposures of 10–20 ppm.

For oral exposures in humans there are only suggestive effects associated with tetrachloroethylene exposure in drinking water, with no reliable data on exposures; therefore, this information does not contribute to the determination of a LOAEL. There is little information on developmental or reproductive effects in animals by the oral route of exposure.

4.7.4. Mode of Action for Developmental Effects

Because of its lipid solubility, tetrachloroethylene can cross both the blood-brain barrier and the placental barrier and, therefore, it can be present in all tissues, including the brain, during development.

Peroxidation of the lipids of the cell membranes (Cojocel et al., 1989), alteration of regulation of fatty acid composition of the membrane (Kyrklund and Haglid, 1991), disturbances in the properties of the nerve membrane (Juntunen, 1986), and progressively increased activity in one or more of the phosphoinositide-linked neurotransmitters (Subramoniam et al., 1989) have all been suggested as MOAs for neurotoxic effects. These mechanisms could be involved during development phases, as well as in adults.

The metabolite TCA may be the causative agent for developmental toxicity expressed as morphological changes, lethality, or growth. Evidence in support of this speculative position is presented in the following discussion. TCA is a weak organic acid, as are many developmental toxicants, such as ethylhexanoic acid and valproic acid. These materials accumulate to a greater extent in the embryo/fetal compartment than in the mother, based on the pKa of the acid and the pH gradient between the maternal plasma and the embryo compartments (O'Flaherty et al., 1992). TCA could induce developmental toxicity by changing the intracellular pH or through peroxisome proliferation. Ghantous et al. (1986) detected TCA in the amniotic fluid of pregnant mice exposed to tetrachloroethylene via inhalation.

Smith et al. (1989) found that oral gavage doses of TCA (330, 800, 1,200, and 1,800 mg/kg-day) delivered on gestation days 6–15 to pregnant Long-Evans rats produced soft tissue

malformations, principally in the cardiovascular system. Johnson et al. (1998) found cardiac defects in rat fetuses whose mothers received 2,730 ppm TCA in drinking water during the period of cardiac development. Saillenfait et al. (1995), using the rat whole embryo (day 10) culture system, found that both tetrachloroethylene and TCA induced embryo toxicity, including mortality, malformations, and delayed growth and differentiation. TCA produced a reduction in the first branchial arch as well as other morphological changes at a lower concentration (2.5 mM) than that at which tetrachloroethylene induced no adverse effect (3.5 mM). TCA also induced a reduction of the yolk sac diameter at 1 mM.

4.8. TOXIC EFFECTS IN OTHER ORGAN SYSTEMS

This section discusses effects in organ systems not covered previously. It does not include effects on the liver, kidney, or nervous system; nor does it include developmental or reproductive effects. To be consistent with other sections of the document, effects in humans are presented separately from those in animals. Immune effects and lymphoid cancer are the most studied, and these are the predominant topics of the noncancer and cancer sections, respectively.

4.8.1. Human Studies

4.8.1.1. *Noncancer Effects*

4.8.1.1.1. *Immune-related effects in humans.* Adverse effects on the immune system resulting from chemical exposure fall within the following principal domains: immunosuppression (host resistance), immunostimulation, autoimmunity, and allergy-hypersensitivity. Various immunologic measurements (e.g., T-cell counts, immunoglobulin (Ig) E levels, specific autoantibodies) may provide evidence of altered immune response that may subsequently be related to risk of clinically expressed diseases such as infections, asthma, or systemic lupus erythematosus. Tetrachloroethylene exposure via air or water may result in immune-mediated organ-specific or systemic effects, as described in a case report of hypersensitivity pneumonitis in a 42-year-old female dry cleaner worker (Tanois et al., 2004). Another case report described severe fatigue, weight loss, myalgia, arthralgia, cardiac arrhythmia, decreased T-cell count, high-titer (1:160) antinuclear antibodies, and neurological symptoms that were linked to an unusual chemical sensitivity to tetrachloroethylene in a municipal water supply (Rea et al., 1991).

4.8.1.1.1.1. *Tetrachloroethylene and immunologic parameters*. Byers et al. (1988) provide data pertaining to immune function from 23 family members of leukemia patients in Woburn, Massachusetts. In 1979, testing of the wells in this town revealed that the water in two of the wells was contaminated with a number of solvents, including tetrachloroethylene (21 ppb) and trichloroethylene (267 ppb; as cited in Lagakos et al., 1986). These wells had been in operation

from 1964 to 1979. Byers et al. collected serum samples in May and June of 1984 and in November of 1985. They determined the total lymphocyte counts and lymphocyte subpopulations (CD3, CD4, CD8), and the CD4/CD8 ratio in these samples, and in samples from a combined control group of 30 laboratory workers and 40 residents of Boston selected through a randomized probability area sampling process. The study authors also assessed the presence of autoantibodies (antismooth muscle, antiovarian, antinuclear, antithyroglobulin, and antimicrosomal antibodies) in the family member samples and compared the results with laboratory reference values. The lymphocyte subpopulations were higher and the CD4/CD8 ratio was lower in the Woburn family members compared to the controls in both of the samples taken in 1984. In the 1985 samples, however, the subpopulation levels had decreased and the CD4/CD8 ratio had increased; the values were no longer statistically different from the controls. None of the family member serum samples had antithyroglobulin or antimicrosomal antibodies, but 10 family member serum samples (43%) had antinuclear antibodies (compared to <5% expected based on the reference value). Because the initial blood sample was taken in 1984, and because of the considerable mixture of exposures that occurred in this setting, it is not possible to determine the patterns at a time nearer to the time of the exposure, or to infer the exact role of tetrachloroethylene in alterations of the immunologic parameters.

Andrýs et al. (1997) examined immunologic parameters in 21 dry cleaning workers (20 women) and 16 office workers in the dry cleaning plant (14 women) and compared them to reference values based on samples from blood donors and "healthy persons in the same region" (n = 14–311, depending on the test). The mean age of the exposed workers and office controls was 45.7 years and 31.9 years, respectively; no information was provided on the age or sex distribution of the reference controls. The tests included measures of Ig, A, G, M, and E levels, complement (C3 and C4) levels, phagocyte activity, C-reactive protein, α-macroglobulin, T-lymphocytes, and a blast transformation test. Several differences were observed between the exposed workers and the office workers (e.g., higher levels of serum complement C3 and C4, and of salivary IgA in the exposed), and between the exposed workers and the reference controls (reduced T-lymphocytes, higher phagocytic activity, higher C3 levels in exposed). However, there were also many differences noted between the office workers and reference group (including reduced T-lymphocytes in office workers). The lack of information about the reference group adds to the difficulty in interpreting these results.

4.8.1.1.1.2. ***Tetrachloroethylene and immunosuppression*****.** In 1982, Lagakos et al. (1986) conducted a telephone survey of residents of Woburn, Massachusetts, collecting information on residential history and history of 14 types of medically diagnosed conditions. The survey included 4,978 children born since 1960 who lived in Woburn before age 19. Completed

surveys were obtained from approximately 57% of the town residences with listed phone numbers. Lagakos et al. used information from a study by the Massachusetts Department of Environmental Quality and Engineering to estimate the contribution of water from the two contaminated wells to the residence of each participant, based on zones within the town receiving different mixtures of water from various wells, for the period in which the contaminated wells were operating. This exposure information was used to estimate a cumulative exposure based on each child's length of residence in Woburn. A higher cumulative exposure measure was associated with history of kidney and urinary tract disorders (primarily kidney or urinary tract infections) and with lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia). There are no other human data that characterize the effects of tetrachloroethylene-only exposure on immunosuppression, as measured by increased susceptibility to infections.

4.8.1.1.1.3. ***Tetrachloroethylene and autoimmune disease*****.** In the 1970s, recognition of a scleroderma-like disease characterized by skin thickening, Raynaud's phenomenon, and acroosteolysis and pulmonary involvement in workers exposed to vinyl chloride (Gama et al., 1978) prompted research pertaining to the role of organic solvents in autoimmune diseases. Exposure to the broad categories of solvents, organic solvents, or chlorinated solvents has been associated with a 2- to 3-fold increased risk of systemic sclerosis (scleroderma) in epidemiologic studies summarized in a recent meta-analysis (Aryal et al., 2001) and in subsequent studies (Garabrant et al., 2003; Maitre et al., 2004). Similar results were seen in studies of other systemic autoimmune diseases including undifferentiated connective tissue disease (Lacey et al., 1999), rheumatoid arthritis (Lundberg et al., 1994; Sverdrup et al., 2005), and antineutrophil-cytoplasmic antibody (ANCA)-related vasculitis (Lane et al., 2003; Beaudreuil et al., 2005). In contrast, there was little evidence of an association between solvent exposure and systemic lupus erythematosus in two recent case-control studies (Cooper et al., 2004; Finckh et al., 2007).

As described in the preceding paragraph, the epidemiologic data in relation to the role of solvents, as a broad category, in systemic autoimmune diseases, varies among these conditions. Much more limited data is available pertaining to specific solvents, including tetrachloroethylene, and risk of autoimmune diseases. Case reports have been published describing a condition similar to vinyl-chloride induced scleroderma in a man who worked as a presser in a dry cleaning plant, and who also helped clean the tetrachloroethylene-containing drums on a weekly basis (Sparrow, 1977), and a localized scleroderma in a man who had worked with tetrachloroethylene as a metal degreaser, with workplace exposures reported to be between 10–25 ppm (Hinnen et al., 1995; in German). Among 279 cases with connective tissue disease, Goldman (1996) observed a higher frequency of individuals who reported employment as a dry cleaner among systemic

sclerosis patients (4 of 33) compared with patients with other connective tissue diseases (1 of 246; $p < 0.01$). Similar patterns were seen with self-reported history of tetrachloroethylene exposure (3 of 33 systemic sclerosis patients compared with 2 of 246 other patients, $p < 0.01$), but the author noted the difficulty in obtaining this type of information.

One registry-linkage study from Sweden of rheumatoid arthritis (Lundberg et al., 1994) and three case-control studies of undifferentiated connective tissue disease (Lacey et al., 1999), scleroderma (Garabrant et al., 2003), and antineutrophil-cytoplasmic antibody (ANCA) related diseases (Beaudreuil et al., 2005) provide data concerning dry cleaning work or tetrachloroethylene exposure (Table 4-11). As expected in population-based studies, the exposure prevalence is low, with approximately 4% of controls reporting work in dry cleaning and 1% reporting exposure to tetrachloroethylene. The observed associations are generally weak (odds ratios for dry cleaning around 1.5 for the 3 large studies of women) and none of the individual studies are statistically significant. The results seen for the exposure to tetrachloroethylene in the three studies that attempted this kind of assessment were more varied (Lacey et al., 1999; Garabrant et al., 2003; Beaudreuil et al., 2005). Only the study of ANCA-related diseases resulted in an elevated odds ratio, but again this estimate was somewhat imprecise (OR = 2.0, 95% CI = 0.6, 6.9; Beaudreuil et al., 2005). These studies are clearly limited by the low prevalence of and difficulty in accurately characterizing occupational exposure to tetrachloroethylene in population-based or clinical settings.

4.8.1.1.1.4. ***Tetrachloroethylene and allergy and hypersensitivity*****.** Allergy and hypersensitivity, as assessed with measures of immune system parameters or immune function tests (e.g., asthma, atopy) in humans, have not been extensively studied with respect to the effects of tetrachloroethylene.

Th2 cytokines (e.g., interleukin-4) stimulate production of IgE and Th1 cytokines (e.g., interferon-γ) act to inhibit IgE production. Lehmann et al. (2001) examined IgE levels and cytokine producing cells (interferon-γ, tumor necrosis factor-α, and interleukin-4) in relation to indoor levels of volatile organic compounds among children (age 36 months) selected from a birth cohort study in Leipzip, Germany. The hypothesis underlying this work is that a shift in Th1 to Th2 cytokine profile is a risk factor for IgE-mediated allergic disease in children (Tang et al., 1994; Warner et al., 1994). Enrollment into the birth cohort occurred between 1995 and 1996. The children in this allergy study represent a higher-risk group for development of allergic disease, with eligibility criteria that were based on low birth weight (between 1,500 and 2,500 g), or cord blood IgE greater than 0.9 kU/l with double positive family history of atopy. These eligibility criteria were met by 429 children; 200 of these children participated in the allergy

Table 4-11. Immune-related conditions in studies of dry cleaning or tetrachloroethylene exposure in humans[a]

Condition, location, diagnosis period, sample size, demographics, source of data	Results	Authors
Autommune diseases		
Rheumatoid arthritis, Sweden (13 counties), hospitalized 1981–1983, 896 male cases, 629 female cases; population comparison (total 370,035 men, 140,139 women), ages 35–74. Registry linkage to 1960 and 1970 census occupation data	launderers and dry cleaning men: 1 exposed cases; OR = 0.8 (95% CI = 0.1–5.0) women: 7 exposed cases; OR = 1.5 (95% CI = 0.7–3.2)	Lundberg et al. (1994)
Undifferentiated connective tissue disease, Michigan and Ohio, diagnosed 1980–1991 (Michigan) 1980–1992 (Ohio). 205 cases, 2095 population controls. Women, ages 18 and older. Structured interview (specific jobs and materials; jobs held 3 or more months)	dry cleaning cases: 4.3%, controls 3.8% OR = 1.4 (95% CI = 0.68, 2.8) tetrachloroethylene: cases: 0%, controls 1% OR = 0.00	Lacey et al. (1999)
Scleroderma, Michigan and Ohio. Diagnosed 1980–1991 (Michigan), 1980–1992 (Ohio). 660 cases, 2227 population controls. Women, ages 18 and older. Structured interview (specific jobs and materials; jobs held 3 or more months)	dry cleaning cases: 4.7%, controls 3.7% OR = 1.4 (95% CI = 0.9, 2.2) tetrachloroethylene: self report cases: 1.1%, controls 1.0% OR = 1.4 (95% CI = 0.6, 3.4) expert review cases: 0.8%, controls 0.8% OR = 1.1 (95% CI = 0.4, 2.9)	Garabrant et al. (2003)
ANCA-related diseases,[b] France. Diagnosed 1999–2000. 60 patients, 120 hospital controls. men and women (50% each), mean age 61 years	tetrachloroethylene cases: 8.3%, controls 4.1% OR = 2.0 (0.6–6.9)	Beudreuil et al. (2005)

Table 4-11. Immune-related conditions in studies of dry cleaning or tetrachloroethylene exposure in humans* (continued)

Condition, location, diagnosis period, sample size, demographics, source of data	Results	Authors
Allergy and hypersensitivity		
IgE levels, Germany, 1995–1996. 121 children (ages 36 months), selected based on high risk profile for allergic diseases, blood sample and indoor air sampling (child's bedroom) of 26 volatile organic chemicals (4 weeks around age 36 months)	no association between tetrachloroethylene measures and total IgE or IgE-specific allergen antibodies	Lehmann et al. (2001)
CD3 T-cell subpopulations from cord blood, Germany 1997–1999. 85 newborns, cord blood and indoor air sampling (child's bedroom) of 28 volatile organic chemicals (4 weeks immediately after birth)	Tetrachloroethylene exposure associated with decreased interferon-γ cells, but no association with interleukin-4, interleukin-2, or tumor necrosis factor-α	Lehmann et al. (2000)
Exacerbation of asthmatic symptoms, Los Angeles, 1999–2000. 21 children (ages 10–16 years), 3 month diaries, ambient levels and exhaled breath measures of 8 volatile organic compounds and 8 criteria pollutant gases	Little evidence of an association between ambient tetrachloroethylene exposure or exhaled tetrachloroethylene measures and asthma symptoms	Delfino et al. (2003a, b)

[a] Includes case-control studies and cross-sectional studies, but does not include case reports.
[b] ANCA = antineutrophil-cytoplasmic antibody. Diseases included Wegener glomerulonephritis (n = 20), microscopic polyangiitis (n = 8), pauci-immune glomerulonephritis (n = 10), uveitis (n = 6), Churg-Strauss syndrome (n = 4), stroke (n = 4) and other diseases (no more than 2 each)

study described below, but complete data (IgE and volatile organic compound measurements) were available for only 121 of the study participants.

Lehmann et al. measured 26 volatile organic compounds via passive indoor sampling in the child's bedroom for a period of 4 weeks around the age of 36 months. The highest exposures were seen for limonene (median 19.1 $\mu g/m^3$), α-pinene (median 16.3 $\mu g/m^3$), and toluene (median 13.3 $\mu g/m^3$). The median exposure of tetrachloroethylene was 2.5 $\mu g/m^3$ (0.87 $\mu g/m^3$ and 5.1 $\mu g/m^3$ for the 25th and 75th percentiles, respectively). The only strong correlation ($r > 0.3$) between tetrachloroethylene and the other volatile organic compounds measured was a correlation of 0.72 with trichloroethylene. Blood samples were taken at the 36-month-study examination and were used to measure the total IgE and specific IgE antibodies directed to egg white, milk, indoor allergens (house dust mites, cat, molds), and outdoor allergens (timothy-perenial grass, birch- tree). There was no association between tetrachloroethylene exposure and any of the allergens tested in this study, although some of the other volatile organic compounds (e.g., toluene, 4-ethyltoluene) were associated with elevated total IgE levels and with sensitization to milk or eggs.

Another study by Lehmann et al. (2002) examined the relationship between indoor exposures to volatile organic compounds and T-cell subpopulations measured in cord blood of newborns. The study authors randomly selected 85 newborns (43 boys and 42 girls) from a larger cohort study of 997 healthy, full-term babies, recruited between 1997 and 1999 in Germany. Exclusion criteria included a history in the mother of an autoimmune disease or infectious disease during the pregnancy. Twenty-eight volatile organic compounds were measured via passive indoor sampling in the child's bedroom for a period of 4 weeks after the birth (a period which is likely to reflect the exposures during the prenatal period close to the time of delivery). The levels were generally similar or slightly higher than the levels seen in the previous study using samples from the bedrooms of the 36-month-old children. The highest levels of exposure were seen for limonene (median 24.3 $\mu g/m^3$), α-pinene (median 19.3 $\mu g/m^3$) and toluene (median 18.3 $\mu g/m^3$), and the median exposure of tetrachloroethylene was 3.4 $\mu g/m^3$ (1.8 $\mu g/m^3$ and 7.3 $\mu g/m^3$ for the 25th and 75th percentiles, respectively). Flow cytometry was used to measure the presence of CD3 T-cells obtained from the cord blood labeled with antibodies against interferon-γ, tumor necrosis factor-α, interleukin-2 and interleukin-4. Tetrachloroethylene was the only one of the measured volatile organic compounds that was associated with a reduced level of interferon-γ. In the univariate analysis, the median percentage of interferon-γ cells was 3.6% and 2.6% in the groups that were below the 75th percentile and above the 75th percentile of tetrachloroethylene exposure, respectively. The odds ratio between high (above the 75th percentile) tetrachloroethylene exposure and reduced (less than the 25th percentile) levels of interferon-γ cells was 2.9 (95% CI = 1.0–8.6), adjusting for family history of

atopy, gender and smoking history of the mother during pregnancy. There was no association between tetrachloroethylene exposure and interleukin-4 cells, but naphthalene and methylcyclopentane were associated with elevated levels of interleukin-4 cells.

Delfino et al. (2003a, b) examined the exacerbation of asthmatic symptoms following exposure to volatile organic compounds that occurred due to variation in air quality over a 3 month period in 1999–2000 in Los Angeles. This study included daily repeated exposures to ambient air pollutants and peak expiratory flow rates over a 3-month period in 21 children (17 males and 4 females) of Hispanic origin ages 10–16 years; an additional child participated in the ambient air but not in the exhaled air portion of the study. Daily diaries were used to record severity of symptoms and asthmatic episodes. Exposure metrics included exhaled breath measures and ambient levels of eight volatile organic compounds (benzene, methylene chloride, styrene, toluene, *m,p*-xylene, *o*-xylene, *p*-dichlorobenzene, and tetrachloroethylene) and eight criteria pollutant gases. An association between criteria air pollutants and subsequent symptoms of asthma in children in the Los Angeles area suggest an increased risk of adverse health outcomes with exposure to SO_2 and NO_2 (Delfino et al., 2003a). Although ambient levels of tetrachloroethylene were associated with bothersome asthma symptoms (OR = 1.37, [95% CI = 1.09, 1.71]) per an interquartile range change), this association was reduced with the adjustment for SO_2 or NO_2 (Delfino et al., 2003a). In the 21 children who partcipated in the peak expiratory flow measurements, the mean breath level of tetrachloroethylene was 4.40 ng/L (sd 10.77 ng/L), the mean ambient level was 3.52 (sd 2.17) ng/L, and the correlation between the same-day measures was 0.31 ($p < 0.01$; Delfino et al., 2003b). There was little relation between asthma symptoms and exhaled breath levels of tetrachloroethylene. The mean exhalation of tetrachloroethylene was 2.50 and 2.69 ng/L, respectively, in the two groups of asthma symptoms (none or not bothersome; bothersome and more severe). Stronger associations were reported between asthma symptoms and some of the other volatile organic chemicals, specifically for benzene, toluene, *m,p*-xylene.

The limited available data from these studies (Lehmann et al., 2001; Lehmann et al., 2002; Delfino et al., 2003a, b), provide weak evidence of an effect of tetrachloroethylene exposure during childhood on allergic sensitization or exacerbation of asthma symptomology. However, the observation of the association between increased tetrachloroethylene exposure and reduced interferon-γ in cord blood samples may reflect a sensitive period of development, and points to the current lack of understanding of the potential immunotoxic effects of prenatal exposures.

4.8.1.1.2. ***Endocrine system effects.*** Only one study of endocrine system effects was found in the published literature. Ferroni et al. (1992) observed an increased serum concentration of

prolactin among tetrachloroethylene-exposed dry cleaners as compared with controls (12.1 ± 6.7 μg/L vs. 7.4 ± 3.1 μg/L). The median tetrachloroethylene concentration to which these workers were exposed was 15 ppm. The variance of serum prolactin concentration was wider for exposed subjects than for unexposed controls, and 4 of 41 subjects had serum prolactin concentrations above the upper normal limit (defined by Ferroni et al. as 25 μg/L), compared with none of 23 controls. The prevalence of abnormal concentrations, however, was not statistically significantly elevated. Positive correlations between the response and tetrachloroethylene were not observed with either exposure duration or biomarker measures. The evaluation of prolactin was part of an overall assessment of neurobehavioral functioning (see the discussion of this study in the section on neurobehavioral effects) for which these investigators hypothesized a relationship between dopamine and serum prolactin concentration (Ferroni et al., 1992; Mutti and Smargiassi, 1998).

Epidemiologic studies of other parameters of endocrine function are lacking. The endocrine system can be considered a potential target for tetrachloroethylene because another like solvent, trichloroethylene, has been shown to induce endocrine system changes in both humans (Goh et al., 1998; Chia et al., 1996, 1997) and experimental animals (Kumar et al., 2000).

4.8.1.2. *Cancer*

The body of literature reporting carcinogenic effects in humans associated with exposure to tetrachloroethylene consists of cohort, proportional mortality, and case-control studies. A small number of studies, including studies of cohorts involved in metal degreasing or in aircraft manufacturing/maintenance (Boice et al., 1999; Anttila et al.; 1995, Spirtas et al., 1991), have assessed tetrachloroethylene exposure explicitly. These cohort studies present risks associated with site-specific cancer mortality (Boice et al., 1999; Spirtas et al., 1991) or incidence (Anttila et al., 1995) for a subcohort of the larger study population who were exposed to tetrachloroethylene. Additionally, a few case-control studies were able to examine the relationship between cancer at specific sites and tetrachloroethylene exposure (Vaughan et al., 1997; Schlehofer et al., 1995; Pesch et al., 2000a; Heineman et al., 1994).

A larger body of evidence on cancer exists for workers employed in dry cleaning. Dry cleaners have potential exposures to a number of solvents, including tetrachloroethylene, which has been in widespread use since the early 1960s (IARC, 1995). Information on the potential for tetrachloroethylene exposure and concentration measurements is lacking for individual study subjects in these studies: however, the cohort studies of Ruder et al. (1994, 2001) and Blair et al. (1990, 2003) are of individuals primarily exposed to tetrachloroethylene (Lynge et al., 1997). The exposure assessment approach of Lynge et al. (2006), a case-control study nested within a cohort of dry-cleaning and laundry workers, relies on job title to increase sensitivity for

tetrachloroethylene exposure identification, with dry cleaners identified as having greater potential for tetrachloroethylene exposure. This study lacks information on tetrachloroethylene concentration for individual study subjects, is unable to classify job title for 20% of study subjects, and has a large percentage of cases from Sweden and Norway with job title provided by next-of-kin.

Although several community-based drinking water studies are available (Aschengrau et al., 1993, 1998, 2003; Paulu et al., 1999; Fagliano et al., 1990; Cohn et al., 1994; MA DPH, 1997; Vartiainen et al., 1993; Lagakos et al., 1986), exposure in most of these studies was to a mixture of solvents, including tetrachloroethylene and trichloroethylene except for the studies by Aschengrau et al. (1993, 1998, 2003) and Paulu et al. (1999) that examined tetrachloroethylene specifically. Summary tables of these analyses are presented in Appendix 4B.

4.8.1.2.1. ***Lymphoid cancer.*** A number of epidemiologic studies including degreaser cohort (tetrachloroethylene subcohorts), dry cleaner and laundry worker cohort, case-control, and community studies have examined tetrachloroethylene exposure and lymphoid cancer. Elevated risks of lymphoid cancer incidence, specifically non-Hodgkin's lymphoma (NHL), were observed in studies of degreasers exposed to tetrachloroethylene; a total of 15 cases were observed in three available studies (Table 4B-2, Appendix 4B) versus 6.8 expected cases or deaths (95% CI = 1.2–3.7). Spirtas et al. (1991) observed a statistically significant elevated risk for NHL for males and females combined (standardized mortality ratio [SMR] = 4.0, 95% CI = 1.1–10.2, four deaths). Two of the four deaths occurred among females, with females having the highest risk. The tetrachloroethylene cohorts of Anttila et al. (1994) and of Boice et al. (1999) support the findings in Spirtas et al. (1991). NHL risk is elevated—but not statistically significantly—in both studies (routine exposed, SMR = 1.7, 95% CI = 0.7–3.3, eight deaths, Boice et al., 1999; SIR = 3.8, 95% CI = 0.8–11.0, three cases, Anttila et al., 1994). Boice et al. (1999) also present an analysis of duration of exposure-response gradient for NHL mortality; however, the inclusion of deaths with either routine or intermittent exposure in this analysis (a total of 16 deaths), with nonsolvents-exposed factory workers as referents prevents a comparison to the excess NHL risk reported for the eight deaths with routine exposure to tetrachloroethylene (Table 8 in Boice et al., 1999). NHL relative risk for subjects with >5 years duration of exposure (intermittent or routine exposure) to tetrachloroethylene was 1.6 (95% CI = 0.8–3.2) in Poisson regression analysis using internal controls (factory workers not exposed to any solvent) and no indication of a linear trend of increasing RR with increasing duration of exposure ($p < 0.20$). The inclusion of subjects with differing exposure patterns in an analysis of exposure duration likely introduces misclassification bias and does not diminish observations of routinely exposed subjects.

The three degreaser cohort studies provide only limited information on lymphoid tumors other than NHL due to few total numbers of observed deaths or incident cases for lymphoid neoplasms and, in general, a high proportion of cancers attributable to NHL. Furthermore, none of these cohort studies provide information on leukemia subtype. For example, Anttila et al. (1994) observed three cases of lymphoid tumors, and all three were attributable to NHL. Noteworthy, however, is the observation in one study of aircraft maintenance workers (Spirtas et al., 1991) of a large but imprecise risk for multiple myeloma and exposure to tetrachloroethylene (SMR = 17.0, 95% CI = 2.1–61.6) among females, which was not seen in another cohort of mostly male aircraft manufacturing workers (Boice et al., 1999).

Eight studies of incidence in dry cleaners and laundry workers in Scandanavian countries, one study of leukemia incidence in Portland, Oregon, and two studies of mortality of dry cleaners in the United States were available for review: Morton and Marjanovic (1984); Lynge and Thygesen (1990); Andersen et al. (1999); Ruder et al. (2001); Cano and Pollán, (2001); Travier et al. (2002); Blair et al. (2003) and Ji and Hemminki, (2005b, 2006). Observations in several of these studies are summarized in Table 4B-1a and Table 4B-1b, Appendix 4B, while others are discussed below. Anderson et al. (1999) examines cancer incidence by occupational title in the 1970 census among citizens of Denmark, Finland, Norway, and Sweden, between 1971 and 1987–1991, depending on country. Site-specific lymphoma incidence, but not leukemia subtype, is reported for launderers and dry cleaners. Further analysis of lymphoma incidence in Swedish subjects, many of whom overlap with the larger cohort of Anderson et al. (1999), was presented by Travier et al. (2002) who examined leukemia subtype and by Cano and Pollán (2001) who examined non-Hodgkin's lymphoma incidence. Ji and Hemminki (2005b, 2006) expanded the Swedish cohort, following launderers and dry cleaners identified on 1960, 1970, 1980, and 1990 censuses another 10 years to 2002. Lynge and Thygesen (1990) examine lymphoma incidence among Danish dry cleaners and launderers who were identified with this job title in the 1970 census and followed for 10 years to 1980.

Overall, association with Hodgkin's disease, NHL, or chronic lymphocytic leukemia are suggested, although site-specific risks are not always elevated or statistically significant in all studies nor were they elevated in both sexes (see Table 4B-1a). Two studies examined Hodgkin's disease (Anderson et al., 1999; Travier et al., 2002) and both reported statistically significant associations in females but not males: SIR = 1.9 (95% CI = 1.1–2.9) in Andersen et al. (1999), RR = 3.6 (95% CI = 1.2–11.1) in Travier et al. (2002). The elevated risk was observed particularly among the subjects who were below 40 years of age in 1960 (Travier et al., 2002). These are subjects who used mainly tetrachloroethylene with possibly some trichloroethylene.

NHL risks in the four-country study of Andersen et al. (1999) were 1.5 (95% CI = 1.0–2.1) for males and 1.0 (95% CI = 0.7–1.2) for females. Separate analyses of Swedish and Danish workers supported these observations: males, RR = 1.4 (95% CI = 0.6–3.4); females, RR = 0.5 (95% CI = 0.2–1.6; Travier et al., 2002); males, RR = 1.9 (95% CI = 0.8–4.1; Cano and Pollán , 2001); males, SIR = 2.8 (95% CI = 0.9–6.5); females, SIR = 0.5 (95% CI = 0.1–1.5; Lynge and Thygesen, 1990).

Three studies examine association with leukemia subtype (Ji and Hemminki, 2005b, 2006; Travier et al., 2002; Morton and Marjanovic, 1984) and all report statistically significant association with chronic lymphocytic leukemia in females but not males. A noteworthy finding in Travier et al. (2002) was a statistically significant elevated risk for leukemia among dry cleaners or launderers in both the 1960 and 1970 Swedish census (RR = 1.8, 95% CI = 1.1–3.1), mostly due to chronic lymphocytic leukemia (CLL); 6 of the 15 total leukemia cases, RR = 1.9, 95% CI = 0.8–4.1; of which 5 of the 6 CLL cases were in females, RR = 2.9, 95% CI = 1.2–7.0. Ji and Hemminki (2005b) also examined associations with leukemia subtypes and reported a similar finding with chronic lymphocytic leukemia in females but not males (females: SIR = 1.5, 95% CI = 1.1–2.1; males: SIR = 0.9, 95% CI = 0.5–1.3). Similarly Morton and Marjanovic (1984) reported leukemia incidence, particularly lymphocytic leukemia incidence, was statistically significantly higher in women laundry and dry cleaners in the Portland, Oregon area. Age-standardized incidence rates (per 100,000) for women dry cleaners and laundry workers compared to all women were: all leukemias, 23.7 compared to 6.7; lymphatic leukemia, 20.9 compared to 2.6. Many chronic lymphocytic leukemias and NHLs may arise from a common cell type (Beers and Berkow, 1999, in Bukowski et al., 2003).

The update of the cohort mortality study by Ruder et al. (2001) found only six deaths attributable to cancer of the lymphatic and hematopoietic system. The few deaths due to lymphatic cancer greatly impact the statistical power in this study. Blair et al. (2003), in an updated mortality analysis of a cohort that is predominately female, present observed and expected number of deaths for categories of lymphomas. Although based on a small number of deaths in several categories, these authors discuss this study as supporting an excess risk of Hodgkin's disease (SMR = 2.0, 95% CI = 0.6–4.6, 5 cases) and this observation is consistent with observations in the Scandinavian studies discussed above. Neither of the mortality studies of United States drycleaners examined leukemia subtype.

Ten case-control studies examine site-specific lymphomas and occupational exposure to tetrachloroethylene or job title of dry cleaner or launderer (Lynge et al., 2006; Mester et al., 2006; Miligi et al, 2006; Kato et al., 2005; Fabbro-Peray et al., 2001; Seniori Costantini et al., 2001; Clevel et al. 1998; Blair et al., 1993; Scherr et al., 1992; and Hardell et al., 1981; Table 4B-5, Appendix 4B). Several studies examine cell type (Mester et al., 2006, Miligi et al., 2006,

Clevel et al., 1998). The only study available on Hodgkin's disease observed a statistically significant elevated risk for male with a job title of dry cleaner or laundry worker (Seniori Costantini et al., 2001). Risks above 1.0 were observed in several studies of NHL and CLL although risks were not statistically significantly elevated, likely due to several factors discussed below (Mester et al., 2006 Miligi et al., 1999; 2006; Kato et al., 2005; Clevel et al., 1998; Blair et al., 1993; Scherr et al., 1992; Hardell et al., 1981).

Overall, case-control studies examining occupational exposure are quite limited for evaluating lymphoma and tetrachloroethylene for a number of reasons. Reviewed case-control studies are typically population-based studies. More recent studies are of large number of cases and controls compared to older studies. These recent studies adopt procedures to blind interviewers and apply more refined exposure assessment methods, examining tetrachloroethylene specifically as opposed to a grouping of dry cleaners and laundry workers (Lynge et al., 2006; Mester et al., 2006; and Miligi et al., 2006). However, the prevalence of exposure to tetrachloroethylene or as a dry cleaner, or launderer, particularly long-duration exposure, exposure, is low in these studies. A consequence of this is few exposed cases and imprecise risks (wide confidence intervals) that reflect lower statistical power to examine lymphoma and tetrachloroethylene exposure (Miligi et al., 2006; Mester et al., 2006; Seniori Costantini et al., 2005). Four other aspects of population case-control studies are important to consider in their interpretation. Risk is difficult to determine for low-prevalence jobs when studying a regional population; associations may be nonlinear because categorical assignment of duration and intensity are arbitrary and do not necessarily represent a linear dose relationship, and duration and cumulative exposure variables do not address age at first exposure, which also affects cancer risk (NRC, 2005). Additionally, missing information either as a result of lower participation rates or missing job history data, can introduce bias—particularly misclassification bias. For example, the lack of information to classify job title for roughly 20% of NHL cases and controls in Lynge et al. (2006), in addition to a large number of next-of-kin interviews, limits this study due to an increased potential for exposure misclassification and, as assessed using two exposure groups (yes/no) in this study, results in risks close to 1.0 (no risk). Lynge et al. (2005) is considered a null or uninformative study for this reason rather than a study supporting no association between tetrachloroethylene and NHL. Furthermore, quantitative exposure information is missing from all studies and leads to substantial misclassification of exposure.

Four case-control studies are available on childhood leukemia (acute lymphocytic leukemia, ALL) and parental occupational exposure to tetrachloroethylene or to drinking water contaminated with trichloroethylene, tetrachloroethylene, and other chlorinated solvents (Infante-Rivard et al., 2005; Costas et al., 2002; Shu et al., 1999; Lowengart et al. 1987; Table 4B-5, Appendix 4B). Many aspects discussed above for case-control studies examining occupational

exposure are found in ALL studies. While some studies appear consistent (Costas et al.,2002; Shu et al., 1999; Lowengart et al., 1987), these studies are insensitive for assessing association, or lack thereof, between ALL and tetrachloroethylene exposure because observations are based on few exposed cases. Other studies are needed to clarify the role of tetrachloroethylene and ALL.

Four studies examine drinking water exposure and lymphoma: a case-control study by Aschengrau et al. (1993), and the ecological analyses by Cohn et al. (1994); Fagliano et al., 1990; and Vartiainen, 1985 (see Table 4B-13 and Appendix 4B). In a study by Aschengrau et al. (1993), where tetrachloroethylene was identified as the putative exposure, an elevated risk of leukemia was observed for those most exposed (90^{th} percentile of exposure; with no latency, OR = 8.3; 95% CI = 1.5–45.3; considering a latency period of 5 years, OR = 5.9; 95% CI = 1.4–24.9). An exposure-response relationship is suggested; the crude unadjusted risk for high-level exposure (exposure at the 90^{th} percentile; OR = 6.0, 95% CI = 0.6–32.0) is larger than the unadjusted risk for any exposure (OR = 1.8, 95% CI = 0.6–4.3).

Moreover, the case-control study of Costas et al. (2002) and the ecologic studies by Fagliano et al. (1990) and Cohn et al. (1994) provide some evidence of an association between NHL or leukemia and drinking water that includes trichloroethylene and tetrachloroethylene. The actual level of exposure to tetrachloroethylene and other solvents in these studies is not known, and in the case of Costas et al. (2002), trichloroethylene was measured in the well water at concentrations an order of magnitude higher than tetrachloroethylene. Each of these solvents is hypothesized to be metabolized and bioactivated to TCA (see metabolism discussion). Thus, exposure to tetrachloroethylene can be considered to add to the level of these metabolites generated through trichloroethylene exposure.

The classification of lymphoid neoplasms, specifically lymphomas, has recently undergone a revision, primarily on the basis of new findings from molecular biology, genetics, and immunology, which have changed older concepts of lymphoid cancer, making them obsolete (Herrinton, 1998). Although lymphomas have been classified in the past into distinct categories (e.g., leukemia, reticulosarcoma/lymphosarcoma), lymphomas can share common biological properties (Weisenburger, 1992) and differentiation pathways. For example, advances in molecular biology have blurred the distinction between lymphoid leukemia and lymphoma (Herrinton, 1998). Few studies assessing tetrachloroethylene exposure have included analyses of diagnostic subcategories and no studies have examined cellular or molecular markers.

In 1994, the International Lymphoma Study Group published the revised European-American Lymphoma (REAL) classification, and this system has been adopted by WHO (Harris et al., 2000a). Modifications in the REAL include the grouping of lymphatic leukemia with NHL (Herrinton, 1998; Miligi et al., 1999); the REAL/WHO Classification considers lymphomas and

lymphoid leukemias of the same cell type as one disease, with different clinical presentation or stages (Harris et al., 2000b). This implies that the classification system used in many of the epidemiologic studies is imprecise for both diseases. The resulting bias related to disease misclassification affects observed risk estimates by masking underlying associations, biasing risks towards the null or RR close to 1.0. Hence, the elevated risks observed for different categories of lymphoid tumors in individual studies are noteworthy because of study insensitivities and, additionally, these risks may not be inconsistent with the pathogenesis of disease and with an etiology associated with tetrachloroethylene.

Rats exposed chronically to tetrachloroethylene for 2 years developed increased incidences of mononuclear cell leukemia (MCL) or large granular lymphocyte leukemia (JISA, 1993; NTP, 1986a). Section 4.8.2.2.1 describes these observations and their interpretation as a human cancer hazard. Large granular lymphocyte (LGL) cells exist in humans that are morphologically, biochemically, and functionally similar to the cells involved in MCL in the F344 rat (Stromberg, 1985). In humans, clonal disorders of LGLs represent a biologically heterogeneous spectrum of lymphoid malignancies thought as originating either from mature T-cell or natural killer (NK) cells (Sokol and Loughran, 2006). LGL disorders can clinically present as an indolent (chronic) or aggressive diseases (Sokol and Loughran, 2006). The indolent form of LGL leukemia is a disease of the elderly, with a median age at diagnosis of 60 years. A number of clinical conditions have been seen in patients with LGL leukemia. These include the following: red cell aplasia and aplastic anemia; other lymphoproliferative disorders such as NHL, Hodgkin's lymphoma, multiple myeloma, hairy cell leukemia, and B-cell lymphoprliferative disorders; and autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematousus (Rose and Berliner, 2004). The etiology of LGL disorders is not known (Rose and Berliner, 2004; Sokol and Loughran, 2006). Several possible etiologies have been proposed including chronic activation of T-cell by a viral antigen or autoantigen in which case LGL leukemia could be considered as an autoimmune disorder (Sokol and Loughran, 2006).

Existing epidemiologic studies of tetrachloroethylene exposure are simply not able to inform human relevance examinations of rat MCL. Lymphoid tumor pathobiology in rats and humans, its historical and current classification, and epidemiology, including observations in tetrachloroehylene-exposed populations, have bearing on examination of the human relevance of rat mononuclear cell leukemia. Important to any examination are the changes in diagnostic and classification criteria of human lymphoid tumors and lack of data on molecular markers in the tetrachloroethylene epidemiologic studies, as discussed above. Diagnostic and classification criteria may not be uniform across studies and hinders comparison of consistency within epidemiologic studies of lymphoid cancers and tetrachloroethylene exposure and, also, between human and rat lymphoid tumor observations. Furthermore, adoption of consensus nomenclatures

of human lymphoid tumors, i.e., the WHO scheme, for rats will facilitate cross-species comparisons, as was recently conducted by the hematopathology subcommittee of the Mouse Models for Human Cancers Consortium (Morse et al., 2002).

4.8.1.2.2. ***Esophageal cancer.*** Both cohort and case-control studies support an association between tetrachloroethylene and excess risk of esophageal cancer. An overall excess in the number of observed deaths was seen in the recent updates of the dry cleaner mortality studies (Ruder et al., 2001; Blair et al., 2003): a total of 31 deaths as compared with 13.7 expected deaths (RR = 2.3, 95% CI = 1.5–3.2; Table 4B-1b, Appendix 4B). This finding is the same as that of Wartenberg et al. (2000), who examined a slightly different set of studies for esophageal cancer. The recent PMR study by Walker et al. (1977) provides further support (aged <65 years at time of death, PMR = 1.7, 95% CI = 1.1–2.5). Both Ruder et al. (2001) and Blair et al. (2003) reported a similar magnitude of risk among workers employed before 1960 and after 1960. No clear picture of increasing risk was seen in either study between duration of exposure and esophageal cancer risk. Except for Boice et al. (1999), studies of the degreasers do not present risks for esophageal cancer (Table 4B-2, Appendix 4B). Boice reported a risk of 1.5 (95% CI = 0.5–3.2), based on six deaths.

The cohort and PMR studies cannot directly address possible effects due to smoking or alcohol, which are risk factors for the squamous cell histologic type of esophageal cancer. It is not known whether elevated risk may reflect smoking and alcohol effects. Data from the National Health Interview Survey (Nelson et al., 1994) suggest that the prevalence of smoking among dry cleaners and laundry operators as equal to that of "blue collar" workers. Moreover, Ruder et al. (2001) and Blair et al. (2003) note that the magnitude of the risks for several smoking-related cancers was greater than could be explained by smoking alone, suggesting a further contribution from another risk factor, such as occupational exposure.

The incidence of esophageal cancer is generally higher for nonCaucasan males than for Caucasan males (Blot and McLaughlin, 1999; Brown et al., 2001). In contrast, Ruder et al. (2001) observed similar SMRs for esophageal cancer across all race-sex groupings (supplementary table at http://www.cdc.gov/niosh/dc-mort.html), providing further support for occupational exposure as a risk factor. For these reasons, the observations in Ruder et al. (2001) and Blair et al. (2003) together suggest that the excess esophageal cancer risks seen in the dry cleaner studies cannot be entirely due to smoking, alcohol, or some other factor that may be associated with race.

Additionally, in the case-control study by Vaughan et al. (1997), the OR for cumulative (ppm-yr) tetrachloroethylene exposure and squamous cell esophageal cancer, which were adjusted for effects of both smoking and alcohol consumption, although imprecise (large

confidence intervals), were significantly elevated (Table 4B-6, Appendix 4B). In fact, the magnitude of risk for tetrachloroethylene exposure after adjustment in the statistical analysis for smoking and alcohol consumption was larger for the effect of tetrachloroethylene exposure when compared with the crude or unadjusted OR, suggesting that the association between occupational exposure and esophageal cancer may be underestimated in those studies that could not control for these factors.

Lacking information to classify job title for 25% and 19% of cases and controls, respectively, Lynge et al. (2006) provides little weight for informing an examination of the presence or absence of association between tetrachloroethylene and esophageal cancer.

4.8.1.2.3. ***Cervical cancer.*** There is some evidence for an excess in risk for cervical cancer mortality. The total number of observed and expected numbers of cancers in the tetrachloroethylene cohort mortality studies was 31 observed deaths versus 19 expected (RR = 1.6, 95% CI = 1.1–2.3; Table 4B-1b, Appendix 4B). An association with dry cleaning is supported by an exposure-response trend in Ruder et al. (2001), although RRs for cervical cancer mortality in Blair et al. (2003) did not appear to differ between subjects with medium/high exposure and those with little or no exposure to tetrachloroethylene. The number of female subjects in studies of workers exposed to tetrachloroethylene as a degreasing agent is few, with a consequence of limited statistical power (Table 4B-2, Appendix 4B). Data availability on socioeconomic and lifestyle factors in the dry cleaner studies precludes an evaluation of these factors.

4.8.1.2.4. ***Suggestive evidence of cancer at other sites.*** More limited are the findings of excess risks from cancers of the bladder, lung, pancreas, and small bowel.

4.8.1.2.4.1. ***Bladder cancer.*** The recent updates of two cohort mortality studies of dry cleaners with tetrachloroethylene as the primary exposure (Blair et al., 2003; Ruder et al., 2001) provide some evidence for an excess risk for bladder cancer mortality (Table 4B-1b, Appendix 4B). Ruder et al. (2001) observed statistically significant differences in bladder cancer risk among the entire cohort (SMR = 2.2, 95% CI = 1.1–4.1, 10 observed deaths). No deaths were observed among subjects employed after 1960, a date corresponding with greater usage of tetrachloroethylene. The magnitude of bladder cancer risk in Blair et al. (2003), on the other hand, was similar regardless of level of exposure (little or no exposure vs. medium/high exposure). The Nordic incidence studies are consistent with the mortality studies (Table 4B-1a, Appendix 4B). The study of Lynge et al. (2006), who examine bladder cancer incidence and tetrachloroethylene exposure using a nested case-control approach in a cohort of Nordic dry

cleaners and laundry workers, contributes little weight to causal evidence. The limitations of this study preclude drawing conclusions on whether this study is suggestive of, or lack of, an association between tetrachloroethylene and site-specific cancers such as bladder cancer. This study is uninformative due to a high percentage of study subjects whose job title could not be classified (for example, 16% of bladder cancer cases and controls) and potential bias resulting from the large number of next-of-kin interviews.

Of the studies of workers exposed to tetrachloroethylene as a degreasing agent, only the study by Boice et al. (1999) reports data for bladder cancer, based on two deaths (Table 4B-2, Appendix 4B). Several case-control studies of bladder cancer (Table 4B-7, Appendix 4B) have examined a job title as a dry cleaner or laundry worker and present risks adjusted for a number of factors, including cigarette smoking, a known risk factor for this cancer. These studies also provide weak support for an association: RRs ranged from 1.3 to 2.8, although increased risks were generally not statistically significant.

Two population case-control studies examined tetrachloroethylene exposures specifically, Pesch et al. (2000b) and Aschengrau et al. (1993). Pesch et al. (2000b) examined occupational exposure to tetrachloroethylene using a job exposure and job task exposure matrix. Urothelial cancer cases (a category that includes cancer of the urinary bladder, ureter, and renal pelvis) were histologically confirmed. A statistically significant excess risk (OR) was reported in both exposure assessment methods for males with substantial exposure to tetrachloroethylene in analyses that adjusted for age, study center, and smoking.

Aschengrau et al. (1993) reported an adjusted OR of 4.9 (95% CI = 0.7–25.1) with high exposure to tetrachloroethylene in drinking water (Table 4B-13, Appendix 4B). Strengths of this study are the use of exposure modeling to reconstruct tetrachloroethylene delivery to a home and adjustment of ORs for sex, age at diagnosis, vital status, educational level, and smoking.

4.8.1.2.4.2. ***Lung cancer.*** Lung cancer risk was elevated in the mortality studies by Blair et al. (2003) and Ruder et al. (2001), where the total number of observed deaths was 144, with 106.6 deaths expected (RR = 1.4, 95% CI = 1.1–1.6; Table 4B-1b, Appendix 4B); in the incidence study by Lynge and Thygesen (1990; RR = 1.2, 95% CI = 0.9–1.6) 60 cases among males and females, 49.4 expected cases (Table 4B-1a, Appendix 4B); and in the degreaser studies, a total of 51 cases observed with 43 expected; RR = 1.2, 95% CI = 0.9–1.6; Table 4B-2, Appendix 4B). The possible effect of smoking cannot be examined in the cohort studies.

Two case-control studies (Pohlabeln et al. 2000; Brownson et al., 1993) examined the association between occupational risk factors and lung cancer among nonsmokers (Table 4B-8, Appendix 4B). Both studies reported an association between lung cancer (among nonsmokers) and dry cleaning work. Additionally, the case-control study by Paulu et al. (1999) provides

further support for an association between tetrachloroethylene exposure and lung cancer (Table 4B-13, Appendix 4B). This study examined oral (drinking water) exposure to tetrachloroethylene, and the statistical analysis adjusted for both active and passive smoking.

4.8.2. Animal Studies

In addition to the toxic effects in animals already mentioned (in liver, kidney, nervous system, and developmental/reproductive system), effects have also been reported on cardiac function, immunosuppression, and cancer at other sites.

4.8.2.1. *Noncancer Effects*

4.8.2.1.1 ***Whole animal toxicity.*** Hayes et al. (1986) administered tetrachloroethylene to SD rats in drinking water at doses of 0, 14, 400, and 1,400 mg/kg-day for 90 days and observed the body weights of all animals weekly throughout the experiment; and the liver, kidney, and brain weights of all animals at necropsy. They also performed measurements of hematological and serum chemistry parameters on 10 animals per group at the end of the period. They found significant ($p < 0.05$) decrements in body weight gain in males at 1,400 mg/kg-day and in females at 400 and 1,400 mg/kg-day. No effects that could be attributed to administered dosing were observed in hematology, serum chemistry, urinalysis, mortality, or organ weights. The body-weight-gain decrements in this experiment signify a LOAEL of 400 mg/kg-day and a NOAEL of 14 mg/kg-day.

4.8.2.1.2. ***Cardiac toxicity.*** Kobayashi et al. (1982) treated animals using intravenous injections of tetrachloroethylene. In the animals examined (rabbits, cats, and dogs), tetrachloroethylene enhanced the vulnerability of the ventricles to epinepherine-induced arrhythmias. The threshold doses were 10, 24, and 13 mg/kg in rabbits, cats and dogs, respectively.

Cardiac effects of some tetrachloroethylene metabolites have been examined in animals. As mentioned in Section 4.6.4, Smith et al. (1989) and Johnson et al. (1998) observed cardiac anomalies in rat fetuses after exposure of pregnant rats to TCA. Epstein et al. (1992) saw cardiac defects in rat fetuses after exposure to DCA. This work indicated a developmental LOAEL of 1,900 mg/kg-day DCA. DCA has also been shown to concentrate in rat myocardial mitochondria (Kerbey et al., 1976). More research into cardiac toxicity resulting from exposures to tetrachloroethylene and its metabolites is needed to fully characterize possible adverse cardiac effects.

4.8.2.1.3. ***Immunotoxicity.*** The animal evidence for immunotoxicity following exposure to tetrachloroethylene is also very limited. These studies consist of a mixed solvent exposures and

some inhalation and oral studies in which experimental animals were dosed with tetrachloroethylene alone.

Immune systems parameters were altered in a mouse study (female B6C3F1) administered tetracholroethylene (maximum concentration 6.8 ppm) along with a mixture 24 contaminants frequently found in ground water near superfund sites. Exposure lasted 14 or 90 days and mice were sacrificed to assess immune system parameters. Evidence of immunosuppression was seen, with a dose related decrease in antibody response to sheep red blood cells and decreased host resistance to following challenge to Plasmodium yoelli. There were no changes in lymphocyte number, T-cell subpopulations, NK cell activity, or in challenge listeria monocytgens or PYB6 tumor cells. While these findings may be attributed to B-cell/humoral immunity these effects cannot be attributed to tetrachloroethylene alone (Germolec et al., 1989).

In another inhalation study, mice were given a single exposure of 170 mg/m^3 tetrachloroethylene (50 ppm) for 3 hrs and then challenged with Klebsiella pneumoniae; an increase in streptococcal pneumonia was observed (Aranyi et al., 1986). Interpretation of the significance of these findings is confounded with a high degree of mortality in the control group.

In a study by Hanioka et al. (1995), atrophy of the spleen and thymus was observed in rats receiving 2,000 mg/kg/d tetrachloroethylene via corn oil gavage for 5 days. No effect was seen in the 1,000 mg/kg/d group. In a 14-day corn oil gavage (1,000 mg/kg/d) study of tetrachloroethylene, no effects were observed on thymus and spleen weights of adult rats at dose that produced liver toxicity (Berman et al., 1995). Another study employed 3 daily ip doses of tetrachloroethylene to mice (Schlichting et al., 1992). No effects were observed on ex vivo natural killer cell activity or humoral responses of T-cells to exogenous mitogens.

A series of experiments in the lupus-prone MRL +/+ mice examined the effect of trichloroethylene on the expression of features of lupus (Griffin et al., 2000a; Gilbert et al., 2004). Activation and expansion of CD4+ T-cells has been demonstrated, through a mechanism that appears to be mediated through the CYP2E1 metabolism of trichloroethylene and inhibition of FasL expression on the surface of the CD4+ T-cells (Griffin et al., 2000b; Blossom et al., 2004; Blossom and Gilbert, 2006). Trichloroethylene exposure via drinking water was also shown to induce an autoimmune hepatitis, characterized by mononuclear infiltration around the portal vein, in the MRL +/+ mice (Griffin et al., 2000c). This evidence of immunological alterations following trichloroethylene exposure provides suggestive evidence for perchloroethylene, a halogenated solvent which shares some common metabolites with trichloroethylene. To date, similar studies have not been conducted with other solvents, so the extent to which these findings pertain to tetrachloroethylene is not known.

Additional data from inhalation, oral, and dermal exposures of different durations are needed to assess the potential immunotoxicity of tetrachloroethylene along multiple dimensions, including immunosuppression, autoimmunity, and allergic sensitization. This lack of data taken together with the concern that other structurally related solvents have been associated with immunotoxicity contributes to uncertainty in the database for tetrachloroethylene.

4.8.2.2. *Cancer Effects*

4.8.2.2.1. ***Mononuclear cell leukemia in rats.*** NTP (1986a) reported that the chronic inhalation administration of tetrachloroethylene to male and female F344/N rats at concentration levels of 0, 200, and 400 ppm caused positive trends in the incidence of MCL in both sexes. The incidence data are shown in Table 5-8 (Chapter 5). For males, there was a statistically significant trend ($p = 0.004$), and for females the trend was marginally significant ($p = 0.053$). Pairwise comparisons of tumor incidences in dosed and control groups of males (life table analysis) disclosed statistically significant increases in both the low- and high-dose groups. Analysis of the data for female rats revealed a significant increase in the low-dose group and a marginally significant increase in the high-dose group. Interpretation of these data is somewhat clouded by the fact that overall incidences of MCL in the concurrent chamber control groups were high relative to historical chamber control groups at the performing laboratory (males 28/50 [56%] vs. 117/250 [47%]; females 18/50 [36%] vs. 73/249 [29%]). The concurrent control group rates were also higher than the NTP program historical rate for untreated control groups (males 583/1,977 [29%]; females 375/2,021 [18%]).

Because of these factors, NTP conducted supplemental analyses of the progression of the disease, the effect of tetrachloroethylene on the time of onset of advanced MCL, and the contribution of MCL to early deaths in control and dosed animals. The results of these supplemental analyses showed the following:

- In both males and females, tetrachloroethylene produced a dose-related increase in the severity of MCL.

- Tetrachloroethylene exposure significantly shortened the time to onset of MCL in female rats.

- Although there was no remarkable effect of tetrachloroethylene exposure on survival of female rats, there was an increased incidence of advanced MCL in female rats that died before the scheduled termination of the study. Thus, a more appropriate statistical analysis was conducted in which only the incidences of advanced MCL in rats were considered. Significantly positive trends and significant increases in the incidences of advanced MCL were observed in both male and female rats in the high-dose groups.

In 1987, the U.S. EPA's Science Advisory Board took exception to the use of these special analyses because they did not represent generally accepted approaches to evaluating increased incidences of MCL. According to the NTP report, however, the interpretation of MCL incidences in the tetrachloroethylene study was based on standard methods of data evaluation (NTP, 1986a). The special analyses were conducted to support rather than to establish the interpretation.

The Japan bioassay (JISA, 1993) in F344/DuCrj rats exposed for 104 weeks at concentrations of 0, 50, 200, and 600 ppm also reported clearly significant trends in MCL in males. In females, MCL showed a marginally significant trend with dose. These data are also shown in Table 5-8 (Chapter 5). In this assay, the control incidences for both males and females were also higher than those for historical controls (for males, study control incidence was 11/50, [22%], whereas for the historical controls it was 147/1,149 [13%]). This higher incidence in concurrent controls versus historical controls also occurred in the NTP assay. The historical control incidence data for the Japan rat studies are shown in Table 5-10 (Chapter 5). The Japan bioassay report did not include an analysis of the tumor latency.

4.8.2.2.1.1. ***Discussion of issues associated with rat mononuclear cell leukemia (MCL).*** Under the conditions of the bioassays, a carcinogenic effect of tetrachloroethylene in male and female rats was evidenced by significant increases of MCL in both sexes. However, the reliability of MCL in the rat in predicting human carcinogenic risk associated with tetrachloroethylene exposure has been questioned for several reasons, such as high spontaneous background incidences, use of special supplemental analyses to aid in data interpretation, and the relevance of MCL in F344/N rats to human hazard. Some of the issues have been reviewed by Caldwell (1999) and others.

4.8.2.2.1.2. ***Background.*** Lymphohematopoietic neoplasms, leukemias, and lymphomas represent uncontrolled proliferation or clonal expansion of bone marrow or lymphoid cells that can no longer differentiate to mature blood cells (U.S. EPA, 1997a; Sawyers et al., 1991; Nowell, 1991). Like other cancers, they are thought to develop via a multi-step process in the transformation of a normal cell to a malignant cell. Although rodent models—especially mouse models—are considered to be highly relevant for understanding most aspects of hematopoiesis (Bagby, 1994), several differences exist between humans and rodents. Differences in cell composition, number, and proliferation rates and organization of the stem-cell compartment likely influence the hematotoxic and carcinogenic effects observed in human and rodent systems following exposure to carcinogenic chemicals (U.S. EPA, 1997b).

In adult humans, hematopoiesis occurs in the bone medullary spaces, with extramedullary hematopoiesis occurring in the spleen, liver, and lymph nodes only under stress conditions. In rodents, however, hematopoietic cells are commonly found in the spleen and, to some extent, the liver. The primary type of lymphohematopoietic cancer induced by chemicals in humans is myeloid leukemia; however, the induction of lymphoma has been associated with immunosuppressive agents. In contrast, lymphohematopoietic tumors in rats and mice originate primarily in lymphoid tissue.

4.8.2.2.1.3. ***Issues.*** The usefulness of increased incidences of MCL in predicting human carcinogenic risk associated with exposure to tetrachloroethylene has been questioned on several grounds, and these issues are discussed below.

MCL is a recognized as a common, spontaneously occurring neoplasm in F344 rats, and its rate of appearance in historical control groups is highly variable. High-incidence MCL occurs only in the F344 rat strain and not in mice. For this reason, Caldwell (1999), for example, has stated that marginal increases in incidences are of questionable biological significance. High and variable control incidence is also an issue with liver tumors in mice, and it has always been a source of uncertainty in risk assessments.

Although the occurrence in a single strain indicates a genetic susceptibility of these rats to spontaneous MCL, the occurrence in humans of a similar genetic susceptibility is by no means ruled out. Humans are more genetically heterogeneous than are inbred rat strains, and some individuals could potentially possess the same inherited susceptibility that is exhibited in F344 rats.

Another issue is the pathobiology of MCL. Some scientists believe it is too poorly understood to allow the tumors to be used to determine human health risk. However, MCL is a relatively well defined and well understood rodent neoplasm that is characterized by infiltration of pleomorphic blastlike mononuclear cells in numerous organs. The disease per se, which is splenic in origin but later infiltrates the liver, lung, bone marrow, lymph nodes, and other organs, is readily and unequivocally diagnosed by standard histopathological techniques. MCL has also been described as large, granular, lymphocytic leukemia and is known to be a rapidly progressing and fatal neoplasm whose incidence is age related. The tumor is transplantable; its etiological factor is unknown.

The similarities and differences in the tissue of origin, precursor cell line, and pathologic characteristics of rat MCL and human lymphoid cancers have been reviewed by Caldwell (1999) Ishmael and Dugard (2006) and EPA (U.S. EPA, 1997b). Both diseases result from abnormal development and maturation of lymphocytes. It is known that the tissue of origin in rats is the

spleen and that lymphomas and leukemias in humans originate in the bone marrow. The precursor cell line is not known for either rat MCL or the human lymphoid cancers.

Large granular lymphocyte cells exist in humans that are morphologically, biochemically, and functionally similar to the cells involved in MCL in the F344 rat (Stromberg, 1985). The pathological characteristics of rat MCL are similar in some respects to one of the human T-cell leukemias (Caldwell, 1999), and some investigators believe MCL serves as a model for T-cell leukemia (Stromberg, 1985). However, discounting a rodent neoplasm simply because it has no exact human counterpart is not a scientifically defensible position. Strict site concordance is not a requirement for relevancy in extrapolation of hazard potential. For example, many aromatic amines are probable bladder carcinogens in humans but are likely to produce Zymbal gland tumors in rats, for which there is no analogous organ in humans.

The specific mechanism of leukemogenesis in rats is not understood, but neither is it well understood in humans. A possible link to MOA for tetrachloroethylene-induced MCL in rats comes from early reports of toxicity of cysteine S-conjugates, where S-(1,2,-dichlorovinyl)-L-cysteine, the trichloroethylene metabolite, was implicated in induction of aplastic anemia and marked biochemical alteration of DNA in bone marrow, lymph nodes, and thymus in calves (Bhattacharya and Schultze, 1971, 1972).

As discussed elsewhere, the GSH conjugate of tetrachloroethylene is hydrolyzed in the kidney to the comparable cysteine S-conjugate, a compound that can be cleaved to form a mutagen. Humans as well as rodents activate the conjugate via FMO3, CYP3A and/or the beta lyase pathway. Thus, the possibility exists that the tetrachloroethylene S-conjugate S-(1,2,2-trichlorovinyl)-L-cysteine may be involved in inducing leukemia in rats and may have the potential to produce blood dyscrasias in humans as well. However, a recent report of a study in which TCVC was given to two calves did not find that it produced bone marrow injury in these animals at dose levels comparable to those of DCVC that caused bone marrow toxicity in calves in the same study (Lock et al., 1996).

4.8.2.2.1.4. ***Summary and conclusions regarding the leukemia finding in rats.*** Leukemia incidences were significantly increased in both male and female rats, in spite of high spontaneous background incidences. Although caution is recommended with regard to interpreting results for species that have high spontaneous incidences for any specific tumor, it is also important to remember that high spontaneous incidences of lymphohematopoietic cancer are not unique to rodent strains—e.g., high incidences of leukemia occur in certain genetically susceptible humans as well (U.S. EPA, 1997a). In addition to causing increased tumor incidences, tetrachloroethylene caused a dose-related increase in severity of MCL in both sexes and shortened the time to tumor in female rats in one of the chronic bioassays.

The principal type of chemically induced lymphohematopoietic cancer in humans is myeloid leukemia, with the exception being the lymphohematopoietic cancers induced by immunosuppressive agents, which are usually associated with development of lymphomas. There is some epidemiologic evidence that occupational exposure to tetrachloroethylene is associated with NHL; thus, lymphohematopoietic cancers are observed in both rats and humans. Although the specific etiology of NHL is unknown at the present time, it is thought likely to be related to imbalances or disturbances in the immune system. Two potent immunosuppressive chemotherapeutic agents, cyclosporin and azathioprine, are associated with NHL.

If a chemical produces a significant increase in MCL in the F344 rat, the finding cannot be ignored. The observation of a significant increase of MCL in rats signals that the chemical may possibly cause similar or different types of tumors in humans.

4.8.2.2.2. ***Tumors at other sites in animal bioassays.*** In the NTP inhalation study, an elevated incidence of rare brain gliomas in rats was observed. In males in the control and the mid- and high-tetrachloroethylene concentration groups, the incidences were 1/50, 0/50, 4/50, respectively, and there was a significantly positive dose-related trend by the life table test but not by the incidental tumor trend test. In females the incidence was 1/50, 0/50, 2/50. These are rare tumors in NTP rat bioassays; the historical control incidence for males and females combined in the laboratory was 2/247 (0.8%), and in the overall NTP program it was 6/1,971 (0.3%). Because these tumors had not been observed in the previous NTP studies of trichloroethylene (NTP, 1990b) or pentachloroethane (NTP, 1983), and because they appeared in the untreated groups, NTP concluded that they were not related to tetrachloroethylene exposure.

On the other hand, the tumors in the high-dose males occurred slightly earlier (88, 96, 102, and 103 weeks) than in the control group (99 weeks); in the high-dose females they occurred more definitively earlier (75 and 78 weeks in the high-dose group vs. 104 weeks in the control group). In addition, a Fisher's exact test of the significance of combined male and female incidences in the tetrachloroethylene-treated animals shows significance with respect to both lab and NTP program historical data, whereas the control incidence is not significant with respect to either of the historical data sets. Therefore, although the data showing that tetrachloroethylene is causing brain gliomas in rats is not strong, it is suggestive.

The incidence of interstitial testicular tumors in male F344 rats treated with tetrachloroethylene was significantly higher than in controls in the study. However, it is common in control rats (the NTP program historical control incidence was 1,729/1,949 [89%]), and the incidence in treated animals was not higher than in historical laboratory or historical program controls. Also, the combined incidence of hyperplasia and tumors was not significant

with respect to that of the study controls. For these reasons, the NTP concluded that the marginally higher incidence was not related to tetrachloroethylene exposure.

Hemangioendotheliomas in the liver and spleen in male mice were observed in the Japan bioassay. These were mentioned in Section 4.4.2.2 in connection with liver cancer, and the data are given in Section 5.2.2. These tumors were not observed in the NTP studies.

4.8.3. Summary of Immunotoxicologic Effects in Humans and Animals and Potential Mode of Action

The epidemiologic evidence pertaining to tetrachloroethylene exposure in relation to immune-related conditions, is limited (Table 4-11). Estimated associations based on population-based case-control studies of various systemic autoimmune diseases have low statistical power (and thus are highly imprecise) because of the relatively low prevalence of occupational exposure to this chemical in the general population. Exposure misclassification is likely in these studies, and in general would be expected to result in an attenuation of the observed effect. To date, no relevant occupational cohorts have examined the risk of developing these diseases. The few studies that have been conducted related to allergy and hypersensitivity have used direct measures of tetrachloroethylene and other compounds in ambient or exhaled air samples, but these studies do not provide much evidence of an adverse effect relating to tetrachloroethylene exposures. However, the only study of asthma severity was quite small (n = 21), and our understanding of the impact of changes in specific T-cell subsets (i.e., interferon-γ) is currently limited.

The immune system is clearly crucial to the prevention of disease caused by infectious agents. Alterations in immune function may also contribute to the development of non-infectious diseases including cancer, autoimmune diseases, and hypersensitivity disorders. Many immunosuppressive agents are human carcinogens (Tomatis et al., 1989), and as described in the previous section, inhibition of the natural immune surveillance could play a role in the hepatocarcinogenic properties of tetrachloroethylene. The numerous immune-mediated activities of relevance to the pathogenesis of a variety of disease include the binding and processing of antigens by B-cells and T-cells, alteration and loss of tolerance to self-antigens, defects in apoptosis which may effect the clearance of cells, and the secretion of pro- and anti-inflammatory cytokines (Seliger, 2005; Ayensu et al., 2004).

Binding of reactive compounds to cellular macromolecules has been proposed as an important step in the pathogenesis of several diseases, both for cancer (Hinson and Roberts, 1992) and for chemically induced autoimmune disease (Uetrecht et al., 1988). The modification of proteins may lead to more immunoreactive products, and these may lead to the development of autoantibodies and the cellular damage seen in alcoholic liver disease and in autoimmune

diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis; Kurien et al., 2006). Reactive metabolites of tetrachloroethylene have been shown to bind irreversibly to cellular macromolecules in vitro (e.g., Costa and Ivanetich, 1980) and in vivo (Pegg et al., 1979; Schumann et al., 1980). Binding occurs proportionally to the amount metabolized, and metabolism is proportional to toxicity (e.g., Buben and O'Flaherty, 1985). Several published studies have demonstrated formation of trichloroacylated protein adducts, for example, in liver and kidney of rats (Birner et al., 1994) and in plasma of rats and humans (Pahler et al., 1999) following exposures to tetrachloroethylene. Another example is the detection of trichloroacetylated protein adducts formed in the liver of MRL-lpr/lpr and MRL +/+ mice treated with tetrachloroethylene (Green et al., 2001). These strains are "lupus-prone" mice, because of their genetic susceptibility to the development of lupus-like disease. Further studies designed to identify the adducted proteins may help to elucidate an MOA for tetrachloroethylene-induced autoimmune response, which, in turn, may be related to cancer-causing activity (not clear how this autoimmune response is related to cancer).

Apoptosis, a form of natural cell death differing from necrosis, is essential for proper functioning of the immune system and clearance of tumor cells. Apoptosis also plays an important role in the pathogenesis of autoimmune diseases. Genetic or environmental exposures leading to increased apoptosis or to decreased clearance of apoptotic debris may stimulate the production of autoantibodies directed against intracellular antigens (Gaipl et al., 2006). The production of free radicals, increased lipid peroxidation, and increased apoptosis was demonstrated in a recent study using human lung adenocarcinoma cells treated with tetrachloroethylene (Chen et al., 2002b). Alternatively, the inhibition of apoptosis of CD4+ T-cells may also effect the development of autoimmune disorders, as demonstrated by the recent studies of trichloroethylene metabolites (Blossom and Gilbert, 2006). Thus, with respect to autoimmune diseases, as well as neurodegenerative and other diseases, the strict regulation of apoptosis signalling is crucial (Ethell and Buhler, 2003; Schattenberg et al., 2006).

4.9. SUSCEPTIBLE POPULATIONS

Variation in response among segments of the population may be due to age, genetics, and ethnicity, as well as to differences in lifestyle, nutrition, and disease status. These could be potential risk factors that play an important role in determining an individual's susceptibility and sensitivity to chemical exposures. Studies on tetrachloroethylene toxicity and MOA in relation to some of these risk factors are discussed below.

4.9.1. Life Stages

Individuals of different life stages are physiologically, anatomically, and biochemically different. Early and later life stages differ greatly from mid-life stages in body composition, organ function, and many other physiological parameters that can influence the absorption, distribution, metabolism, and elimination of chemicals and their metabolites from the body (ILSI, 1992). The limited data on tetrachloroethylene exposure suggest that these subpopulations—particularly individuals in early life stages—may have greater susceptibility than does the general population. This section presents and evaluates the pertinent published literature available to assess how individuals of differing life stages may respond differently to tetrachloroethylene.

4.9.1.1. *Life Stage-Specific Exposures*

Section 2.2 describes the various exposure routes of concern for tetrachloroethylene. For all postnatal life stages, the primary exposure routes of concern include inhalation (see Section 2.2.1) and contaminated water (see Section 2.2.2). In addition, certain exposure pathways to tetrachloroethylene are unique to early life stages, such as through placental transfer or via breast milk ingestion, or may be increased during early or later life stages. In utero, there is biological plausibility of transfer of tetrachloroethylene across the human placental barrier as seen in rodents (Ghantous et al., 1986; Szakmáry et al., 1997). Fetal blood concentrations have been modeled for human exposure (Gentry et al., 2003).

For infants, a unique exposure route of concern is ingestion of breast milk (see Section 2.2.4). The breast milk of one woman was found to contain 10 mg/L tetrachloroethylene 1 hr following a visit to her spouse working at a dry cleaning establishment, dropping to 3 mg/L after 24 hrs (Bagnell and Ellenberger, 1977). Tetrachloroethylene has also been measured in the breast milk of a woman living in an apartment located in a building housing a dry cleaning facility (Schreiber et al., 2002; NYS DOH, 2005b). PBPK models have been used to estimate the dose a nursing infant might receive from an exposed mother's breast milk (Gentry et al., 2003; Schreiber, 1993). Using different exposure scenarios, Schrieber predicted that breast milk concentrations could range from 1.5 μg/L for a typical residential scenario, 16–3,000 μg/L for a residential scenario near a dry cleaner, to 857–8,440 μg/L for an occupational scenario. Assuming that a 7.2 kg infant ingests 700 mL of breast milk per day, Schreiber estimated dose to the infant could range from 0.0001 to 0.82 mg/kg/day (Schreiber, 1993). Therefore, the dose an infant could receive through breast milk may exceed the previous EPA RfD level (0.01 mg/kg-day). Byczkowski and Fisher (1995) refined the approach used by Schrieber (1993) and found that with the same exposure conditions, the results predicted lower doses to the infant (0.0009–0.202 mg/kg/day).

For infants on formula, ingestion of contaminated water may be of concern. Taking into account tetrachloroethylene volatilization in boiling water, Letkiewicz et al. (1982) estimated that 22% of formula-fed infants received fluids contaminated with tetrachloroethylene levels found in the water supply. Data showed that about 11% (0.5 × 22%) of formula-fed infants could receive an increased exposure as compared with adults on a mg/kg basis through drinking contaminated water.

Dairy products have been found to have elevated concentrations of tetrachloroethylene (see Section 2.2.3), and children ingest larger quantities of dairy products compared to adults (NRC, 1993). Therefore, there may be concern for ingestion of contaminated dairy products in early life stages, although this exposure route for tetrachloroethylene has not been well characterized for any life stage.

Inhalation exposures may be increased for both early and later life stages compared to adults, since children and the elderly have increased ventilation rates per kg body weight compared to adults (NRC, 1993; U.S. EPA, 2006) and since they spend the majority of their time indoors (NRC, 1993; U.S. EPA, 2002), where increased concentrations of tetrachloroethylene have been found (U.S. EPA, 2001b). Section 2.2.1 describes increased indoor air concentrations measured inside apartments containing dry cleaned clothing (Thomas et al., 1991), in apartments above or adjacent to dry cleaners (Altmann et al., 1995; Chien, 1997; Garetano and Gochfeld, 2000; McDermott et al., 2005; Schreiber et al., 1993, 2002), and in daycare centers adjacent to dry cleaners (NYS DOH, 2005b, c). In addition, inhalation may also occur during showering or bathing as dissolved tetrachloroethylene in the warm tap water is volatilized (Rao and Brown, 1993).

Dermal exposures may be increased for both early and later life stages compared to adults, since infants have increased skin area per kg body weight (NRC, 1993) and the elderly experience changes in permeability (U.S. EPA, 2006). Dermal exposure may occur in an occupational setting from direct handling of tetrachloroethylene or in a residential setting from showering, bathing, or swimming in contaminated water (Rao and Brown, 1993; U.S. EPA 2001). (see Section 2.2.2) While dermal exposure is generally not considered a major route of exposure, this route of exposure is not well characterized for early life stages (prenatal or postnatal), or later life stages.

4.9.1.2. *Early Life Stage Effects*

Although limited data exist on tetrachloroethylene toxicity as it relates to early life stages, there is enough information to discuss the qualitative differences. In addition to the evidence described below, Section 4.7 contains information on both human and animal evidence for reproductive and developmental outcomes such as spontaneous abortion/fetal loss, low birth

weight, IUGR, SGA, congenital abnormalities, sperm quality, developmental delays, and behavioral changes. Together, Sections 4.4 on liver toxicity, 4.5 on kidney toxicity, 4.6 on neurotoxicity, and 4.8 on toxic effects in other organ systems characterize a wide array of postnatal developmental effects.

4.9.1.2.1. ***Differential effects in early life stages.*** Preconception exposure has been associated with altered semen quality in occupationally exposed humans (Eskenazi et al., 1991b; Rachootin and Olsen, 1983; Sallmén et al., 1995), as well as in mice, but not in rats (Beliles et al., 1980). Additionally, reduced testes weight was seen in rats after inhalation exposure (Tinston, 1994).

A number of human studies have shown spontaneous abortion among women employed as laundry workers (Hemminki et al., 1980) or dry cleaners (Ahlborg, 1990; Bosco et al., 1987; Doyle et al., 1997; Olsen et al., 1990; Kyyrönen et al., 1989), married to men employed as dry cleaners (Taskinen et al., 1989; Eskenazi et al., 1991a), exposed to other occupational solvents (Windham et al., 1991; Lindbohm et al., 1990), or living in residences receiving contaminated water (Lagakos et al., 1986; Bove et al., 1995; ATSDR, 1998). However, another study population of women working as laundry or dry cleaning workers did not experience spontaneous abortion (McDonald et al., 1986, 1987). Reduced fertility has been seen in women and men exposed occupationally (Eskenazi et al, 1991a, b; Sallmén et al., 1995; Rachootin and Olsen, 1983).

In the literature concerning animal studies, there is evidence of increased pre- and post-implantation loss (Szakmáry et al., 1997) and increased resorption of rodent pups after maternal inhalation (Schwetz et al., 1975; Szakmáry et al., 1997), reduction in litter size after maternal gavage (Narotsky and Kavlock, 1995), and litters with dead pups (Tinston, 1994). However, fetal loss was not seen in other animal studies (Carney et al., 2006; Hardin et al., 1981). In vitro studies show decreased fertilized oocytes (Berger and Horner, 2003), and increased mortality, malformations, and delayed growth and differentiation of embryos (Saillenfait et al., 1995) when exposed to tetrachloroethylene.

After residential exposure to contaminated water, birth outcomes related to in utero exposure in humans include perinatal death, birth defects (eye and ear anomalies, and CNS/oral cleft anomalies; Lagakos et al., 1986), and IUGR (Windham et al., 1991) or SGA (Sonnenfeld et al., 2001). Also, childhood leukemia has been associated with in utero exposure to tetrachloroethylene due to maternal ingestion of contaminated water (MA DPH, 1997; see Section 4.9.1.2.4). The study population reported in Sonnenfeld et al., (2001) is currently being further examined to determine any association between maternal ingestion of contaminated water and the incidences of birth defects (e.g., neural tube defects and oral clefts; ATSDR, 2003). However, other human studies have not shown effects after occupational exposure for other birth

outcomes, such as stillbirths, congenital anomalies, or decreased birth weight (Bove et al., 1992; Olsen et al., 1990, Kyyrönen et al., 1989, Taskinen et al., 1989, Windham et al., 1991). In addition, preconception or prenatal exposure may lead to other latent outcomes such as an increased risk for schizophrenia as seen in a large prospective study of parental occupational exposure to tetrachloroethylene (Perrin et al., 2007).

Birth outcomes in animals exposed to tetrachloroethylene in utero include skeletal retardation and malformations, decreased body weight and weight gain, altered brain fatty acid composition, and developmental delay. Skeletal retardation and malformations were increased in rodent pups after maternal inhalation exposure 7 days per week (Carney et al., 2006; Szakmáry et al., 1997), but no birth defects were seen in other animal studies using a similar dose but for 5 days per week (Hardin et al., 1981). Exposure resulted in decreased fetal or pup body weights (Carney et al., 2006; Szakmáry et al., 1997; Tinston, 1994), along with reduction in weight gain (Nelson et al., 1980). Also, Kyrklund and Haglid (1991) noticed slightly altered brain fatty acid composition after maternal exposure during pregnancy was also noted (Kyrklund and Haglid, 1991). Developmental delay was seen in rat offspring after maternal exposure during pregnancy (Nelson et al., 1980). In addition, cardiac anomalies have been seen in rats exposed to the metabolites TCA (Smith et al., 1989; Johnson et al., 1998) and DCA (Epstein et al., 1992; see Sections 4.6.2, 4.7.2, and 4.8.2).

Neurotoxicological effects in children have been reported after low exposure levels to tetrachloroethylene (see Section 4.6 and Table 4-4). While other neurotoxic effects are seen in adults (see Table 4-5), decreased VCS has been the main observation in children, including in those who resided in an apartment building with a dry cleaning establishment (Schreiber et al., 2002; NYS DOH, 2005a). Children who attended a day care center adjacent to a dry cleaner were too young to take a visual exam given to the adult workers that demonstrated decreased VCS. Other neuropsychological tests conducted on the children attending this day care center 5 weeks after exposure ceased did not consistently find any effect on cognition or behavior (NYS DOH, 2005b). A follow-up evaluation of a different set of children attending the same day care center 4 to 5 years after exposure showed no residual changes in VCS or color vision, although these children were not tested immediately after exposure (NYS DOH, 2005c). A case study reported reduced VCS in a 2½ year old boy after prenatal exposure to tetrachloroethylene (Till et al., 2003). Sections 4.6.2 and 4.7.2 discuss studies of postnatal neurological effects in animals after prenatal exposure. Altered brain biochemistry was seen in the offspring of exposed rodents (Kyrklund & Haglid, 1991; Nelson et al., 1980), and the offspring showed signs of developmental delay (Nelson et al, 1980), altered motor activity (Szakmáry et al., 1997; Tinston, 1994), decreased muscular strength (Szakmáry et al., 1997), and short-term reduced response to sound in pups (Tinston, 1994). In addition, young animals have been directly exposed

postnatally to tetrachloroethylene. One gavage study on young 45–50 gram rats showed behavioral and locomotor effects (Chen et al., 2002a), and another gavage study on 10-day old mice showed increased locomotor activity and decreased rearing behavior (Fredriksson et al., 1993). Following i.p. dosing, 8-week-old male mice showed effects on the righting reflex and balancing (Umezu et al., 1997), and 6-week-old rats showed effects on locomotor activity (Motohashi et al., 1993). Both human and animal evidence supports an association between neurodevelopmental effects and tetrachloroethylene exposure.

Section 4.8.1.1.1 and Table 4-10 describe studies relating tetrachloroethylene to immune response in children. Lehmann et al. (2002) examined cord blood samples for T-cell subpopulations and associated them with indoor exposure to VOCs measured 4 weeks after birth (likely to reflect late-prenatal exposures); however, another study examining indoor exposure to VOCs and allergic sensitization and cytokine secretion in 3-year-old children at high risk for development of allergic disease (low birth weight, high cord blood IgE, family history of atopy) found no association between tetrachloroethylene exposure and any of the allergens tested in this study (Lehmann et al. 2001). In a study of inhalation exposure, Delfino et al. (2003a, b) measured the concentration of ambient air pollutants, including tetrachloroethylene, and correlated it with subsequent symptoms of asthma in children in the Los Angeles area. The results suggest an increased risk with exposure to tetrachloroethylene (Delfino et al., 2003a). However, another analysis of the data examined the amount of tetrachloroethylene and other volatile organic compounds in exhaled breath of asthmatic children (Delfino et al., 2003b). Although there was a significant correlation between ambient and exhaled concentrations, the investigators did not find any association with exhalation concentrations and asthma symptoms or ambient air concentrations and asthma symptoms, although the OR for exhaled breath was larger than for ambient air exposure (OR = 1.94, 95% CI = 0.8–4.7; Delfino et al., 2003b). An 18-year-old without personal or family history of bronchial asthma developed respiratory symptoms (cough, dyspnea, altered forced expiratory volume) after maintaining dry cleaning machines (Boulet, 1988). The limited, available data from these studies provide weak evidence of an effect of tetrachloroethylene exposure during childhood on allergic sensitization or exacerbation of asthma symptomology. However, the observation of the association between increased tetrachloroethylene exposure and reduced interferon-γ in cord blood samples may reflect a sensitive period of development, and points to our current lack of understanding of the potential immunotoxic effects of prenatal exposures.

Other postnatal health effects after tetrachloroethylene exposure have been seen in children. In one case study with inexact exposure information, tetrachloroethylene vapors off-gassing from dry-cleaned fabrics were implicated in causing the death of a 2-year-old boy after sleeping in a room with curtains that had been incorrectly dry cleaned (Garnier et al., 1996).

Bagnell and Ellenberger (1977) reported that a child suffered from obstructive jaundice and hepatomegaly after consuming contaminated breast milk, with conditions improving when breastfeeding was discontinued. In the one case of a child's direct ingestion of tetrachloroethylene, a 6-year-old boy who swallowed 12–16 g tetrachloroethylene lost consciousness and lapsed into a coma (Koppel et al., 1985). This 6-year-old also experienced drowsiness, vertigo, agitation, and hallucinations, but he later recovered. Follow-up testing on the boy was not reported; therefore, any potential long-term effects of the exposure are unknown (see Section 2.2.5).

4.9.1.2.2. ***Toxicokinetics and tetrachloroethylene in early life stages.*** Chapter 3 describes the toxicokinetics of tetrachloroethylene. Early life stage-specific information regarding absorption, distribution, metabolism, and excretion needs to be considered for a child-specific and chemical-specific PBPK model. To adequately address the risk to infants and children, age-specific parameters for these values should be used in PBPK models that can approximate the internal dose a infant or child receives based on a specific exposure level (Byczkowski and Fisher, 1994; Clewell et al., 2004; Gentry et al., 2003; Rao and Brown, 1993; see Section 3.5).

As discussed in Section 3.1, exposure may occur via inhalation, ingestion, and skin absorption. In addition, prenatal exposure may result in absorption via the transplacental route. Exposure via inhalation is proportional to the ventilation rate, duration of exposure, and concentration of expired air, and children have increased ventilation rates per kg body weight compared to adults, with an increased alveolar surface area per kg body weight for the first two years (NRC, 1993). For lipophilic compounds such as tetrachloroethylene, percent adipose tissue, which varies with age (NRC, 1993), will affect absorption and retention of the absorbed dose. It is not clear to what extent dermal absorption may be different for pregnant women and children compared to adults, given their increased surface areas and thinner outer skin layers.

The distribution of tetrachloroethylene to specific organs will depend on organ blood flow and the lipid and water content of the organ (NRC, 1993), which may vary between life stages. Rodent studies demonstrate that tetrachloroethylene crosses the placental barrier when pregnant dams are exposed (Ghantous et al., 1986; Szakmáry et al., 1997), and in humans it has been shown that during lactation, tetrachloroethylene distributes to breast milk (NYS DOH, 2005b; Schreiber, 1993; Sheldon et al., 1985). However, a noticeable difference exists between the milk/blood partition coefficients for rats (12) and for humans (2.8; Byczkowski and Fisher, 1994), reflecting the higher fat content of rat milk.

Tetrachloroethylene can also cross the blood-brain barrier during both prenatal and postnatal development; this may occur to a greater extent in younger children. Based on the modeled dose of tetrachloroethylene to the brain after a showering/bathing scenario, a study by

Rao and Brown (1993) showed that for a given set of exposures, the younger a person is, the greater the estimated concentration of tetrachloroethylene in the brain. Modeling showed that after a 30-minute bathing scenario, a 3-year-old child accumulated higher brain tissue concentrations of tetrachloroethylene as compared with a 10-year-old and an adult. An autopsy conducted on the previously mentioned 2-year-old boy found dead after exposure to dry-cleaned curtains revealed the highest levels of tetrachloroethylene in the brain, 77 mg/kg. Levels in his blood, heart, and lungs were 66 mg/L, 31 mg/kg, and 46 mg/kg, respectively (Gaillard et al., 1995; Garnier et al., 1996).

Animal studies provide clear evidence that tetrachloroethylene distributes widely to all tissues of the body (see Section 3.2) but it is not clear whether distribution may vary differentially with lifestage.

Section 3.3.3 describes the production of CYP enzymes involved in the metabolism of tetrachloroethylene. Expression of these enzymes changes during various stages of fetal development (Hakkola et al., 1996a, 1996b, 1998) and during postnatal development (Tateishi et al., 1997). One study modeled the role of the age-dependent development of CYP2E1 in oxidative metabolism (TCA) in the mother and lactating infant (Vieira et al., 1996). A number of other human studies suggest that CYP2B6 may also play a role in the metabolism of tetrachloroethylene (White et al., 2001), although this enzyme was not detected in placental or fetal liver samples (Hakkola et al., 1996a, b), and differences between a group of 10 perinatal and infant patients showed significantly lower CYP2B6 protein expression in placental hepatic microsomes as compared with an adult group (Tateishi et al., 1997).

The major processes of excretion of tetrachloroethylene and its metabolites are discussed in Section 3.3 and 3.4. Tetrachloroethylene or its metabolites have been measured in blood (NYS DOH, 2005a; Popp et al., 1992), exhaled breath (Delfino et al, 2003b; Schreiber et al., 2002; NYS DOH, 2005a), and urine of children (NYS DOH, 2005b; Schreiber et al., 2002; Popp et al., 1992).

4.9.1.2.3. ***Toxicodynamics and tetrachloroethylene in early life stages.*** Toxicodynamic responses to chemical exposures can change throughout different life stages. TCA, a metabolite of tetrachloroethylene, is hypothesized to be the causative agent for developmental toxicity expressed as morphological changes, lethality, and/or growth. TCA could accumulate to a greater extent in the embryo/fetal compartment than in the mother, based on the pKa of the acid and the pH gradient between the maternal plasma and the embryo compartments (O'Flaherty et al., 1992). TCA could induce developmental toxicity by changing the intracellular pH or through peroxisome proliferation. Ghantous et al. (1986) detected TCA in the amniotic fluid of pregnant mice exposed to tetrachloroethylene via inhalation (see Section 4.7.4).

4.9.1.2.4. ***Susceptibility to cancer in early life stages.*** The epidemiologic and experimental animal evidence is limited regarding susceptibility to cancer from exposure to tetrachloroethylene during early life stages. The human epidemiological evidence is summarized above for cancer in the liver (see Section 4.4.1.2), kidney (see Section 4.5.1.2), and other organ systems (see Section 4.8.1.2). The animal research is summarized above for cancer in the liver (see Section 4.4.2.2), kidney (Section 4.5.2.2), and other organ systems (see Section 4.8.2).

Few studies have examined cancer in children after exposure to tetrachloroethylene; however, those few have found evidence for concern for leukemia (see Section 4.8.1.2.1). One case-control study of children residing in Woburn, Massachusetts diagnosed with leukemia examined exposure to drinking water contaminated with multiple solvents, including tetrachloroethylene (Costas et al., 2002; MA DPH, 1997). This study reported a large but imprecise association and a dose-response relationship between maternal exposure during pregnancy and childhood leukemia in the offspring when compared with exposure prior to pregnancy or postnatal exposure to the infant via lactation (Costas et al., 2002; MA DPH, 1997), and altered immune response was found in family members of the cases (Byers et al., 1988; see Section 4.8.1.1.1). However, it is difficult to uniquely identify tetrachloroethylene as the causative agent given the higher concentrations of trichloroethylene reported in these studies. Similarly, in another case-control study of childhood leukemia, paternal exposure to chlorinated solvents have been associated with increased risk (Lowengart et al., 1987), although this and other case-control studies do not show an increased risk from paternal (Lowengart et al., 1987; Shu et al., 1999) or maternal (Infante-Rivardet al., 2005; Shu et al., 1999) occupational exposure to tetrachloroethylene, possibly due to the relatively small sample size. Another study population is currently being further examined to determine any association between maternal ingestion of contaminated water and the incidence of childhood cancers (ATSDR, 2003). One in vitro study of human mononuclear cord blood cells exposed to tetrachloroethylene found that pathways involved in cancer induction were affected through altered gene expression of inflammatory responses, tumor and metastatis progression, and the apoptotic process (Diodovich et al., 2005). Leukemia has also presented in adult humans after tetrachloroethylene exposure (see Section 4.8.1.2.1, Appendix 4B, and Tables 4B-5 and 4B-13), and MCL has been seen in exposed adult rats in spite of high spontaneous background incidences (see Section 4.8.2.4). While interspecies extrapolation of rat MCL to humans has been questioned (see Section 4.8.2.4.1), the findings in humans suggest relevance for the leukemia findings in animals. However, no data are available on leukemia risk in young animals exposed to tetrachloroethylene.

4.9.1.3. *Later Life Stages*

Few studies examine the effects of tetrachloroethylene exposure in elderly adults. One study found elevated blood tetrachloroethylene levels (310–1770 μg/L) and urine trichloroacetic acid levels (22–1650 μg/L) in an elderly couple living above a dry cleaning facility (Popp et al., 1992). Another residential study examined two individuals over the age of 60 years and found that the mean scores of VCS were lower than the 12th percentile of all control subjects (Schreiber et al., 2002). These studies suggest that older adults may experience increased exposure to tetrachloroethylene and resulting increased VCS deficits than younger adults. However, there is no further evidence for elderly individuals exposed to tetrachloroethylene beyond these two studies.

4.9.2. Other Susceptibility Factors

Aside from age, many other factors may affect susceptibility to tetrachloroethylene toxicity. A partial list of these factors includes gender, genetic polymorphisms, pre-existing disease status, nutritional status, diet, and previous or concurrent exposures to other chemicals. The toxicity that results due to changes in multiple factors may be quite variable, depending on the exposed population and the type of exposure. Qualitatively, the presence of multiple susceptibility factors will increase the variability that is seen in a population response to tetrachloroethylene toxicity.

4.9.2.1. *Health and Nutritional Status*

It is known that kidney diseases can affect the clearance of chemicals from the body, and therefore poor kidney health may lead to increased half-lives for tetrachloroethylene and its metabolites. Similarly, liver disease may change the metabolic profiles in the liver, thus potentially altering tetrachloroethylene metabolism.

Co-exposure to α-tocopherol (vitamin E) along with tetrachloroethylene resulted in decreased rat (Costa et al., 2004) and mouse (Ebrahim et al., 1996, 2001) liver cell toxicity. A similar protective effect was also seen with co-exposure to 2-deoxy-D-glucose in mice (Ebrahim et al., 1996, 2001) and taurine in mice (Ebrahim et al., 2001). However, no associations were found for blood levels of vitamin E and beta-carotene in rats (Toraason et al., 2003; see Sections 4.3 and 4.4.4.4.3).

4.9.2.2. *Gender*

In humans, it has not been determined whether there is a gender difference in response to exposure to tetrachloroethylene. However, because gender also affects metabolic capabilities, which vary throughout development, it is important to consider sex-specific changes.

In the case of tetrachloroethylene, there is some indication that tetrachloroethylene metabolism is different between males and females. One PBPK model found gender-specific differences that were small (although significant) in tetrachloroethylene blood concentrations but considerable (2-fold at age 40) with regard to TCA blood concentration levels (Clewell et al., 2004; see Section 3.5.2 and Figure 3-3.). Opdam and Smolders (1986) exposed six human subjects to concentrations ranging from 0.5–9 ppm and found alveolar concentrations in male subjects to be only slightly less than those in females (see Figures 3-6a, b). It is not known whether gender variation of beta lyase activity (see Section 3.3.3.2.3), the most important activator of toxic products in the conjugation pathway, exists in humans as it does in rats, with metabolism in males being faster than in females (Volkel et al., 1998), although there seems to be little gender difference in the concentrations of metabolites in blood, regardless of age (Sarangapani et al., 2003).

Ferroni et al. (1992) evaluated neurological effects of tetrachloroethylene exposure among female dry cleaners and concluded that tetrachloroethylene exposure in dry cleaning shops may impair neurobehavioral performance and affect pituitary function. The pituitary is controlled in part by hypothalamic dopamine, which is important to neurotransmission. Study participants were tested during the proliferation phase of menstruation which may better capture changes in prolactin secretion but also may potentially confound findings if there are individual differences in severity of menstruation and in the timing of test session relative to the day of menstruation (U.S. EPA, 2004; see Section 4.6.1.2.5).

In a study of aircraft maintenance employees, Spirtas et al. (1991) observed an increased risk for NHL in females compared to males (see Section 4.8.1.2.1). Although quantitative exposure information on tetrachloroethylene was not obtained in this study, differences in exposure potential and level of exposure may explain the difference in risk between women and men. Differences in physiological parameters may also explain the observed gender difference in risk.

The studies by Pesch et al. (2000a) and Dosemeci et al. (1999) suggest that there may be gender differences in risk to renal cell carcinoma with occupational exposure to tetrachloroethylene; in both studies the risks were higher in males than in females (see Section 4.5.1.2). In a rat inhalation study, tubule cell hyperplasia was observed in eight males at various doses, but in only one female at high dose. Also, renal tubule adenomas and adenocarcinomas were observed only in males; however, chronically induced tetrachloroethylene neoplastic

kidney lesions do not exhibit sex specificity (NTP, 1986a). In a rat gavage study, there was no gender difference for toxic nephropathy (NCI, 1977). A marked gender difference was seen between male and female rats in the severity of acute renal toxicity with male rats being more affected than female rats (Lash et al., 2002), but otherwise no gender variation was observed for chronic nephrotoxicity not associated with alpha-2μ-globulin nephropathy (see Sections 4.5.2.2 and 4.5.4.3.3).

In the liver, male rats showed an increased incidence of spongiosis hepatitis as compared with females, but there was no gender difference in hepatocellular adenomas and carcinomas; however, the spleen showed increased effects in males versus females (JISA, 1993; see Sections 4.4.2.1 and 4.4.2.2).

4.9.2.3. *Race/Ethnicity*

One residential study found that buildings with >100 $\mu g/m^3$ tetrachloroethylene were more likely in minority neighborhoods (OR = 6.7; 95% CI = 1.5–30.5; NYS DOH, 2005a). In addition to possible increased exposure, different racial or ethnic groups may express metabolic enzymes in different ratios and proportions due to genetic variability.

In a follow-up study on the mortality of a cohort of dry cleaners, bladder cancer was elevated among Caucasian men and women, and kidney cancer was elevated among black men and women; however, these associations were not strongly related to duration or estimated level of exposure to tetrachloroethylene (Blair et al., 2003). One study found that following tetrachloroethylene exposure, TCA concentration in the urine of six Asian subjects was no different from the levels found in six Caucasians; however, this study was confounded by significant differences in alcohol consumption between the Caucasian and Asian populations (Jang and Droz, 1997).

Eskenazi et al. (1991a) noted a slightly lower per-cycle pregnancy rate among wives of men who received higher level exposure to tetrachloroethylene, but the potential contribution of tetrachloroethylene exposure to time to conception was small when compared with the contribution observed from Hispanic ethnicity and smoking, which were found to be stronger and statistically significant predictors of time to conception.

4.9.2.4. *Genetics*

Human variation in response to tetrachloroethylene exposure may be associated with genetic variation. For example, in a study of six adults, Monster et al. (1979) found that the mean coefficient of interindividual variation for tetrachloroethylene uptake was 17%. Human genetic polymorphisms in metabolizing enzymes involved in biotransformation of tetrachloroethylene are now known to exist (U.S. EPA, 1991; IARC, 1995; Lash and Parker,

2001). Section 3.3.3.1.5 discusses CYP isoforms and genetic polymorphisms, Section 3.3.3.2.1 covers GST isoenzymes and polymorphisms, and Section 3.3.4 describes differences in enzymatic activity.

Reitz et al. (1996) examined tetrachloroethylene metabolism in seven adult human liver samples and found a fivefold difference in the rate of tetrachloroethylene metabolism between the 50^{th} and 99^{th} percentiles. Opdam (1989) found a 2-fold spread in tetrachloroethylene blood concentrations in a study population of nine adult human subjects. In this study, the amount of fat and the blood concentrations seemed to be positively correlated but could not be confirmed; the author suggested that if the subjects had a wider range of body fat levels (range in this study was only 7–22 kg), a larger amount of interindividual variation would be expected. Computer modeling was used to examine the toxicokinetic variability of tetrachloroethylene (Bois et al., 1996; Chiu and Bois, 2006). However, whether CYP or GSH polymorphisms account for interindividual variation in tetrachloroethylene metabolism among humans, and thus differences in susceptibility to tetrachloroethylene-induced toxicities, is not known.

4.9.3. Multiple Exposures and Cumulative Risks

When considering health risks, it is important to consider the cumulative impact of effects that may be due to multiple routes of exposure. EPA published *Framework for Cumulative Risk Assessment* (U.S. EPA, 2003c) to address these issues. A human aggregate exposure model developed by McKone and Daniels (1991) incorporated likely exposures from air, water, and soil media through inhalation, ingestion, and dermal contact. They asserted that the aggregate exposure may be age dependent, but did not present any data for persons of differing life stages.

The limited data summarized by the ATSDR in its draft interaction profile on tetrachloroethylene, trichloroethylene, 1,1-dichloroethane, and 1,1,1-trichloroethane suggest that additive joint action is plausible (ATSDR, 2001). Co-exposure to other pollutants, including trichloroethylene and methylchloroform which produce some of the same metabolites and similar health effects as tetrachloroethylene, is likely to occur in occupational settings as well as in non-occupational sources such as in ground water contamination (e.g., Bove et al., 1995; Lagakos et al., 1996; MA DPH, 1997; ATSDR, 1998; Sonnenfeld et al., 2001). However, no evidence was among available studies indicates greater-than-additive effects for liver and kidney toxicity.

Due to the effects that many chemicals have on inducing and/or repressing metabolic enzymes as well as on organ systems, co-exposures may alter the way in which tetrachloroethylene is metabolized and cleared from the body. Inhibition or induction of the enzymes responsible for tetrachloroethylene metabolism can—and likely does—alter

susceptibility to toxicity (U.S. EPA, 1985a; IARC, 1995; Lash and Parker, 2001). Numerous environmental pollutants and therapeutic agents have the potential to induce or inhibit tetrachloroethylene-metabolizing enzymes. For example, tetrachloroethylene metabolism is increased by inducers of CYP enzymes such as toluene, phenobarbital, and pregnenolone-16-α-carbonitrile, whereas CYP inhibitors such as SKF 525A, metyrapone, and carbon monoxide decrease tetrachloroethylene metabolism (Moslen et al., 1977; Ikeda and Imanura, 1973; Costa and Ivanetich, 1980). Likewise, tetrachloroethylene exposure may increase the effects of exposures to other chemicals or stressors. For instance, adverse effects due to exposure to chlorinated solvents and alcohol may be increased because tetrachloroethylene may induce shared metabolic enzymes (see Section 3.3.4).

The acute effects of tetrachloroethylene share much in common functionally with those of other solvents (e.g., toluene, volatile anesthetics, and alcohols) such as changes in reaction time, nerve conduction velocity, and sensory deficits. There is emerging evidence that such agents act on the ligand-gated ion channel superfamily in vitro (Shafer et al., 2005), particularly on the inhibitory amino acids NMDA, nicotinic, and GABA receptors in vivo (Bale et al., 2005). Other organic solvents induce effects on memory and color vision (Altmann et al., 1995; Mergler et al., 1991; Hudnell et al., 1996a, b). The consistency of these observations suggests a common MOA of organic solvents to altered vision pattern. Hence, a concern exists for neurobehavioral effects from interaction or competitive inhibition between tetrachloroethylene and exposures with similarly hypothesized MOAs.

The interaction between tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane (methylchloroform) was modeled in rats (Dobrev et al., 2001) and in computer models for humans (Dobrev et al., 2002) and were shown to compete for metabolic capacity. The interaction between tetrachloroethylene and trichloroethylene showed a less-than-additive effect on the liver and kidney through inhibition of TCA formation (Pohl et al., 2003). Similarly, when exposed to tetrachloroethylene, rat liver cells had increased toxicity when co-exposed to peroxidation drugs such as cyclosporine A, valproic acid, and amiodarone (Costa et al., 2004), and n-hexane and ethylbenzene inhibited the metabolism of tetrachloroethylene in rats (Skowron et al., 2001).

Alcohol and smoking are generally regarded as confounders, although the additive or interactive effects of these exposures along with tetrachloroethylene are not well characterized. Exposure to alcohol changes the metabolic profiles in the liver, thus increasing metabolism to TCA through the CYP2E1 pathway (Pastino et al., 2000). Alcohol by itself cannot account for the observed deficits in neurobehavioral functions, because statistical analyses of the epidemiologic observations accounted for this covariate. Meskar et al. (2001) also contended that alcohol induces CYP2E1, which in turn has the ability to activate other compounds such as

halogenated solvents. This could potentially cause higher toxicity of tetrachloroethylene for those who use alcohol, if the oxidative metabolism leads to a proto-oxidant. Valic et al. (1997) showed greater decrements in color vision among subjects with both exposures as compared with individuals with solvent exposure only or with neither exposure (see Sections 4.6.1.3 and 4.6.3).

Regarding esophageal cancer, occupational observations suggest that the magnitude of the risks for several smoking-related cancers among dry cleaners was greater than could be explained by smoking alone, suggesting a further contribution from another risk factor, such as occupational exposure (Blair et al., 2003; Ruder et al., 2001; see Section 4.8.1.2.2).

4.9.4. Uncertainty of Database for Susceptible Populations

There is a need to better characterize the implications of tetrachloroethylene exposures to susceptible populations. A number of areas where the data base is currently insufficient are identified below.

4.9.4.1. *Uncertainties of Exposure*

A number of uncertainties exist regarding exposure to subpopulations. Further evaluation of the effects of multiple routes and pathways of exposures and aggregate risks is needed. Similarly, the effects due to co-exposures to other compounds with similar or different MOAs need to be evaluated. An estimate of multiple exposures is needed to know where along the dose-response curve to place an incremental exposure to tetrachloroethylene. The size of any increased risk will be different in dissimilar regions of the dose-response curve. This means that a dose that is safe for an unexposed population will not necessarily be a safe dose if background and other exposures are considered. Until quantitative conclusions can be made for each susceptibility factor, it will be very hard to consider the impacts of changes in multiple susceptibility factors.

Although there is more information on early life exposure to tetrachloroethylene than on other potentially susceptible populations, there remain a number of uncertainties regarding children's susceptibility. For example, it is not clear to what extent tetrachloroethylene may pass through the placenta in humans, as shown in a rodent study (Ghantous et al., 1986). Also, there is limited information that evaluates nonoccupational exposures to tetrachloroethylene (e.g., in homes and automobiles) for all susceptible populations or on additional exposures that may modify an individual's exposure to tetrachloroethylene. Improved PBPK modeling and validation of these models will aid in determining how variations in metabolic enzymes affect tetrachloroethylene metabolism.

Although inhalation is believed to be of most concern for tetrachloroethylene, the pathways of exposure as well as the MOA for children are not well characterized. Inhalation

exposures may occur when tetrachloroethylene vapors are released from treated clothing or the clothing worn by occupationally exposed individuals, as well as when vapors are exhaled in the breath of exposed workers (ATSDR, 1997; Aggazzotti et al., 1994a, b). Dry-cleaned garments transported in an automobile may also lead to unexpectedly high levels of exposure to children who sit in the rear seats of cars, nearest to where most items are stored (Park et al., 1998; Chien, 1997). Inhalation exposure may also occur during showering or bathing as dissolved tetrachloroethylene in the warm tap water becomes volatilized (Rao and Brown, 1993; see Section 2.2.1).

Although there is more information on early life exposure to tetrachloroethylene than on other potentially susceptible populations, a number of uncertainties remain regarding children's susceptibility. For example, though demonstrated in a study of placental transport by Ghantous et al. (1986), it is not clear to what extent tetrachloroethylene may pass through the human placenta. Also, there is limited information that evaluates nonoccupational exposures to tetrachloroethylene (e.g., in homes and automobiles) for all susceptible populations or on additional exposures that may modify an individual's exposure to tetrachloroethylene. Improved PBPK modeling and validation of these models will aid in determining how variations in metabolic enzymes affect tetrachloroethylene metabolism.

Although ingestion of tetrachloroethylene through breast milk may be a significant pathway of exposure for some infants (see Sections 2.2.4 and 3.2), it has been suggested that if these infants live adjacent to or in close proximity of dry cleaning facilities, the dose received through ingestion of breast milk will become insignificant when compared with the inhalation exposure and subsequent dose (Schreiber, 1997).

Certain foods have been found to be contaminated with tetrachloroethylene (see Section 2.2.3). Because children consume a high level of dairy due to their need for calcium for bone growth, the lipophilicity of tetrachloroethylene may pose a higher concern for children than for adults. Dairy intake is generally highest during infancy and decreases throughout life (NRC, 1993).

It is not clear to what extent dermal absorption is possible for children. Although an infant's skin has similar permeability to adults, a premature infant may have increased permeability (Guzelian et al., 1992). Also, an infant has approximately double the ratio of surface area to body weight compared to adults (NRC, 1993), which could imply increased exposure during bathing and swimming, which has already been modeled for adults by Rao and Brown (1993).

It is not known to what extent tetrachloroethylene is absorbed by a child and to which organs the chemical and its metabolites may be distributed. A validated PBPK model is needed

that contains physiologic parameter information for infants and children, including the effects of maternal inhalation exposure and the resulting concentration in breast milk.

4.9.4.2. *Uncertainties of Effects*

More studies specifically designed to evaluate effects in early and later life stages are needed in order to more fully characterize potential life stage-related tetrachloroethylene toxicity. Because the neurological effects of tetrachloroethylene constitute the most sensitive endpoints of concern for noncancer effects, it is quite likely that the early life stages may be more susceptible to these outcomes than are adults. Life stage-specific neurotoxic effects, particularly in the developing fetus, need further evaluation. It is important to consider the use of age-appropriate testing for assessment of these and other outcomes, both for cancer and noncancer outcomes.

The reduction in fertility seen in some studies (Eskenazi et al., 1991a, b; Rachootin and Olsen, 1983; Sallmén et al., 1995) occurs by an unknown mechanism. Altered sperm quality is one possibility (Beliles et al., 1980; Eskenazi et al., 1991b), as is spontaneous abortion/fetal loss occurring early in gestation without maternal knowledge of the pregnancy, thereby being misclassified as infertility (see Section 4.7.1).

Data specific to the carcinogenic effects of tetrachloroethylene exposure during the critical periods of development of experimental animals and humans also do not exist. The perinatal period, which encompasses the end of pregnancy and the early postnatal period, may be the most susceptible window for exposure for tetrachloroethylene across species (Beliles, 2002). Several of the adverse pregnancy outcome studies evaluated exposure during a critical window, the first trimester of the pregnancy, a critical window for exposure (MA DPH, 1997; Kyyrönen et al., 1989; Ahlborg, 1990; Taskinen et al., 1989). Exposure during another developmental period may not result in certain outcomes that occur from exposure during this critical window. Alternately, another window of exposure may result in a different outcome than occurs during the first trimester.

4.9.5. Conclusions on Susceptibility

There is some evidence that certain subpopulations may be more susceptible to exposure to tetrachloroethylene. These subpopulations include early and later life stages, health and nutrition status, gender, race/ethnicity, genetics, and multiple exposures and cumulative risk.

Cancer outcomes of concern for perinatal exposure are not well characterized in either the human epidemiological or the experimental animal literature. Data-derived noncancer outcomes of concern in early life stages are spontaneous abortion/fetal loss, mortality, and neurological impairment. As described above, the evidence for spontaneous abortion following prenatal exposures to tetrachloroethylene is well characterized in humans, and fetal loss is well

characterized in experimental animals. However, the human epidemiological data that support this conclusion do not provide information on the maternal dose to tetrachloroethylene that may have resulted in spontaneous abortion. Further, data from the experimental animal studies suggest that this finding may be a high-dose effect. Together, this evidence suggests that reference values that are established on the basis of more sensitive neurological endpoints should mitigate potential risk of fetal lethality.

Regarding postnatal hazard, the correlation of tetrachloroethylene exposure to childhood mortality is based on a single report in which a 2-year-old toddler died following exposure to dry-cleaned curtains (Garnier et al., 1996). Ambient dose was not well characterized, although tissue levels of tetrachloroethylene indicated the possibility of bioaccumulation in the brain. Yet, it is noted that early postnatal mortality was not observed in animal studies. The overall confidence in this endpoint, observed in a single individual, is minimal relative to predicting risk for the broader population.

Likewise, evidence of neurobehavioral impairment in children is based on a minimal data set, consisting of only four children who resided in an apartment building with a dry cleaning establishment and who demonstrated visual system impairment (Schreiber et al., 2002). Confidence in the results of this study for the assessment of risk to the broader population of children is minimal due to the size of the studied population (i.e., only four individuals). Given the lack of information on these factors in referent children, it is difficult to evaluate the possible contribution of other factors that may have contributed to the observed visual system impairment in children (Storm and Mazor, 2004). The lack of associations reported in Storm and Mazor does not contradict the findings of Schreiber et al., given the differences in study protocol and procedures (Hudnell and Schreiber, 2004). Decreased VCS has been observed in children who resided in an apartment building with a dry cleaning establishment (Schreiber et al., 2002; NYS DOH, 2005a). Children who attended a day care center adjacent to a dry cleaner did not consistently show any effect on cognition or behavior when assessed 5 weeks after exposure ceased (NYS DOH, 2005b), and no effect on VCS was seen 5 years after exposure (NYS DOH, 2005c). Moreover, the findings of Till et al. (2001a, b, 2005) and Laslo-Baker et al. (2004), although not definitive, further suggest that the developing fetus is susceptible to maternal organic solvent exposures (see Section 4.6.1).

Other subpopulations with potential for susceptibility to tetrachloroethylene include the elderly, diminished health status, gender, race/ethnicity, and multiple/cumulative exposures. There is suggestive evidence that there may be greater susceptibility for exposures to the elderly. Diminished health status (e.g., impaired kidney liver or kidney) will likely affect an individual's ability to metabolize tetrachloroethylene, whereas certain nutrients may have a protective effect on exposure. Gender and race/ethnic differences in susceptibility are likely due to variation in

physiology and exposure, and genetic variation likely has an effect on the toxicokinetics of tetrachloroethylene. Multiple and cumulative exposures are likely to cause competition in metabolic capacity. Future research should better characterize possible susceptibility for certain life stages or subpopulations.

4.10. SUMMARY OF HAZARD IDENTIFICATION

4.10.1. Description of Effects and Exposure Levels at Which They Occur

In the previous sections of this document the effects in each organ system were discussed pairwise in three categories: humans/animals, noncancer/cancer, and inhalation/oral. The summaries in Sections 4.4.3, 4.5.3, 4.6.3, and 4.7.3 pertain to each of the organ systems individually. In this section, the same effects are integrated across organ systems, with the primary subdivision being humans/animals, the secondary subdivision being noncancer/cancer, and later subdivisions being the exposure route and organ system. Section 4.10.2 summarizes the potential modes of action. The dose levels, effect levels, and concentrations discussed here are those observed in the studies and are not corrected for continuous exposure or for human equivalency. In Chapter 5, these corrections are made before deriving the RfCs and RfDs.

4.10.1.1. *Summary of Effects in Humans*

4.10.1.1.1. ***Human noncancer effects.*** The epidemiologic evidence indicates that the primary targets of tetrachloroethylene noncancer toxicity are the CNS, kidneys, liver, and developing fetus. The epidemiologic evidence supporting these inferences is derived primarily from studies of tetrachloroethylene-exposed dry cleaners—with two studies reporting neurobehavioral effects in residents living in housing located in close proximity to a dry cleaning facility—and from studies reporting effects to the developing fetus in populations exposed to drinking water contaminated with tetrachloroethylene and other solvents. In the drinking water studies, several of the contaminants are congeners (e.g., tetrachloroethylene and trichloroethylene), which are metabolized in the body to TCA and DCA.

The epidemiologic database is primarily composed of studies of a prevalence or cross-sectional design. Although a cohort study, by definition, is able to identify that exposure has indeed occurred before disease, the available epidemiologic studies, including those of a cross-sectional design, support a causal role of tetrachloroethylene.

In most cases, the number of study subjects was not large; however, the issue of sample size affects the power of the study to detect underlying risk. Hence, observed effects are considered noteworthy if chance and bias are minimized. Furthermore, the number of studied individuals in the epidemiologic studies of tetrachloroethylene is not any smaller than that of

epidemiologic studies for many chemicals identified in the U.S. EPA's IRIS. In fact, it is a rare case when hazard inferences are based on a large human population.

Studies have adopted a number of methods to infer exposure to tetrachloroethylene. Exposure was ascertained in many cases indirectly by questionnaire, by job title, or by the subject living in a residence receiving drinking water containing tetrachloroethylene. There is higher confidence of exposure potential to tetrachloroethylene in those studies using the occupational title of dry cleaner because tetrachloroethylene is the solvent of choice. Atmospheric montoring of dry cleaning facilities show 8-hr TWA concentrations in the range of 10–20 ppm, with short-term exposures many times this value. However, the analyses that combined dry cleaners with laundry workers carry more uncertainty than do studies whose analyses included only dry cleaners, because laundry workers have a lower probability for exposure to tetrachloroethylene. More rarely, studies incorporated biological measures such as tetrachloroethylene excretion in breath or urinary TCA.

When looked for, exposure-response gradients have not been observed, generally, and this is another uncertainty associated with the inferences regarding a causal association. However, exposure misclassification may partially explain the lack of exposure-response associations, hence, the lack of an exposure-response gradient does not diminish the observed associations between tetrachloroethylene exposure and adverse effects. Moreover, observed effects cannot be considered to arise from confounding; investigators have taken great effort to take into account the effects of smoking, age, and other factors through matching exposed subjects with like controls or through statistical analysis of the data. This synthesis of the epidemiologic evidence places greatest weight on those studies where confounding has been adequately controlled and identifies those studies where confounding may be a possible explanation for observed results. It is not possible to examine residual confounding or effects not explained by variables adjusted for in the study's design or statistical analysis. Residual confounding is an issue for both epidemiologic and toxicologic studies and may explain observed study findings.

In general, observations from the epidemiologic studies are consistent with the biology of tetrachloroethylene. Tetrachloroethylene is lipophilic and would distribute to organs rich in lipids, e.g., the CNS. The liver and kidney are considered target organs due to their ability to metabolize tetrachloroethylene. Moreover, systemic effects, specifically to the kidney, liver, CNS, and the developing organism, have been observed in experimental animals. Thus, humans do not appear as an exception to the systemic toxicity elicited by tetrachloroethylene. The pattern of effects seen with tetrachloroethylene exposure is similar to that seen with other solvents, such as trichloroethylene. These findings together support a causal role of tetrachloroethylene in the development a number of systemic effects in humans.

4.10.1.1.2. ***Human cancer effects.*** Overall, the epidemiologic evidence considered as a whole has associated tetrachloroethylene exposure with excess risks for a number of site-specific cancers. Studies of tetrachloroethylene and cancer showed positive associations between exposure and cancer of the lymphoid system, esophagus, and cervix, with more limited evidence for cancer of the bladder, kidney, and lung. For both lymphoid and esophageal cancer, excess risk was observed in studies of dry cleaners and in degreasers, populations who have exposure to tetrachloroethylene and other solvents. In both cases, average risks were doubled as compared with those of referents. Studies of drinking water exposure also support an association between lymphoid cancer and tetrachloroethylene and other solvents, as do case-control studies that assessed employment as a dry cleaner or laundry worker. Chance and confounding by smoking are unlikely explanations for the observed excesses is risks. Furthermore, the finding of elevated risk for lymphohematopoietic system cancer incidence in a Swedish cohort of subjects who developed suspected solvent-related disorders from organic solvent exposures supports the findings of the tetrachloroethylene studies (Berlin et al., 1995). EPA judged that these data, though limited and not consistently observed across all studies, suggested an association between lymphoma and tetrachloroethylene.

For esophageal cancer, indirect evidence suggests that esophageal risk in these studies is larger than that expected due to smoking. Blair et al. (2003) stated that if the magnitude of the difference in smoking for dry cleaners and the general population is in the range of 10% or less, confounding from smoking in their study is unlikely to result in an RR greater than 1.2, a finding similar to that of Kriebel et al. (2004). Hence, the finding of a doubling in risk strongly suggests occupational exposure as a contributing (etiologic) factor. Observations from one case-control study that was able to adjust for the effects of smoking support the cohort study findings.

The epidemiologic evidence also is suggestive of excess risks for cervical cancer, based on observations in dry cleaner and laundry worker cohorts, with few cases in the degreaser studies. Unfortunately, information is not available on possibly confounding factors such as socioeconomic and lifestyle factors. Associations with kidney, bladder, and lung cancers and dry cleaning employment or, more specifically, with tetrachloroethylene, were reported in recent updates of the American and Nordic cohorts and in case-control studies. Conclusions are more uncertain for these sites, because they are based either on heterogenous observations between differing study designs or on a small number of available studies. Overall, EPA judged these findings as suggestive of an association.

Other reviews of the tetrachloroethylene epidemiologic evidence have concluded that "little consistent evidence existed for an association with a specific cancer such as kidney" (McLaughlin and Blot, 1997) or that there is "limited evidence" for cancers of the cervix,

esophagus, bladder, or kidney or for NHL (IARC 1995; Weiss 1995; Lynge et al., 1997; Ulm et al., 1996). The Institute of Medicine (IOM) reviewed a similar—but not the same—body of epidemiologic literature as EPA (IOM, 2002). For example, the large four-country Nordic cohort of dry cleaners and laundry workers in Andersen et al. (1999) was not considered, nor was the most recent update of the cohort of American dry cleaner and laundry workers in Blair et al. (2003). The IOM committee concluded limited or suggestive evidence of an association between bladder and kidney cancers and tetrachloroethylene and dry cleaning solvents. No conclusions were presented on the epidemiologic evidence on esophageal and lung cancers and tetrachloroethylene, given that the committee could not reach a consensus opinion. Some committee members believed that the overall evidence was limited by potential confounding from smoking in cohort studies, whereas other committee members considered, for esophageal cancer, the lack of other smoking-related cancers in cohort studies or, for lung cancer, the presence of exposure-response relationships as supportive of an conclusion of limited/suggestive evidence. For other cause-specific cancers, the committee concluded that there was inadequate or insufficient evidence to determine whether an association existed.

U.S. EPA's analysis is similar to those of the IOM committee's on kidney and bladder cancer, and, like some committee members, EPA considered the evidence on esophageal and lung cancers as suggestive of an association. U.S. EPA's conclusions on lymphoma are supported, in part, by observations from studies not considered by the IOM committee.

Mundt et al. (2003) reviewed a body of epidemiologic studies similar to U.S. EPA's and presented conclusions as to whether an association was "likely" or "not likely." The authors reported that little support existed on which to base a conclusion that tetrachloroethylene was a strong occupational risk factor, but that "because of a number of positive findings suggested from some of these epidemiological studies, one cannot definitely rule out the possibility that associations between PCE [tetrachloroethylene] and some cancers exist in humans." This conclusion is consistent with conclusions in this assessment, although it is expressed differently.

Although epidemiologists acknowledge that using guidelines to assess causation is an imperfect process, some find that the aspects developed by A.B. Hill (1965) are helpful in making these difficult judgments. Making this determination may precede an understanding of the underlying mechanisms and involves consideration of several aspects that would be characteristic of a cause-and-effect relationship (Hill, 1965; Rothman and Greenland, 1998).

1. *Strength of the observed association*. The finding of large and precise risks increases confidence that the association is likely not due to chance, bias, or other factors. For tetrachloroethylene, observed risks are generally modest, 2-fold or less. The observed risks for esophageal cancer are not thought to be attributable to smoking or alcohol,

although insufficient data exist on socioeconomic factors important to cervical cancer to assess their impact on observed elevated risks for these site-specific cancers.

2. *Consistency of the observed association*. An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. Excess risks for lymphoid cancers are seen in studies of dry cleaners, degreasers, and populations exposed to drinking water containing tetrachloroethylene—and in some cases trichloroethylene—and for esophageal cancer in studies of dry cleaners and degreasers. Excess risks for the other site-specific cancers are less consistently observed across these populations.

3. *Specificity of the observed association*. Traditionally, specificity has been described in terms of one cause, one disease (Hill, 1965). This implies that one factor is associated with the observed effect and no other effects are associated with the putative factor. Tetrachloroethylene causes cancer at several sites in rats and mice; hence, there is no expectation that tetrachloroethylene would be associated with only one human cancer. Furthermore, many agents cause cancer at multiple sites, and many cancers have multiple causes. Specificity has little meaning in this case, and therefore the lack of specificity does not detract from the weight of the overall epidemiologic evidence.

4. *Temporal relationship of the observed association*. Causal relationships have temporality, i.e., the cause precedes the effect. Associations between tetrachloroethylene exposure and several forms of cancer are established primarily by cohort and case-control studies, in which the temporal relationship is well described. Many drinking water studies are ecologic or prevalence studies, in which knowledge of the temporal relationship is lacking. The exceptions are those studies assessing exposure to residents of Cape Cod, MA, Woburn, MA, and Camp Lejeune, NC. For this reason, the conclusions place greater weight on the cohort and case-control studies.

5. *Biological gradient (exposure-response relationship)*. A clear exposure-response relationship often suggests cause and effect. For tetrachloroethylene, biological gradients are only sporadically observed, though most studies identify exposure only as a dichotomous variable (yes/no), or the number of site-specific cancers is often too small to identify biological gradients. For esophageal cancer, the dry cleaner studies of tetrachloroethylene exposure (Blair et al., 2003; Ruder et al., 2001) showed no clear picture of exposure response relationships. Exposure response analyses in the drinking water studies collectively suggest that greater exposure to drinking water contaminated with tetrachloroethylene—and in a smaller number of studies, with chlorinated solvents both tetrachloroethylene and trichloroethylene—is associated with lymphoid cancer, particularly leukemia and NHL (Aschengrau et al., 1993; MA DPH 1997; Fagliano et al., 1990; Cohn et al., 1994).

6. *Biological plausibility.* The mechanistic studies (discussed in another section in this assessment) investigating tetrachloroethylene carcinogenic effects in rats or mice and their relevance to humans indicate that carcinogenesis is complex and likely involves multiple mechanisms. Overall, the MOA for site-specific cancer is not know at this time.

7. *Coherence*. Coherence means that the causal interpretation of the data should not seriously conflict with generally known facts about the natural history and biology of the disease. The strongest associations between tetrachloroethylene and human cancer are for the lymphopoietic system and esophagus, with more limited evidence for cervix, bladder, kidney, and lung. Several of these organ systems are also targets for noncancer toxicity. The associations between cervical and esophageal cancer have no suitable animal counterparts.

8. *Experimental evidence (from human populations)*. Experimental evidence (e.g., a "natural experiment" that measures effects with exposure and in the absence of exposure) is seldom available from human populations and exists only when conditions of exposure are altered to create a kind of quasi-experiment. There are few data to evaluate this criterion. The only study that does present information notes that childhood leukemia cases appeared to be more evenly distributed throughout Woburn, MA, after closure of the two wells contaminated with trichloroethylene and tetrachloroethylene (MA DPH, 1997).

9. *Analogy*. The pattern of effects associated with tetrachloroethylene, particularly cancers of the lymphoid system, cervix, kidney, and pancreas, has similarities to that of several other chlorinated solvents and to mixed-solvent exposures.

Together, the evidence on tetrachloroethylene partially fulfills several of these criteria and is suggestive of a cause-and-effect relationship between tetrachloroethylene and human cancer. The body of human evidence is not sufficient to regard tetrachloroethylene as a known human carcinogen.

4.10.1.1.3. ***Susceptibility.*** Many of tetrachloroethylene's metabolites are formed through the enzyme system that also metabolizes ethanol and other drugs and environmental pollutants. Exposures to these chemicals can alter tetrachloroethylene's toxicity, not only altering the pharmacokinetics of tetrachloroethylene but also the pharmacodynamics for toxicity. For tetrachloroethylene's effects on the nervous system, kidney, and liver, the available limited data suggest that a joint effect that reflects the addition of all exposures is plausible. In addition, susceptibility to tetrachloroethylene's toxicity may vary among individuals because of both intrinsic factors, (age, sex, and genetic factors, including metabolic polymorphisms) and acquired factors (disease status, nutritional status).

4.10.1.2. ***Summary of Effects in Animals***

4.10.1.2.1. ***Animal noncancer effects.*** Tetrachloroethylene exposure in animals results in toxicity to the liver, kidney, and nervous system and also causes developmental and reproductive effects. These are all sites of high metabolic activity, and the CNS is also a lipid accumulation

site. The immune system is potentially affected, but there are very few studies of these effects, and none of them are in intact animals. No information is available on the effects of tetrachloroethylene on the endocrine system in animals. The effects have been discussed in several other review documents and are described in Sections 4.4.2.1, 4.5.2.1, 4.6.2.1, 4.6.2.2, and 4.7.2.1.

In the liver, several measures of toxicity have been observed, such as increased liver weight, infiltration of fat, necrosis, peroxisome proliferation, polyploidy of hepatocytes, and increased triglycerides. In kidney, increased weight, hyperplasia, hyaline droplets, and protein cast formation in tubules have been observed. In the CNS, alteration of brain neurotransmitter levels, increased motor activity, and delayed reaction times to visual stimuli have been observed. Fetal growth retardation, increased fetal mortality, and behavioral changes occurring after birth to animals exposed in utero have been observed. Section 4.10.1.3 describes the doses at which these effects occurred.

4.10.1.2.2. ***Animal cancer effects***. Carcinogen bioassays in rats and mice have shown benign and malignant tumors at various sites, as summarized in Sections 4.4.2.2, 4.5.2.2, and 4.8.2. Data on the incidence and dose levels at which these effects occur are presented in Section 5.3.2 and Tables 5-6, 5-8, and 5-9 (Chapter 5). One study that used the oral route found liver adenomas and carcinomas in mice. Two inhalation bioassays, both of which used both mice and rats, found tetrachloroethylene-induced excess incidence of hepatocellular adenomas and carcinomas (mice) and mononuclear cell leukemia (rats). One of these studies found hemangioendothelioma (mice) and the other found brain glioma, kidney tubular cell tumors, and testicular interstitial cell tumors, all in male rats. Brain and kidney tumors are rare in unexposed animals, but they were found to be slightly elevated above control levels in only one of the two inhalation studies.

Testicular tumors are extremely common in control animals, and the statistically significant elevation in one of the bioassays was not considered by the investigators as related to tetrachloroethylene exposure; however, the testicular tumors and kidney tumors are consistent with exposure of rats to trichloroethylene, a structural analogue of tetrachloroethylene. Mononuclear cell leukemias in rats were elevated in both inhalation bioassays. As discussed in Section 4.8.2.4.1, this is a relatively common tumor in nonexposed animals, with a possible site concordance with human lymphoid cancer, but because the mechanism of formation for both human and animal hematopoietic neoplasms is not understood in all of its complexity, they are of possible relevance to humans.

4.10.1.3. *Summary of Effect Levels*

Table 4-12 summarizes the lower ranges of air concentrations and oral doses at which effects occur in each of the organ systems discussed in this document. The lowest of these air concentrations is 0.7 ppm (mean), which is associated with neurological effects observed in residents living above dry cleaning facilities (Altmann et al., 1995) in Germany. This is close to the mean concentration measured by Schreiber et al. (2002) for a similar exposure situation in the United States (0.4 ppm). The lowest concentration showing effects in animals is 9 ppm, where liver toxicity was observed in mice which are more sensitive than the rat test strains.

Table 4-12. Summary of low-effect levels of exposure to tetrachloroethylene

Organ System	Humans		Animals	
	Inhalation (ppm)	Oral (µg/kg/day)	Inhalation (ppm)	Oral (mg/kg/day)
Liver	12–16 (Table 4-1)	–	9–50, mice (Table 4-2) 100 for cancer in mice[a]	100 (Table 4-2) 386 in mice for cancer[a]
Kidney	1.2 and 8.8 (Table 4-3)	–	100 ppm, mice[b]	No studies[b]
Neurological	0.3 (Table 4-5)	–	37–90, mice and gerbils (Table 4-6)	No chronic study (Table 4-7)
Developmental, reproductive	1.2 (Table 4-8)	6, uncertain (Table 4-8)	100, rats (Table 4-9)	–
Other organs	Exposure uncertain[c]	–	No conclusion[d]	–

[a] See Section 4.4.2.2.
[b] See Section 4.5.2.1.
[c] See Section 4.8.1.
[d] See Section 4.8.2.

– = No studies available

For subchronic oral exposures, the lowest dose for which adverse effects occurred in animals is 100 mg/kg-day. These data come from the Buben and O'Flaherty (1985) gavage study, where the exposure duration was 6 weeks. There are no reliable data for humans exposed orally.

4.10.2. Characterization of Cancer Hazard

Tetrachloroethylene is "Likely to be a human carcinogen by all routes of exposure" within the framework of the 2005 carcinogen risk assessment guidelines (U.S. EPA, 2005b). This conclusion is based on reported associations in epidemiologic studies between tetrachloroethylene exposure and site-specific cancers and by the induction of site-specific tumors in rodents given tetrachloroethylene by oral gavage and inhalation. Several metabolites of tetrachloroethylene also are considered rodent carcinogens. Metabolites from the oxidative pathway, TCA and DCA, produce liver tumors in mice, and DCA also induces liver tumors in rats. Metabolites from the GST pathway have not been tested in a standardized 2-year bioassay. This hazard characterization is discussed in more detail in Section 4.10.2.2. The context for this statement is described in the following section.

4.10.2.1. *Background*

As specified in the guidelines, the descriptor "Likely to be carcinogenic to humans" expresses the conclusion regarding the weight of evidence for carcinogenic hazard potential, and it is presented only in the context of a weight of evidence narrative. Although the term "likely" can have a probabilistic connotation in other contexts, its use as a weight of evidence descriptor does not correspond to a quantifiable probability of whether the chemical is carcinogenic. The five recommended standard hazard descriptors are as follows:

"Carcinogenic to humans"
"Likely to be carcinogenic to humans"
"Suggestive evidence of carcinogenic potential"
"Inadequate information to assess carcinogenic potential"
"Not likely to be carcinogenic to humans"

These descriptors are not unlike those used by the IARC, NTP, and other health agencies that weigh carcinogenicity evidence. If there are no or insufficient pertinent data, then the descriptors "Inadequate information to assess carcinogenic potential" or "Suggestive evidence of carcinogenic potential" are used. If the evidence is stronger, as is the case with tetrachloroethylene, the descriptor "Likely to be carcinogenic to humans" is used; convincing evidence, usually conclusive demonstration of causality in epidemiological studies, would support "Carcinogenic to humans." On the other hand, if the conclusion is negative (*i.e.*, strong, consistent and compelling information indicating the absence of human health hazard), the agent would be described as "Not likely to be carcinogenic to humans." Thus, going down the list of descriptors from "Carcinogenic to humans" to "Inadequate information to assess carcinogenic potential" indicates a decrease in the level of evidence or of a human health hazard. In summary,

use of the weight of evidence descriptor "Likely to be carcinogenic to humans" for tetrachloroethylene is intended to communicate that the available information indicates the presence of a human health hazard.

The weight-of-evidence conclusion represented by the top three levels of evidence is related to but distinct from the quantitative dose-response assessment/conclusions in that the judgment that an agent is a human carcinogen does not guarantee adequate data to quantitatively estimate human risk. Notably, evaluation of an agent that is judged a likely human carcinogen may offer data conducive to estimating human risk. Indeed, dose-response assessments are generally completed for agents considered "Carcinogenic to humans" and "Likely to be carcinogenic to humans." Section 5.4 provides the dose-response analyses for tetrachloroethylene.

4.10.2.2. *Hazard Characterization for Tetrachloroethylene*

Overall, the epidemiologic evidence considered as a whole has associated tetrachloroethylene exposure with excess risks for a number of site-specific cancers. Lymphoid cancer is now recognized as a combination of NHL, Hodgkin's disease, lymphosarcoma, multiple myeloma, and lymphatic leukemia. Cohort studies of dry cleaner and laundry workers and of degreasers suggest excess risks of lymphoid cancers, as do case-control studies of drinking water exposure and occupational exposure. Exposure to a number of solvents is likely in most of the case-control studies; however, these solvents have a qualitatively similar profile of metabolites, although quantitative differences are expected. One study of exposure only to tetrachloroethylene in drinking water reported a statistically significant association, based on a small number of exposed cases, between leukemia and a residence receiving tetrachloroethylene-contaminated water (Aschengrau et al., 1993).

Both cohort and case-control studies of dry cleaning workers support an association between tetrachloroethylene and excess risk of esophageal cancer. Recent updates of dry cleaners and laundry worker cohorts (Ruder et al., 2001; Blair et al., 2003) carry great weight in this evaluation because dry cleaners are predominately exposed to tetrachloroethylene and a statistically significant elevated mortality from this cancer continued to be observed. Little weight is given to the Lynge et al. (2006) study due to potential biases that likely dampen their observations and because of these biases it is considered a null study. No clear patterns are seen in the tetrachloroethylene studies for either level or duration of exposure and response.

The possibility that other exposures such as smoking and alcohol consumption may potentially confound the associations observed in Blair et al. (2003) and Ruder et al. (2001) cannot be directly addressed. Indirect evidence suggests that the esophageal risk in these studies is larger than that expected due to smoking. Moreover, the case-control study by Vaughan et al.

(1997) provides support for an association with tetrachloroethylene; a statistically significant association was observed between tetrachloroethylene exposure and esophageal cancer after adjustment for smoking, alcohol, and socioeconomic status. Support by analogy is derived from the finding of excess esophageal cancer incidence in a cohort that was occupationally exposed to trichloroethylene (Hansen et al., 2001).

More deaths from cervical cancer were observed among American and Nordic female dry cleaners or laundry workers than were expected. The observation of exposure-response trends in the studies that presented this information (Blair et al., 1990; Ruder et al., 1994, 2001) support an association with dry cleaning. Lack of data on socioeconomic status—a proxy for exposure to the human papilloma virus, a known risk factor for cervical cancer—indicates great uncertainty for asserting this association with tetrachloroethylene exposure.

There is also some support, albeit less than for the sites above, for an association between dry cleaning occupations and other cancers, specifically, cancers of the kidney, bladder, and lung. These findings are based on heterogenous observations of differing study designs, on a small number of available studies, or on small numbers of study subjects.

An open question in the dry cleaner studies is the specificity of exposure to tetrachloroethylene. Elevated mortality for cancer of the esophagus and cervix were observed in two cohorts that were considered to have primarily tetrachloroethylene exposures. However, individuals who may have had exposures to other dry cleaning solvents were also included in these studies. There are only three studies of cancer incidence or mortality among degreasers exposed to tetrachloroethylene, and they are of a small number of subjects with tetrachloroethylene exposure and, consequently, of few site-specific cases. These studies are only now collectively beginning to provide insight on associations between tetrachloroethylene exposures and site-specific cancers.

In rodents, hepatocellular carcinomas in both male and female B6C3F1 mice have been observed following inhalation and oral gavage exposure, and the same tumor response was observed in male and female Crj:BDF1 mice after inhalation exposure. MCL, a common tumor site in treated and untreated F344 rats, was significantly increased in both males and females in inhalation bioassays carried out in both Japan and the United States. Malignant liver hemangiosarcomas and splenic hemangioendotheliomas were also observed in male mice in the Japan bioassay. In the U.S. inhalation study in F344 rats, a small excess incidence of rare renal tubule cell carcinoma and adenoma was observed in males. Testicular interstitial cell tumors, a common tumor in treated and untreated F344 rats, were significantly elevated in the U.S. bioassay, and an elevation of rare brain glioma incidence was also observed in these rats.

The major metabolite of tetrachloroethylene in humans and rodents, TCA, is carcinogenic by gavage in male mice, and another metabolite in rodents, DCA, is also carcinogenic by gavage

in male mice. The MOA of tetrachloroethylene or its metabolites in the likely causation of cancer is not known. Extensive testing of tetrachloroethylene showed that it does not damage DNA except in a few studies of conditions where the GSH metabolites would be generated, and it induces chromosome aberrations in some studies. Several of the known or putative oxidative metabolites are mutagenic. Metabolism through kidney GSH conjugation produces trichlorovinyl GSH and trichlorovinyl cysteine, which were mutagenic in the Salmonella test but which have not been tested in mammalian genotoxicity assays. The latter metabolite reacts with beta lyase in the kidney to produce reactive thiol compounds. Other metabolites, including reactive sulfoxides, can be also be produced by FMO3 or CYP3A metabolism of TCVC. This is a plausible MOA for the rare rat kidney tumors observed in one bioassay. However, the MOAs for human tumors and the mice liver tumors is still unknown. Therefore, there is little mechanistic basis for choosing a low-dose extrapolation model.

4.10.3. Mode-of-Action Summary

The MOA for tetrachloroethylene-induced carcinogenesis is not yet fully characterized, completely tested, or understood. The database for hepatocarcinogenesis is especially limited with regard to chemical-specific studies. The available evidence points to multiple MOAs being involved. Furthermore, although there is some evidence for common MOAs, there is also evidence indicating differences in the potential MOA across organ systems.

Tetrachloroethylene exposure has been associated with peroxisome proliferation in rodent liver and kidney. Compelling insight into the hypothesized MOA by which certain chemicals induce proliferation of peroxisomal organelles and possibly cancer—specifically, the focus has been on liver cancer—was disclosed by the discovery of the PPAR receptors, a class of nuclear receptors closely related to the thyroid hormone and retinoid receptors that were first shown to be activated by peroxisome proliferators by Issemann and Green (1990). To date, three known subtypes of PPAR have been described in mammals: PPAR gamma, PPAR-δ, and PPAR-α.

Evidence exists to support PPAR-α as being the specific receptor that is necessary for transient cell proliferation and its role in hepatocarcinogenesis has been the subject of several investigations, although most studies have explored the potent agonist Wy-14,643 (Lee et al., 1995; Peters et al., 1997a; Corton et al., 2000). Activation of the steroid-like PPAR receptor regulates transcription of the genes. The PPAR target genes encode enzymes involved in peroxisomal and mitochondrial beta-oxidation and ketone body synthesis as well as certain P450 4A enzymes, fatty-acid binding proteins, apolipoproteins, lipoprotein lipase, malic enzyme, and phosphoenolpyruvate carboxykinase (Issemann et al., 1993; Desvergne and Wahli, 1995; Reddy

et al., 1986). The PPAR genes are expressed in a wide range of tissues, and PPAR occurs across species.

Several recent studies have expanded the scientific understanding of the PPAR-α mode of action proposed by Klaunig et al (2003; see Caldwell et al., 2008). First, Yang et al (2007) demonstrated that PPAR-α activation in hepatocytes induces peroxisome proliferation but not liver tumors. The approach entailed targeting expression of PPAR-α to hepatocytes by placing the VP16 PPAR-α transgene gene under control of the liver enriched activator protein (LAP) promoter. LAP-VP16 PPAR-α transgenic mice showed a number of PPAR-α -mediated effects: decreased serum triglycerides and free fatty acids, peroxisome proliferation, enhanced hepatocyte proliferation, and induction of cell-cycle and PPAR-α target genes. However, compared with wild-type mice exposed to Wy-14,643, the extent of hepatomegaly was reduced and no hypertrophy or eosinophilic cytoplasms was seen in LAP-VP16 PPAR-α mice. Also in contrast with wild-type mice exposed to Wy-14,643, no evidence of non-parenchymal cell proliferation was observed in the LAP-VP16 PPAR-α transgenic mice. Moreover, at one year of age no evidence of preneoplastic hepatic lesions or hepatocellular neoplasia was observed in LAP-VP16 PPAR-α transgenic mice. As noted by the authors, PPAR-α activation only in mouse hepatocytes is sufficient to induce peroxisome proliferation and hepatocyte proliferation but "…is not sufficient to induce liver tumors."

Secondly, Ito et al. (2007) found that DEHP, a proposed robust example of PPAR-α agonism-induced hepatocarcinogenesis, yields liver tumors in a 2-year study in PPAR-α knock-out mice. This study demonstrates the limitations, cited by the FIFRA SAP, of drawing conclusions from the one-year bioassays of high doses of Wy-14,643 referenced above (e.g., Peters 1997). It supports the view that knock-out mouse bioassays should be carefully characterized and conducted for 2 years to assess whether PPAR-α activation is indeed necessary for induction of liver cancer. Thus, although a weak peroxisome proliferator, chemical-specific data supporting the hypothesis that PPAR-α activation plays a prominent or essential role in tetrachloroethylene tumor induction are lacking. Critical review of the scientific literature reveals significant data gaps regarding the relationship between the PPAR-α activation and neoplasia induced by peroxisome proliferators as a group and tetrachloroethylene specifically. If PPAR-α does play a role in tetrachloroethylene-induced tumorigenesis, available information suggests relevance to humans cannot be ruled out.

Although accumulation of alpha-2μ-globulin has been suggested as an MOA leading to nephropathy that culminates in the formation of renal tumors, the available data do not support this MOA for tetrachloroethylene. Indeed, the available data suggest that alpha-2μ-globulin accumulation following tetrachloroethylene exposure occurs only at doses higher than those used in the carcinogenicity bioassays. In addition, tetrachloroethylene does not meet all the criteria to

suggest that alpha-2μ-globulin accumulation is the MOA. Therefore, an important role for alpha-2μ-globulin accumulation in tetrachloroethylene-induced renal tumors is highly unlikely.

The role of genotoxicity in tetrachloroethylene liver cancer, an effect that is thought to be related to products of CYP metabolism, is uncertain. The available data suggest that several of the chloroacid metabolites are mutagenic. In particular, tetrachloroethylene oxide, the primary metabolite hypothesized to be formed during CYP metabolism, is a known bacterial mutagen; it has not been tested in mammalian systems, although genotoxicity in such tests could be anticipated based on the expected DNA reactivity of the epoxide moiety. GSH-derived intermediates also exhibit genotoxicity. The glutathione conjugation of tetrachloroethylene in the kidney leads sequentially to S(1,2,2-trichlorovinyl)glutathione and S(1,2,2-trichlorovinyl)cysteine—TCVG and TCVC. TCVC can be further processed by beta-lyase to yield an unstable thiol, 1,2,2-trichlorovinylthiol, that may give rise to a highly reactive thioketene which can form covalent adducts with cellular nucleophiles including DNA. TCVC can also undergo FMO3 or P450 oxidation to reactive intermediates; additionally, sulfoxidation of both TCVC and its N-acetylated product occurs, resulting in reactive metabolites (Ripp et al, 1997, 1999; Werner et al., 1996). While most of these intermediates have not been characterized for mutagenic potential, TCVG, TCVC and NAcTCVC are clearly mutagenic in Salmonella tests. In addition, tetrachloroethylene exhibited mutagenicity in Salmonella in the few studies of conditions that could generate GSH-derived metabolites and, following in vivo exposures, induces SSB and DNA binding in kidney. A mutagenic MOA is therefore likely to play a role in the development of tetrachloroethylene-induced renal cancer. A mutagenic MOA could also play a role in the formation of other tumors such as brain gliomas and MCL in rats, if sufficient concentration of the potentially genotoxic metabolites arising from the initiation of GSH conjugation occurs at target sites. In the kidney, the conjugates are concentrated after being transported from the liver, in addition to being generated on site in that target tissue. Further processing by beta lyase, FMO3 or CYPs yields mutagenic products in what could be sufficiently high target tissue concentrations. Beta lyase is also found in the brain and other tissues.

In summary, cancers resulting from tetrachloroethylene exposures are likely due to multiple MOAs that vary from target tissue to target tissue. The MOAs for tetrachloroethylene-induced cancers are not yet well understood. The potential MOAs for cancer discussed in this section are summarized in Table 4-13 below. The implications of these potential MOAs to risk extrapolation to concentrations lower than those producing effects in animal bioassays are explored in Table 4-14. These considerations underlie the discussion in Chapter 5 of the uncertainties in modeling risk at low concentrations.

Table 4-13. Summary of potential modes of action for cancer

Organ, tumor type	Potential MOA	Evidence for MOA	Limitations/evidence against MOA	Weight of evidence
Kidney adenocarcinoma in male rats.	Mutagenicity. Via the GSH pathway in liver and kidney, glutathione and cysteine conjugates are produced. Identification of the urinary mercapturate metabolite in humans and rodents shows that this pathway is operative in both species.	Reactive thiol and sulfoxide compounds are formed in kidney from TCVG/TCVC that are mutagenic.	Several metabolites (TCVG/TCVC, NAcTCVC) are mutagenic in bacteria but are not characterized in other systems; other GSH metabolites have not been identified and characterized. Mutagenicity is commonly assumed to contribute to cancer.	Experimental evidence for this pathway and identification of its urinary mercapturate metabolites supports the MOA.
	Tubular cell necrosis and nephrotoxicity followed by hyperplasia and neoplastic transformation.	In vitro kidney damage from processing of GSH metabolites to cytotoxic species.	Relationship between non-genotoxic kidney necrosis and carcinogenicity has not been studied.	Inadequate tetrachloroethylene-specific data exist to support this potential MOA.
	Accumulation of alpha-2μ-globulin, a male rat specific protein, in hyaline droplets of tubule cells leading to a specific pattern of nephropathy (involving tubule degeneration, compensatory hyperplasia, neoplastic transformation).	Hyaline droplets observed in short-term (42-day) experiments BUT ONLY at high dose (1,000 mg/kg-day).	Tetrachloroethylene-induced renal nephropathy also occurs in female rats and mice of both sexes. Hyaline droplets are not observed in 28-day experiments at lower concentrations (400 ppm) that induce kidney tumors. alpha-2μ-globulin protein not identified. Two features characteristic of this MOA do not occur: (1) mineralization of tubules in the chronic bioassay; (2) male-rat-only nephrotoxicity.	Kidney tumors are not specific to the male rat. This MOA only occurs at concentrations greater than those necessary for tumor induction.
	PPAR-α receptor activation in kidney.	Peroxisome proliferation occurs in kidney, but no evidence supports causal association with tumorigenesis	Peroxisome proliferation is greater in mouse kidney than rat kidney; however male rats develop kidney tumors.	Little evidence to support this MOA.

Table 4-13. Summary of potential modes of action for cancer (continued)

Organ, tumor type	Potential MOA	Evidence for MOA	Limitations/evidence against MOA	Weight of evidence
Liver hepatocellular carcinoma in male and female mice.	PPAR-α receptor activation	PPAR-α is found in human liver, testis, pancreas.	Perc[1] only weakly activates PPAR-α. There are no studies in PPAR-α null mice evaluating PERC-induced tumors.	Some, but not conclusive, evidence for this MOA
	Mutagenicity produced by P450 pathway in liver.	Tetrachloroethylene oxide and DCA are mutagenic and other metabolites (trichloroacetyl chloride) are capable of binding to DNA.	Genotoxicity is commonly assumed to contribute to carcinogenesis.	Some, but not conclusive, evidence for this MOA
	Necrosis of liver cells leads to compensatory hyperplasia, excess mutation rate and neoplastic transformation.	No evidence, but plausible hypothesis.	Cytotoxicity and compensatory hyperplasia are not observed at bioassay doses.	Some evidence against this MOA.
Liver and spleen hemangiosarcoma in male mice.	Mutagenicity of P450 and/or GSH-derived reactive intermediates.	Tetrachloroethylene oxide and DCA are mutagenic and other metabolites (trichloroacetyl chloride) are capable of binding to DNA.	Genotoxicity is commonly assumed to contribute to carcinogenesis.	No studies have explored this MOA for tetrachloroethylene-induced hemangiosarcomas.
Blood mononuclear cell leukemia in male and female rats.	Mutagenicity. There is a potential for circulating genotoxic metabolites, or their precursors produced by liver metabolism, to be further processed to reactive products in other tissues.	DCVC, a metabolite of trichloroethylene, induced blood dyscrasias (aplastic anemia, DNA alteration in bone marrow, lymph nodes and thymus in calves.	TCVC, the analogous perc metabolite, did not produce bone marrow injury in calves in a single study.	No further studies have explored this MOA.
Brain glioma in male rats. (Rare)	Perc incorporation into brain cell membranes, disruption of membrane ion channels, interference with neurotransmitters.	Perc induces persistent changes in fatty acid composition of brain in rodents.	No information about cell membrane changes at low doses or longer than acute exposures. Connection with carcinogenesis is speculative.	No studies have explored this MOA.

Table 4-13. Summary of potential modes of action for cancer (continued)

Organ, tumor type	Potential MOA	Evidence for MOA	Limitations/evidence against MOA	Weight of evidence
Brain glioma in male rats. (Rare) (continued)	Mutagenicity of metabolites generated in situ.	Beta lyase has been observed in rat brain tissue. Therefore a potential exists for metabolizing TCVG/TCVC to reactive compounds.	Pathway from genotoxic metabolites to brain cancer is not defined.	Little evidence for this MOA.

[1] Perc = Tetrachloroethylene

Table 4-14. Quantitative Implications of different modes of action: candidate modeling approaches

Organ	Potential MOA	Weight of evidence	Inference about low-dose shape	Candidate models	Limitations of extrapolation procedure
Kidney adenocarcimoma in male rats.	Mutagenicity of GSH-derived metabolites.	Evidence for MOA.	Linear no threshold.	POD, linear extrapolation.	Model is commonly used. As implemented in the current BMDL software, upper confidence limits on dose are not calculated.
	Necrosis, hyperplasia, neoplastic transformation.	No direct evidence.	Non-linear model resulting from distribution of thresholds for individuals in a population.	Log probit or Log logistic.	Variability of thresholds is not the same in humans as in animals. BMDS implementation of models is not believed to be accurate.
	Alpha-2μ in hyaline droplets in male rats.	Not likely to occur at bioassay doses.	MOA does not occur in humans.		
	PPAR-α activation.	Little evidence.	Too little evidence to inform modeling approach.		
Liver hepatocellular carcinoma in male and female mice.	PPAR-α receptor activation, peroxisome proliferation, hyperplasia, neoplastic transformation.	Some, but not conclusive, evidence for this MOA.	Receptor binding shape could be linear, sub-linear or supra-linear, depending on binding constants.	Model for receptor binding induced by gene activation. (Kohn et al., 1993).	Binding data to inform modeling approach not available.
	Mutagenicity of P450 metabolites.	Some, but not conclusive, evidence for this MOA.	Linear no threshold.	POD, linear extrapolation.	Model is commonly used. As implemented, upper confidence limits on dose are not given.
	Necrosis, hyperplasia, neoplastic transformation.	Evidence against this MOA. Probably not occurring.	Too little evidence to inform modeling approach .		
Liver and spleen hemangiosarcoma in male mice.	Mutagenicicity (e.g., of GSH metabolites).	No studies have explored this MOA.	Linear no threshold.	POD, linear extrapolation.	Model is commonly used. As implemented, upper confidence limits on dose are not given.

Table 4-14. Quantitative Implications of different modes of action: candidate modeling approaches (continued)

Organ	Potential MOA	Weight of evidence	Inference about low-dose shape	Candidate models	Limitations of extrapolation procedure
Blood mononuclear cell leukemia in male and female rats.	Mutagenic GSH metabolites.	Only two conflicting, inconclusive reports.	Linear no threshold.	POD, linear extrapolation.	Model is commonly used. As implemented, upper confidence limits on dose are not given.
Brain glioma in male rats (rare tumor).	Perc[1] incorporation into cell membranes.	No evidence.	Linear no threshold.	POD, linear extrapolation.	Model is commonly used. As implemented, upper confidence limits on dose are not given.
	Mutagenicity of metabolites generated in situ.	Little evidence but plausible.	Linear no threshold.	POD, linear extrapolation.	Model is commonly used. As implemented, upper confidence limits on dose are not given.

[1] Perc = Tetrachloroethylene

4.10.4. Rationale for Selection of Dose Metric

4.10.4.1. *Liver*

There are several possible choices to consider as the dose metric for tetrachloroethylene-induced liver toxicity and carcinogenicity. First is administered dose or exposure concentration. Tetrachloroethylene hepatotoxicity is associated, however, with cytochrome P450 metabolism occurring in the liver. Several investigators have reported hepatotoxicity in rodent studies to be directly related to metabolism. Because liver toxicity, including carcinogenicity, is generally considered to be caused by metabolites rather than by the parent compound, choosing the specific chemical species responsible for adverse effects in the liver would be the preferred choice over administered dose/exposure concentration, particularly since tetrachloroethylene metabolism is nonlinear with dose of parent compound, with the percent metabolized decreasing with increasing dose.

TCA is considered a key product of this P450 oxidation pathway. TCA is the major urinary metabolite from tetrachloroethylene biotransformation, and it is the principal metabolite in the systemic circulation. TCA, like the parent compound, also causes liver toxicity and carcinogenicity in mice. Therefore, the second plausible dose metric for use with the liver target tissue is the concentration and AUC for TCA.

The MOA for tetrachloroethylene-induced liver toxicity and carcinogenicity is not clear, however, and whether TCA is the sole contributory metabolite to tetrachloroethylene-induced hepatotoxicity and cancer is unknown. Other possible P450 oxidation products, such as DCA, are also associated with liver toxicity when administered directly. In addition, it is not known whether reactive intermediates such as tetrachloroethylene oxide and trichloroacetyl chloride are involved in tetrachloroethylene-induced liver toxicity.

Hepatic toxicity correlates better with metabolism than with administered dose. In other words, a better linear relationship exists between metabolism and hepatotoxicity than between administered dose and hepatotoxicity. Because of the uncertainty about which metabolite species are involved in causing liver toxicity and the degree to which they are involved, the most appropriate dose metric is considered to be total metabolism. Production of the putative metabolites is then considered to be directly proportional to the total amount of tetrachloroethylene metabolized, a reasonable assumption.

4.10.4.2. *Kidney*

More than one choice was considered for the kidney target organ dose metric. The most simplistic dose metric is administered dose or exposure concentration. However, renal toxicity, including kidney cancer, is associated with metabolism. It is specifically associated with GSH-dependent metabolism, although P450 metabolism could potentially contribute to renal toxicity.

It is generally accepted that the interorgan GSH-dependent pathway, which also occurs completely in the kidney target organ, results in the production and accumulation of mutagenic metabolites. Unfortunately, the measurements of GSH-dependent metabolism are from in vitro studies or are of urinary excretion products, and are not representative of the toxic species in vivo.

The total production of the thioketene reactive intermediate divided by the volume of the kidney has been proposed as the dose metric for use in the PBPK model for kidney target organ. In order to use this dose metric, however, several assumptions must be made. One assumption might be that all GSH conjugate formed in the liver is transported to the kidney. Excretion of N-acetyl TCVC is the measurement used to represent flux through the pathway. Clearance of TCVC would be modeled, and production of toxic metabolites would be assumed to be proportional to overall flux. Unfortunately, the flux through the beta lyase and FMO3/CYP3A—or sulfoxide-producing branches of the pathway—has not been measured in vivo.

Better methods are needed to quantitate the reactive species that are generated during tetrachloroethylene metabolism, particularly in the beta lyase, FMO3 and CYP3A sections of the pathway, to improve the usefulness of data in development and validation of PBPK models. The amounts of N-acetyl TCVC excreted in urine represent only a portion of the flux through the overall GSH-dependent pathway. This excretion of mercapturate does not represent the processing of TCVC and also N-acetyl TCVC to the reactive and toxic products important to toxicity. The fraction of overall flux represented by the excretory product is simply unknown, and how it is related to the fraction processed through the beta lyase branch of the path is also unknown. Several products formed in the pathway are unstable and reactive, and, therefore, they are difficult to quantitate. Because the quantitative information about toxic metabolites from the GSH-dependent pathway is not available, and there is no way of knowing whether the measurement of excretory mercapturate is proportional to production of the toxic species produced in this pathway, the administered exposure and total metabolites are both considered as dose metrics (see Section 5.3.3.2). Production of the putative metabolites, then, is considered to be directly proportional to the total amount of tetrachloroethylene metabolized.

4.10.4.3. *Hematopoietic Target Organ*

Tetrachloroethylene causes mononuclear cell leukemia in rats. Although the specific mechanism of leukemogenesis in rats is not understood, neither is it well understood in humans. Whether the parent compound, a metabolite, or several metabolites are involved in the tetrachloroethylene induction of the leukemia is not known. In the case of the tetrachloroethylene congener trichloroethylene, the comparable DCVC conjugate metabolite has been associated with causing adverse effects to the hematopoietic system. A possible link to

MOA for tetrachloroethylene-induced MCL in rats comes from early reports of toxicity of cysteine S-conjugates where DCVC, the trichloroethylene metabolite, was implicated in induction of aplastic anemia and marked biochemical alteration of DNA in bone marrow, lymph nodes, and thymus in calves (McKinney et al., 1957; Schultze et al., 1959; Bhattacharya and Schultze, 1971, 1972). To the contrary, however, the single study of TCVC, the tetrachloroethylene conjugate, in calves, did not result in the adverse effects observed in studies of exposures to DCVC. Therefore, because considerable uncertainty surrounds the identification of the causative chemical species, the administered exposure and total metabolites are both considered as dose metrics (see Section 5.3.3.2). Production of the putative metabolites is considered to be directly proportional to the total amount of tetrachloroethylene metabolized.

4.10.4.4. *Central Nervous System*

As discussed in Section 4.6.4, the best surrogate for internal dose is blood tetrachloroethylene concentration.

APPENDIX 4A: CONSISTENCY OF TETRACHLOROETHYLENE AND TRICHLOROACETIC ACID HEPATOCARCINOGENICITY

TCA, a metabolite of tetrachloroethylene, is associated with hepatocarcinogenicity in male and female mice (Bull et al., 1990, 2002; Daniel et al., 1993; DeAngelo et al., 2008; Herren-Freund et al., 1995; Ferreira-Gonzalez, 1987; Pereira, 1996), as is tetrachloroethylene (NCI, 1977; NTP, 1986; JISA, 1992).

There has been some suggestion that TCA does not account for all of the toxicity observed with tetrachloroethylene exposure (Buben and O'Flaherty, 1985; Clewell et al., 2005). The purpose of this investigation was to compare the incidence of hepatocarcinogenicity observed with tetrachloroethylene exposure to that observed with TCA exposure, in order to examine whether the TCA that is expected to be generated by tetrachloroethylene can account for tetrachloroethylene's hepatocarcinogenicity. This was carried out by pooling the separate TCA studies, fitting a time-to-tumor model to the TCA data, and comparing the incidence of hepatocellular tumors expected based on the TCA studies with that observed in the tetrachloroethylene bioassays.

4A.1. METHODS

Table 4A-1 summarizes data from the available TCA studies considered for carrying out dose-response modeling. As detailed below, a number of these TCA studies lack information for a complete comparison of hepatocarcinogenicity between tetrachloroethylene and TCA.

4A.1.1. Response Data

EPA generally emphasizes combining hepatocellular adenomas and carcinomas in developing cancer risk values, for three reasons: (1) Hepatocellular adenomas develop from the same cell lines as carcinomas and can progress to carcinomas; (2) Adenomas are often distinguished from carcinomas only on the basis of size; and (3) histopathologic decision criteria may vary between laboratories or over time.

However, most of the TCA studies either did not consider adenomas or did not report combined incidence of adenomas and carcinomas. Lacking data on adenomas, the studies that only provided carcinoma incidence may under-represent hepatocellular tumor incidence. For studies not reporting combined incidence of adenomas and carcinomas, there could be some double-counting of animals when the separate totals of adenomas and carcinomas are added together.

Therefore, for the purposes of this analysis, only the chronic data of DeAngelo et al. (2008) and the chronic data of Pereira (1996) were considered further for comparing with the tetrachloroethylene bioassays in male and female mice, respectively. The DeAngelo et al. study was conducted at the lowest TCA levels of all the available studies, with exposures spanning about 6 to 60 mg/kg-day; these exposure levels span the range of TCA equivalents in the tetrachloroethylene bioassays. Comparison of the Pereira data with the responses of the female mice in the tetrachloroethylene bioassays is limited by the availability of carcinoma data only, and by the study being conducted only through Week 82, not through Week 104.

Table 4A-3 provides the hepatocellular adenoma or carcinoma incidence data from the two tetrachloroethylene bioassays considered in this assessment, NTP (1986) and JISA (1993) (for convenience, the studies will be referred to in the remainder of this appendix as the NTP and JISA studies). For comparison across data sets, all incidences were normalized by converting each to extra risk, [P(d)-P(0)]/[1-P(0)].

4A.1.2. Exposure Level Conversions

TCA bioassay exposures were generally reported in terms of water concentration, in mg/L or mmol/L. Table 4A-1 provides the exposure levels as reported by each set of authors. Some reports provided mg/kg-day equivalents. TCA exposures in mg/kg-day for the Pereira (1996) study were interpolated from the other TCA studies which reported exposures in mg/kg-day (see Table 4A-2).

The Reitz et al. (1996) PBPK model was used to estimate total metabolites corresponding to the bioassay exposures in the NTP and JISA studies. Then it was assumed that 60% of the total metabolites were TCA, as assumed in the model of Gearhart et al. (1993). Although it is possible that the extent of metabolism to TCA may be dose dependent, as for trichloroethylene-induced TCA, there were insufficient data to characterize a dose dependency of TCA formation for tetrachloroethylene.

The estimates of TCA induced by tetrachloroethylene exposure are internal doses, while the exposures in the TCA bioassays were administered doses. Because orally administered TCA has been estimated to be 95% absorbed in mice, the tetrachloroethylene-induced TCA estimates were adjusted by dividing by 0.95, in order to approximate administered TCA exposures that would be compatible with the dose-response modeling of the TCA drinking water studies.

4A.1.3. Dose-Response Model

The TCA data sets for male and female mice were fit separately. The male mice TCA were modeled using the multistage model (BMDS 1.4.1; U.S.EPA, 2007), given by:

$$P(d) = 1 - \exp[(-q_0 - q_1 \times d - q_2 \times d^2 \times \ldots q_6 \times d^6),$$

where d = exposure level.

The TCA data set for female mice was fit using a multistage-Weibull model because the only available data were limited to two time points less than the 104-week length of the tetrachloroethylene bioassays; this model provided a means of including both time points in the same analysis and facilitated extrapolation to 104 weeks. The multistage-Weibull model is given by:

$$P(d,t) = 1- \exp[(-q_0 - q_1 \times d - q_2 \times d^2 \times \ldots q_6 \times d^6) \times t^z,$$

where:

d = exposure level

t = time to observation of the tumor

q_i, z = parameters estimated in fitting the model

Time of scheduled sacrifice was input as the time to observation of each tumor. All tumors were taken to be incidental to the death of affected animals. The software used was Tox_Risk (see Section 5.4.4.1).

For comparison with the observed tetrachloroethylene data, model predictions were also adjusted to estimate extra risk.

4A.2. RESULTS

4A.2.1. Trichloroacetic Acid (TCA), Male Mice

Figure 4A-2 provides the result of fitting a multistage model to the DeAngelo et al. data. The responses at the control and low dose levels did not follow a monotonically increasing pattern (the low-dose response was lower than the control), but a nearly linear one-stage model provided an adequate fit (p = 0.15; model output included with Figure 4A-2).

4A.2.2. Trichloroactic Acid (TCA) Data, Female Mice

The evaluation of the model fit of the female mouse TCA data (Pereira, 1996) followed the same steps as for the male mice. The hepatocellular tumor data for female mice exposed to TCA in drinking water are shown in Table 4A-2 and Figure 4A-2. These data include groups of animals evaluated at three exposure levels plus control at two time points, for a total of eight groups. The response at the high dose (463 mg/kg-day) was very similar for both time points, at 25 - 28%. A one-stage model also provided the best fit to these data, with the two highest doses

at week 82 fitting least well. Because the fit at the lower doses was relatively good, no other attempts were made to refine the dose-response model for the TCA female mouse data.

4A.2.3. Comparison of Tetrachloroethylene Hepatocellular Tumor Data With Predictions Based on Trichloroacetic Acid Data

For the male mice, the extra risk of adenomas or carcinomas observed following 104 weeks of inhalation exposure to tetrachloroethylene in the two bioassays is provided in Table 4A-4, for comparison with the predicted extra risk of adenomas or carcinomas from the TCA-based dose-response modeling for male mice (based on the data of DeAngelo et al., 2008). For each male exposure group in the tetrachloroethylene bioassays, the observed proportion responding is higher than that predicted using the TCA drinking water study, by 2- to 12-fold. Mitigating factors to investigate further include possible differences in histopathology protocols between laboratories and adequacy of the assumptions used to derive the TCA-equivalents corresponding to the tetrachloroethylene exposure levels. Comparison between the tetrachloroethylene and TCA studies for the male mice at Week 104 suggests concordance, but "inconclusive" appears to be a plausible conclusion as well.

For the female mice, the extra risk of carcinomas observed following 104 weeks of inhalation exposure to tetrachloroethylene in the two bioassays is provided in Table 4A-5, for comparison with the predicted extra risk of carcinomas from the TCA-based dose-response modeling for female mice (based on the data of Pereira, 1996). The female mouse TCA model appears to agree with the lack of carcinomas in female mice at the lower two exposures in the JISA study, at approximately 3 and 11 mg/kg-day, but underestimates the observed incidence at 30 mg/kg-day of 29% by 90-fold. In contrast, the TCA model underpredicts the observed carcinomas in both exposed groups of female mice in the NTP study by more than 200-fold. In addition to the mitigating factors mentioned above, note that the tetrachloroethylene bioassays were conducted at exposures associated with lower TCA levels than were used in the female mouse TCA study. That is, for JISA female mice, the highest bioassay exposures were associated with 28 mg/kg-day TCA (NTP) and 30 mg/kg-day, and the lowest exposure level in the TCA study was approximately 47 mg/kg-day. Consequently, there is a degree of extrapolation beyond the TCA data set that may impact the predictions. Also note that the tetrachloroethylene bioassays do not have sufficient resolution (let alone statistical power) to detect response levels as low as those predicted by the TCA model in this range of exposures (bioassays with 50 animals/group cannot provide estimates below 1/50 or 2%).

4A.3. DISCUSSION AND CONCLUSIONS

This analysis suggests that TCA may not explain the incidence of carcinomas observed in the available tetrachloroethylene bioassays, at least at TCA levels near 2 mg/kg-day in male mice and 20 mg/kg-day in female mice. Otherwise, these data are inconclusive.

As mentioned earlier in this appendix, some of the assumptions made in quantifying the dose-response relationships may have contributed to an overestimate of TCA's carcinogenicity. The Pereira study involved planned sacrifices at the reported time points, while the tetrachloroethylene studies did not. This would have led to earlier detection of tumors in the TCA studies relative to the tetrachloroethylene bioassays due to the detection of some tumors before they may have become fatal, and, therefore, a slightly higher estimate of carcinoma incidence in the time-to-tumor model. Therefore, dose-response estimates based on the female TCA study may contribute to overestimating risk, all else being equal.

And as mentioned earlier, another uncertainty is the use of PBPK estimates of TCA levels resulting from inhalation or oral exposure to tetrachloroethylene. Another interpretation of tetrachloroethylene-induced TCA levels has been provided by Clewell et al. (2005), who provided TCA levels corresponding to the bioassay levels in the NTP bioassay, but not the JISA bioassay. These levels were 16.3 mg/kg-day for the low-dose males, 30.6 mg/kg-day for the high-dose males, 16.9 mg/kg-day for the low-dose females, and 31.6 mg/kg-day for the high-dose females. These levels differ from those estimated here by no more than 15%, which does not explain the differences in response levels compared in this analysis. Given the current state of the science, the impact of this source of uncertainty is not well understood.

The differing results from the other TCA studies underscore the need to consider the joint incidence of adenoma and carcinomas, which could have a substantial impact on this analysis. The relative time courses of and correlation between adenomas and carcinomas in the TCA bioassays are less clear, because relevant data were not included in the TCA reports. This is perhaps the most uncertain part of this analysis. Additional information should be obtained from the original investigators for further evaluation if possible, perhaps in a meta-analysis.

Table 4A-1. Trichloroacetic Acid (TCA) drinking water studies in male mice: incidence of hepatocellular adenomas and carcinomas

Source	Weeks of exposure	TCA exposure, g/L	Equivalent TCA exposure (mg/kg-day)	N	Incidence of adenomas	Incidence of carcinomas	Incidence of adenomas or carcinoma	Proportion responding with carcinomas
Bull et al. (1990)[a]	37	2	330	11	0	3	3	0.27
	52	0 1 2	0 170 330	35 11 24	0 2 1	0 2 4	0 NR NR	0.0 0.18 0.17
Bull et al. (2002)	52	0 0.5 2	0 NR NR	20 20 20	0 5 6	0 3 3	0 6 8	0.0 0.15 0.15
Herren-Freund et al. (1987)	61	0 5	0 NR	22 22	2 8	0 7	2 NR	0.0 0.32
Ferreira-Gonzalez et al. (1995)	104	0 4.5	0 NR	16[c] 11	NR NR	3[c] 8	NR NR	0.19 0.73
DeAngelo et al. (2008)	104	0 0.05 0.5	0 8 68	56 48 51	10 10 20	26 14 32	31 21 36	0.55 0.44 0.71

a Cumulative TCA exposures were provided in g/kg for the mice evaluated at 52 weeks. Those exposures were converted to mg/kg-day by multiplying by (1,000 mg/g)/(7 days/week * 52 weeks).

[c] Estimated from the reported proportion responding by selecting the smallest group size and incidence value consistent with the precision of the reported proportion.

NR = not reported

Table 4A-2. Trichloroactic acid (TCA) drinking water study in female mice—incidence of hepatocellular adenomas and carcinomas

Weeks of exposure	TCA exposure, g/L[a]	Equivalent TCA exposure[b] (mg/kg-day)	N	Incidence of adenomas	Incidence of carcinomas	Incidence of adenomas or carcinomas	Proportion responding with carcinomas
52	0.0	0	40	1	0	1	0.0
	0.3	47	40	3	0	3	0.0
	1.1	189	19	3	0	3	0.0
	3.3	463	20	2	5	NR	0.25
82	0.0	0	90	2	2	NR	0.022
	0.3	47	53	4	0	4	0.0
	1.1	189	27	3	5	NR	0.19
	3.3	463	18	7	5	NR	0.28

[a] Exposure concentration was reported in mmol/L.
[b] Estimated by interpolating exposures in Table 4A-1.

NR = not reported
Source: Adapted from Pereira (1996).

Table 4A-3. Incidence of hepatocellular adenomas and carcinomas in B6C3F1 mice exposed to tetrachloroethylene in two inhalation bioassays

Sex	Bioassay	Administered exposures, (ppm)	Cumulative liver tumor incidence at week 104			Total at risk[a]
			Adenomas	Carcinomas	Adenomas or carcinomas	
Male	NTP (1986)	0	12	7	17	49
		100	8	25	31	47
		200	19	26	41	50
	JISA (1993)	0	7	7	13	46
		10	13	8	21	49
		50	8	12	19	48
		250	26	25	40	49
Female	NTP (1986)	0	3	1	4	45
		100	6	13	17	42
		200	2	36	38	48
	JISA (1993)	0	3	0	3	50
		10	3	0	3	47
		50	7	0	7	48
		250	26	14	33	49

[a] Animals dying before the first appearance of a hepatocellular tumor, but no later than week 52, were omitted from the totals because these animals were presumed not to have adequate time on study to develop tumors.

Table 4A-4. Comparison of cumulative hepatocellular tumor incidence in male mice exposed for 104 weeks to tetrachloroethylene in chronic inhalation bioassays, to predictions based on trichloroacetic acid (TCA) exposure via drinking water

Study	N	Tetrachloro-ethylene exposure (ppm)	Total metabolites (mg/kg-day)	Tetrachloro-ethylene-induced TCA[a] (mg/kg-day)	Observed proportion responding[b,c]	Predicted proportion responding[b]	Observed/ predicted
NTP (1986)	49	0	0	0	0.0	0.0	-
	47	100	27	17	0.479	0.139	3
	50	200	41	26	0.724	0.204	4
JISA (1993)	46	0	0	0	0.0	0.0	-
	49	10	3	2	0.203	0.017	12
	48	50	14	9	0.158	0.076	2
	49	250	36	23	0.744	0.182	4

[a] Estimated using PBPK model of Reitz et al. (1996) and adjusted for use with the TCA dose-response model by dividing by 0.95 to approximate a drinking water exposure to TCA (see Section 4A.1.2).
[b] Extra risk.
[c] Calculated from Table 4A-1.

Table 4A-5. Comparison of cumulative hepatocellular carcinoma incidence in female mice exposed for 104 weeks to tetrachloroethylene in chronic inhalation bioassays, to predictions based on trichloroacetic acid (TCA) exposure via drinking water

Study	N	Tetrachloro-ethylene exposure (ppm)	Total metabolites (mg/kg-day)	Tetrachloro-ethylene-induced TCA[a] (mg/kg-day)	Observed proportion responding[b,c]	Predicted proportion responding[b]	Observed/ predicted
NTP (1986)	45	0	0	0	0.0	0.0	-
	42	100	31	20	0.287	0.0014	210
	48	200	45	28	0.728	0.0029	260
JISA (1993)	50	0	0	0	0.0	0.0	-
	47	10	4	3	0.0	0.00002	-
	48	50	18	11	0.0	0.00041	-
	49	250	47	30	0.286	0.0031	90

[a] Estimated using PBPK model of Reitz et al. (1996) and adjusted for use with the TCA dose-response model by dividing by 0.95 to approximate a drinking water exposure to TCA (see Section 4A.1.2).
[b] Extra risk.
[c] Calculated from Table 4A-2.

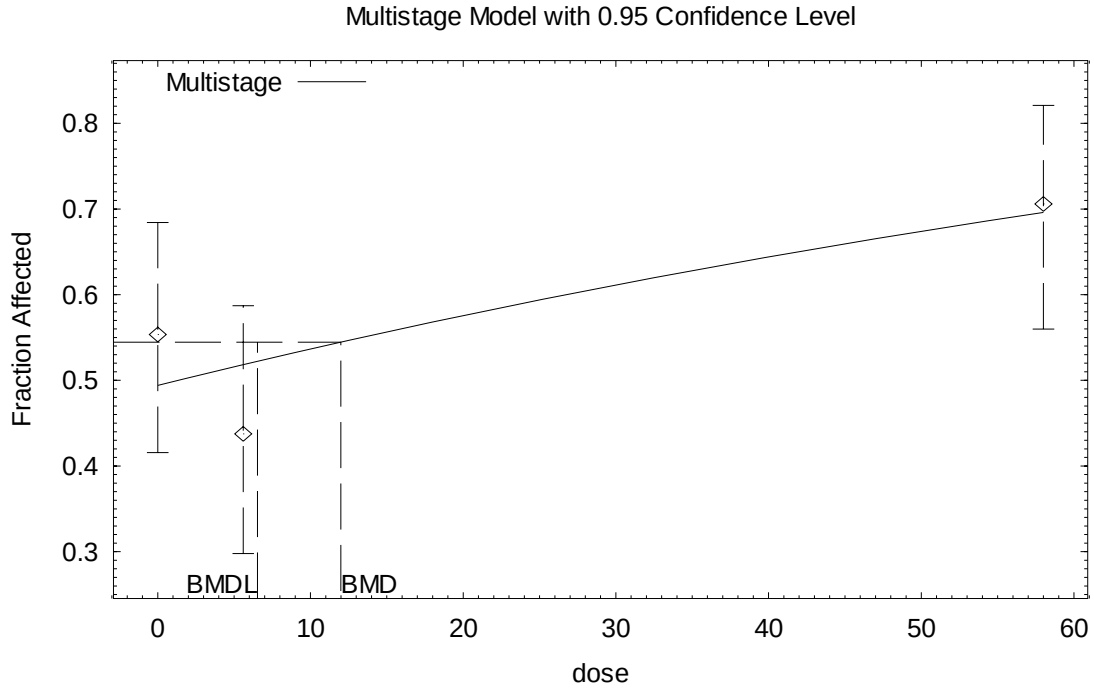


Figure 4A-1. Multistage dose-response fit of male mouse hepatocellular tumor incidence associated with exposure to trichloroacetic acid in drinking water; data from DeAngelo et al. (2008).

```
=================================================================
         Multistage Model. (Version: 2.7;  Date: 01/18/2007)
         Input Data File: C:\BMDS\UNSAVED1.(d)
         Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
  ================================================================

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

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = hep_a_c
   Independent variable = mg_kg_d

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values
Background = 0.489396
Beta(1) = 0.00926311

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.54
Beta(1)	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.493963	*	*	*
Beta(1)	0.00878738	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-102.285	3			
Fitted model	-103.323	2	2.07524	1	0.1497
Reduced model	-106.011	1	7.4518	2	0.02409

AIC: 210.645

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.4940	27.662	31	56	0.892
5.6000	0.5183	24.877	21	48	-1.120
58.0000	0.6960	35.497	36	51	0.153

Chi^2 = 2.07 d.f. = 1 P-value = 0.1499

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 11.99

BMDL = 6.53618

BMDU = 43.6117

Taken together, (6.53618, 43.6117) is a 90 % two-sided confidence interval for the BMD

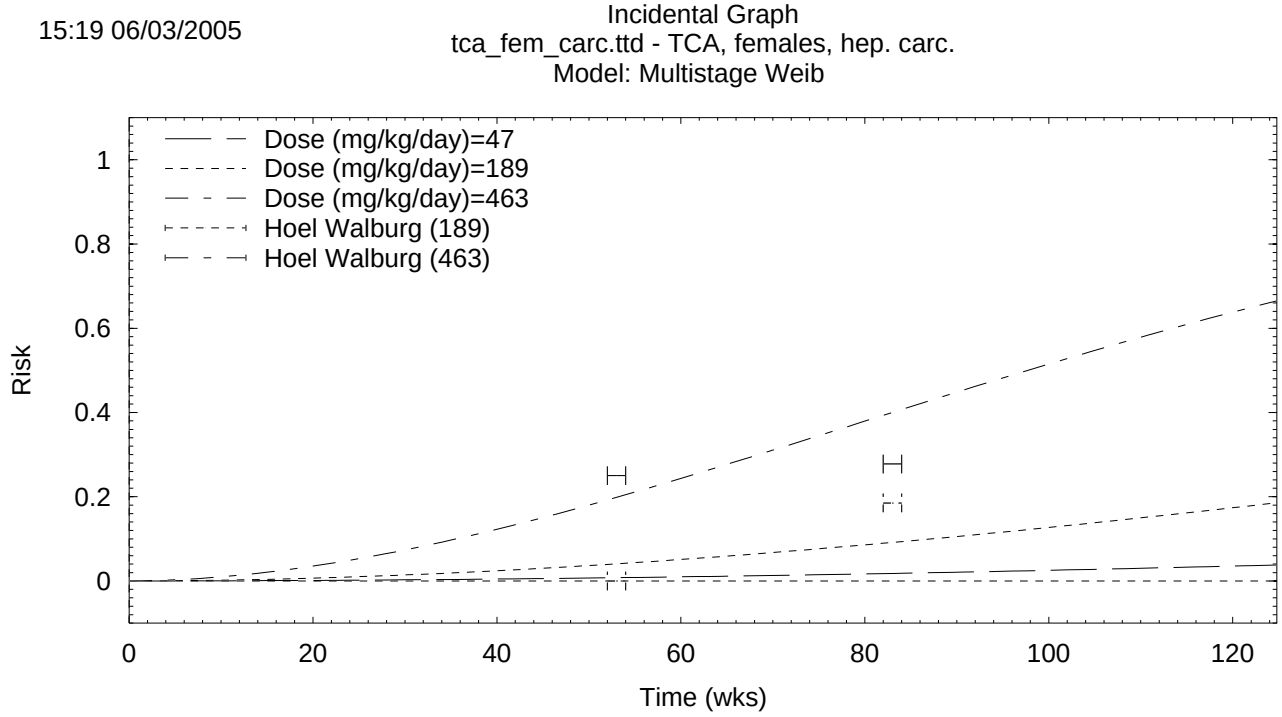


Figure 4A-2. Multistage-Weibull dose-response fit of female mouse hepatocellular carcinoma incidence associated with exposure to trichloroacetic acid in drinking water; data in Table 4A-2. Multistage-Weibull model parameters: $q_0 = 3.39 \times 10^{-6}$, $q_2 = 6.10 \times 10^{-5}$, $z = 1.9$.

APPENDIX 4B:
HUMAN STUDIES OF CANCER

The body of literature assessing carcinogenic effects associated with exposure to tetrachloroethylene is composed of cohort, proportionate mortality, and case-control studies. A small number of studies, including studies of cohorts involved in metal degreasing or in aircraft manufacturing/maintenance (Boice et al., 1999; Anttila et al., 1995; Spirtas et al., 1991), have assessed tetrachloroethylene exposure explicitly. These cohort studies present risks associated with site-specific cancer mortality (Boice et al., 1999; Spirtas et al., 1991) or incidence (Anttila et al., 1995) for a subcohort of the larger study population who were exposed to tetrachloroethylene. Additionally, a few case-control studies were able to examine the relationship between cancer at specific sites and tetrachloroethylene exposure (Vaughan et al., 1997; Schlehofer et al., 1995; Pesch et al., 2000a; Heineman et al., 1994).

A larger body of evidence on cancer exists for workers employed as dry cleaners. Dry cleaners have potential exposures to a number of solvents, including tetrachloroethylene, which began to be widely used in the early 1960s (IARC, 1995). Two cohorts, Ruder et al. (1994, 2001) and Blair et al. (1990, 2003), are of individuals primarily exposed to tetrachloroethylene (Lynge et al., 1997).

Last, several community-based drinking water studies are available (Aschengrau et al., 1993, 1998; Paulu et al., 1999; Fagliano et al., 1990; Cohn et al., 1994; MA DPH, 1997; Vartiainen et al., 1993; Lagakos et al., 1986). Exposure in most of these studies was to a mixture of solvents, including tetrachloroethylene and trichloroethylene, although the studies by Aschengrau et al. and Paulu et al. examined tetrachloroethylene specifically.

Tables 4B-1a–4B-13 summarize the observations from these epidemiologic studies. Table 4B-1a presents results of incidence studies of dry cleaners and laundry workers and Table 4B-1b those of mortality studies of dry cleaners. Three studies of aircraft maintenance/manufacturing workers or metal degreasing workers identified workers (a subcohort) exposed to tetrachloroethylene. Tables 4B-3–4B-13 present results of case-control studies of specific organ sites (e.g., lymphoma, liver, kidney, esophagus). A few studies assessed exposure to tetrachloroethylene, with the majority of studies evaluating the relationship between site-specific cancer and employment in dry cleaning.

Table 4B-1a. Standardized incidence ratios (95% confidence intervals) in cohort studies of dry cleaners and laundry workers

Site	Andersen et al. (1999) Male	Andersen et al. (1999) Female	Lynge and Thygesen (1990) Male	Lynge and Thygesen (1990) Female	Chow et al. (1995)	McLaughlin et al. (1987) Male	McLaughlin et al. (1987) Female
Bladder	1.1 (0.9–1.5) 62 obs/ 54.4 exp	0.9 (0.7–1.2) 57 obs/ 64.0 exp	0.6 (0.2–1.3) 6 obs/9.7 exp	0.9 (0.4–1.7) 8 obs/9.1 exp			
Breast		0.9 (0.8–0.97) 634 obs/ 712 exp		0.8 (0.7–1.1) 94 obs/110.7 exp			
Cervix		1.2 (1.01–1.4) 155 exp/ 131.4 exp		0.8 (0.6–1.2) 34 obs/40.3 exp			
Esophagus	0.8 (0.3–1.7) 7 obs/8.5 exp	1.0 (0.5–1.6) 14 obs/14.4 exp			1.0 (0.2–2.9) 3 obs/3 exp		
Hodgkin's disease	0 obs/4.0 exp	1.9 (1.1–2.9) 19 obs/10.1 exp					
Kidney	1.0 (0.7–1.5) 24 obs/ 23.3 exp	0.9 (0.7–1.2) 57 obs/64.8 exp	1.5 (0.6–3.3) 6 obs/4 exp	0.6 (0.2–1.4) 5 obs/8.6 exp		1.0 (0.6–1.6)[a] 18 obs/18.2 exp	0.9 (0.6–1.3)[a] 25 obs/29.1 exp

Table 4B-1a. Standardized incidence ratios (95% confidence intervals) in cohort studies of dry cleaners and laundry workers (continued)

Site	Andersen et al. (1999) Male	Andersen et al. (1999) Female	Lynge and Thygesen (1990) Male	Lynge and Thygesen (1990) Female	Chow et al. (1995)	McLaughlin et al. (1987) Male	McLaughlin et al. (1987) Female
Leukemia	0.7 (0.03–1.2) 12 obs/ 17.8 exp	0.9 (0.7–1.2) 46 exp/50.9 exp	0.7 (0.1–2.6) 2 obs/2.8 exp	0.8 (0.2–1.7) 5 obs/6.7 exp			
Primary liver	1.3 (0.6–2.3) 11 obs/ 8.7 exp	1.3 (0.9–1.9) 28 obs/21.2 exp	1.2[b] (0.3–3.5) 3 obs/2.5 exp	2.7[b] (1.5–4.5) 14 obs/5.2 exp			
Lung	1.2 (1.1–1.5) 141 obs/ 113.7 exp	1.2 (1.08–1.4) 172 obs/148.3 exp	1.1 (0.8–1.7) 28 obs/24.5 exp	1.3 (0.9–1.8) 32 exp/24.9 obs			
Multiple myeloma	1.4 (0.8–2.3) 14 obs/ 10.1 exp	0.9 (0.6–1.3) 31 obs/ 34.8 exp	3.3 (0.9–8.5) 4 obs/1.2 exp	1.1 (0.2–3.1) 3 obs/2.8 exp			
Non-Hodgkin's	1.5 (0.96–2.1) 27 obs/ 18.5 exp	1.0 (0.7–1.2) 55 obs/57.9 exp	2.8 (0.9–6.5) 5 obs/1.8 exp	0.5 (0.1–1.5) 3 obs/6 exp			
Pancreas	1.4 (0.98–2.0) 35 exp/ 24.8 exp	1.0 (0.8–1.3) 85 obs/83.3 exp	2.4 (1.1–4.5) 9 obs/3.8 exp	1.4 (0.7–2.4) 13 obs/9.3 exp			

Table 4B-1a. Standardized incidence ratios (95% confidence intervals) in cohort studies of dry cleaners and laundry workers (continued)

Site	Andersen et al. (1999) Male	Andersen et al. (1999) Female	Lynge and Thygesen (1990) Male	Lynge and Thygesen (1990) Female	Chow et al. (1995)	McLaughlin et al. (1987) Male	McLaughlin et al. (1987) Female
Prostate	1.1 (0.9–1.3) 118 obs/ 11.3 exp		1.5 (0.7–2.6) 11 obs/7.6 exp				
Rectum	0.9 (0.6–1.3) 33 obs/ 37.1 exp	1.0 (0.8–1.2) 106 obs/ 110.4 exp	1.4 (0.6–2.6) 9 obs/6.5 exp	0.7 (0.4–1.3) 11 obs/15.5 exp			
Skin	1.0 (0.8–1.3) 60 obs/ 60.7 exp	0.9 (0.8–1.04) 174 obs/ 196.1 exp	1.1 (0.6–1.9) 14 obs/12.7 exp	0.7 (0.5–1.1) 23 obs/32.1 exp			
Stomach	1.3 (0.9–1.7) 47 obs/ 37.6 exp	1.0 (0.8–1.2) 89 obs/93.7 exp	1.3 (0.5–2.7) 7 obs/5.3 exp	1.3 (0.6–2.3) 11 obs/8.6 exp			

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5. DOSE-RESPONSE EVALUATION

5.1. INHALATION REFERENCE CONCENTRATION (RfC)

Although the RfD is commonly presented first in the IRIS toxicological reviews, the RfC is presented in Section 5.1 and the RfD in Section 5.2 because the available data were primarily from inhalation exposure and pharmacokinetic modeling was available to carry out route-to-route extrapolation of the RfC to the oral route of exposure.

The RfC[1] for tetrachloroethylene is derived through a process of (1) considering all studies and selecting the adverse health effects that occur at the lowest exposure concentration, (2) selecting the point of departure (POD)[2] at which the adverse health effect either is not observed or would occur at a relatively low prevalence (e.g., 10%), (3) deriving the POD in terms of the human equivalent concentration (HEC), and (4) reducing this exposure concentration by uncertainty factors (UFs) to account for uncertainties in the extrapolation from the study conditions to an estimate of human environmental exposure. This is EPA's first attempt to define a tetrachloroethylene RfC for IRIS. Health assessments from other agencies, more fully described in Appendix A, have included a criterion for noncarcinogenic effects associated with inhalation exposure based on neurotoxic effects observed in human epidemiologic studies (NYS DOH, 1997; ATSDR, 1997).

5.1.1. Choice of Principal Study and Critical Effect

The database of human and animal studies on inhalation toxicity of tetrachloroethylene is adequate to support derivation of inhalation reference values. A number of targets of toxicity from chronic exposure to tetrachloroethylene, include the nervous system, liver, kidney, reproductive system, and developing fetus, with published reports in both animals and humans. Greatest consideration is given to human data, if adequate, to develop an RfC.

Neurological effects were judged to be associated with lower tetrachloroethylene concentrations. This finding is in agreement with Rao and Brown (1993), who, using categorical analysis methods, identified neurological effects as the most sensitive noncancer toxicity

[1]The RfC is 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 appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, a LOAEL, or a benchmark concentration, with uncertainty factors generally applied to reflect limitations of the data used. It is generally used in EPA's noncancer health assessments. The RfC, like the RfD for oral exposure, is based on the assumption that thresholds exist for certain toxic effects, such as liver pathology, but may not exist for other toxic effects, such as carcinogenicity.

[2]The POD denotes a dose at the lower end of the observed dose-response curve where extrapolation to lower doses begins. For effects other than cancer, the POD is either a NOAEL, a LOAEL if no NOAEL can be identified, or a modeled point (e.g., a $BMCL_{10}$ or an LED_{10}) if the data are suitable for dose-response modeling.

endpoint. A number of studies assessing neurobehavioral effects in both humans and rodents are available for RfC analysis. The epidemiologic body of evidence is characterized by studies that used standardized neurobehavioral batteries. In addition, some studies employed assessment of visual function (Cavalleri et al., 1994; Schreiber et al., 2002; Echeverria et al., 1994, 1995), a neurological outcome known to be sensitive to volatile organic compounds. Most epidemiological studies have examined occupational exposure to tetrachloroethylene. Two epidemiological studies examined residential exposure to tetrachloroethylene (Altmann et al., 1995; Schreiber et al., 2002). Together, the epidemiologic evidence supports an inference of a broad range of cognitive, behavioral, and visual functional deficits following tetrachloroethylene exposure (U.S. EPA, 2004).

The research in animal models on the effects of tetrachloroethylene on functional neurological endpoints consists of screening studies (functional observation battery, motor activity) or effects on sensory system function as assessed by evoked potential. Some consistency is seen in the animal models, with effects on motor activity and motor function following exposure to tetrachloroethylene in either the adult or the developmental period, changes in evoked potentials following acute and subchronic exposures, and replication of observed alterations in brain DNA, RNA, or protein levels and brain weight changes.

Of the studies discussed in Chapter 4, a number of epidemiologic studies of neurological effects in either occupational workers or residential subjects with tetrachloroethylene exposure are considered for the principal study with which to identify the POD. No single epidemiologic study stands out as a superior candidate for identifying the POD, as all of the available studies have limitations. However, some studies are considered more desirable as a principal or critical study than other studies for the reasons below. The epidemiologic studies by Ferroni et al. (1992) and Spinatonda et al. (1997) have associated uncertainties related to incomplete reporting and are methodologically weaker than other epidemiologic studies, such as those of Seeber (1989), Altmann et al. (1995), or Echeverria et al. (1994, 1995), that assessed neurobehavioral functions. The studies by Echeverria et al. (1994, 1995) are not informative for supporting a POD because the authors attribute observed visual function effects to past higher tetrachloroethylene concentrations and historical exposure data that are not available. Table 5-1 identifies study characteristics and the rationale for the principal study considered in the RfC analysis.

Seeber (1989) reports effects on visuo-spatial function, as does the residential study by Altmann et al. (1995). Both studies are considered adequate for quantitative analysis, given the numbers of study subjects (with Seeber et al., 1989, having the larger number of study subjects), and their use of appropriate statistical methods, including methods to adjust for potentially

Table 5-1. Summary of rationale for principal study selection

Consideration/ approach	Type of data	Decision
Quality of study	Animal neurotoxicity studies	Animal neurotoxicity studies are considered as supporting studies. An RfC/RfD from human data, if available and of adequate quality, is preferred for reducing interspecies extrapolation uncertainties.
Quality of study	Human neurotoxicity studies	Both occupational and residential studies on tetrachloroethylene exposure contain uncertainties regarding their use for quantitative analysis. Some of these epidemiology studies carry greater weight for quantitative analysis than other studies. The occupational studies of Seeber (1989) and Cavelleri et al. (1994), and the residential study of Altmann et al. (1995) are considered to carry greater weight for quantitative analysis than Ferroni et al. (1992), Echeverria et al. (1994, 1995), and Spinatonda et al. (1997).
Measurement tool	Standardized neurobehavioral battery	Both occupational and residential epidemiology studies assessed neurobehavioral function using a standardized neurobehavioral battery. The battery has been widely administered to occupational population in different setting with a reasonably high degree of validity. WHO and ATSDR recommend these test methods to evaluate nervous system deficits in adults and children.
Endpoint	Deficits in neurological domains such as attention, motor function, vigilance,or visuo-spatial function.	There is congruence of neurological effects observed in studies of both residential and occupational populations. These domains are also sensitive to acute tetrachloroethylene exposure in controlled human studies. The consistency of observed effects between occupational and residential populations and their persistence with lower tetrachloroethylene concentration, as experienced by residential populations, provide a strong rationale for a study of lower-level exposures as the basis for the RfC.
Relevance of exposure scenario	Epidmiology studies of residential populations	Tetrachloroethylene exposure to residential populations is of lower concentration and of chronic duration compared to acute duration and higher concentration exposure to occupational populations. Additionally, potential to tetrachloroethylene peak or intensity concentrations is more common with occupational exposures. A study of residential exposure, if adequate and of similar quality as an occupational epidemiology study, is preferred for supporting the RfC because it better represents exposure scenarios of interest to EPA.

confounding factors. However, in both studies, statistical analyses that adjusted for potential confounders may have not have been fully complete due to the use of categorical variables.

The report by Cavalleri et al. (1994) is consistent with the growing body of literature indicating that chronic exposure to a variety of volatile organic solvents, including tetrachloroethylene, toluene, styrene, and carbon disulfide, is associated with deficits in visual perception measured either as deficits in color vision or deficits in VCS (see Section 4.6.1); visual perception is a sensitive test of neurological impairment. The study authors reported poorer performance on a test of color vision among dry cleaning operators. A statistically significant lower prevalence of tetrachloroethylene-exposed dry cleaners had perfect scores on a

test of color perception, and their mean score was higher as compared with those of controls, indicating impaired color vision among dry cleaners. No effects were observed in laundry workers with exposure to a lower mean TWA concentration than that of dry cleaning operators. These effects of tetrachloroethylene-induced deficits in color vision are supported by other studies (Muttray et al., 1997; Gobba et al., 1998).

Deficits in visual function were also reported in Schreiber et al. (2002), a study originally designed as a pilot for a study composed of a larger number of subjects, assessed tetrachloroethylene with air monitoring and markers in biological samples (biomarkers). This pilot study was expanded after its inception to include control subjects and tests of VCS (NYS OAG, 2004). The findings in Schreiber et al. (2002), a first report of VCS deficits, need replication. For these reasons, although this study contributes to the weight of evidence for hazard identification, it is less desirable than the residential study of Altmann et al. (1995) as a critical study for developing an RfC.

The study by Altmann et al. (1995) is chosen as the principal study for a number of reasons. First, it adopted a standardized neurobehavioral battery to evaluate neurological effects in residents. Tests in this battery have been widely administered to occupational populations in different settings with a reasonably high degree of validity (Anger et al., 2000). Additionally, several public health organizations, such as the World Health Organization and the ATSDR in the United States, recommend these test methods to evaluate nervous system deficits in adults and children (Anger et al., 1994, 2000, 2003; ATSDR, 1996; Amler et al., 1994). Second, there is congruence of neurological effects observed in studies of both residential and occupational populations. As shown in Table 4-1 (Chapter 4), decrements in a number of neurological domains such as attention, motor function, and vigilance reported by Altmann et al. (1994) are also reported for occupationally exposed populations. The consistency of these effects between the two populations and their persistence with lower tetrachloroethylene concentration, as experienced by residential populations, provide a strong rationale for a study of lower-level exposures as the basis for the RfC. Last, a study of residential exposures is preferred for quantitative analysis because it better represents exposure scenarios of interest to EPA. Table 5-2 identifies studies and outcomes considered for quantitative analysis.

Table 5-1 summarizes chronic, subchronic or longer-term, and developmental toxicity studies considered for derivation of the inhalation RfC and are a subset of the body of evidence on tetrachloroethylene more fully described in Chapter 4. These studies are considered supportive of a POD and an RfC because they report effects associated with lower exposure concentrations or are studies with multiple experimental exposures, allowing exploration of benchmark dose (BMD) approaches. For each study, Table 5-1 identifies the species; the

Table 5-2. Inhalation studies considered in the development of an RfC

Organ/ system	Study	Species	Duration/ exposure route	NOAEL/LOAEL[a] (ppm)	Effect	Human equivalent continuous concentrations[b] (ppm)	
						NOAEL/LOAEL	BMCL[c]
Liver	Brodkin et al. (1995)	Human	20 years mean duration	4.6, 19.8 (overall mean, 16)	Hepatic parenchymal changes	LOAEL: 2	$BMCL_{10}$: 0.5
	Gennari et al. (1984)	Human	12 years mean duration	11.3 (mean)	Gamma glutamyl transpeptidase	LOAEL: 4	NA
	Kjellstrand et al. (1984)	Mouse	Subchronic (4 weeks) continuous	0, 9, 37, 75, 150	Increased liver weight	LOAEL: 9	$BMCL_S$: 0.6 $BMCL_{S/P}$: 1.4–10
	NTP (1986)	Mouse	Chronic bioassay (104 weeks)	0, 100, 200	Increased liver degeneration, necrosis	LOAEL: 18	NA
	JISA (1993)	Mouse	Chronic (104 weeks)	0, 10, 50, 250	Increased angiectasis	NOAEL: 2	$BMCL_{10}$: 2 $BMCL_{10/P}$: 4.3–23
Kidney	Verplanke et al. (1999)	Human	4 years duration (geometric mean)	1.2 (mean)	Retinol binding protein	LOAEL: 0.3	NA
	Trevisan et al. (2000)	Human	15 years duration (geometric mean)	8.8 (mean)	Glutamine sythetase	LOAEL: 3	NA
	Franchini et al. (1983)	Human	14 years mean duration	10	Lysozyme	LOAEL: 4	NA
	Mutti et al. (1992)	Human	10 years duration	15 (median)	Urine and serum markers of nephrotoxicity	LOAEL: 5	
	NTP (1986)	Rat	Chronic bioassay (104 weeks)	0, 200, 400	Increased karyomegaly, megalonuclear-cytosis	LOAEL: 36	$BMCL_{10}$: 2.2
CNS	Altmann et al. (1995)	Human	10.6 years median duration	0.7 (mean) 0.2 (median)	Verbal function , cognitive function, vigilance, vision	LOAEL: 0.7	NA
	Schreiber et al. (2002)	Human	5.8 years mean duration	0.1 (residents, median and mean), maybe as high as 0.4 (mean) and 0.3 (median	Visual contrast sensitivity	LOAEL: 0.4	NA

Table 5-2. Inhalation studies considered in the development of an RfC (continued)

Organ/ system	Study	Species	Duration/ exposure route	NOAEL/LOAEL[a] (ppm)	Effect	Human equivalent continuous concentrations[b] (ppm)	
						NOAEL/LOAEL	BMCL[c]
CNS (cont.)	Schreiber et al. (2002)	Human	4 years mean duration	0.3 (daycare workers, mean and median)	Visual contrast sensitivity	LOAEL: 0.1	NA
	Cavalleri et al. (1994)	Human	8.8 years mean duration	7	Dyschromatopsia	LOAEL: 3	NA
	Spinatonda et al. (1997)	Human	Inhalation (no information on duration)	8 (median)	Reaction time	LOAEL: 3	NA
	Seeber (1989)	Human	>10 years mean duration	12, 53	Visuospatial function, information processing speed	LOAEL: 4	NA
	Ferroni et al. (1992)	Human	10.6 years mean duration	15	Reaction time, continuous performance	LOAEL: 5	NA
					Endocrine: prolactin	LOAEL: 4	NA
	Echeverria et al. (1995)	Human	15 (high-exposure group) years mean duration	41 (operators)	Visuospatial function	LOAEL: 15	NA
	Kjellstrand et al. (1984)	Mouse	Subchronic (4 weeks) continuous	0, 9, 37, 75, 150	Butyryl cholinesterase	NOAEL: 9	NA[e]
	Rosengren et al. (1986)	Gerbil	Subchronic (12 weeks, with 16-week follow-up) continuous	0, 60, 300	Brain: protein, DNA concentration	LOAEL: 60	NA
	Mattsson et al. (1998)	Rat	Subchronic (13 weeks) 6hrs/d, 5d/w	0, 50, 200, 800	Flash-evoked potential	NOAEL: 36	NA
	Wang et al. (1993)	Rat	Subchronic (12 weeks) continuous	0, 300, 600	Reduced brain weight, DNA, protein	NOAEL: 300	NA

Table 5-2. Inhalation studies considered in the development of an RfC (continued)

Organ/ system	Study	Species	Duration/ exposure route	NOAEL/LOAEL[a] (ppm)	Effect	Human equivalent continuous concentrations[b] (ppm)	
						NOAEL/LOAEL	BMCL[c]
Reproductive/ developmental	Eskenazi et al. (1991)	Human	9.5 years mean duration, occupational	1.2	Sperm quality	LOAEL: 0.4	NA
	Nelson et al. (1980)	Rat	7 hrs/day on gestation day 7–13 or 14–20	0, 100, 900	Decreased weight gain in offspring; CNS: behavior, brain acetylcholine	NOAEL: 29	NA
	Beliles et al. (1980)	Mouse	5 days exposure; 1, 4, and 10 week follow-up	0, 100, 500	Sperm quality	NOAEL: 21	NA
	Tinston (1994)	Rat	Developmental—multigeneration; 6 hrs/day, 5 days/wk	0, 100, 300, 1,000	F2A pup deaths by day 29; F1 and F2 generations: CNS depression	NOAEL: 18	$BMCL_{01}$: 1.8
	Carney et al. (2006)	Rat	Developmental—6 hrs/day on GD 6–19	0, 65, 250, 600	Decreased fetal and placental weight and incomplete ossification of thoracic veretebral centra	NOAEL: 16	NA

[a] Experimental/observational NOAEL is underlined, LOAEL is double-underlined.

[b] Calculated using RfC methodology for a Category 3 gas, extrathoracic effects, and adjusted to equivalent continuous exposure; occupational exposures were multiplied by 5/7(days) × 10/20 (m^3/day, breathing rate).

[c] $BMCL_{10}$ is the lower bound on concentration associated with a 10% response over background. $BMCL_S$ is the lower bound on dose associated with a one standard deviation change in the mean over the control response. This corresponds to an excess risk of approximately 10% for developing a level of the endpoint above the 98th percentile (or below the 2nd percentile) of the control distribution for normally distributed effects. $BMCL_{X/P}$ is the estimation, based on total metabolism, for either type of benchmark response (percent change or standard deviation).

Table 5-2. Inhalation studies considered in the development of an RfC (continued)

[d] Atmospheric monitoring indicated slightly higher exposure levels were experienced by subjects. Schreiber et al. (2002) found mean tetrachloroethylene concentrations of 0.2 ppm (0.09 ppm, median) of four families living in apartments above active dry cleaning and two families living in an apartment building where dry cleaning had ceased 1 month earlier. Ambient monitoring of these six apartments during a period of active dry cleaning indicated exposure to higher concentrations, mean = 0.4 ppm (median 0.2 ppm).

[e] Benchmark modeling not feasible; exposure-response relationship showed very little gradation among responses aside from apparently maximal response in mid- and high-dose groups relative to control and low-dose groups.

exposure duration; the ambient (experimental) concentration or, for epidemiologic studies, the mean concentration; the observed effect; and the exposure concentration, identified as the NOAEL or the LOAEL. Additionally, HECs for LOAELs or NOAELs are presented so as to better allow examination of effect levels across studies and species. The HECs in Table 5-1 are calculated using the RfC methodology for a category 3 gas, extrathoracic effects, and adjusted to equivalent continuous exposure (U.S. EPA, 1994).

$$NOAEL^*_{[HEC]} = NOAEL^*_{[ADJ]}\ (ppm) \times (H_{b/g})_A / H_{b/g})_H$$

where:

$NOAEL^*_{[HEC]}$ = the NOAEL or analogous effect level such as the benchmark concentration (BMC)

$NOAEL^*_{[ADJ]}$ = the NOAEL or analogous effect level adjusted for duration of experimental regimen; experimental exposure times duration (number of hrs exposed/24 hrs) times week (number of days of exposure/7 days)

$(H_{b/g})_A / H_{b/g})_H$ = the ratio of the blood/gas (air) partition coefficient of the chemical for the laboratory animal species to the human value. The value of 1 is used for the ratio if $(H_{b/g})_A > H_{b/g})_H$

Response levels in Table 5-1 are presented as either a NOAEL, a LOAEL if the study did not identify a NOAEL, or a modeled BMCL[3] if the study results were suitable for modeling, using BMD methodology (U.S. EPA, 2000). Five studies (Brodkin et al., 1985; Kjellstrand et al., 1984; JISA, 1993; NTP, 1986; Tinston, 1994) reported toxicity in other organs besides the nervous system and are presented for the comparative purposes. These studies had experimental designs with multiple exposure concentrations and quantitative information that were sufficient for quantitative analysis using BMD methodologies.

Ideally, an examination using pharmacokinetically derived dose metrics is preferred when well validated models are available. An alternative procedure for deriving a POD using pharmacokinetic modeling to estimate metabolite production for liver toxicity data sets is also examined in this document.

[3] A $BMCL_{10}$ is the lower 95% bound on the dose or concentration associated with a 10% extra risk compared to background. In general, the benchmark approach is a superior methodology to the LOAEL/NOAEL approach because it makes more complete use of exposure/ response data rather than being limited by the sample size of the study group that happened to be exposed at the LOAEL or NOAEL. The BMD approach identifies doses that are not restricted to being one of the study exposure levels, another improvement over the LOAEL/NOAEL approach, particularly when doses are widely spaced. BMDs correspond to specific response levels, such as 10% extra risk, facilitating comparisons across studies and endpoints. Because the BMD corresponds to an adverse effect level, it should be treated conceptually as a LOAEL.

5.1.2. Method of Analysis

The present analysis defines a POD using the traditional NOAEL/LOAEL approach in addition to using BMD modeling where feasible. Further, PBPK modeling was used with suitable studies in animals in order to inform the process of extrapolating to human equivalent exposures. The use of these alternative approaches has the potential to add information to the NOAEL/LOAEL approach.

Altmann et al. (1995) reports a mean 8-hr TWA of 0.7 ppm (4.8 mg/m^3) for residents exposed to tetrachloroethylene from living in close proximity to a dry cleaning establishment. This mean concentration is used as the POD for the RfC derivation. The POD is not adjusted for exposure duration as is the general practice when using an occupational study. Instead, an assumption was made that residents were continuously exposed. In other words, no further adjustments to the estimated exposure level to approximate continuous exposure levels were considered necessary due to the lack of information concerning the duration and length of exposure of the study population.

The application of BMD methodologies offer advantages over traditional LOAEL/NOAEL approaches, however, exposure in Altmann et al. (1995) is reported as a group mean concentration and does not allow fitting of BMD models. Data sufficient for BMD modeling generally came from animal experiments, with the exception of one human study. This was the Brodkin et al. (1985) study, which found an increasing incidence of hepatic parenchymal changes in laundry workers with increasing exposure to perchloroethylene. There was no concurrent control group, so substitution of a background level was necessary (Hartwell et al., 1985). BMD modeling of the two reported groups plus the substituted control group yielded a $BMCL_{10}$ of 0.5 ppm (see Table 5-1). In addition to the lack of a control group, another limitation of the result modeled from the Brodkin et al. (1985) study is that hepatic parenchymal changes appear to be a less severe endpoint, as all of the participants had normal liver function measurements, and its relationship to frank liver disease is not known. Despite these uncertainties, the result provides support for the POD derived from the Altmann et al. (1995) study. Furthermore, Eskenazi et al. (1991), who observed effect on sperm quality at a similar mean exposure concentration as that of Altmann et al. (1995) and Schreiber et al. (2002), support the POD of the critical study. Table 5A-1 in the appendix provides details of the BMD modeling.

The animal studies suitable for BMD modeling addressed liver and kidney toxicity and pup death. For liver toxicity in mice (increased liver weights [Kjellstrand et al., 1984]), a human equivalent $BMCL_S$[4] of 0.6 ppm was estimated using administered concentration, and a $BMCL_{S/P}$

[4] $BMCL_S$ = Lower bound on dose (concentration) associated with a one standard deviation change in the mean over the control response. This corresponds to an excess risk of approximately 10% for the proportion of individuals above the 98th percentile (or below the 2nd percentile) of the control distribution for normally distributed effects (U.S. EPA, 2000).

of 1.4–10 ppm (9.6–69 mg/m^3) was derived using pharmacokinetic models, assuming that total metabolism was the relevant dosimeter for animals and humans (see Tables 5A-2 and 5A-3 for modeling details). For liver toxicity in rats (increased angiectasis [JISA, 1993]), BMD modeling yielded a $BMCL_{10}$ of 3.7 ppm (25 mg/m^3) using administered concentration and a $BMCL_{10/P}$ of 4.3–23 ppm (29–156 mg/m^3) using pharmacokinetic modeling (see Table 5A-6). PBPK models are described in Section 3.5 and are more fully considered in the cancer dose-response analysis (see Section 5.4.4.2). Karyomegaly (kidney) was observed in male rats in the chronic study by the NTP (1986). BMD modeling yielded a $BMCL_{75}$ of 29 ppm (near the lowest exposure tested) and a $BMCL_{10}$ of 2.2 ppm (15 mg/m^3). Because the lowest exposure was associated with a relatively high response, estimation of the $BMCL_{10}$ is somewhat tenuous, and modeling using total metabolism was not pursued. Last, BMD modeling of pup deaths through Day 29 in the F2A generation of a multigeneration study (Tinston, 1994) yielded a $BMCL_{01}$ of 1.8 ppm (12 mg/m^3). These BMD analyses are more fully presented in Tables 5A-2 through 5A-9 and are provided in support of the choice of the Altmann et al. (1995) study as the most relevant data source for developing the RfC.

5.1.3. Reference Concentration (RfC) Derivation, Including Application of Uncertainty Factors

The NOAEL of 0.7 ppm (4.8 mg/m^3) from Altmann et al. (1995) is the POD, as described above. The POD is reduced by the following UFs.[5]

$BMCL_{X/P}$ = Lower bound on dose (concentration), where X denotes the benchmark response (either in percent or one standard deviation), based on dose metric of total metabolism as estimated by a pharmacokinetic model. This subscript distinguishes these BMCLs from those based on administered exposure.

[5]RfCs apply to lifetime human environmental exposure, including exposures of sensitive subgroups. Differences between study conditions and conditions of human environmental exposure may make a dose that appears safe in an experiment not safe in the environment. UFs account for differences between study conditions and conditions of human environmental exposure. These include the following:

1. Variation from average humans to sensitive humans: RfCs apply to the human population, including sensitive subgroups, but studies rarely target sensitive humans. Sensitive humans could be adversely affected at doses lower than those that affect a general study population; consequently, general population NOAELs are reduced to cover sensitive humans.
2. Uncertainty in extrapolation from animals to humans: If an RfC is developed from animal studies, the animal NOAEL is reduced to reflect pharmacokinetic and pharmacodynamic factors that may make humans more sensitive than animals.
3. Uncertainty in extrapolating from subchronic NOAELs to chronic NOAELs: RfCs apply to lifetime exposure, but sometimes the best data come from shorter studies. Lifetime exposure can have effects that do not appear in a shorter study; consequently, a safe dose for lifetime exposure can be less than the safe dose for a shorter period. If an RfC is developed from less-than-lifetime studies, the less-than-lifetime NOAEL is adjusted to estimate a lifetime NOAEL.
4. Uncertainty in extrapolation from LOAELs to NOAELs: RfCs estimate a dose with appreciable risks, but sometimes adverse effects are observed at all study doses. If an RfC is developed from a dose where there are adverse effects, that dose is adjusted to estimate a NOAEL.
5. Other factors to reflect professional assessment of scientific uncertainties not explicitly treated above, including completeness of the overall database, minimal sample size, or poor exposure characterization.

1. *Human variation*: The UF of 10 is applied for human variation. Although human residential data were used as the basis for the POD (Altmann et al., 1995), the overall database does not support the use of a value other than the default 10-fold UF_H. The rationale for this determination is based on several considerations. First, Altmann et al. (1995) excluded subjects with disorders such as hypertension, neurological or endocrinological diseases (e.g., diabetes), impaired vision, or impairment of joints. Hence, subjects in Altmann et al. (1995) are considered to be a select population, analogous to an occupational population and subject to selection bias known as the "healthy worker effect." The use of these exclusion criteria and the small numbers of subjects in Altmann et al. (1995; $n = 37$, 14 exposed and 23 control subjects) suggest that the range of human variation in a larger and more diverse population is not likely represented by this study. Second, no information is presented in Altmann et al. (1995) with which to examine variation between subjects. Third, the sparse data available on tetrachloroethylene indicate the presence of pharmacokinetic variation in the human population. One report described variation in tetrachloroethylene blood concentrations among nine subjects exposed acutely to tetrachloroethylene and observed a twofold spread in the ratio of alveolar air concentrations to atmospheric concentrations (Opdam, 1989).

   Gentry et al. (2003), Clewell et al. (2004), and Pelekis et al. (2001) present pharmacokinetic modeling simulations of pharmacokinetic variation between adults and children in tetrachloroethylene parent and its metabolites. As the authors themselves indicated, validation of these results for various life stages and further refinement of the parameters in the model are necessary before the results of such an analysis can be considered for use in risk assessment. Further investigation of variability in the parameters used in the Clewell et al. (2004) analysis is also needed before their results can be used to address pharmacokinetic uncertainty for age and gender variability.

   Given an adequate database, or after adjustment in the assessment for deficiencies in the database, a reference value incorporating the default 10-fold factor for human variation is believed to adequately address likely susceptibilities in children. A thorough evaluation of the animal and human hazard data for tetrachloroethylene identified the developing fetus and the young child as susceptible life stages (populations). As described in Section 4.9, data-derived noncancer outcomes of concern in children for perinatal exposure are (1) spontaneous abortions, (2) childhood mortality, and (3) neurological impairment. This assessment contains a database uncertainty factor addressing, in part, limitations in life stage-related data in human and rodent studies.

   Section 4.9 also describes differential opportunities for exposure to children. However, susceptibilities of this nature are not addressed in the determination of the UF for human variation, but rather are used to establish a context for the hazard and dose-response evaluation and are further addressed in the exposure assessment and subsequent risk characterization.

2. *Animal-to-human uncertainty*. This UF is used when the POD is supported by an animal study. When the POD is supported by a human study, this UF is not needed.

3. *Subchronic-to-chronic uncertainty*. A factor to address the potential for more severe toxicity from chronic or lifetime exposure to tetrachloroethylene is not used in this assessment. The epidemiologic studies, except for Schreiber et al. (2002), are all of median duration of exposures of more than 15% of a 70-year lifespan. There are no data to suggest that continuing exposure to tetrachloroethylene can increase the severity of effects; duration-response trends are not generally evident in the human studies.

4. *LOAEL-to-NOAEL uncertainty*. The default value of 10 is applied for use of a LOAEL because of the lack of a NOAEL in Altmann et al. (1995).

5. *Database uncertainty*. A threefold database UF has been applied to address the lack of data to adequately characterize the hazard and dose-response in the human population. The rationale for this database UF is based on several considerations. There is human evidence of neurotoxicity following tetrachloroethylene exposure, with both visual system dysfunction and cognitive performance deficits. However, these studies have limitations, and in particular lack adequate data to address childhood or other life stage susceptibility. There is also a lack of animal studies (including in developing animals) designed to clearly investigate these neurotoxicity findings and define and characterize the exposure-response relationship.

   A broad range of animal toxicology data are available for the hazard assessment of tetrachloroethylene, as described throughout this document. Included in these studies are short-term and long-term bioassays in rats and mice (see Chapter 4 and Table 4-2); neurotoxicology studies in rats, mice, and gerbils (see Tables 4-6 and 4-7); prenatal developmental toxicity studies in rats, mice, and rabbits and a two-generation reproduction study in rats (see Table 4-10); and numerous supporting genotoxicity and metabolism studies. Nevertheless, critical data gaps have been identified. Data from acute studies in animals (Warren et al., 1996; Umeza et al., 1997) suggest that cognitive function is affected by exposure to tetrachloroethylene. These studies do not address the exposure-response relationship for subchronic and chronic tetrachloroethylene exposures on cognitive functional deficits observed in humans (e.g., Seeber, 1989; Echeverria et al., 1994; and Altmann et al., 1995).

   Even more importantly, there is a lack of cognitive testing in both developmentally exposed animals and adult animals following exposures to tetrachloroethylene that are longer than acute durations of exposure. For another critical outcome, visual function, there has been a limited evaluation of visual function in rodents, with the exception of the evoked potential studies by Mattsson et al. (1998). Visual system dysfunction and processing of visual spatial information are sensitive endpoints in human studies. The exposure-response relationship of these functional deficits could be evaluated more definitively with studies using homologous methods that examine retinal and visual function in experimental animals. These types of studies could help elucidate whether there are both peripheral and central effects of tetrachloroethylene exposure on visual perception, and they could be used as an animal model to better define the exposure-response relationships.

Additionally, the database of human epidemiological studies includes studies of liver and kidney toxicity (see Tables 4-1 and 4-3), neurotoxicity (see Tables 4-4 and 4-5), and developmental/reproductive effects (see Table 4-8). The reference value is established on the basis of a sensitive neurological effect in adult humans (Altmann et al., 1995), and some characterization of the response of children to tetrachloroethylene exposure was found in limited data for a similar neurological (visual system) parameter (Schreiber et al., 2002).

Although the toxicological database is considered adequate for establishing a reference value, some uncertainties remain. In both the Altmann et al. (1995) and the Schreiber et al. (2002) studies, there was a lack of robust sample size and an inadequate dose-response characterization for potentially susceptible human populations following tetrachloroethylene exposures. Although the Altmann et al. (1995) study (with a LOAEL of 0.7 ppm for healthy adult subjects) was used in setting the reference value (based on a number of considerations that are summarized in Section 5.2.1), the Schreiber et al. (2002) study, using an alternative visually based testing paradigm, identified adverse visual effects at 0.4 ppm (see Section 4.6.1.2.11). Additionally, in a postnatal neurotoxicity study in mice (Fredriksson et al., 1993), persistent neurological effects (i.e., increased locomotion and decreased rearing behavior at 60 days of age, measured 43 days after exposure ceased) were observed at an oral dose of 5 ppm, with no NOAEL, although this study did not conform to traditional toxicity testing guidelines (see Section 4.6.2.2). The possibility exists that if adequate, robust, dose-response data based on the most appropriate neurophysiological and cognitive tests were available, the exposure eliciting an adverse response (and hence the POD for the reference value) could be lower than that established on the basis of deficits in visuo-spatial and cognitive function following tetrachloroethylene exposure in healthy adults (Altmann et al., 1995).

A total UF of 300 was applied to this effect level: 10 for human variation, 10 for consideration of LOAEL to NOAEL, and 3 for database uncertainties.

$$RfC = NOAEL / UF$$
$$= 0.7 \text{ ppm } (4.8 \text{ mg/m}^3) / 300$$
$$= 0.002 \text{ ppm } (0.02 \text{ mg/m}^3, \textit{rounded to 1 significant digit})$$

where:

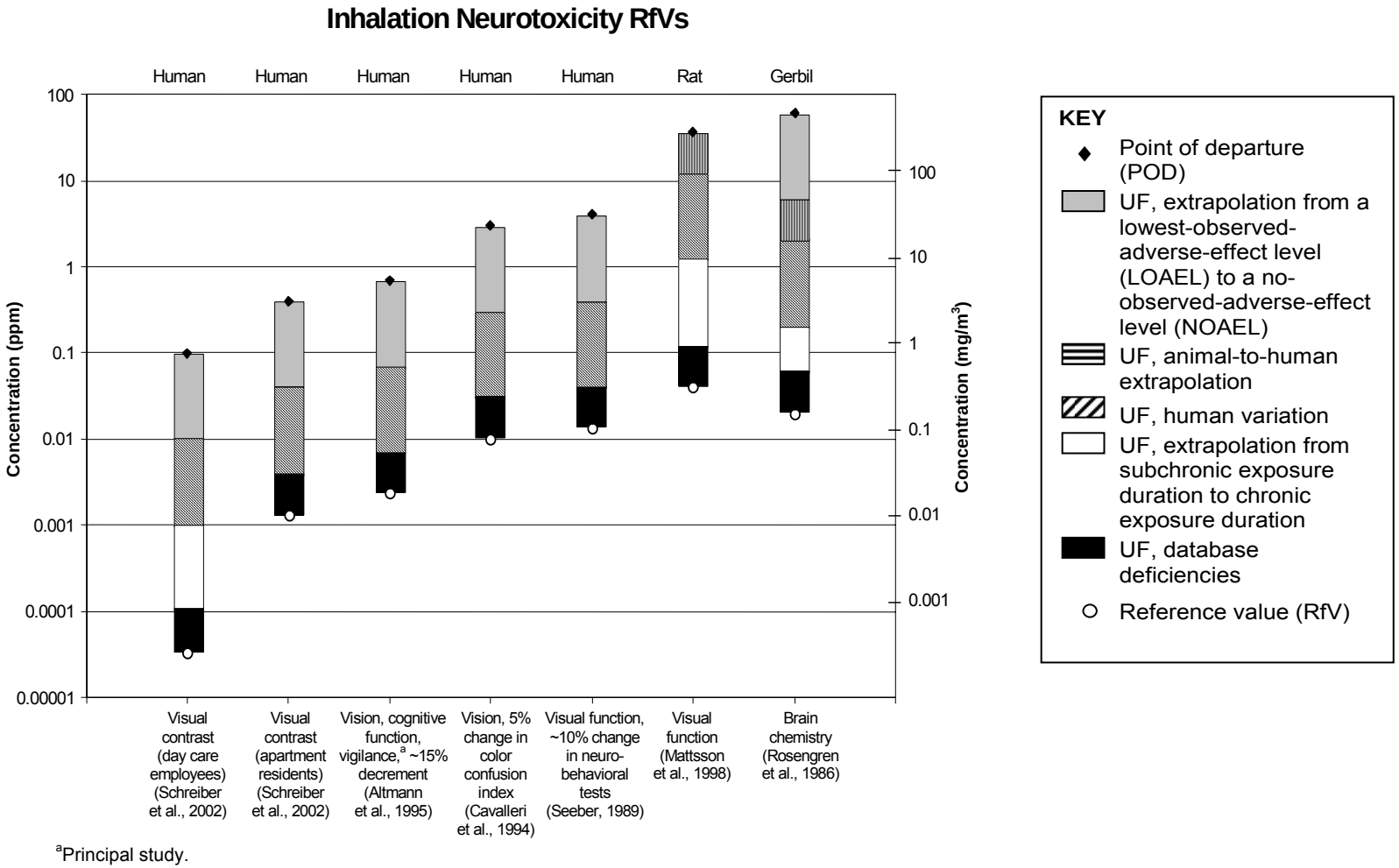
0.7 ppm = LOAEL for neurological effects in residents exposed to tetrachloroethylene (Altmann et al., 1995)

300 = composite UF chosen to account for extrapolation from a LOAEL to a NOAEL in humans, intra-individual variability in humans, and uncertainties in the database

5.1.4. Supporting Studies

PODs and reference values (RfVs) that could be derived from supporting neurotoxicity studies identified in Table 5-2 (see Section 5.1.1) are presented below in Figure 5-1 to allow a comparison with the critical study. Not all studies of neurotoxic effects identified in Table 5-2 are presented in Figure 5-1; however, these studies are a sample of human and animal data sets for some of the more sensitive measures of neurotoxic endpoints. Vision or visual function effects are observed in the human and rodent studies and one study in gerbils reports changes in brain chemistry (Schreiber et al., 2002; Altmann et al., 1995; Cavalerri et al., 1994; Seeber, 1989; Mattsson et al., 1998; Rosengren et al., 1986). Effect magnitudes could be identified for three studies and ranged from a 5% change in color vision index to roughly a 15% decrement in several tests on a neurobehavioral evaluation battery (summarized in Figure 5-1). In the absence of tetrachloroethylene data to inform uncertainty factors, the analysis uses the default values as discussed in Section 5.1.3: a factor of 10 to extrapolate from a LOAEL to a NOAEL; a factor of 10 for human variation; and a factor of 3 for database deficiencies. For the rodent studies of Mattson et al. (1998) and Rosengren et al. (1986), PODs represent human equivalent concentrations for a category 3 gas adopting EPA's RfC methodology (U.S. EPA, 1994) and an uncertainty factor of 3 addresses uncertainties associated with extrapolating from animal data to an average human. Three studies are of subchronic exposure duration, and extrapolation to chronic exposure duration is achieved using a factor of 10 for the studies of Mattsson et al. (1998) and Schreiber et al. (2002; daycare employees). A subchronic to chronic factor of 3, rather than 10, was applied for Rosengren et al. (1986) in light of the large overall uncertainty for this study associated with extrapolating from a LOAEL to NOAEL, from animal to humans, for human variation, and for database deficiencies; the total uncertainty factor was 3,000.

One study in Figure 5-1 used a neurological test for visual contrast sensitivity and yields LOAELs (PODs) of 0.1 and 0.4 ppm for day care workers or apartment dwellers adjacent to dry cleaners, respectively (Schreiber et al., 2002). An RfV developed from this study would be lower than that of Altmann et al. (1995) but, as more fully discussed in Section 5.1.1, this study was not considered as the critical study due to its design as a pilot for a larger study conducted by the NYS DOH (see Sections 5.2.1 and 5.2.2). LOAELs are higher than that of Altmann et al. (1995) for the occupational studies of Cavalleri et al. (1994) and Seeber (1989) and reflect higher tetrachloroethylene concentrations in the occupational setting when compared to residential concentrations.

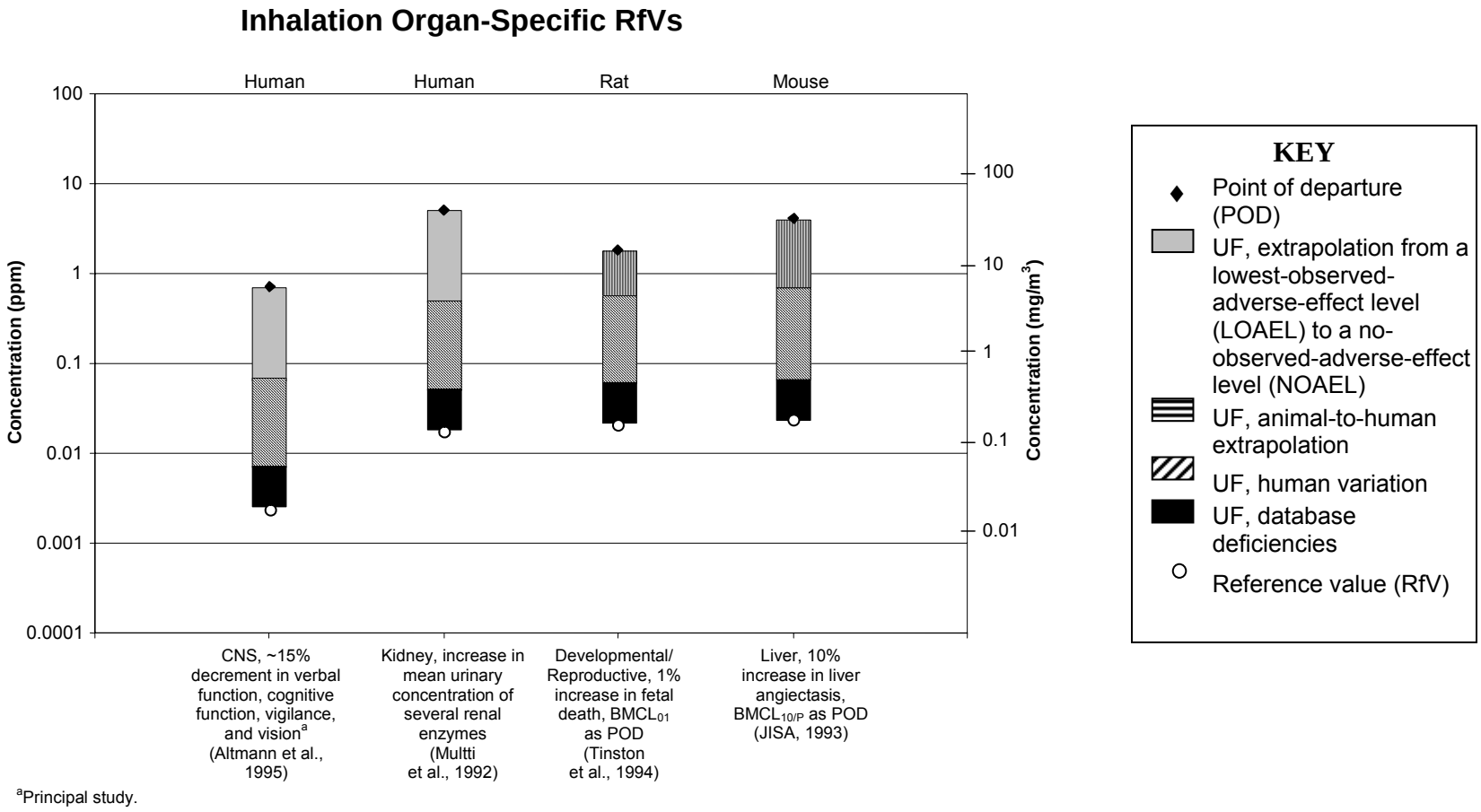


[a]Principal study.

Figure 5-1. Array of PODs and reference values for a subset of neurotoxic effects of studies in Table 5-2.

PODs and inhalation organ-specific RfVs are presented for selected studies in Figure 5-2 to give perspective on the RfC derived from the adverse neurotoxic effects in Altmann et al. (1995) and to provide information on other systemic effects associated with tetrachloroethylene exposure. Toxicity to the liver, kidney, developing fetus, and reproductive organs are observed at higher mean or median tetrachloroethylene concentrations than Altmann et al. (1995). The POD for a given study is the tetrachloroethylene concentration associated with the LOAEL, NOAEL, or lower bound on a benchmark concentration (BMCL). Benchmark concentration models are fit to JISA (1993) and Tinston (1994; see Appendix 5, Tables 5A-5 and 5A-7). Furthermore, a pharmacokinetic model of Bois et al. (1996) and scaling of the body weight to the $3/4^{th}$ power is adopted for liver weight changes in JISA (1993) to obtain human equivalent concentrations, a treatment consistent with the cancer dose-response analysis of liver tumors and more fully described in Section 5.4.3.1. PODs developed from pharmacokinetic models of Rao and Brown (1993) and Reitz et al. (1996), reflecting uncertainties associated with tetrachloroethylene metabolism, are up to an order of magnitude higher than that of Bois et al. (1996). The POD for JISA (1993) using the Bois et al. (1996) pharmacokinetic model is shown on Figure 5-2.

Organ-specific inhalation RfVs for endpoints besides neurotoxicity are developed by a procedure similar to that for neurotoxicity and was carried out for Altmann et al. (1995). Default values in the absence of data-informed adjustment factors are adopted to account for uncertainties in the analysis. With human studies, the default values as discussed in Section 5.1.3 are a factor of 10 to extrapolate from a LOAEL in Altmann et al. (1995) and Mutti et al. (1992) to a NOAEL; a factor of 10 for human variation; and a factor of 3 for database deficiencies. Another uncertainty factor is adopted to account for uncertainty in extrapolating laboratory animal data to the case of average healthy humans (U.S. EPA, 1994). Typically, this factor addresses residual uncertainties not associated with default dosimetric adjustment like the human equivalent concentration. The POD for Tinston (1994) is a human equivalent concentration for a category 3 gas and an uncertainty factor of 3 addresses uncertainty associated with extrapolating animal data to the average healthy human case. In the case of liver angiectasis, cross-species scaling of total rate of metabolism using body weight to the $3/4^{th}$ power was used for describing toxicological equivalence because of the extensive rationale supporting it (U.S. EPA, 1992). The methodology achieves a human equivalent concentration that is expected to approximate AUC or ppm equivalence across species for a category 3 gas. An animal to human uncertainty factor of 3 addresses non-pharmacokinetic uncertainties such as pharmacodynamics as suggested in the RfC framework (U.S. EPA, 1994).



[a]Principal study.

Figure 5-2. Organ-specific reference values for inhalation exposure to tetrachloroethylene.

5.1.5. Previous Inhalation Assessment

There is no previous EPA RfC assessment for tetrachloroethylene with which to compare and contrast the RfC developed in this assessment. Other assessments identified in Appendix A have derived a noncancer reference value from the human evidence. California's drinking water assessment on tetrachloroethylene (Cal EPA, 2001) derived a public health goal (PHG)[6] for noncancer effects from a geometric mean in Altmann et al. (1995) and two occupational studies: Spinatonda et al. (1997) and Ferroni et al. (1992). The most recent assessment of tetrachloroethylene by the NYS DOH (1997) used a slightly different set of neurotoxicity studies than did California and presented reference criteria for neurotoxicity derived from Cavalleri et al. (1994), Altmann et al. (1995), and Seeber (1989). NYS DOH (1997) considered the reference criterion of Seeber (1989) as best providing a sufficient margin of exposure over the air levels of tetrachloroethylene associated with CNS effects.

ATSDR (1997), on the other hand, based its chronic MRL on Ferroni et al. (1992). ATSDR (1997) considered Altmann et al. (1995) to provide a NOAEL, a conclusion inconsistent with the assessments by New York State and California. ATSDR, however, noted that the Altmann et al. (1995) study suggested a need to characterize neurotoxic effects in populations exposed to very low levels of tetrachloroethylene. A second report (Schreiber et al., 2002) of visual functional deficits in two populations exposed to tetrachloroethylene at lower ambient concentrations that were similar to those of Altmann et al. (1995) has become available since the publication of the ATSDR toxicological profile.

A difference between this and previous assessments is in the previous assessments' treatment of human variation, particularly the residential study by Altmann et al. (1995). A choice other than the UF of 10 has been adopted in the assessments by California and New York State. A presumption underpinning this choice is that the residential population studied by Altmann et al. (1995) is more reflective of the general adult population than of an occupational population and any accompanying selection bias that is often associated with a healthier worker population. Although there is some merit in this opinion, observations in Altmann et al. (1995) are of a German population: 14 adults with exposure to tetrachloroethylene. These individuals likely do not represent the full range of human variation found in a large and ethnically diverse population such as the United States population. Furthermore, as noted, the Altmann et al. (1995) study excluded subjects with disorders such as hypertension, neurological or endocrinological diseases (e.g., diabetes), impaired vision, or impairment of joints; hence, these subjects can be considered as having an overall good health status, and individuals whose

[6]PHG is conceptually similar to an RfD.

diseases may have increased their susceptibility to tetrachloroethylene effects were not included in this study.

5.2. ORAL REFERENCE DOSE (RfD)

Ideally, the studies of greatest duration of exposure and conducted via the oral route of exposure have the most confidence for derivation of an RfD.[7] An earlier assessment of tetrachloroethylene oral noncancer toxicity by EPA, for example, identified liver toxicity in Buben and O'Flaherty (1985) as the critical effect for developing an RfD (U.S. EPA, 1988). However, the application of pharmacokinetic models for a route-to-route extrapolation of the inhalation studies expands the oral database. Cal EPA (2001), for example, carried out a route-to-route extrapolation of the human inhalation studies of neurotoxic effects to develop a PHG for oral tetrachloroethylene exposure, based on a route-to-route extrapolation of inhalation neurotoxicity studies.

5.2.1. Choice of Principal Study and Critical Effects

Toxicity to several targets, including the liver, kidney, nervous system, and reproductive system and to the developing fetus is seen in rodents with oral tetrachloroethylene exposure. Effects have been observed at these targets in acute studies (28 days or less), longer term/subchronic studies (90 days), or chronic studies (1 year or more). At higher doses (above approximately 1,000 mg/kg-day), targets of oral tetrachloroethylene toxicity include the liver, kidney, nervous system, lymphatic system, reproductive system, and developing fetus (ATSDR, 1997). There are few studies at lower doses as compared to the number of studies of inhalation exposure. Several targets of toxicity from oral exposure are similar to targets observed with inhalation exposures, i.e., liver and kidney.

No epidemiologic studies of oral exposure were suitable for quantitative analysis, although these studies did provide information for hazard identification. Four studies of subchronic oral exposure in mice or rats (Buben and O'Flaherty, 1985; Jonker et al., 1996; Berman et al., 1995; Hayes et al., 1986) are available, as is a developmental study in mice of oral exposure to tetrachloroethylene (Fredriksson et al., 1993). As discussed above, EPA previously developed an RfD from Buben and O'Flaherty (1985). A significant effect on liver weight was seen in this study.

[7]The RfD is expressed in units of milligrams per kilogram body weight per day (mg/kg-day). In general, the RfD is 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 noncancer effects during a lifetime.

The CNS is a sensitive target for tetrachloroethylene inhalation toxicity, as discussed in Section 5.1.1. This assessment has attempted to expand the database for derivation of an RfD using relevant inhalation data and route-to-route extrapolation with the aid of a PBPK model (see Section 3.5) to the POD of Altmann et al. (1995). The nervous system is an expected target with lower oral tetrachloroethylene exposures, in view of the fact that other organ systems such as the liver the kidney are also common targets associated with both inhalation and either oral routes of subchronic or chronic exposure. The similarity of effects in these organ systems with either oral or inhalation exposure to tetrachloroethylene supports the use of route extrapolation to compare PODs for oral and inhalation exposure. For these reasons, the inhalation study in humans by Altmann et al. (1995) is chosen as the principal study for supporting the RfD.

5.2.2. Methods of Analysis, Including Models

The present analysis defines a POD using the traditional NOAEL/LOAEL approach in addition to using BMD modeling where feasible. This assessment has attempted to expand the database for derivation of an RfD using relevant inhalation data and route-to-route extrapolation with the aid of a PBPK model (see Section 3.5). Several factors support the use of route-to-route extrapolation for tetrachloroethylene. Tetrachloroethylene has been shown to be rapidly and well absorbed by both the oral and inhalation routes of exposure (ATSDR, 1997). Additionally, the metabolic pathways and kinetics of excretion with oral exposure are similar to those of inhalation exposure (ATSDR, 1997). Furthermore, the data for oral administration indicate a pattern of effects similar to that of inhalation exposure, including effects on the liver and kidney. PBPK modeling was also used with suitable studies in animals in order to inform the process of extrapolating to HECs. The use of these alternative approaches has the potential to add information to the NOAEL/LOAEL approach.

PBPK modeling was used to derive the oral dose that would result in the same tetrachloroethylene in blood AUC as that following a continuous inhalation exposure of 0.7 ppm, the LOAEL from the inhalation study by Altmann et al. (1995). A hypothetical drinking water scenario of 9 equal drinking water incidents, spaced 2 hrs apart allowing for an 8-hr sleep period, was judged to be a reasonable baseline. Use of this scenario generated an estimated total oral ingestion of 1.1 mg/kg-day of tetrachloroethylene, leading to the same steady-state blood tetrachloroethylene AUC as a continuous inhalation exposure of 0.7 ppm using the PBPK model of Rao and Brown (1993).

A route-to-route extrapolation based on a venous blood dose metric is more robust than one based on another dose metric such as the amount of metabolized tetrachloroethylene, and it provides a strong rationale for using blood AUC as a dose metric for extrapolating between

exposure routes. Venous blood concentration is well-validated in the Rao and Brown (1993) model; hence, little model uncertainty is associated with its estimation (see the pharmacokinetic discussion in Chapter 3). Furthermore, as noted in Section 5.1.1, the use of blood tetrachloroethylene provides some attempt to account for breathing rates and to adjust for kinetic nonlinearities related to tetrachloroethylene absorption, and it is assumed to better reflect tetrachloroethylene pharmacokinetics than use of default methodologies. The chemical species responsible for tetrachloroethylene-induced neurotoxic effects has not been demonstrated, but blood tetrachloroethylene is presumed to be one step in the MOA pathway and is used as a marker for the dose metric associated with neurologic effects.

The route-to-route extrapolation starts with the estimation of the average venous blood tetrachloroethylene AUC resulting from continuous inhalation exposure at the LOAEL of 0.7 ppm. The venous blood tetrachloroethylene AUC at steady state resulting from continuous exposure to 0.7 ppm tetrachloroethylene is estimated to be 68.3 mg/min/L, according to the Rao and Brown (1993) model. This model does not address pharmacokinetic variation in the human population. An analogous curve corresponding to a drinking water scenario is provided in Figures 5-3 and 3-10. The drinking water scenario models a subject consuming water every 2 hrs except during sleep, which was assumed to be for 8 hrs. An assumption of the amount of water consumed is also not necessary, because blood concentrations of tetrachloroethylene are solely dependent on the amount of compound ingested during each drinking episode. The curve depicted in Figures 5-3 and 3-10 yields the same AUC as does continuous inhalation exposure to 0.7 ppm and corresponds to ingestion of 76 mg/day. Therefore, the extrapolation of an inhalation exposure of 0.7 ppm (4.8 mg/m^3) using the PBPK model of Rao and Brown (1993) yields the same blood concentration of tetrachloroethylene as does ingestion of 1.1 mg/kg-day.

Table 5-3 summarizes the results of animal studies of oral exposures that represent the lower end of the dose-response curve. The doses shown in Table 5-3 are expressed in human equivalent terms—using $mg/kg^{3/4}$-day scaling—to enable interspecies comparisons (U.S. EPA, 1992). For liver effects, an alternative procedure of using the pharmacokinetic model of total metabolism as the dose metric for extrapolating between species was carried out. Potential PODs are presented as either a NOAEL or a modeled LED_x when the study results were suitable for modeling. Among the four studies identified in Table 5-2, a significant effect on liver weight is seen in the study by Buben and O'Flaherty (1985), with a NOAEL at a duration-adjusted human equivalent dose of 2 mg/kg-day. The modeled human equivalent $BMDL_S$ was 5 mg/kg-day in terms of administered exposure and 3.4–32 mg/kg/day using the available pharmacokinetic models. As discussed above, EPA previously developed an RfD from Buben and O'Flaherty (1985).

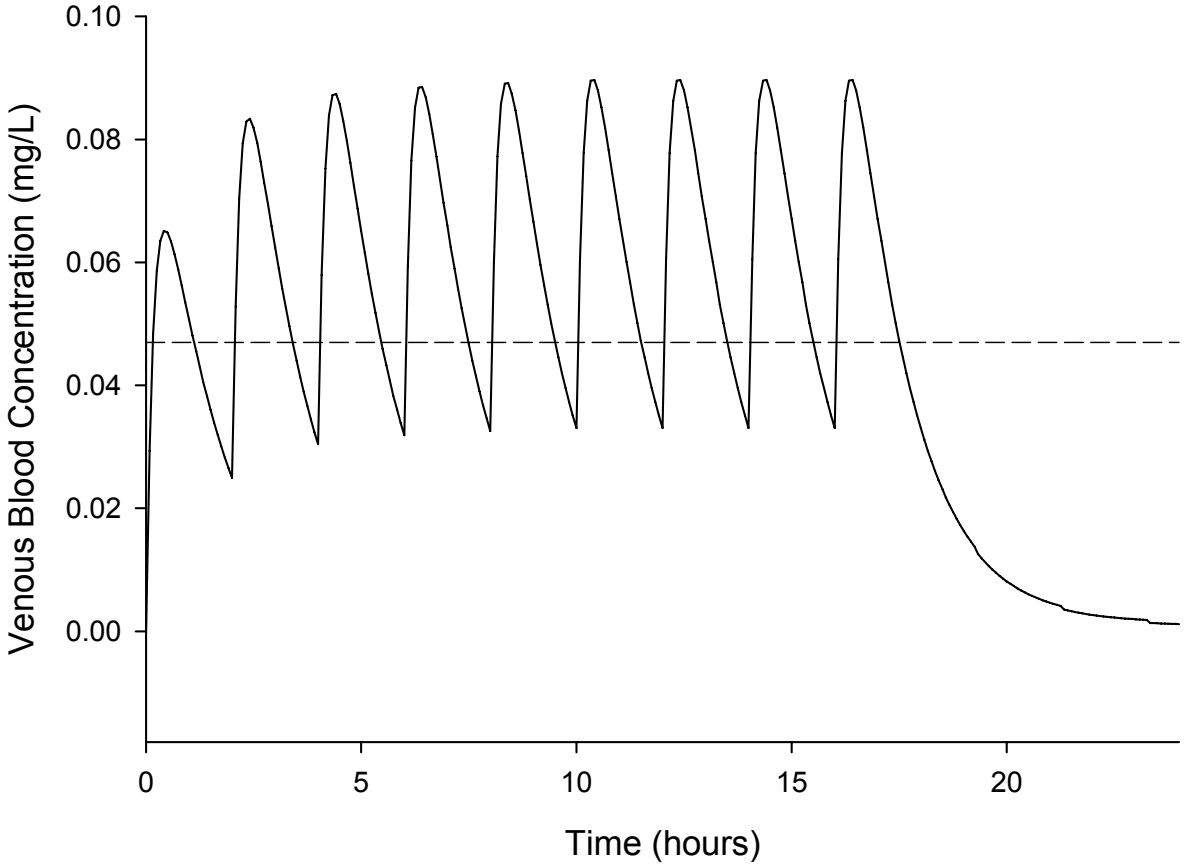


Figure 5-3. Time course of venous blood concentration in humans as predicted by the Bois et al. (1996), Rao and Brown (1993), and Reitz et al. (1996) PBPK models for ingested tetrachloroethylene. A total of 76 mg of tetrachloroethylene was orally delivered via drinking water in nine bolus doses spaced 2 hrs apart for a duration of 18 hrs, followed by 8 hrs of no dosing. The dashed line indicates the steady-state blood concentration level due to inhaled tetrachloroethylene of 0.7 ppm exposure concentration that results in the same area under the curve as above the curve, integrated over a 24-hr period. The alveolar ventilation rate was 9.3 L/min (total inspiratory rate 13.9 L/min) and the ventilation-to-perfusion ratio was equal to 1.3. The three models result in nearly equal concentrations at this exposure concentration.

5.2.3. Reference Dose (RfD) Derivation, Including Application of Uncertainty Factors

The POD of 1.1 mg/kg-day obtained from route-to-route extrapolation from Altmann et al. (1995) is similar to the POD of 2 mg/kg-day observed for liver toxicity in mice orally exposed to tetrachloroethylene (see Table 5-4). Several studies have identified similar PODs for adverse liver effects, indicating some degree of confidence in Altmann et al. (1995) as the basis for the POD and for calculating an RfD. Studies of inhalation exposure show that humans respond to tetrachloroethylene's neurotoxic effects at lower concentrations than do rodents, indicating humans as a potentially more sensitive species for these effects. Humans are also expected to be sensitive to neurotoxicity from oral tetrachloroethylene exposure, and there are no data to indicate otherwise. Hence, the LOAEL of 1.1 mg/kg-day derived from Altmann et al. (1995) represents the POD for the RfD for oral exposure to tetrachloroethylene.

To address differences between study conditions and conditions of lifetime human environmental exposure, the POD is reduced by UFs that consider specific areas of uncertainty. The following areas of uncertainty were evaluated for this RfD:

1. *Human variation*. Because the critical study is the same for this RfD derivation as for the RfC, the 10-fold factor applied to the POD for the RfC is used as a default in this assessment for human variation.

2. *Animal-to-human uncertainty*. Because the critical study is in humans, this factor is not needed. For animal data, a factor of 3 would be used for extrapolation of pharmacodynamics, because body weight scaling was used for calculating the human equivalent doses (see Table 5-4).

3. *Subchronic-to-chronic uncertainty*. As with the RfC derivation, described in Section 5.1.3, the POD is based on a study of 10.6-year median duration of exposure, or 15% of a 70-year lifespan. There are no data to suggest that continuing exposure to tetrachloroethylene can increase the severity of effects; exposure-response trends are not evident in the human studies, and there is no systematic trend in the relation between the LOAEL and treatment duration for neurotoxicity in animals. A factor to address the potential for more severe toxicity from lifetime exposure to tetrachloroethylene is not used in this assessment.

4. *LOAEL-to-NOAEL uncertainty*. LOAELs are observed in this human study, and, as in the RfC derivation, a 10-fold factor is adopted in this assessment to approach the range where a negligible response could be expected.

5. *Database uncertainty*. A factor of 3 is used for the database uncertainties for the same reasons as those for the derivation of the inhalation RfC.

Table 5-3. Oral studies considered in analysis of the oral RfD

Organ/ system	Study	Species	Duration/exposure route	Dose/exposure (NOAEL/LOAEL)[a] (mg/kg-day)	Effect	Human equivalent doses [b] (mg/kg-day)	
						NOAEL/ LOAEL	BMDL[c]
Liver	Buben and O'Flaherty (1985)	Mouse (40 g)	Subchronic (6 weeks)/oral gavage	0, 20, 100, 200, 500, 1,000, 1,500, 2,000	Liver weight, triglycerides	NOAEL: 2	$BMDL_S$: 5.0 $BMDL_{S/P}$: 3.4–32
Liver, kidney	Jonker et al. (1996)	Rat	Subchronic (4 weeks)/oral gavage	0, 600, 2,400	Liver weight, enzyme levels; kidney weight, kidney enzyme levels	LOAEL: 133	NA
Liver	Berman et al. (1995)	Rat	14 days/oral gavage	0, 50, 150, 500, 1,500, 5,000	Liver weight, ALT	NOAEL: 77	NA
Central nervous system	Fredriksson et al. (1993)	Mouse	Post-natal days 10–16/oral, gavage	0, 5, 320	Day 60: increased locomotion, decreased rearing	LOAEL: 0.5[c]	NA
Whole animal	Hayes et al. (1986)	Rat	Subchronic (91 days)/oral drinking water	0, 0 (vehicle control), 14, 400, 1,400	Body weight[d]	NOAEL: 4	NA

[a] NOAELs are underlined once, LOAELs are double-underlined.

[b] Human equivalent doses calculated using RfD methodology (Barnes and Dourson, 1988) and scaled to the ratio of body weight to the 0.75 power, i.e., multiplied by [(animal body weight in kg)/human body weight (70kg)]$^{0.25}$. Also adjusted for daily exposure by multiplying by (5 days)/(7 days) where relevant.

[c] BMDL is the lower bound on dose associated with a one standard deviation change in the mean over the control response. This corresponds to an excess risk of approximately 10% for developing a level of the endpoint above the 98th percentile (or below the 2nd percentile) of the control distribution for normally distributed effects. $BMDL_{S/P}$ is the $BMDL_S$ using pharmacokinetic modeling for relating metabolites in the experimental animals to the responses, and for reflecting extrapolation to humans (see Appendix 5A for details).

[c] For Fredriksson et al. (1993), a regression was fit to the data with body weight estimated as 8g, the body weight at day 13, the midpoint of 10–16 days. The human equivalent NOAEL is $5 \times (0.008/70)^{0.25} = 0.5$ mg/kg-day.

[d] Female rats (LOAEL was 400 mg/kg-day). Authors describe lower body weight gain as significant; however, statistical testing is not presented in the published paper (only that there was a statistical difference [$p \leq 0.05$] between treatment and control groups).

Table 5-4. Oral RfV: point of departure and uncertainty factors

Study	Human equivalent concentration[a]	Oral human equivalent dose (mg/kg-day)	Human variation	Animal to human	Subchronic to chronic	LOAEL to NOAEL	Database	Composite uncertainty factor	RfV (mg/kg-day)
Altmann et al. (1995): humans	0.7 ppm (LOAEL)	1.1[b]	10	1	1	10	3	300	4×10^{-3}
Buben and O'Flaherty (1985): mice, increased liver/body weight	NA	3.4–32[c]	10	3[c]	10	1	3	1000	3×10^{-3} to 3×10^{-2}
Fredriksson et al. (1993)	NA	0.5	10	3	1	10	3	1000	5×10^{-4}
Hayes et al. (1986)	NA	4	10	3	10	1	3	1000	4×10^{-3}

[a] Ambient concentration is assumed to represent continuous exposure in the residential studies.
[b] Equivalent oral exposure from application of PBPK model of Rao and Brown (1993), on the basis of equivalent AUC of blood tetrachloroethylene for humans.
[c] See Table 5A-9 for the dose-response modeling summary and extrapolation to human equivalent exposure. These human equivalent doses lie in the nonlinear portion of the exposure versus rate of metabolism relationship. However, for the purpose of comparing alternate reference doses, the calculation presented here is equivalent to applying the uncertainty factors prior to conversion from human equivalent metabolized dose to human equivalent administered dose.

A total UF of 300 was applied to this effect level: 10 for human variation, 10 for consideration of LOAEL to NOAEL, and 3 for uncertainties in the database.

RfD = *NOAEL* / *UF*
= 1.1 mg/kg-day / 300
= 0.0037 mg/kg-day (rounded to 0.004 mg/kg-day)

where:

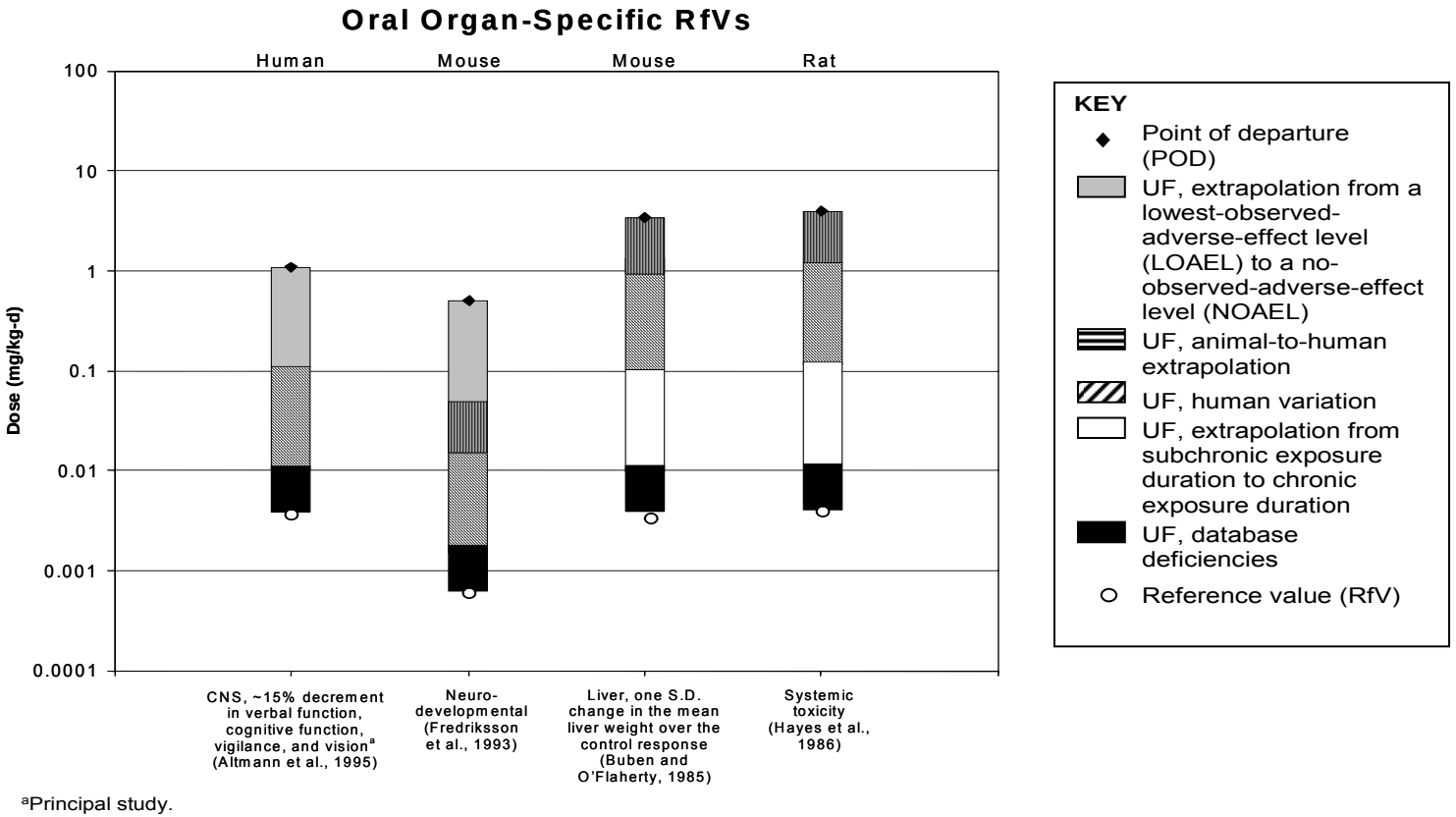
1.1 mg/kg-day = the oral exposure POD equivalent of 0.7 ppm (4.8 mg/m^3) continuous inhalation exposure LOAEL for neurologic effects in residents exposed tetrachloroethylene (Altmann et al., 1995) estimated via PBPK modeling

300 = composite UF chosen to account for extrapolation from a LOAEL to a NOAEL in humans, intra-individual variability in humans, and uncertainties in the database

In summary, an RfD for tetrachloroethylene was developed through a route-to-route extrapolation from the POD in Altmann et al. (1995), which reports neurological toxicity in residents exposed to tetrachloroethylene. The oral exposure POD equivalent to the 0.7 ppm (4.8 mg/m^3) continuous inhalation exposure LOAEL was estimated via PBPK modeling to be 1.1 mg/kg-day. A composite UF of 300 was obtained by multiplying factors of 10 for average to sensitive human variation, 10 for starting from an effect level instead of a NOAEL, and 3 for database uncertainties. Dividing the POD by a composite UF of 300 yields an RfD of 4×10^{-3} mg/kg-day. This RfD is equivalent to a drinking water concentration of 0.12 mg/L, assuming a body weight of 70 kg and a daily water consumption of 2 L.

5.2.4. Supporting Studies

PODs and oral RfVs from studies in Table 5-3 are arrayed in Figure 5-4 to give a perspective on the oral RfD supported by Altmann et al. (1995). Effects include liver weight changes (POD [$BMDL_{S/P}$] of 3.4 mg/kg-day from Buben and O'Flaherty [1985]), body weight changes (POD [NOAEL] of 4 mg/kg-day from Hayes et al. [1986]), and developmental neurotoxicity (POD [LOAEL] of 0.5 mg/kg-day from Fredriksson et al. [1993]). These PODs span a range which includes the oral-equivalent POD (LOAEL) of 1.1 mg/kg-day of Altmann et al. (1995), converted using a pharmacokinetic model for route-to-route extrapolation (Rao and Brown [1993]). The POD from Buben and O'Flaherty (1985) is a standard deviation change in mean liver-to-body weight over control using benchmark dose models and using



[a]Principal study.

Figure 5-4. Oral organ-specific reference values for exposure to tetrachloroethylene.

pharmacokinetic models for relating metabolites in the experimental animals to the responses, and for reflecting extrapolation to humans (see Appendix 5A and Table 5A-8 for details). Default uncertainty factor values in the absence of data-informed adjustment factors are adopted to account for uncertainties in the analysis (see Table 5-3). A factor of 10 is used to extrapolate from a LOAEL in Altmann et al. (1995), and Fredriksson et al. (1993) to a NOAEL because an effect occurred at the lowest dose studied; this factor is 1.0 for the Buben and O'Flaherty (1985) and Hayes et al. (1986) studies because there was no effect observed at the lowest dose studied. In all studies a factor of 10 is used for human variation and a factor of 3 for database deficiencies. For rodent studies, an uncertainty factor of 3 is adopted to account for uncertainty in extrapolating laboratory animal data to the case of average healthy humans (U.S. EPA, 1994). Typically, this factor addresses residual uncertainties not associated with default dosimetric adjustment like the human equivalent concentration. Scaling experimental doses in the rodent studies to the $3/4^{th}$ power achieves a human equivalent dose that is considered toxicologically equivalent. For Buben and O'Flaherty (1985), specifically, body weight scaling of the pharmacokinetically derived total rate of metabolism similarly produces a human equivalent dose that is assumed to be toxicologically equivalent. The uncertainty factor of 3 is used to account for residual uncertainty such as pharmacodynamic processes. For studies of subchronic exposure duration, a factor of 10 accounts for uncertainty associated with extrapolating to chronic exposure duration.

RfVs in the oral rodent studies of weight changes in Buben and O'Flaherty (1985) and Hayes et al. (1986) were 3×10^{-3} and 4×10^{-3} mg/kg-day, respectively. The RfD of Altmann et al. (1995) at the higher end of the range and with a total uncertainty factor (300) less than total uncertainty factor of 1000 was applied to the two rodent studies. A developmental neurotoxicity study in animals with persistent effects on motor activity yielded a NOAEL of 5 mg/kg-day and a RfV of 5×10^{-4} mg/kg-day (Fredriksson et al., 1993). However, this study was not considered as the principal study for chronic exposure due to uncertainties associated with study design and its level of confidence (see Section 4.6.2.2).

5.2.5. Previous Oral Assessment

EPA previously suggested an RfD of 1×10^{-2} mg/kg-day (U.S. EPA, 1988), which was supported by an adjusted NOAEL of 14 mg/kg-day in Buben and O'Flaherty (1985), and a composite UF of 1,000 (10 for extrapolation from the rat to humans, 10 for human variation, and 10 for extrapolating to chronic exposure conditions). A human study is now available, using route-to-route extrapolation, and is preferred to animal data. The composite UF of 300 in the current analysis is smaller than that used in the previous analysis, reflecting fewer uncertainties

associated with using human data. More recently, Cal EPA (2001) developed a PHG for oral exposure to tetrachloroethylene from the studies by Altmann et al. (1995), Spinatonda et al. (1997), and Ferroni et al. (1992) and conversion factors for breathing and absorption rates for an extrapolation from the inhalation to the oral exposure route. The PHG, calculated by taking the geometric mean of these three studies, of 0.032 mg/kg-day is higher than the current RfD (U.S. EPA, 1987) and the RfD developed in this assessment. On the other hand, ATSDR (1997) did not develop subchronic- and chronic-duration oral MRLs, although an acute MRL was developed from the study by Fredriksson et al. (1993). ATSDR (1997) noted neurological effects as the principal effect of tetrachloroethylene in humans and the scarcity of data in animals from subchronic and chronic studies on this endpoint.

One difference between this assessment and the health assessment from California (Cal EPA, 2001) are choices of studies and UFs. The Schreiber et al. (2002) and NYSDOH (2005) studies, which support the findings of Altmann et al. (1995) of neurotoxic effects in residentially exposed populations, were not available at the time of the California assessment. Another difference between this assessment and previous assessments is their treatment of human variation. A choice other than the default of 10 was adopted in the California assessment, based on a presumption that the residential population studied by Altmann et al. (1995) is more reflective of the general adult population than of an occupational population and any accompanying selection bias that is often associated with a healthier worker population.

Although there is some merit in this opinion, the observations in Altmann et al. (1995) are of a German population of 14 adults with exposure to tetrachloroethylene. These individuals do not likely represent the full range of human variation found in a large and ethnically diverse population such as the UNITED STATES population. The study excluded subjects with disorders such as hypertension, neurological or endocrinological diseases (e.g., diabetes), impaired vision, or impairment of joints; therefore, the subjects in Altmann et al. (1995) likely had overall good health status. Individuals with diseases that may have increased their susceptibility to tetrachloroethylene effects were not included in the study.

5.3. UNCERTAINTIES IN INHALATION REFERENCE CONCENTRATION (RFC) AND ORAL REFERENCE DOSE (RfD)

Risk assessments need to portray associated uncertainty. The following discussion identifies uncertainties associated with the RfC or RfD for tetrachloroethylene. As presented earlier in this chapter (see Sections 5.1.2, 5.1.3, 5.2.2, and 5.2.3), the uncertainty factor approach, following EPA practices and RfC and RfD guidance (U.S. EPA, 1993, 1994), was applied to a POD, a LOAEL from an epidemiologic study of neurobehavioral effects. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for

extrapolating the POD, the starting point in the analysis, to a no-adverse-effect concentration or dose (LOAEL to NOAEL) given insufficient data in the principal study for benchmark dose modeling, to a diverse population of varying susceptibilities, and to account for database deficiencies. These extrapolations are carried out with default approaches instead of factors derived from data on tetrachloroethylene given the paucity of experimental tetrachloroethylene data to inform individual steps. As further explained below, limited information is available on human variation in blood tetrachloroethylene concentration and can provide qualitative information on uncertainties associated with human variation. Evaluation of a tetrachloroethylene exposure dose or concentration likely to be without an appreciable risk of chronic adverse health effects over a lifetime and associated uncertainties relies on chemical-specific data to describe dose-response curves, on the breadth of the database for evaluating toxicity in a number of organs, and on characteristics of these data.

A broad range of animal toxicology and human epidemiologic data is available for the hazard assessment of tetrachloroethylene, as described throughout the previous section (Chapter 4). Included in these studies are short-term and long-term bioassays in rats and mice (see Table 4-2, Chapter 4); neurotoxicology studies in humans, rats, mice, and gerbils (see Tables 4-4, 4-5, 4-6, and 4-7); prenatal developmental toxicity studies in rats, mice, and rabbits and a two-generation reproduction study in rats (see Table 4-10); and numerous supporting genotoxicity and metabolism studies. Toxicity associated with inhalation exposure to tetrachloroethylene is observed in the liver, kidney, central nervous system, reproductive organs, and the developing fetus (see Chapter 4, Table 5-1, and Figure 5-2). Liver, kidney, and neurodevelopmental effects are observed with oral exposure (see Chapter 4, Table 5-2, and Figure 5-4). Nevertheless, critical data gaps have been identified and uncertainties associated with data deficiencies are more fully discussed below.

Neurotoxicity appears to be a sensitive organ system as previously identified in more limited analyses of Rao and Brown (1993) and Guth et al. (1997). The neurotoxic effects observed in a residential population (Altmann et al., 1995) are similar to those observed in occupational populations exposed at higher mean tetrachloroethylene concentration (Seeber, 1989, Echeverria et al., 1994). Schreiber et al. (2002) observed visual effects (visual contrast sensitivity) among residents co-located near dry cleaning establishments; however, this study was a pilot for a larger study. The larger study (NYS DOH, 2005) has become available as a final report and appears supportive of this pilot study

5.3.1. Point of Departure

A POD based on a LOAEL or NOAEL is, in part, a reflection of the particular exposure concentration or dose at which a study was conducted. It lacks characterization of the dose-response curve and for this reason is less informative than a POD defined as a BMC or a BMD obtained from benchmark dose-response modeling. With respect to neurotoxicity of tetrachloroethylene, benchmark dose-response models are fit to five data sets (Buben and O'Flaherty, 1985; JISA, 1993; NTP, 1986; Brodkin et al., 1995; Tinston, 1994) with sufficient information. The choice of benchmark dose model does not lead to significant uncertainty in estimating the POD since benchmark effect levels were within the range of experimental data. Parameter uncertainty can be assessed through confidence intervals and probabilistic analysis. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. Uncertainty in the animal dose-response data can be assessed through the ratio of BMCs to their BMCLs. These generally do not exceed a factor of two at the POD identified in Tables 5-1 and 5-2.

Effects in the CNS and in other organ systems (liver, kidney, reproductive, and developmental) in occupational populations and in animals are observed at higher average tetrachloroethylene concentrations than the Altmann et al. (1995) residential study. As more fully discussed in Section 5.1, uncertainties in other studies of neurotoxicity and of other organ systems differ from those of Altmann et al. (1995). For both occupational and residential populations, studies do not describe a NOAEL and human variation is not well characterized in study subjects. Uncertainties associated with the occupational studies include the following: (1) potential for neurobehavioral effects at lower exposures and (2) exposure pattern differences between occupational and residential studies with peaks characterizing occupational exposures. For animal studies, uncertainties are associated with extrapolating high concentration exposure typically of subchronic duration to genetically inbred rodents to infer a concentration of tetrachloroethylene that is likely to be without an appreciable risk of adverse health effects over a lifetime to a diverse human population.

5.3.2. Extrapolation from Laboratory Animal Studies to Humans

Extrapolating from animals to humans embodies further issues and uncertainties. First, the effect and its magnitude associated with the concentration at the POD in rodents is extrapolated to human response. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing. This was possible for liver toxicity where limited MOA information suggests metabolism as important to toxicity. The ranges of BMCLs presented for liver effects (a 10-fold range of estimates of tetrachloroethylene metabolism)

demonstrates the uncertainty in tetrachloroethylene pharmacokinetic models. The discrepancies between the models and with experimental data may point to large uncertainties in the parameters used in these models. Because the accuracy of the models has been evaluated only against blood and breath concentrations of the parent compound, their reliability for predicting total metabolites is an unknown. The use of all three of these pharmacokinetic models to provide a range of risk estimates is intended to capture some of this uncertainty.

5.3.3. Human Variation

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation needs consideration, also, in extrapolating dose from a subset or smaller sized population, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population.

In the absence of tetrachloroethylene-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation. Human variation may be larger or smaller; however, tetrachloroethylene-specific data to examine the potential magnitude of over- or under-estimation are few. The pharmacokinetic model of Clewell et al. (2004) of mean physiological parameters to explore age-dependent pharmacokinetic differences is suggestive of a 2-fold variation in blood tetrachloroethylene levels (see Chapters 3 and 5). Bois et al. (1996), revised by Chiu and Bois (2006), have examined uncertainty and variation in a tetrachloroethylene pharmacokinetic model describing the amount of tetrachloroethylene metabolism. This analysis suggests large uncertainty is associated with estimating the quantity of tetrachloroethylene metabolism in humans.

5.3.4. Database Uncertainties

Critical data gaps have been identified: uncertainties associated with database deficiencies on developmental, immunological, and neurotoxic effects. Most notably, data characterizing dose-response relationships and chronic visual-spatial functional deficits and the cognitive effects of tetrachloroethylene exposure under controlled laboratory conditions are lacking. Several halogenated organic solvents have been linked with altered immune system function in both animals and humans (e.g., toluene, TCE). Additional data from inhalation, oral, and dermal exposures, at different durations, are needed to assess the potential immunotoxicity of tetrachloroethylene. This lack of data combined with the concern that other structurally related solvents have been associated with immunotoxicity contributes to uncertainty in the database for tetrachloroethylene.

Data from acute studies in animals (Warren et al., 1996; Umeza et al., 1997) suggest that cognitive function is affected by exposure to tetrachloroethylene. These studies do not address the exposure-response relationship for subchronic and chronic tetrachloroethylene exposures on cognitive functional deficits observed in humans (e.g., Seeber, 1989; Echeverria et al., 1994; and Altmann et al., 1995). Even more importantly, there is a lack of cognitive testing in both developmentally exposed animals and adult animals following exposures to tetrachloroethylene that are longer than acute durations of exposure. Visual system dysfunction and processing of visual spatial information are sensitive endpoints in human studies. The exposure-response relationship of these functional deficits could be evaluated more definitively with studies using homologous methods that examine retinal and visual function in experimental animals. However, there has been a limited evaluation of visual function in rodents, with the exception of the evoked potential studies by Mattsson et al. (1998). These types of studies could help determine whether there are both peripheral and central effects of tetrachloroethylene exposure on visual perception, and they could be used as an animal model to better define the exposure-response relationships.

Subjects in the epidemiologic studies comprise adults, and some characterization of the response of children to tetrachloroethylene exposure was found in limited data for a similar neurological (visual system) parameter (Schreiber et al., 2002) and in a larger number of subjects (NYS DOH, 2005) using other visually based testing paradigms. Additionally, in a postnatal neurotoxicity study in mice (Fredriksson et al., 1993), persistent neurological effects (i.e., increased locomotion and decreased rearing behavior at 60 days of age, measured 43 days after exposure ceased) were observed at an oral dose of 5 ppm, with no NOAEL, although this study did not conform to traditional toxicity testing guidelines (see Section 4.6.2.2). There is uncertainty that if adequate, robust, dose-response data based on the most appropriate neurophysiological and cognitive tests were available, the exposure eliciting an adverse response (and hence the POD for the reference value) could be lower than that established on the basis of deficits in visuo-spatial and cognitive function following tetrachloroethylene exposure in healthy adults (Altmann et al., 1995).

5.4. CANCER DOSE-RESPONSE ASSESSMENT

The following dose-response assessment was developed following the guidelines in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). As discussed in Section 4.10.1, there is some indication from epidemiologic investigations that a human cancer risk is associated with exposure to tetrachloroethylene. Sufficient human data linked with exposure

characterizations from these studies have not been available, but estimating cancer risk from these studies might be feasible in the future.

Further, as detailed in Section 4.10.4., the available body of MOA information is not sufficient to derive quantitative, biologically based, or toxicodynamic models for low-dose extrapolation from animal data. Moreover, current literature does not identify a nonlinear MOA for tetrachloroethylene carcinogenicity. Therefore, consistent with the 2005 cancer guidelines, a default low-dose linear model is indicated for use with the animal data to estimate human cancer risk.

There is evidence that one or more tetrachloroethylene metabolites may be involved in some of the carcinogenicity associated with tetrachloroethylene exposure (see Section 4.10.4). PBPK models are available to estimate total metabolism in laboratory rodents and humans from inhalation and oral exposure to tetrachloroethylene. The dose-response discussion below describes where the PBPK models have been used to estimate human carcinogenic risk arising from tetrachloroethylene exposure through their impact on high-dose to low-dose extrapolation in animals, interspecies extrapolation, and route-to-route extrapolation.

5.4.1. Choice of Study/Data with Rationale and Justification

As discussed in Chapter 4, there are several chronic studies in rats and mice: an oral gavage study in mice and female rats by NCI (1977) and two inhalation studies in mice and rats (NTP, 1986; JISA, 1993). These studiesestablished that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, results in increased incidence of tumors. In at least two studies, several tumor sites showed statistically significantly increased rates with increasing tetrachloroethylene administration: MCL in male and female rats and hepatocellular adenomas and carcinomas in male and female mice. Other cancer dose response assessments of tetrachloroethylene have relied on the tumor data from the NCI and NTP studies (see Appendix A).

This analysis considers all three bioassays but focuses primarily on the JISA (1993) study results. First, the JISA (1993) study included lower exposures than did the two earlier bioassays for both species tested, which makes it a stronger study for deriving dose-response relationships for risk assessment purposes, insofar as all other aspects of these studies can be considered comparable. For mice, the lowest exposure concentration of 10 ppm was 10-fold lower and the mid-dose of 50 ppm was 2-fold lower than the lower exposure concentration in the NTP (1986) inhalation study (100 ppm). For rats, the low-exposure concentration of 50 ppm was fourfold lower than in the NTP study (200 ppm). Second, no other dose response modeling appears to be

available for the JISA (1993) study, whereas the incidence of hepatocellular tumors and MCL in the NTP (1986) study have been extensively analyzed for previous assessments.

5.4.2. Dose-Response Data

5.4.2.1. Liver Tumors in Mice

All three bioassays showed increases in hepatocellular tumors in male and female mice. Table 5-5 summarizes these incidence patterns. Because hepatic adenomas and carcinomas are considered part of the same continuum of tumor development, and adenomas may be differentiated from carcinomas only on the basis of size, this analysis emphasizes the combined incidence of these two tumor types. Historical data from the Japan Bioassay Research Center (JBRC), where the JISA (1993) study was conducted, indicate that the control liver tumor incidences in this study were fairly typical for this laboratory (see Table 5-6). Specifically, the incidence in controls was 28% for males and 6% for females; the averages for the laboratory were 23% and 2% and the upper bounds were 42% and 8%, respectively, for carcinomas.[8]

The results of the inhalation studies are reasonably consistent when adjusted for background tumor incidence (see Figures 5-5a and 5-5b). Liver tumor incidence among male mice in the JISA (1993) study did not follow a clearly monotonic pattern, with a higher response in the low-dose group than seems consistent with the pattern in responses in the other dose groups. Taking into account this variability in the responses, however, the dose-response patterns for the male and female mice in the NTP (1986) and JISA (1993) studies appear reasonably concordant.

Several issues complicate comparisons of the NCI (1977) gavage study results with those of the other chronic bioassays. First, dosing lasted 78 weeks rather than 104 weeks as in the inhalation studies, so in making direct comparisons it might be expected that the observed tumor incidence in the NCI (1977) study would underestimate the incidence associated with 104 weeks of exposure. Second, this oral gavage study had a variable dosing schedule, with doses that were increased by 100 mg/kg-day in the low-dose group and by 200 mg/kg-day in the high-dose group after 11 weeks of study. Consequently, association of a constant level of exposure with the observed effects must be inferred rather than measured. In addition, surviving animals were maintained without further exposure until final sacrifice in week 90.

The NCI (1977) exposures were recalculated on a basis consistent with other EPA estimates of chronic toxicity, in which the cumulative exposure received over the full period of

[8]Combined historical incidence of adenomas and carcinomas was not available. Presumably the incidence of carcinomas slightly underestimates the combined incidence of adenomas and carcinomas.

Table 5-5. Tumor incidence and estimated metabolized doses in mice exposed to tetrachloroethylene

Bioassay	Doses/Exposures: Administered	Doses/Exposures: Continuous Equivalent	Sex	Body Weight[a] (kg)	Total Metabolism[b] (mg/kg-day)	Survival-Adjusted Tumor Incidence[c] (%)
Hepatocellular adenomas and carcinomas						
NCI (1977)[d] B6C3F$_1$ mice Gavage: 5 days/wk, 78 wks	Vehicle control 450 mg/kg-day 900 mg/kg-day	0[e] 332 mg/kg-day 663 mg/kg-day	Male	0.030	0 35 47	2/20 (10) 32/48 (67) 27/45 (60)
	Vehicle control 300 mg/kg-day[e] 600 mg/kg-day	0[e] 239 mg/kg-day 478 mg/kg-day	Female	0.025	0 34 46	0/20 (0) 19/48 (40) 19/45 (42)
NTP (1986) B6C3F$_1$ mice Inhalation: 6 hrs/day, 5 days/wk, 104 wks	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Male	0.037	0 27 41	17/49 (35) 31/47 (70) 41/50 (82)
	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Female	0.032	0 31 45	4/45 (9) 17/42 (40) 38/48 (79)
JISA (1993) Crj:BDF1 mice Inhalation: 6 hrs/day, 5 days/wk, 104 wks	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Male	0.048	0 3.4 14 36	13/46 (28) 21/49 (43) 19/48 (40) 40/49 (82)
	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Female	0.035	0 4 18 47	3/50 (6) 3/47 (6) 7/48 (15) 33/49 (67)
Malignant hemangiosarcomas[f], liver or spleen						
JISA (1993) Crj:BDF1 mice 6 hrs/day, 5 days/wk, 104 wks	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Male	0.048	0 3.4 14 36	2/46 (4) 1/49 (2) 6/48 (13) 9/49 (18)

[a] Average body weight reached during adulthood.

[b] As calculated using the Reitz et al. (1996) pharmacokinetic model for mice, using alveolar ventilation rate, at 67% of total ventilation (see Section 3.5). Total metabolism was estimated from the simulated bioassay exposure pattern, i.e., the amount estimated to be metabolized following an increment of exposure (gavage dose or 6-hr inhalation exposure). Adjustment for continuous exposure followed by multiplying the exposure by (5 days/7 days). Figure 3-9 illustrates the correspondence of total metabolism with administered exposure estimated by this model for mice weighing 0.025 kg: at 100 ppm, approximately 47 mg-equivalent (eq)/kg-day of metabolites are estimated to be produced. For the purposes of this assessment, this is assumed to be equivalent to 47 mg-eq/kg-day × 5/7 = 34 mg-eq/kg-day of metabolites resulting from continuous exposure. Note that this level is higher than the 31 mg-eq/kg-day estimated for 0.032 kg female mice and the 27 mg-eq/kg-day for 0.037 kg male mice in the NTP (1986) study in this table, illustrating the dependence of the PBPK model on body size. This dependence is not tabulated or graphed in this document.

Table 5-5. Tumor incidence and estimated metabolized doses in mice exposed to tetrachloroethylene (continued)

[c] Animals dying before the first appearance of the tumor of interest but no later than week 52 were omitted from the totals because these animals were presumed not to have adequate time on study to develop tumors.

[d] No adenomas were reported in this study. Because hepatic adenomas and carcinomas are considered part of the same continuum of tumor development, and adenomas have been distinguished from carcinomas only on the basis of size, the correspondence of this observation to the other studies is not clear.

[e] Gavage doses listed were increased after 11 weeks by 100 mg/kg-day in each low-dose group or by 200 mg/kg-day in each high-dose group. Animals surviving the 78-week exposure period were observed until the week 90 study termination. Lifetime average daily (administered) doses (LADDs) were calculated as follows:

$$\begin{aligned}\text{LADD (mg/kg-day)} &= \text{Cumulative administered dose (mg/kg)/(total days on study)} \\ &= \{[(\text{initial dose level} \times 11 \text{ weeks}) + (\text{increased dose level} \times 67 \text{ weeks})]/90 \text{ weeks}\} \\ &\quad \times (5 \text{ days}/7 \text{ days})\end{aligned}$$

Male mice received LADDs of 332 and 663 mg/kg-day, and female mice received 239 and 478 mg/kg-day.

[f] These tumors were reported as hemangioendotheliomas in the JISA (1993) report. The term has been updated to hemangiosarcoma. Note that these incidences do not match those tabulated in Table 12 of the JISA report summary. The incidences reported here represent a tabulation of malignant hemangioendotheliomas from the individual animal data provided in the JISA report.

Table 5-6. Historical control data of the Japan Bioassay Research Center, Crj/BDF1 mouse, 104-week studies

Tumor types	Inhalation, feeding, and drinking studies (19 studies)		Inhalation studies only (9 studies)	
	Total incidence (%)	Range (%)	Total incidence (%)	Range (%)
Male mice				
Liver				
hepatocellular adenoma	165/947 (17.4)	4.0–34.0	92/448 (20.5)	10.0–30.6
hepatocellular carcinoma	215/947 (22.7)	2.0–42.0	105/448 (23.4)	10.0–36.7
Spleen				
hemangioma[a]	17/946 (1.8)	0–10.0	8/448 (1.8)	0–8.0
hemangiosarcoma[a]	30/946 (3.2)	0–8.0	12/448 (2.7)	0–6.0
Female mice				
Liver				
hepatocellular adenoma	50/949 (5.3)	2.0–10.0	18/449 (4.0)	2.0–6.0
hepatocellular carcinoma	22/949 (2.3)	0–8.0	14/449 (3.1)	0–8.0
Spleen				
hemangioma[a]	8/949 (0.9)	0–6.0	5/449 (1.1)	0–6.0
hemangiosarcoma[a]	3/949 (0.3)	0–2.0	3/449 (0.7)	0–2.0

[a] The terms "hemangioendothelioma: benign" and "hemangioendothelioma" in the original study have been changed to "hemangioma" and "hemangiosarcoma," respectively.

Source: Attachment to letter dated September 5, 2001, from K. Nagano, Japan Bioassay Research Center, Japan Industrial Safety and Health Association, to R. McGaughy, U.S. EPA. Available from hotline.iris@epa.gov.

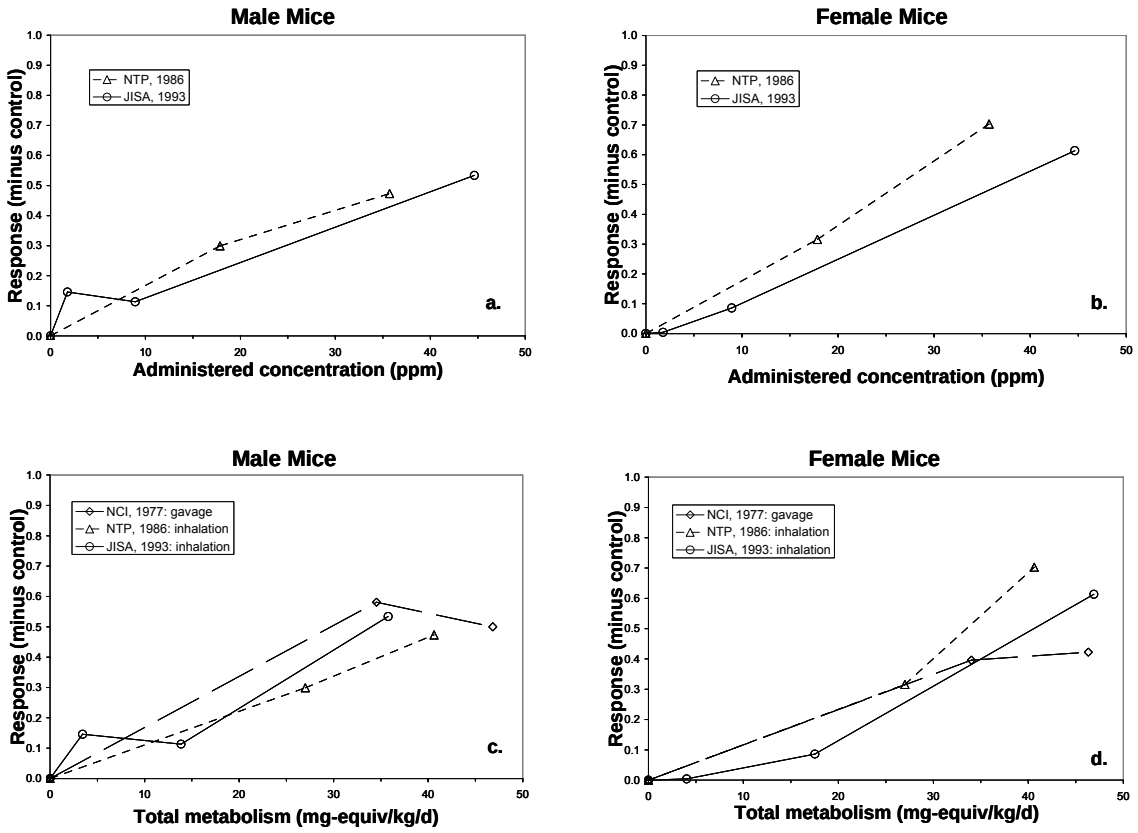


Figure 5-5. Mouse liver tumor responses (hepatocellular adenomas and carcinomas) for three chronic bioassays (Table 5-5), plotted against continuous equivalent concentration (ppm) and total tetrachloroethylene metabolism (mg-equivalents/kg-day), for male and female mice.

observation is prorated to obtain an average lifetime daily exposure. Table 5-5 provides the recalculated exposures. It is not clear in this case, however, that a simple TWA over the period of observation is the most suitable representation of tetrachloroethylene exposure in the NCI (1977) study, due to the substantial changes in the dosing pattern, as noted above. In addition, mortality was significantly increased in both treated groups over that of controls, suggesting that the maximum tolerated dose had been exceeded. Note that although no adenomas were reported in the NCI (1977) study, some of the reported carcinomas may have been adenomas. This factor should be taken into account when comparing the incidence of carcinomas in the NCI (1977) study with the combination of adenomas and carcinomas in the inhalation studies (see Table 5-5). Consequently, it was not feasible to compare this dose-response with that from the inhalation studies on an administered mg/kg-day basis.

In addition to hepatocellular adenomas and carcinomas, the JISA (1993) study demonstrated increased hemangiomas and hemangiosarcomas in the liver and spleen of mid- and high-dose male mice (see Table 5-5; Cochran-Armitage trend test, $p = 0.004$). The incidence in control and low-dose male mice was similar to the JBRC historical control incidence for spleen only (3.2%, range 0–8%; see Table 5-6). This finding was not replicated in the NCI (1977) or NTP (1977) studies (tumors noted in the NTP male mice livers: controls, 3/49; low dose, 2/49; high dose, 2/50; tumors noted in the NTP historical controls for all sites: 4.4%, range 2–8% (http://ntp.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/m_inhar.txt). Both the JBRC and NTP historical controls showed similar background levels of hemangiomas and hemangiosarcomas, although the JBRC data included only the spleen, whereas the NTP data included all sites.

Because tetrachloroethylene's metabolites have been implicated in its liver toxicity (see Section 4.10.4.1), and because a pharmacokinetic model was available to estimate metabolism levels in mice (based on the work of Reitz et al., 1996; see Section 3.5), the hepatocellular tumor responses in the three chronic bioassays were compared in terms of total metabolism of tetrachloroethylene (see Figures 5-5c and 5-5d). Here it can be seen that the hepatocellular tumor dose-response in the gavage study appears to be quite similar to that of the inhalation studies. Note further that, from an empirical point of view, the dose-response patterns for the inhalation studies collectively appear to follow an approximately linear relationship, whether the exposure measure is the administered concentration or total metabolism. In other words, these data do not clearly suggest one dose metric over the other as being more closely associated with the liver tumors or that some other dose metric would be preferred for characterizing cancer incidence in this range of exposure.

5.4.2.2. Mononuclear Cell Leukemia in Rats

The NTP (1986) and JISA (1993) studies demonstrated increased MCL incidences for male and female rats (see Table 5-7). The NCI study did not demonstrate any MCL increases in rats. However, the investigators considered this study inconclusive because of low survival, so the NCI study neither confirms nor refutes the findings of the NTP and JISA studies.

The responses in the NTP (1986) study were approximately twofold higher than for the corresponding groups in the JISA (1993) study, including the control groups. Control groups for both laboratories were consistent with their respective historical controls (see Table 5-8 for the JISA historical controls). Like the hepatocellular tumor results in mice (see Section 5.4.2.1), the MCL results from the NTP and JISA studies were plotted in terms of additional risk versus administered concentration (see Figure 5-6). Note that MCL risk has been considered previously

Table 5-7. Incidence of mononuclear cell leukemia, kidney tumors, and brain gliomas in rats exposed to tetrachloroethylene by inhalation

Bioassay	Exposure concentration (ppm)		Sex	Body weight (kg)	Total metabolism[a] (mg/kg-day)	Survival-adjusted tumor incidence[b] (%)
	Administered	Continuous equivalent				
Mononuclear cell leukemia						
NTP (1986)	0	0	Male	0.44	0.0	28/50 (56)
F344/N rats	200	36			3.6	37/48 (77)
Inhalation	400	72			5.0	37/50 (74)
6 hrs/day,	0	0	Female	0.32	0.0	18/50 (36)
5 days/wk,	200	36			5.1	30/50 (60)
104 wks	400	72			6.9	29/50 (58)
JISA (1993)	0	0	Male	0.45	0.0	11/50 (22)
F344/DuCrj rats	50	9			1.4	14/50 (28)
Inhalation	200	36			3.6	22/50 (44)
6 hrs/day,	600	108			6.1	27/50 (54)
5 days/wk,	0	0	Female	0.3	0.0	10/50 (20)
104 wks	50	9			2.0	17/50 (34)
	200	36			5.1	16/50 (32)
	600	108			8.7	19/50 (38)
Kidney: tubular cell adenoma or adenocarcinoma						
NTP (1986)	0	0	Male	0.44	0.0	1/49 (2)
	200	36			3.6	3/47 (6)
	400	71			5.0	4/50 (8)
JISA (1993)	0	0	Male	0.45	0.0	1/50 (2)
	50	9			1.4	2/50 (4)
	200	36			3.6	1/50 (2)
	600	110			6.1	2/50 (4)
Brain gliomas						
NTP (1986)	0	0	Male	0.44	0.0	1/50 (2)
	200	36			3.6	0/48 (0)
	400	71			5.0	4/50 (8)
JISA (1993)	0	0	Male	0.45	0.0	2/50 (4)
	50	9			1.4	0/50 (0)
	200	36			3.6	0/50 (0)
	600	110			6.1	0/50 (0)

[a] As calculated by the Reitz et al. (1996) pharmacokinetic model for rats using alveolar ventilation rate at 67% of total ventilation (see Section 3.5). Total metabolism was estimated from the simulated bioassay exposure pattern, that is, the amount estimated to be metabolized following an increment of exposure (6-hr inhalation exposure). Adjustment for continuous exposure followed by multiplying the exposure by (5 days/7 days). Figure 3-9 illustrates the correspondence of total metabolism with administered exposure estimated by this model for rats weighing 0.3 kg: at 200 ppm, approximately 7.1 mg-equivalent (eq)/kg-day of metabolites are estimated to be produced. For the purposes of this assessment, this is assumed to be equivalent to 7.1 mg-eq/kg-day × 5/7 = 5.1 mg-eq/kg-day of metabolites resulting from continuous exposure (see metabolite levels above for the JISA female rats). Note that this level is higher than the 3.6 mg-eq/kg-day estimated for 0.45 kg rats in the JISA study, illustrating the dependence of the PBPK model on body size. This dependence is not tabulated or graphed in this document.

[b] Animals dying before the first appearance of the tumor of interest but no later than week 52 were omitted from the totals because these animals were presumed to have had inadequate time on study to develop these tumors.

Sources: NTP (1986) and JISA (1993).

Table 5-8. Historical control data of the Japan Bioassay Research Center, F344/DuCrj (Fischer) rat, 104-week studies

Tumor types	Inhalation, feeding, and drinking studies (23 studies)		Inhalation studies only (11 studies)	
	Total incidence (%)	Range (%)	Total incidence (%)	Range (%)
Male rats				
Mononuclear cell leukemia	147/1149 (12.8)	6.0–22.0	76/549 (13.8)	6.0–22.0
Kidney				
Renal cell adenoma	2/1149 (0.2)	0–2.0	1/549 (0.2)	0–2.0
Renal cell carcinoma	2/1149 (0.2)	0–2.0	2/549 (0.4)	0–2.0
Female rats				
Mononuclear cell leukemia	147/1048 (14.0)	2.0–26.0	68/448 (15.2)	8.0–20.0
Kidney				
Renal cell adenoma	1/1048 (0.1)	0–2.0	1/448 (0.2)	0–2.0
Renal cell carcinoma	0/1048 (0.0)	NA	0/448 (0.0)	NA

Source: Attachment to letter from K. Nagano to R. McGaughy 9/5/01. Available from IRIS Information Desk.

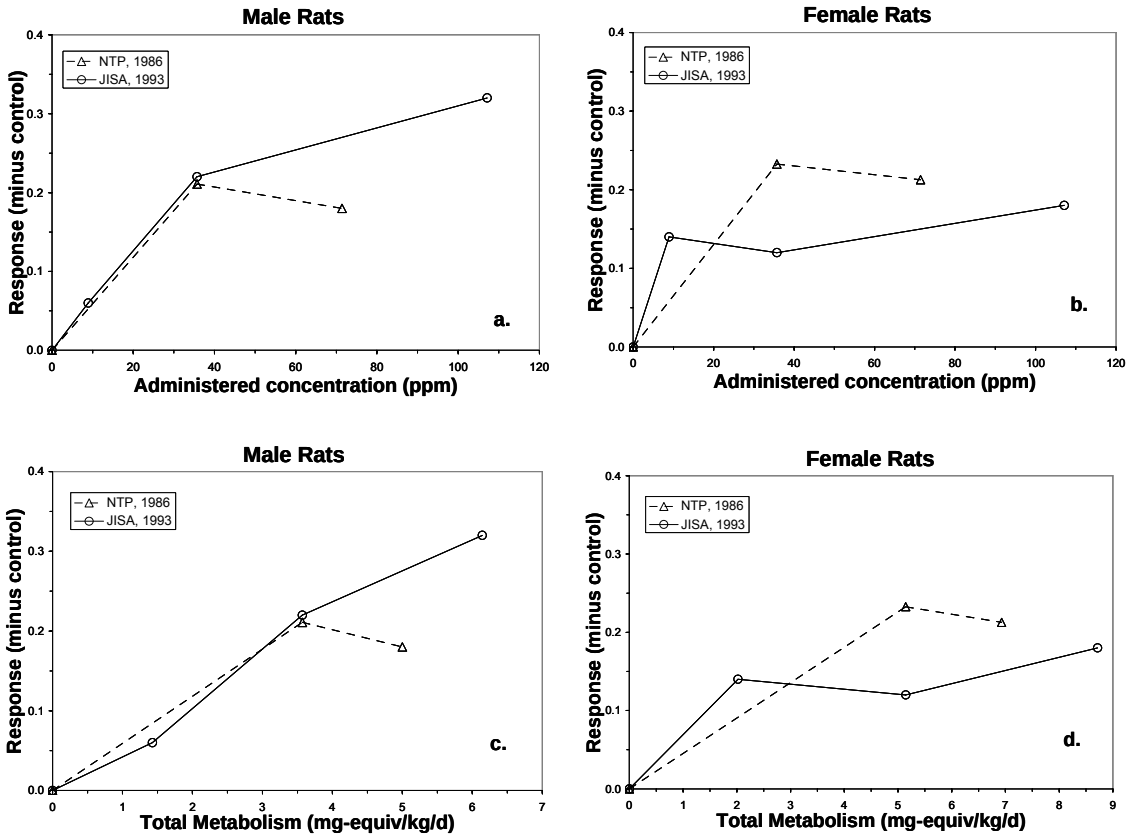


Figure 5-6. Rat mononuclear cell leukemia responses (minus control) in two chronic bioassays (Table 5-7), plotted against continuous equivalent exposure (ppm) and total tetrachloroethylene metabolites, in mg-equivalents/kg-day, for male and female rats.

to be associated with total metabolized dose as the dosimeter (see U.S. EPA, 1986; CARB, 1991).

Although it is not known whether the parent compound, one or more metabolites, or a combination are involved in the induction of MCL by tetrachloroethylene, available evidence indicates that a metabolite of the GST pathway may be involved (see Section 4.10.4.3). Consequently, Figure 5-6 also includes plots of additional risk of MCL versus total metabolism.

The NTP and JISA studies are reasonably consistent for male rats in terms of the relative increases in tumors over background incidences, whether the dosimeter is total metabolism or administered tetrachloroethylene. For this site, the dose-response relationship appears more linear for total metabolism than with administered dose, at least for the JISA study (see Figures 5-6a and 5-6c).

For female rats (see Figures 5-6b and d), the responses adjusted for control rates do not show as much concordance between studies as those for the male rats, with the JISA study still showing lower adjusted responses than the NTP study at comparable exposures. The low-dose females in the JISA study had a higher response relative to the pattern of responses in the other three groups and a higher response than would be expected from the dose-response pattern in the NTP study. The dose-response relationship for the JISA study appears to be slightly more linear for total metabolism than for administered concentration; however, both studies suggest some degree of saturation of effects in the available range of the dose metrics considered. Although F344 rats were used in both studies, it is possible that there could be some differences attributable to the specific lines of animals used at each laboratory and laboratory-specific procedures.

5.4.2.3. Other Tumor Sites in Male Rats

Other elevated tumor incidences—brain gliomas and kidney tubule adenomas and adenocarcinomas—were observed in male F344/N rats in the NTP study but not in the JISA study (again, there were no corresponding data available for the NCI male rats). Table 5-7 summarizes the incidence data from both laboratories for these sites. Brain gliomas in rats in the NTP inhalation study were elevated. In males, the incidences were 1/50, 0/48, and 4/50 in the control, 200 ppm, and 400 ppm tetrachloroethylene groups, respectively. This was a statistically significant dose-related trend by the life table test ($p = 0.039$) but not by the incidental tumor trend test. A similar trend was seen in female rats (1/50, 0/50, 2/50), but this trend was not statistically significant. Brain gliomas are rare tumors in NTP rat bioassays; in male rats the historical control incidence is 2/247 (0.8%) in the laboratory where this study was conducted, and 4/1971 (0.2%) in the overall program. Because these tumors had not been observed in previous NTP studies of tetrachloroethylene, trichloroethylene, or pentachloroethane, and because they appeared in the untreated groups, the NTP investigators concluded that they were not related to tetrachloroethylene exposure.

On the other hand, the previous study of tetrachloroethylene was the NCI (1977) gavage study, not an inhalation study; route-to-route differences are not implausible. Although the other solvents mentioned have been associated with similar adverse effects, there are other differences among them. Also, the brain tumors in the high-dose males started occurring earlier (weeks 88, 96, 102, and 103) than in the control group (99 weeks), and in the high-dose females they occurred even earlier (75 and 78 weeks in the high-dose group vs. 104 weeks in the control group). Therefore, although the association between tetrachloroethylene exposure and brain gliomas in rats in this data set is not strong, it is still suggestive, especially considering that the

nervous system is a target of tetrachloroethylene exposure in humans and animals (see Sections 4.5.3 and 5.1.1).

In the JISA study, brain gliomas were observed only in male control rats. Historical brain tumor incidence data for this laboratory were not available, however. Although F344 rats were used in both studies, it is possible that there could be some differences between laboratories attributable to the specific lines of animals used and laboratory-specific procedures. Given the low overall incidences relative to the other tumor sites, these data were not modeled.

Kidney tubule cell adenomas and adenocarcinomas (see Table 5-7) were elevated in the exposed male rats, but they were not statistically significantly elevated. Statistical significance is a secondary consideration in determining the biological significance of these tumors because they are considered to be uncommon in NTP studies of rats. The investigators noted that these tumors were observed among historical controls at about 0.2% in 1968 untreated control rats. Further support for considering the relevance of this site comes from the evidence relating trichloroethylene and rat kidney tumors (U.S. EPA, 2001).

There was no apparent trend in the incidence of kidney tubule cell adenomas and adenocarcinomas among JISA male rats. The incidence in all groups was consistent with JISA historical control data (see Table 5-8).

5.4.3. Estimation of Dose Metrics for Dose-Response Modeling

The sequence of steps in estimating human equivalent risks is illustrated in Figure 5-7, with estimation of the dose inputs to the dose-response modeling being the first step. Considerations for estimating continuous exposure levels equivalent to the intermittent animal bioassay exposures differ according to whether administered exposures or metabolized doses were used as the measure of dose, and they are discussed following the selection of each dose metric.

5.4.3.1. Dose Metric for Hepatocellular Carcinogenicity

There are several possible rationales to consider for the appropriate dose metric for tetrachloroethylene-induced liver toxicity and carcinogenicity. The specific chemical species responsible for adverse effects on the liver would be the preferred choice. As discussed in Chapter 3 and Section 4.10.4.1, several metabolites associated with P450 metabolism occurring in the liver have been identified. TCA, which is associated with liver toxicity when administered directly, is considered a key product of this P450 oxidation pathway. However, the MOA for tetrachloroethylene-induced liver toxicity and carcinogenicity is not clear. Further, TCA does not appear to explain the liver carcinogenicity observed with tetrachloroethylene, because a

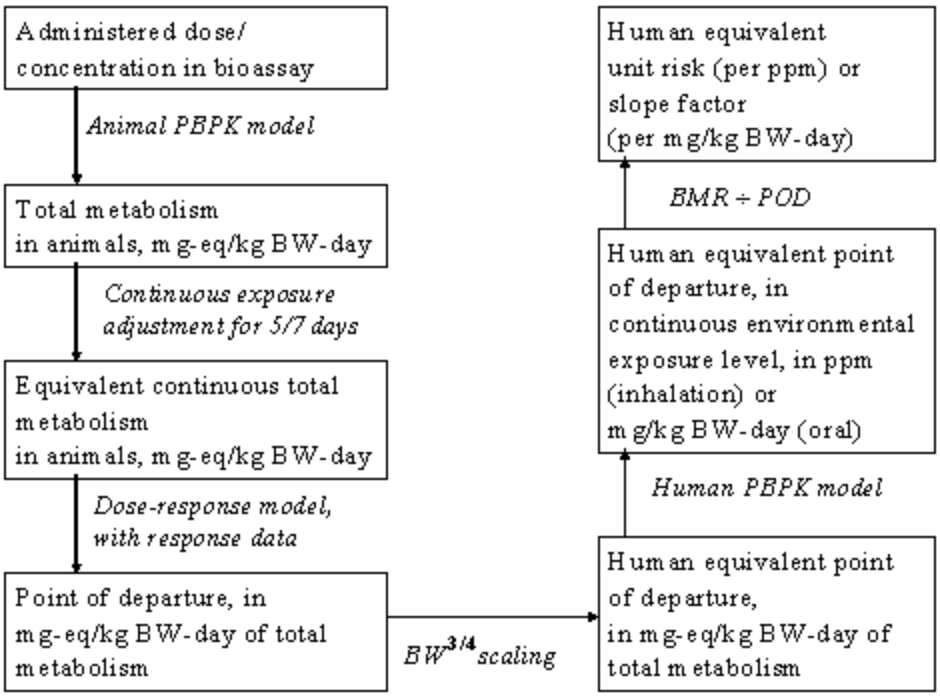


Figure 5-7. Sequence of steps for extrapolating from tetrachloroethylene bioassays in animals to human-equivalent exposures expected to be associated with comparable cancer risk.

comparison of hepatocellular tumor incidence associated with direct TCA exposure appears to underpredict the hepatocellular carcinoma incidence in the NTP and JISA studies when characterized in terms of equivalent TCA exposures (see Appendix 4A in Chapter 4).

Additional metabolites could play a role. For instance, reactive intermediates such as tetrachloroethylene oxide or trichloroacetyl chloride are hypothesized to be precursors to TCA, and as reactive compounds they would be likely candidates. However, their involvement in liver toxicity remains unknown, and they have not been confirmed in the tetrachloroethylene metabolic pathways. Consequently, although it appears plausible that at least another compound besides TCA contributes to tetrachloroethylene-induced hepatocarcinogenicity, none has been identified nor can the amounts be estimated.

Because of the uncertainty over which metabolite species are involved in causing liver toxicity—and to what degree they are involved—total metabolism was considered the most appropriate dose metric. Use of this dose metric does not require assuming that all of the metabolites are responsible for tetrachloroethylene's liver carcinogenicity, however, only that the rate of total metabolite production is proportional to the actual target dose in the target tissue, at least at very low exposures. That is, if there is a constant relationship between the surrogate dose

measure and the relevant measure of the actual carcinogen, at least at very low exposures, then dose-response modeling of each measure (if both were available) would yield the same cancer risk value in terms of environmental exposure. For instance, the concentration of the actual liver carcinogen(s) could be in equilibrium with the circulating blood, or it could be primarily generated and active in the liver; as long as proportionality of the surrogate dose measure is a reasonable assumption, cancer risk values can be estimated and interpreted accordingly. Consequently, the daily production rate of all metabolites of tetrachloroethylene corresponding to the bioassay exposure patterns, as estimated using the PBPK model of Reitz et al. (1996), was used for the dose-response modeling of mouse liver tumors.

The second adjustment made prior to dose-response modeling was to characterize the intermittent bioassay exposures in terms of equivalent continuous exposure. Because the pharmacokinetic model cannot generate an AUC for total metabolism, due to the lack of clearances for the individual metabolites, the daily production rate of all metabolites for five days was averaged over seven days by multiplying it by 5/7 (0.71), under the assumption that concentration multiplied by time maintains a constant effect ($C \times t = k$), is likely to hold for very low tetrachloroethylene exposures. The metabolism rates reported in Tables 5-5 and 5-7 reflect this adjustment.

5.4.3.2. Dose Metric for Rat Leukemias and Kidney Tumors

Experimental evidence suggests that a GST-pathway metabolite (TCVC) is more likely to be associated with the kidney tumors and possibly the leukemias than the P450 pathway (see Sections 4.9.4.2 and 4.9.4.3). However, the measurements of glutathione-dependent metabolism are from in vitro studies or they are measures of urinary excretion products and are, therefore, not representative of the toxic species in vivo. Consequently, insufficient data exist to incorporate the GST-derived metabolites explicitly in the PBPK models.

Given the approximately linear dose-response relationship observed between leukemias and total metabolism for male rats (see Figure 5-6c), it appears plausible that the carcinogen responsible for the leukemias may be approximately proportional to total metabolism. The situation is somewhat less clear for female rats due to the nonmonotonic dose-response patterns, although the degree of saturation was less pronounced when the dose-response relationship was considered in terms of total metabolism (Figure 5-6d). Accordingly, total metabolism was considered a better surrogate than administered concentration for the proximate carcinogen. Because kidney tumors are associated with the same GST-metabolite as the leukemias—somewhat more definitively than the leukemias—total metabolite production was also considered as a dose metric for estimating the male rat kidney dose-response relationship.

Adjustment for continuous exposure was the same as for the liver tumors (see Section 5.4.3.1). That is, the only continuous exposure adjustment to the total metabolite dose metric that was needed was to average the metabolic rate for five days over seven days (by multiplying each by 5/7).

5.4.3.3. Dose Metric for Sites Not Addressed by Physiologically Based Pharmacokinetic (PBPK) Modeling

For tumor sites for which the MOA is not clear, such as for male rat brain tumors (see Section 4.10.4), the administered concentration of tetrachloroethylene was used as the dose metric. Use of this dose metric should provide plausible results as long as the concentration of the proximate carcinogen(s) is proportional to administered concentration—at least at low concentrations. Because there is uncertainty in the identification of the carcinogenic agent for all the sites, the dose-response relationships for all tumor sites were also estimated using this default dose metric, for comparison purposes.

Allowance for extrapolation to continuous exposures was made before dose-response modeling. In all cases, administered inhalation concentrations (in ppm) were adjusted for continuous exposure by averaging the five 6-hr daily exposures over the full week. That is, administered concentrations were multiplied by 6 hrs/24 hrs × 5 days/7days (0.179) to yield equivalent continuous concentrations. Tables 5-5 and 5-7 provide these adjusted concentrations.

5.4.4. Extrapolation Methods

Extrapolation of tetrachloroethylene cancer risks observed in animal bioassays to humans with continuous environmental exposure involved a number of methods, including dose-response modeling in the range of observation, interspecies extrapolation, extrapolation to low exposures, and route-to-route extrapolation. Section 5.4.4.1 and Figure 5-7 summarize the methods used to extrapolate from the experimental data to humans.

5.4.4.1. *Dose-Response Models and Extrapolation to Low Doses*

As discussed in Section 4.10.3, the available body of MOA information is not sufficient to derive biologically based quantitative models for low-dose extrapolation. No key events in the tumor development process for tetrachloroethylene have been identified that would determine the overall dynamics of such a model, nor are there experimental data specific to tetrachloroethylene describing any of the underlying toxicodynamic processes, such as cell replication rates.

The multistage model (and the multistage-Weibull) has been used by EPA in the vast majority of quantitative cancer assessments because it has some parallelism to the multistage carcinogenic process and it fits a broad array of dose-response patterns. Occasionally the multistage model does not fit the available data, in which case an alternate model should be considered. The related multistage-Weibull model has been the preferred model when individual data are available for time-to-tumor modeling, which considers more of the observed response than does the simpler dichotomous response model. Use of this decision scheme has contributed to greater consistency among cancer risk assessments.

Consequently, the multistage model was the primary tool considered for fitting the dose-response data and is given by:

$$P(d) = 1 - exp(-q_0 - q_1 \times d - q_2 \times d^2 - \ldots - q_6 \times d^6)$$

where:

d = exposure level and

q_i = parameters estimated in fitting the model

The multistage model in BMDS (Benchmark Dose Software, version 1.3.2; U.S. EPA, 2000) was used for all multistage model fits.

Two tumor sites with statistically significantly decreased time to tumor were noted: brain gliomas in NTP male rats and MCL in the NTP female rats, especially for the most severe stage of leukemia observed (Stage 3). The multistage-Weibull model, given by the following equation, was also used to evaluate the importance of decreased time to tumor and intercurrent mortality in interpreting these responses.

$$P(d,t) = 1 - exp[(-q_0 - q_1 \times d - q_2 \times d^2 - \ldots - q_6 \times d^6) \times t^z]$$

where:

d = exposure level

t = time to observation of the tumor

q_i, z = parameters estimated in fitting the model

Note that when the time to observation of the tumor is not a significant contributor to the dose-response relationship, the parameter z is estimated to have a value of 1, and the model reduces to the simpler multistage model described just before the multistage-Weibull. Tox_Risk (K.S.

Crump Group, Inc., ICF Kaiser International, Ruston, LA) was used for all multistage-Weibull model fits.

Following dose-response modeling in the range of observation, the cancer risk values for extrapolation to low doses were derived from the lower bound on the dose/concentration associated with a level of risk from the low end of the observed range, usually 10% extra risk, consistent with the 2005 cancer assessment guidelines. Extra risk has been used consistently throughout EPA risk assessments and is given by:

$$Extra\ risk = [P(d) - P(0)] / [1 - P(0)]$$

where:

P(d) = estimated response at dose d and

P(0) = estimated response in the control group

The slope factor (risk per mg/kg-day for oral exposure) and risk per unit concentration (risk per mg/L for drinking water exposure, or per $\mu g/m^3$ for inhalation exposure) are estimated by dividing the risk level by its associated POD:

Risk/(unit of exposure) = Extra risk/(lower confidence bound on associated exposure)

5.4.4.2. *Extrapolation to Human Equivalent Environmental Exposure*

For extrapolation of risk to humans, this assessment used two approaches that were dependent on the relevant dose metric: the EPA RfC methodology (U.S. EPA, 1994), which applies when chemical-specific pharmacokinetic data are lacking, and EPA's cross-species scaling methodology (U.S. EPA, 1992), which applies to exposures characterized in mg/kg-day, whether parent chemical or metabolite.

5.4.4.2.1. ***Metabolized dose.*** Because of the availability of PBPK models to estimate a plausible dose metric that addresses the differential metabolism of tetrachloroethylene between laboratory rodents and humans, extrapolation to human equivalent environmental exposure entailed two steps. First, consistent with the 2005 cancer guidelines (U.S. EPA, 2005a), EPA's methodology for cross-species scaling (U.S. EPA, 1992) was considered when toxicological equivalence for the relevant tumor sites was addressed. Then, the human equivalent exposure in terms of metabolized dose was estimated via the human PBPK models. These considerations are further described below.

EPA's cross-species scaling methodology was used for describing toxicological equivalence because of the extensive rationale supporting it (U.S. EPA, 1992). Briefly, the methodology maintains that, in the absence of adequate information to the contrary, toxicological equivalence across species is determined through equal average lifetime concentrations or AUCs of the carcinogen. The most typical application of this methodology is to oral exposures in mg/kg-day, with no pharmacokinetic or pharmacodynamic information. In this circumstance, the correspondence of equal AUCs is equivalent to considering the exposures in terms of $mg/kg^{3/4}$-day, and is achieved by multiplying animal exposures by $(BW_{animal}/BW_{human})^{1/4}$. Note that this equivalence across species entails the cross-species correspondence of *internal* doses in terms of AUCs or $mg/kg^{3/4}$-day, which is implicit in the frequent default case, i.e., oral carcinogens without chemical-specific pharmacokinetic data. In other words, each time a carcinogen is scaled from animals to humans on the basis of $mg/kg^{3/4}$-day, an implicit assumption is that internal doses are equipotent in terms of $mg/kg^{3/4}$-day ("cross-species scaling"), not mg/kg-day ("body-weight scaling"). Accordingly, when pharmacokinetic data are available that relate administered concentration to the overall metabolized dose of the carcinogen, this methodology is still applicable; internal doses, as a fraction of administered dose, should still tend to produce equivalent effects when considered in terms of AUCs or $mg/kg^{3/4}$-day because metabolites are also subject to scale-affected clearance processes. In other words, the scaling may be thought of as applied to the administered dose adjusted by the fraction metabolized. There is a wide body of empirical evidence that overall metabolic rates associated with enzymatic processes scale with body weight to the 3/4 power (U.S. EPA, 1992). Furthermore, because in this assessment the scaling is applied to an internal dose (namely, the overall metabolic rate), it is applicable regardless of the route of exposure.

EPA has experience applying cross-species scaling methodology in a number of carcinogen assessments that have relied on pharmacokinetic modeling to characterize risks from inhalation exposure. Further, the vast majority of EPA carcinogen assessments have relied on this method—all oral slope factor estimates developed from animal bioassay data and all cancer risk values developed from bioassay data and relying on PBPK models. Specific assessments relying on PBPK models include the previous tetrachloroethylene assessment, dichloromethane, vinyl chloride, and trichloroethylene. In all cases, a scientific rationale was provided for the cross-species scaling approach taken.

The previous tetrachloroethylene assessment (U.S. EPA, 1986) also used cross-species scaling of total rate of metabolism for the liver tumors and leukemias. The dichloromethane assessment used cross-species scaling ($BW^{2/3}$) of the daily amount of inhaled dichloromethane metabolized by a GST pathway (U.S. EPA, 1987, 1995). The vinyl chloride inhalation risk per

unit concentration involved a reactive metabolite whose AUC was judged to be proportional to the metabolite's tissue concentration (U.S. EPA, 2000); that is, AUCs (and responses) for this metabolite would tend to be equal for doses in terms of mg/kg-day rather than mg/kg$^{3/4}$-day.[9] Most recently, EPA's trichloroethylene assessment (external draft) used AUCs of metabolites produced in the liver following inhalation exposure as being predictive of human risk using the liver tumors observed in mice; for kidney tumors, the human equivalent risk was estimated using $BW^{3/4}$ scaling of daily production of thiol in the kidney (U.S. EPA, 2001).

Following the cross-species scaling methodology, metabolized tetrachloroethylene was scaled using mg/kg$^{3/4}$-day in order to estimate equivalent toxic effects in humans (U.S. EPA, 1992). This determination followed consideration of the reactivity of the dose metric and the ability to estimate AUCs for the dose metric. The involvement of reactive metabolites through which all other metabolites may follow has been hypothesized; however, body-weight scaling was not considered pertinent for tetrachloroethylene because the possible reactive metabolites have not been confirmed and because the majority of the metabolites formed is accounted for by TCA, a stable metabolite. Concerning estimation of AUCs, the PBPK models for tetrachloroethylene provide the rate of overall metabolism in units of mg-equivalents/kg-day, which is a rate or flux. The models do not describe the kinetics of the overall metabolism and therefore cannot provide an AUC. As discussed in Section 3, this is because the clearances of all but one of tetrachloroethylene's metabolites are unknown, and many of the metabolites themselves have not been identified. The metabolite whose clearance has been estimated is TCA. While TCA is the predominant metabolite, it is not clear that TCA is responsible for all the observed toxicity (see Appendix 4A). For animals, the study-specific body weights were used (see Tables 5-5 and 5-7), and for humans the default of 70 kg was used.

It might appear that the use of such a procedure constitutes a "double counting" of allometric scaling. This is not the case as is evident from the following explanation. The AUC of the circulating stable metabolite (if available) leads to an equivalent average tissue concentration of the metabolite X, C_X, for both species. This average concentration, when applied over the lifetime of a species, leads to equivalent risk across species. For simplicity, consider a one-compartment model. At steady-state, the production of X will be equal to the clearance of X, so that

[9]Available human incidence data were judged to be concordant with this interpretation of the animal data for vinyl chloride; consequently, no cross-species scaling factor was considered necessary. The cross-species scaling methodology (U.S. EPA, 1992) points out that, in general, body-weight scaling for reactive metabolites entails assuming that the metabolite is removed from its target by spontaneous action, never leaves the tissue in which it is formed, does not form toxicologically active macromolecular adducts, and that there are no species differences in persistence. That is, body-weight scaling of a reactive metabolite would not be expected to result in cross-species toxicological equivalence in all cases.

$$R_{met} = V_d \times BW \times C_X \times k_{cl},$$

where:

R_{met} = rate of production of X
V_d = fractional volume of distribution
BW = body weight (converted to liters)
C_X = concentration of X and
k_{cl} = clearance of X in units of 1/time

Then, for the concentration C_X equivalent in both species:

$$C_X = [R_{met}/BW \times k_{cl} \times V_d\ ]_H = [R_{met}/BW \times k_{cl} \times V_d]_A$$

where H and A refer to human and animal. It is safe to assume that V_d is the same across species. Then, $[R_{met}/BW \times k_{cl}]_H = [R_{met}/BW \times k_{cl}]_A$. Now, k_{cl} (with units of 1/time) is known to scale according to $BW^{1/4}$ (U.S. EPA, 2005a). Thus, the AUC approach leads to

$$R_{met\ (H)}/BW_H^{3/4} = R_{met\ (A)}/BW_A^{3/4}$$

This is the scaling approach used in this assessment due to lack of data to pursue an AUC approach explicitly.

In the last step of the extrapolation to human equivalent PODs, the PODs in terms of metabolized dose were extrapolated to environmental inhalation and oral exposures using pharmacokinetic modeling. As discussed in Section 3.5, three human PBPK models were considered, owing to insufficient data to distinguish between these models at low environmental concentrations, especially concerning validation of total metabolite levels. These models represent the work of Reitz et al. (1996), Rao and Brown (1993), and Bois et al. (1996), as adapted by EPA (see Section 3.5 for more details). Because use of the human PBPK models indicated that the correspondence between total metabolism and administered concentration was linear below 0.1–1 ppm (see Figure 3-10), conversion factors (slopes) derived from Figure 3-10 were applied to estimate the human equivalent PODs in terms of administered concentration; e.g., human equivalent POD (ppm) = human equivalent POD (mg-eq/kg-day) × conversion factor ([mg-eq/kg-day]/ppm). See footnotes d–f in Table 5-9 and footnotes b–d in Table 5-11 for the inhalation and oral conversion factors.

5.4.4.2.2. *Administered concentration as dose metric.* For those sites for which pharmacokinetic-adjusted doses were not available or not otherwise relevant, EPA's default RfC methodology was used (U.S. EPA, 1994). Tetrachloroethylene is considered a Category 3 gas because it is water soluble and perfusion limited, and it has systemic (extra-respiratory) effects. Because the ratio of blood/air partition coefficients for the experimental animal species relative to humans is greater than or equal to 1 (for F344 rats, 18.9/10.3 = 1.8; for $B6C3F_1$ mice, 17.5/10.3 = 1.7), a default value of 1 was used for this ratio (U.S. EPA, 1994). Consequently, when administered inhalation concentrations were used as the dose metric, the concentrations were considered equipotent across species for extrapolating risk to humans. Therefore, no further extrapolation was necessary with the resulting PODs in the units of human equivalent environmental exposure levels.

5.4.5. Cancer Risk Values

Human cancer risk was assessed using six different sex-species animal tumor data sets and three different human PBPK models of total metabolism. The results of the dose-response modeling using the data from the inhalation animal studies are discussed below, followed by route-to-route extrapolation for estimating human cancer risk via oral exposure to tetrachloroethylene. Finally, a discussion of quantitative and qualitative uncertainties underlying the risk estimation process is provided.

5.4.5.1. *Dose-Response Modeling Results*

The dose-response modeling relying on total metabolism as the dosimeter is illustrated in Figures 5-8a through 5-13a, and it is summarized in Table 5-9. The estimation of risk per unit concentration associated with each tumor site is summarized in Tables 5-9 (identification of PODs) and 5-10 (conversions of PODs to risk per unit concentration). The dose-response modeling relying on administered concentration is illustrated in Figures 5-8b through 5-13b and summarized in Table 5-11. Site-specific modeling results and conversions to human equivalent risk values are discussed below. In all cases, linear extrapolation from the PODs was carried out because of the lack of information supporting another extrapolation approach (U.S. EPA, 2005a).

5.4.5.1.1. *Mouse tumors.*

5.4.5.1.1.1. *Hepatocellular tumors, male mice*. The dose-response modeling results from the hepatocellular adenomas or carcinomas in male mice of the JISA bioassay using total metabolism (via PBPK modeling) led to human equivalent PODs ($BMCL_{10}$s) ranging from 1.8 ppm (Bois model) to 18 ppm (Rao and Brown model) tetrachloroethylene in air (see Table 5-9

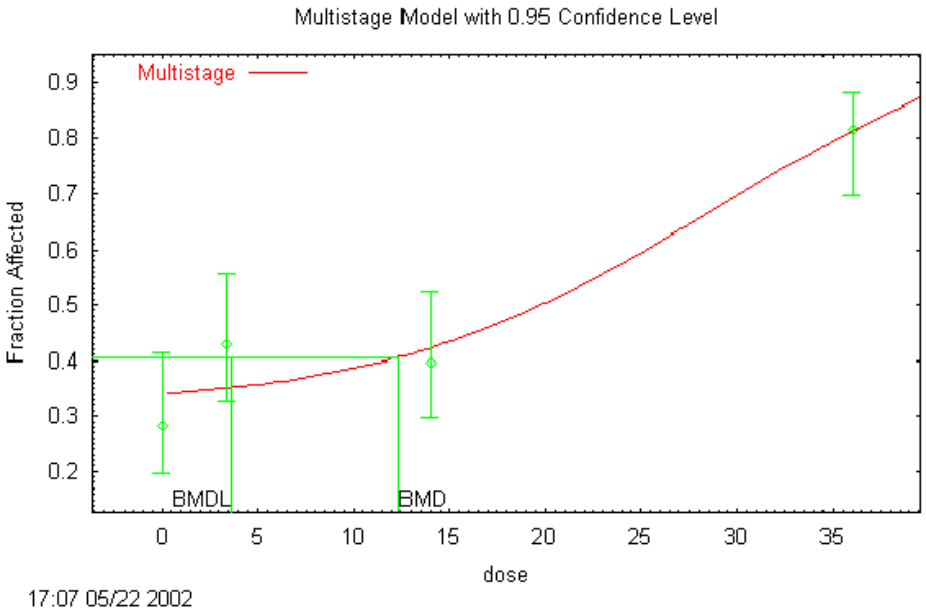


Figure 5-8a. Incidence of hepatocellular adenomas and carcinomas in male mice (JISA, 1993) corresponding to total tetrachloroethylene metabolism (mg-eq/kg-day) and multistage model fit showing BMC and BMCL at 10% extra risk. Data from Table 5-5.

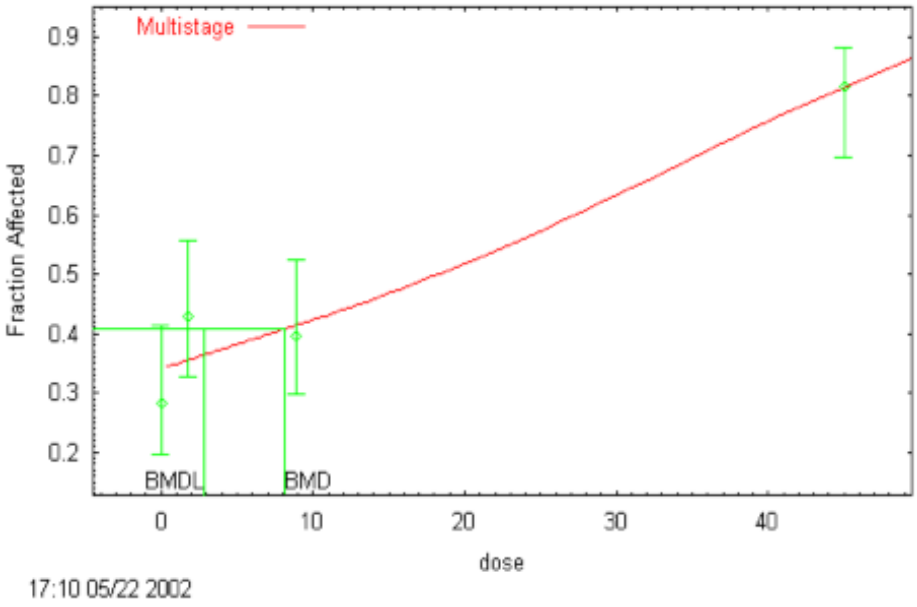


Figure 5-8b. Incidence of hepatocellular adenomas and carcinomas in male mice (JISA, 1993) corresponding to human equivalent continuous tetrachloroethylene exposure (ppm) and multistage model fit showing BMC and BMCL at 10% extra risk. Data from Table 5-5.

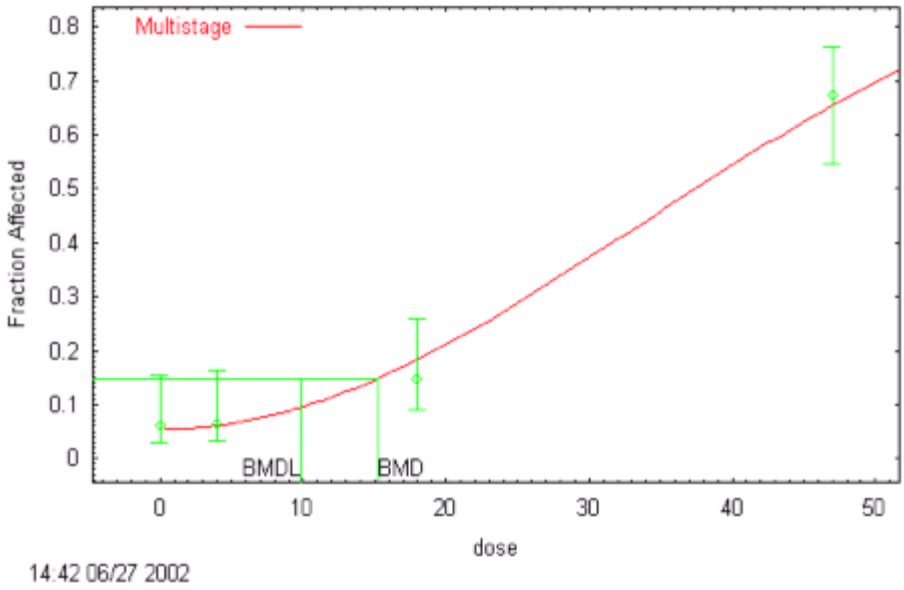


Figure 5-9a. Incidence of hepatocellular adenomas and carcinomas in female mice (JISA, 1993) corresponding to total tetrachloroethylene metabolism (mg-eq/kg-day) and multistage model fit showing BMC and BMCL at 10% extra risk. Data from Table 5-5.

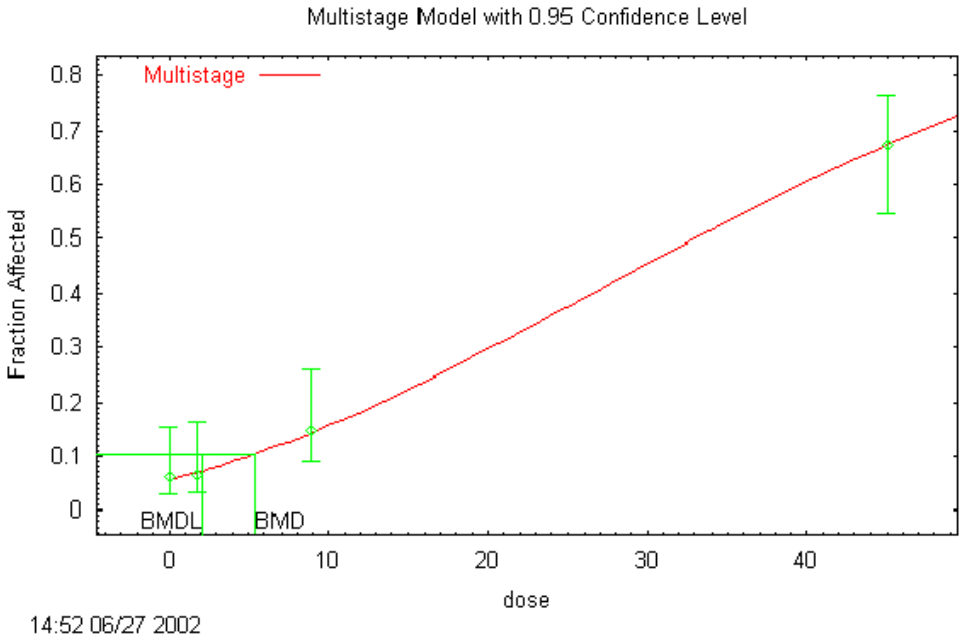


Figure 5-9b. Incidence of hepatocellular adenomas and carcinomas in female mice (JISA, 1993) corresponding to human equivalent continuous tetrachloroethylene exposure (ppm) and multistage model fit showing BMC and BMCL at 5% extra risk. Data from Table 5-5.

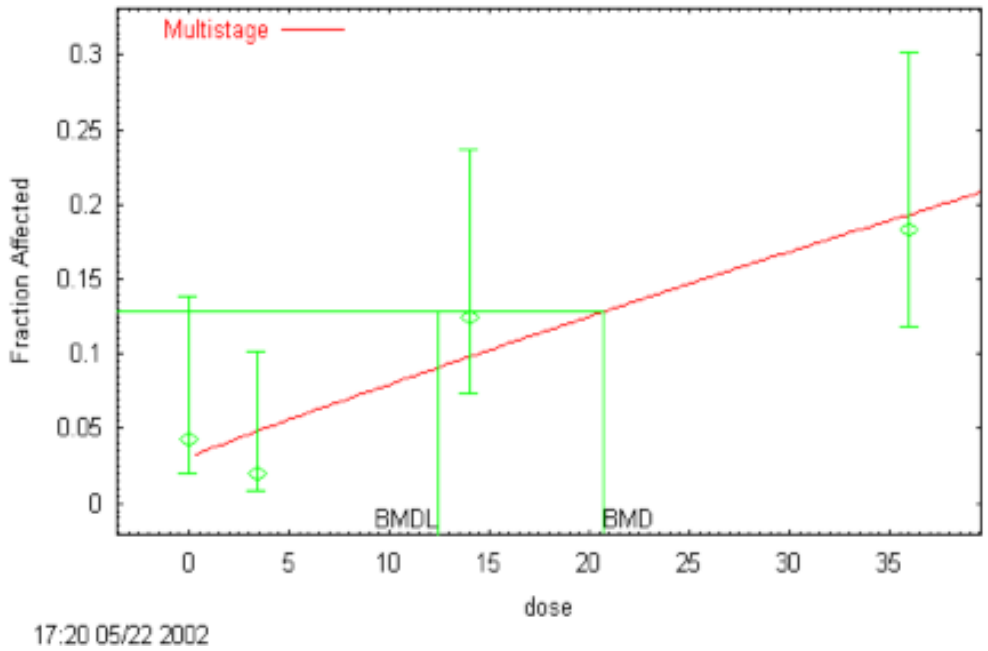


Figure 5-10a. Incidence of malignant hemangiosarcomas in male mice (JISA, 1993) corresponding to total tetrachloroethylene metabolism (mg-eq/kg-day) and multistage model fit showing BMC and BMCL at 10% extra risk. Data from Table 5-5.

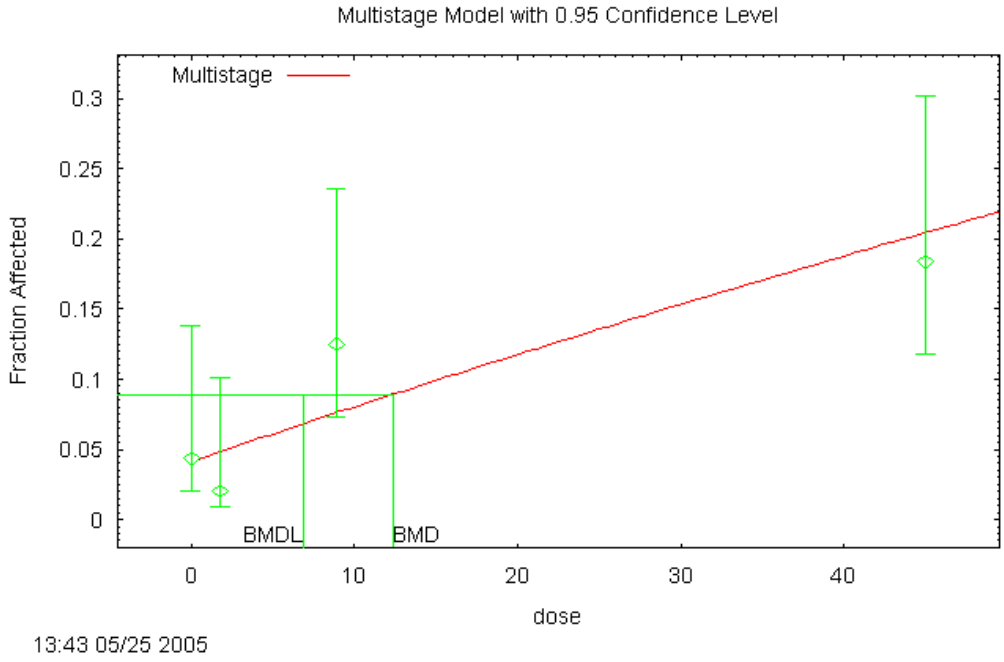


Figure 5-10b. Incidence of malignant hemangiosarcomas in male mice (JISA, 1993) corresponding to human equivalent continuous tetrachloroethylene exposure (ppm) and multistage model fit showing BMC and BMCL at 5% extra risk. Data from Table 5-5.

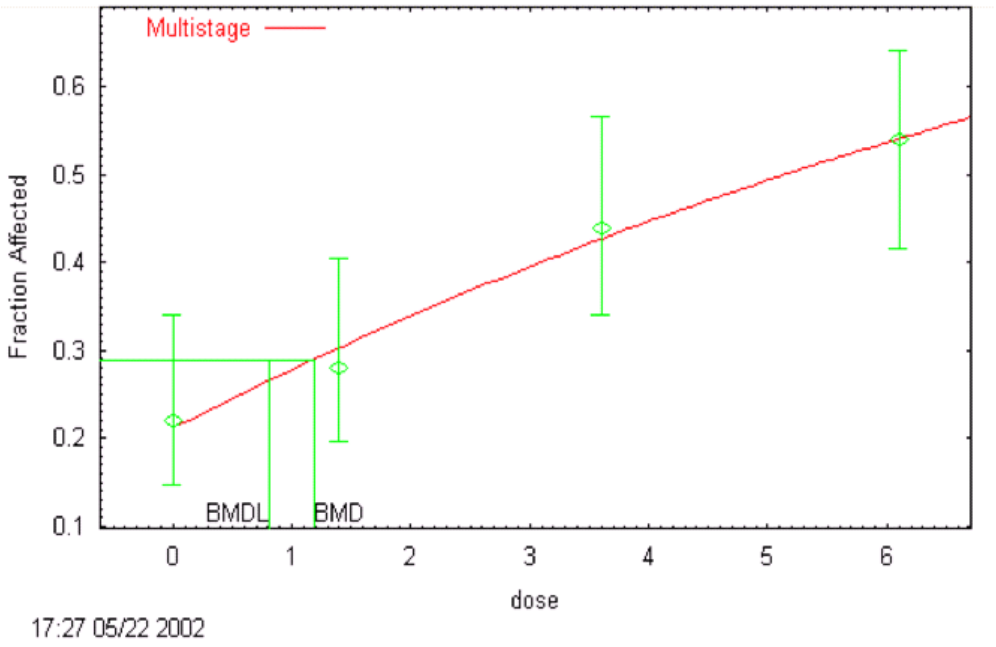


Figure 5-11a. Incidence of mononuclear cell leukemia in male rats (JISA, 1993) corresponding to total tetrachloroethylene metabolism (mg-eq/kg-day) and multistage model fit showing BMC and BMCL at 10% extra risk. Data from Table 5-7.

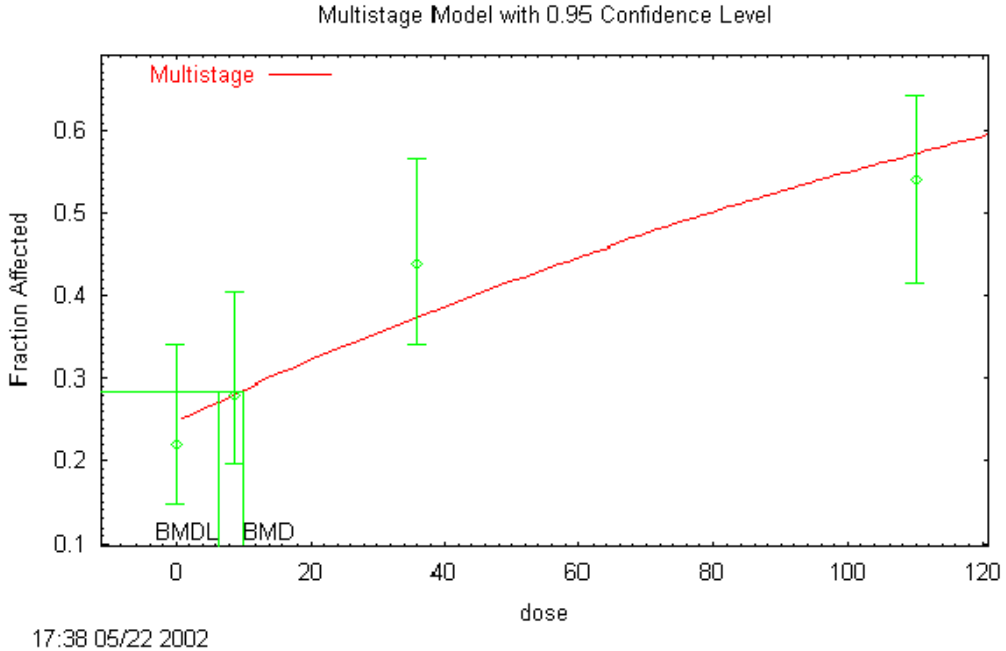


Figure 5-11b. Incidence of mononuclear cell leukemia in male rats (JISA, 1993) corresponding to human equivalent continuous tetrachloroethylene exposure (ppm) and multistage model fit showing BMC and BMCL at 5% extra risk. Data from Table 5-7.

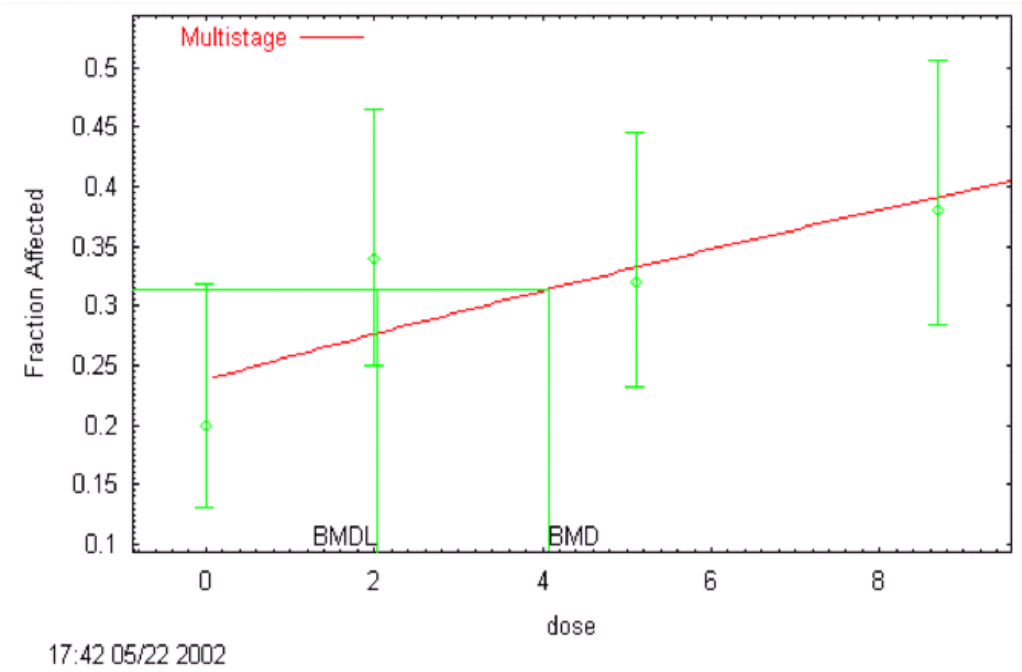


Figure 5-12a. Incidence of mononuclear cell leukemia in female rats (JISA, 1993) corresponding to total tetrachloroethylene metabolism (mg-eq/kg-day) and multistage model fit showing BMC and BMCL at 10% extra risk. Data from Table 5-7.

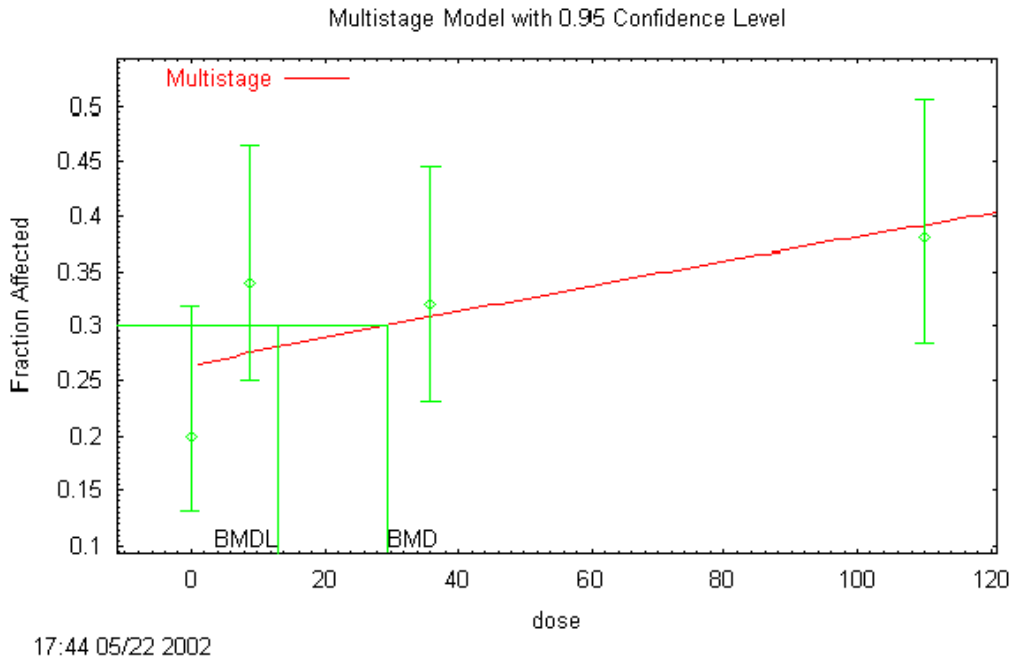


Figure 5-12b. Incidence of mononuclear cell leukemia in female rats (JISA, 1993) corresponding to human equivalent continuous tetrachloroethylene exposure (ppm) and multistage model fit showing BMC and BMCL at 5% extra risk. Data from Table 5-7.

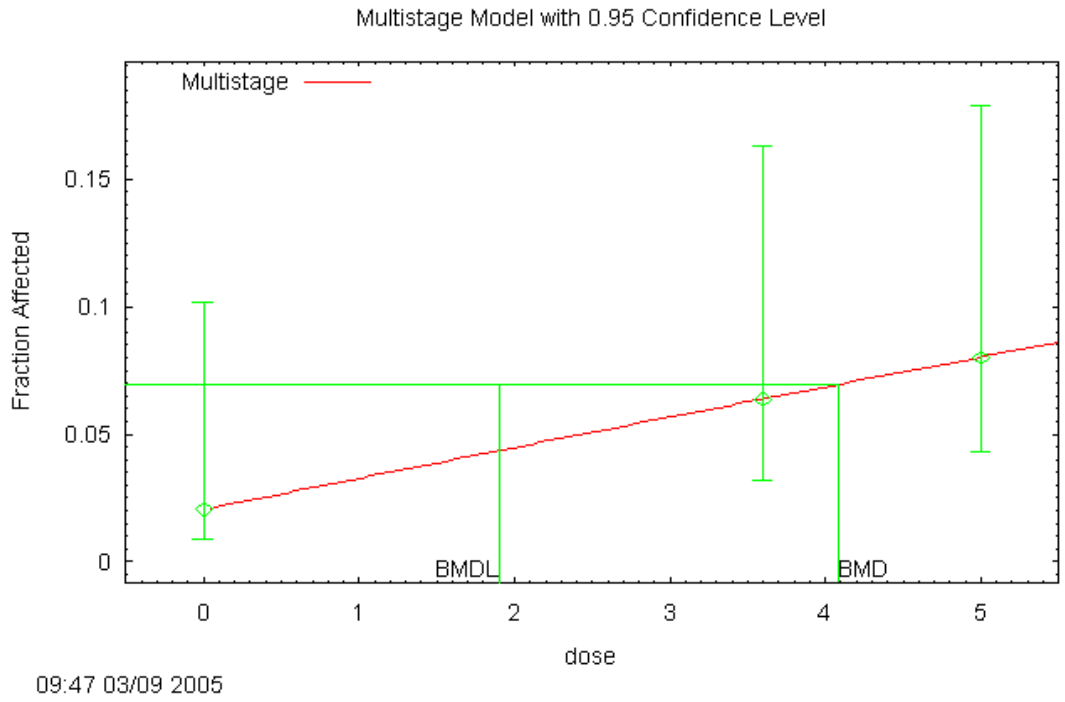


Figure 5-13a. Incidence of kidney adenomas and adenocarcinomas in male rats (NTP, 1986) corresponding to total tetrachloroethylene metabolism (mg-eq/kg-day) and multistage model fit showing BMC and BMCL at 5% extra risk. Data from Table 5-7.

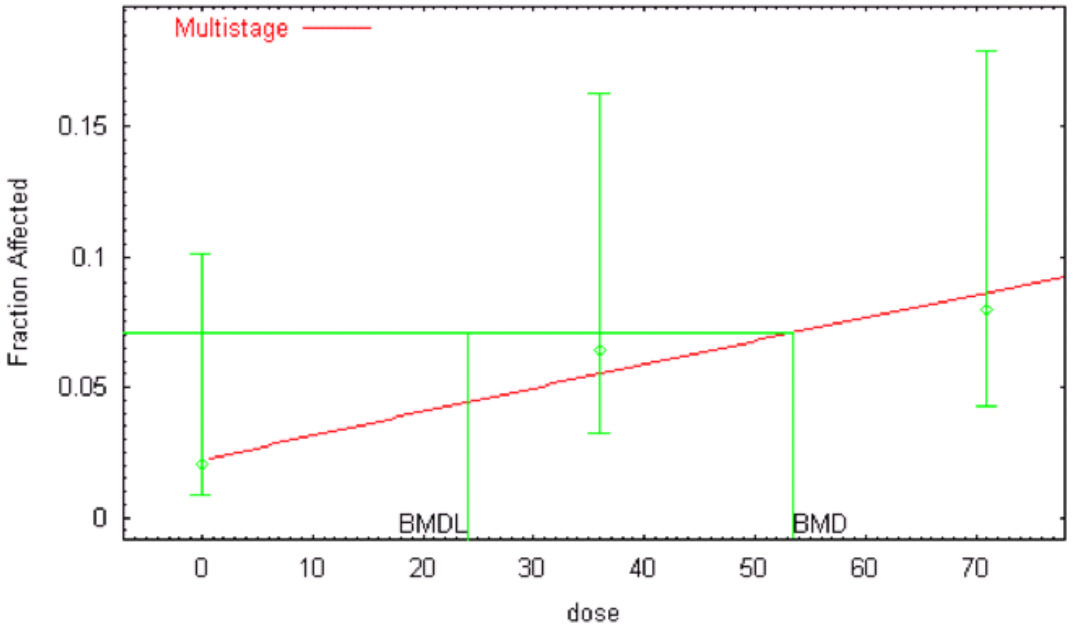


Figure 5-13b. Incidence of kidney adenomas and adenocarcinomas in male rats (NTP, 1986) corresponding to human equivalent continuous tetrachloroethylene exposure (ppm) and multistage model fit showing BMC and BMCL at 5% extra risk. Data from Table 5-7.

Table 5-9. Dose-response modeling summary for tumor sites using total tetrachloroethylene metabolites as the dosimeter; tumor incidence data from JISA (1993) and NTP (1986)

Tumor type	Group	Modeling summary[a]		POD in terms of metabolized dose (mg-eq/kg-day)			Human equivalent POD, in terms of environmental exposure (ppm)		
		MLE coefficients	*p*-value		Bioassay estimate[b]	Human equivalent[c]	Rao and Brown (1993)[d]	Reitz et al. (1996)[e]	Bois et al. (1996)[f]
Hepatocellular adenomas or carcinomas (JISA, 1993)	Male mice	$q_0 = 0.34$ $q_1 = 0.0049$ $q_3 = 2.3 \times 10^{-5}$	0.15	BMC_{10} $BMCL_{10}$	12 3.6	1.9 0.58	59 18	14 4.2	5.9 1.8
	Female mice	$q_0 = 0.055$ $q_2 = 4.6 \times 10^{-4}$	0.75	BMC_{10} $BMCL_{10}$	15 9.8	2.2 1.5	68 44	16 11	6.8 4.4
Hemangiosarcomas, spleen or liver (JISA, 1993)	Male mice	$q_0 = 0.032$ $q_1 = 0.0051$	0.48	BMC_{10} $BMCL_{10}$	21 12	3.4 1.9	100 59	24 14	10 5.9
Mononuclear cell leukemia, rats (JISA, 1993)	Male rats	$q_0 = 0.21$ $q_1 = 0.078$ $q_2 = 0.0020$	0.68	BMC_{10} $BMCL_{10}$	1.3 0.81	0.37 0.23	11 7	2.6 1.6	1.1 0.7
	Female rats	$q_0 = 0.24$ $q_1 = 0.026$	0.48	BMC_{10} $BMCL_{10}$	4.1 2.0	1.1 0.51	32 16	7.5 3.7	3.2 1.6
Kidney tumors (NTP, 1986)	Male rats	$q_0 = 0.020$ $q_1 = 0.013$	1	BMC_{05} $BMCL_{05}$	4.1 1.9	1.2 0.53	35 16	8.2 3.8	3.5 1.6

[a]Model: multistage model, extra risk. Coefficients estimated in terms of mg-equivalents/kg-day, as estimated for the experimental animals, and adjusted to estimate equivalent continuous exposure.

[b]Bioassay estimates illustrated in Figures 5-8a through 5-13a.

[c]Human equivalent points of departure were derived by dividing the bioassay estimate by [70 kg/ animal body weight (kg)]$^{0.25}$. Animal body weights provided in Tables 5-5 and 5-7.

[d]At exposures below 1 ppm, approximately 0.033 (mg-eq/kg-day)/ppm inhaled tetrachloroethylene was estimated to be metabolized at steady state (see Figure 3-10).

[e]At exposures below 1 ppm, approximately 0.14 (mg-eq/kg-day)/ppm inhaled tetrachloroethylene was estimated to be metabolized at steady state (see Figure 3-10).

[f]At exposures below 0.1 ppm, approximately 0.33 (mg-eq/kg-day)/ppm inhaled tetrachloroethylene was estimated to be metabolized at steady state (see Figure 3-10).

Table 5-10. Human equivalent risk per unit concentration, in terms of continuous environmental exposure, derived using total tetrachloroethylene metabolites as the dosimeter; tumor incidence data from JISA (1993) and NTP (1986)

Tumor type	Group	Human equivalent risk per unit concentration[a], continuous environmental exposure, $(ppm)^{-1}$		
		Rao and Brown (1993)	Reitz et al. (1996)	Bois et al. (1996)
Hepatocellular adenomas or carcinomas (JISA, 1993)	Male mice	5.7×10^{-3}	2.4×10^{-2}	5.7×10^{-2}
	Female mice	2.3×10^{-3}	9.6×10^{-3}	2.3×10^{-2}
Hemangiosarcomas, spleen or liver (JISA, 1993)	Male mice	1.7×10^{-3}	7.2×10^{-3}	1.7×10^{-2}
Mononuclear cell leukemia, rats (JISA, 1993)	Male rats	1.4×10^{-2}	6.1×10^{-2}	1.4×10^{-1}
	Female rats	6.4×10^{-3}	2.7×10^{-2}	6.4×10^{-2}
Kidney tumors (NTP, 1986)	Male rats	3.1×10^{-3}	1.3×10^{-3}	3.1×10^{-2}
Concentrations above which these risks per unit concentration should not be used due to nonlinearity of metabolism and dose-response		1 ppm	0.1 ppm	1 ppm

[a]Risk per unit concentration calculated by dividing the risk level by the lower bound on its risk-specific environmental concentration. See Table 5-9 for the risk levels and risk-specific concentrations.

Table 5-11. Dose-response summary and cancer risk estimates using continuous equivalent administered tetrachloroethylene levels as dosimeter, from NTP (1986) and JISA (1993)

Tumor type	Source	Modeling summary[a]		MLE POD[b] (ppm)	Lower bound on POD (ppm)	Risk per unit concentration $(ppm)^{-1}$ [a,c,d]
		MLE dose coefficients	*p*-value			
Hepatocellular adenomas or carcinomas, male mice	JISA (1993)	$q_0 = 0.34$ $q_1 = 0.013$ $q_3 = 7.8 \times 10^{-6}$	0.17	$BMC_{10} = 8.1$	$BMCL_{10} = 2.8$	3.6×10^{-2}
Hepatocellular adenomas or carcinomas, female mice	JISA (1993)	$q_0 = 0.056$ $q_1 = 0.0076$ $q_2 = 3.6 \times 10^{-4}$	0.83	$BMC_{05} = 5.4$	$BMCL_{05} = 2.1$	2.4×10^{-2}
Hemangiosarcomas, spleen or liver, male mice	JISA (1993)	$q_0 = 0.041$ $q_1 = 0.0041$	0.27	$BMC_{05} = 12$	$BMCL_{01} = 6.9$	7.2×10^{-3}
Mononuclear cell leukemia, male rats	JISA (1993)	$q_0 = 0.25$ $q_1 = 0.0051$	0.51	$BMC_{05} = 10$	$BMCL_{05} = 6.4$	7.8×10^{-3}
Mononuclear cell leukemia, female rats	JISA (1993)	$q_0 = 0.26$ $q_1 = 0.0017$	0.34	$BMC_{05} = 29$	$BMCL_{05} = 13$	3.8×10^{-3}
Kidney tubular cell adenoma or adenocarcinoma, male rats	NTP (1986)	$q_0 = 0.022$ $q_1 = 9.6 \times 10^{-4}$	0.75	$BMC_{05} = 53$	$BMCL_{05} = 24$	2.1×10^{-3}

[a]Using dose coefficients in terms of administered ppm of tetrachloroethylene adjusted to equivalent continuous exposure, consistent with RfC methodology (U.S. EPA, 1994), and the multistage model, extra risk: $P(d) = 1 - \exp(-q_0 - q_1 \times d - q_2 \times d^2 \times \ldots q_6 \times d^6)$. See Tables 5-5 and 5-7 for input data.

[b]POD results (MLEs and lower bounds) illustrated in Figures 5-8b through 5-13b.

[c]Consistent with 2005 cancer guidelines; risk per unit concentration calculated by dividing the appropriate risk level by its risk-specific total metabolite level.

[d]Risks per unit concentration, which are approximations for low-dose extrapolation, should not be used with exposures greater than the POD from which they were derived without considering the curvature of the dose-response function (at left).

Dose-response modeling of the male mouse liver tumor data using administered exposure fit the data points as well as when using total metabolism, with the control and lowest exposure groups again having the poorest fit. This dose-response modeling led to a human equivalent POD ($BMCL_{10}$) of 2.8 ppm tetrachloroethylene in air (see Table 5-11 and Figure 5-8b). The corresponding central tendency estimate was approximately threefold higher, at 8.1 ppm. Linear

extrapolation from this POD led to a human equivalent risk per unit concentration of 3.6×10^{-2} per ppm, about twofold lower than the upper end of the range obtained using total metabolism.

Hepatocellular tumors, female mice. The dose-response modeling of the hepatocellular adenomas or carcinomas in female mice from the JISA bioassay and the consideration of total metabolism (via the PBPK models) led to human equivalent PODs ($BMCL_{10}$s) ranging from 4.4 ppm (Bois et al. [1996] model) to 44 ppm (Rao and Brown [1993] model) tetrachloroethylene in air (see Table 5-9; Figure 5-9a). The corresponding central tendency estimates are approximately 1.5-fold higher, at 6.8–68 ppm.

Linear extrapolation from the PODs above for hepatocellular tumors in female mice was carried out because of the lack of information supporting another extrapolation approach. This led to risks per unit concentration that were approximately 2.5-fold lower than those for the male mice, at 2.3×10^{-3} per ppm (Rao and Brown [1993] model 0.1/44 = 0.0023) to 2.3×10^{-2} per ppm (Bois et al. [1996] model; 0.1/0.4.4 = 0.023; see Table 5-9 and Figure 5-9a).

The dose-response modeling results from these same tumor data—but using administered inhalation exposure as the dose metric (without PBPK modeling)—led to a human equivalent POD ($BMCL_{05}$) of 2.1 ppm (see Table 5-11 and Figure 5-9b). Note that, because the range of experimental data extended below 10% extra risk, the risk per unit concentration was based on 5% extra risk. The corresponding central tendency estimate is approximately 2.5-fold higher, at 5.4 ppm. Linear extrapolation from this POD led to a risk per unit concentration of 2.4×10^{-2} per ppm (0.05/2.1 = 0.024), which is virtually identical to the upper end of the risk per unit concentration range obtained using total metabolism as the dose metric.

The dose-response relationship in terms of administered exposure (Figure 5-9b) appears somewhat more linear than when expressed in terms of metabolized dose (Figure 5-9a), but the PODs relying on the PBPK models have relatively narrower confidence intervals. However, the confidence intervals associated with both dose metrics are fairly typical of adequate dose-response fits, and neither dose metric is clearly better on a purely empirical basis.

5.4.5.1.1.2. ***Hemangiosarcomas***. Hemangiosarcomas of the liver and spleen were also observed in the JISA male mice. Because these tumors differ etiologically from the hepatocellular adenomas and carcinomas, they were modeled separately. Dose-response modeling using total metabolism led to human equivalent PODs ($BMCL_{10}$s) ranging from 5.9 ppm (Bois et al. [1996] model) to 59 ppm (Rao and Brown [1993] model) tetrachloroethylene in air (see Table 5-9; Figure 5-10a). The corresponding central tendency estimates are approximately 1.7-fold higher, at 10–100 ppm.

Linear extrapolation from the PODs above for hemangiosarcomas in male mice led to human equivalent risk per unit concentration ranging from 1.7×10^{-3} per ppm (Rao and Brown [1993] model; 0.1/59 = 0.0017) to 1.7×10^{-2} per ppm (Bois et al. [1996] model; 0.1/5.9 = 0.017) tetrachloroethylene in air (see Table 5-10), approximately 3.5-fold less than the corresponding risks per unit concentration for the other male mouse liver tumors. These results raise some concern that total cancer risk based on the male mice data may be underestimated slightly by considering only the hepatocellular adenomas and carcinomas. An analysis combining the risks from these two sites indicates an overall risk from the male mice data of 3.4×10^{-2} per mg-equiv/kg-day, about 20% higher than the risk per unit concentration estimated for hepatocellular adenomas and carcinomas alone.[10]

Dose-response modeling using human equivalent continuous administered concentration led to a human equivalent POD ($BMCL_{05}$) of 6.9 ppm tetrachloroethylene in air (see Table 5-11 and Figure 5-10b). The corresponding central tendency estimate is approximately 1.7-fold higher, at 12 ppm. However, although this fit was technically adequate ($p > 0.1$), the model did not fit as well as the model using total metabolism in the region of the low and middle exposures; the dose-response relationship is essentially a straight line between the high-dose group and the control group.

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[10]In order to gain some understanding of the total risk from multiple tumor sites in male mice, a sum of risks across tumor sites was considered. This combined risk does not constitute double-counting if it can be assumed that the hepatic adenomas and carcinomas were mechanistically independent from the hemangiosarcomas. If there is some dependence between the tumor types, then the combined risk would tend to be an overestimate of the total risk.

A statistically appropriate approach was used to sum the maximum likelihood estimates (MLE) of unit potency across these tumor sites for male mice in the JISA study, assuming independence of the tumor sites. Specifically, an estimate of the 95% upper bound on the summed unit risk, corresponding to the region of 10^{-4} extra risk in the two dose-response curves, where the slopes were reasonably constant and stable. Assuming a normal distribution for the individual risk estimates, the variance of the risk estimate for each tumor site can be derived from its 95% upper confidence limit (UCL) according to the formula

$$95\%\ \text{UCL} = \text{MLE} + 1.645 \times \text{standard error (MLE)} \quad (1)$$

after solving for the standard error

$$\text{standard error (MLE)} = (95\%\ \text{UCL} - \text{MLE})/1.645 \quad (2)$$

where 1.645 is the z-statistic corresponding to a one-sided 95% confidence interval. Then the result is squared the result to obtain the variance of each MLE.

The variances of the MLEs for the two tumor sites were summed to obtain the variance of the sum of the MLEs. Then the standard error of the summed risk was obtained by taking the square root of the variance. The 95% UCL on the sum of the MLEs was then calculated using equation (1) above.

The resulting 95% UCL on the summed unit risk was 3.4×10^{-2} per mg-equiv/kg-day, about 20% higher than the unit risk estimated at the POD at 10% for hepatocellular adenomas and carcinomas alone. That is, at 3.6 mg-equiv/kg-day (see Table 5-8), the extra risk for hepatocellular adenomas and carcinomas in male mice is 2.8×10^{-2} per mg-equiv/kg-day (0.1/3.6 mg-equiv/kg-day).

Linear extrapolation from the POD based on administered concentration led to a human equivalent risk per unit concentration of 7.2×10^{-3} per ppm (0.01/1.4 = 0.0074) tetrachloroethylene in air (see Table 5-11), approximately twofold less than the risk per unit concentration obtained for male mouse hepatocellular tumors and administered concentration.

5.4.5.1.2. ***Rat leukemias.***

5.4.5.1.2.1. ***Male rats.*** Table 5-9 provides the dose-response model coefficients for the curve fit of the male rat MCL data shown in Figure 5-11a. The dose-response modeling using total metabolism (via the PBPK models) led to human equivalent PODs ($BMCL_{10}$s) ranging from 0.7 ppm (Bois et al. [1996] model) to 7 ppm (Rao and Brown [1993] model) tetrachloroethylene in air. The corresponding central tendency estimates are approximately 1.5-fold higher, at 1.1–11 ppm.

Linear extrapolation from the PODs above for MCL in male rats led to human equivalent risks per unit concentration ranging from 1.4×10^{-2} per ppm (Rao and Brown [1993] model; 0.1/7 = 0.014) to 1.4×10^{-1} per ppm (Bois et al. [1996] model; 0.1/0.7 = 0.14) tetrachloroethylene in air (see Table 5-10).

The dose-response modeling results from these same tumor data but using administered inhalation exposure as the dose metric (without PBPK modeling) led to a human equivalent POD ($BMCL_{05}$) of 6.4 ppm (see Table 5-11 and Figure 5-11b). The corresponding central tendency estimate is approximately 1.5-fold higher, at 10 ppm. Linear extrapolation from this POD led to a risk per unit concentration of 7.8×10^{-3} per ppm (0.05/6.4 = 0.0073). Although the model fit in terms of administered concentration was technically adequate ($p = 0.51$), the model overestimated the control response and underestimated the mid-dose group response, leading to a risk per unit concentration 2- to 20-fold lower than those obtained using total metabolism as the dose metric.

5.4.5.1.2.2. ***Female rats.*** It was noted earlier (see Section 5.4.2.2) that the dose-response pattern of MCL for female rats in the JISA study also was not monotonic. In this case, the "best" model fit in terms of both dose metrics (see Figures 5-12a and 5-12b) provided adequate fits overall ($p = 0.48$ and 0.27), but fit the control and low-exposure group responses least well. The model fit in terms of metabolized dose provided a better fit of the control response, although both dose metrics lead to approximately the same estimated response for the low dose, at ~27%, compared with the 34% observed. Although both models would appear to underestimate extra risk in this region of the dose-response for female rat leukemias, it is not clear in this particular set of

responses that the fit to the low dose should be emphasized over fitting as many of the responses as possible.

The dose-response modeling of MCL in female rats using total metabolism (via the PBPK models) led to human equivalent PODs ($BMCL_{10}$s) ranging from 1.6 ppm (Bois et al. [1996] model) to 16 ppm (Rao and Brown [1993] model) tetrachloroethylene in air. The corresponding central tendency estimates are twofold higher, at 3.2–32 ppm.

Linear extrapolation from the PODs above for MCL in female rats led to human equivalent risks per unit concentration ranging from 6.4×10^{-3} per ppm (Rao and Brown [1993] model; 0.1/7 = 0.014) to 6.4×10^{-2} per ppm (Bois et al. [1996] model; 0.1/0.7 = 0.14) tetrachloroethylene in air (see Table 5-10). The dose-response modeling using administered inhalation exposure as the dose metric led to a human equivalent POD ($BMCL_{10}$) of 13 ppm (see Table 5-11 and Figure 5-12b). The corresponding central tendency estimate is approximately twofold higher, at 29 ppm. Linear extrapolation from this POD led to a risk per unit concentration of 3.8×10^{-3} per ppm (0.1/13 = 0.0038), about 1.6- to 16-fold lower than those obtained using total metabolism as the dose metric. Although the risks per unit concentration for female rats were about twofold lower than those for the male rats, this relationship among the dose metrics is very similar to that seen with male rat MCL.

There was an indication of accelerated occurrence of leukemias in female rats in the NTP study, but the addition of time-to-tumor in the multistage model did not significantly affect the estimate from that study. There was no similar observation of earlier leukemia incidence with increasing exposure in the JISA study.

5.4.5.1.3. ***Rat kidney tumors.*** Table 5-9 provides the dose-response model coefficients for the curve fit of the male rat kidney adenocarcinomas and carcinomas seen in the NTP study (see Figure 5-13a). The dose-response modeling using total metabolism (via the PBPK models) led to human equivalent PODs ($BMCL_{05}$s) ranging from 1.6 ppm (Bois et al. [1996] model) to 16 ppm (Rao and Brown [1993] model) tetrachloroethylene in air. Note that, because the 10% extra risk response level fell above the range of experimental data, the POD was based on 5% extra risk which the available data did span. The corresponding central tendency estimates are approximately twofold higher than their lower bounds, at 3.5–35 ppm.

Linear extrapolation from the PODs above for kidney tumors in male rats led to human equivalent risks per unit concentration ranging from 3.1×10^{-3} per ppm (Rao and Brown [1993] model; 0.05/1.6 = 0.0031) to 3.1×10^{-2} per ppm (Bois et al. [1996] model; 0.05/16 = 0.031) tetrachloroethylene in air (see Table 5-10). These risks per unit concentration were the lowest of those estimated for all sites.

The dose-response modeling results from these same tumor data but using administered inhalation exposure as the dose metric (without PBPK modeling) led to a human equivalent POD ($BMCL_{05}$) of 24 ppm (see Table 5-11, Figure 5-13b). The corresponding central tendency estimate is approximately twofold higher, at 53 ppm. Linear extrapolation from this POD led to a risk per unit concentration of 2.1×10^{-3} per ppm (0.05/24 = 0.0021).

5.4.5.1.4. ***Summary and discussion of site-specific dose response modeling.*** Dose-response modeling of the candidate data sets presented no particular difficulties. As noted in the preceding descriptions of modeling results, lower bounds on the central tendency estimates (maximum likelihood estimate [MLEs]) of the PODs tended to be within twofold of the central estimates. The only exception was for male mice, with a threefold difference between the MLEs and their lower bounds.

The slopes of the dose-response curves at the PODs were estimated and found to be within 1.6-fold of the corresponding risks per unit concentration in all cases, reflecting the mostly low-dose linear dose-response relationships estimated within the lower region of the observed data ranges. Because of the similarity of the slopes to the risks per unit concentration and the apparent lack of potential for sublinear dose response behavior in the range of exposure below the experimental data, these slopes are not shown.

Figure 5-14 shows the relative magnitudes of the risks per unit concentration associated with each tumor site. It is interesting to note that the risks per unit concentration estimated using administered concentration are not consistently the lowest or highest risk values among the different estimates for each tumor site.

For example, the risk per unit concentration estimated from female mouse hepatocellular tumors using administered concentration (2.4×10^{-2} per ppm) is approximately equal to the upper end of the range estimated using metabolized tetrachloroethylene (2.3×10^{-2} per ppm). Similarly for the male mice, the risk per unit concentration using administered concentration is about twofold lower than the upper end of the range using metabolized dose (3.6×10^{-2} per ppm vs. 5.7×10^{-2} per ppm, respectively). In contrast, the risks per unit concentration for MCL estimated using administered concentration (7.8×10^{-3} per ppm, males; 3.8×10^{-3} per ppm, females) are about twofold lower than the lower end of the range estimated using metabolized tetrachloroethylene (1.4×10^{-2} per ppm, males; 6.4×10^{-3} per ppm, females). Some of this variation is attributable to the differing shapes of the dose-response curves for the two different dose metrics for each site and the variability in the bioassay responses. Overall, the interleaving of the results from the two types of dosimetric, administered concentration and PBPK-estimated metabolism, underscores some uncertainty in identifying the appropriate dosimetric(s).

5.4.5.1.5. ***Concordance of animal and human risk estimates.*** Although sufficient human data linked with exposure characterizations are not available to derive cancer risk values, an analysis by van Wijngaarden and Hertz-Picciotto (2004) provides a limited perspective on the human cancer risk values estimated from animal bioassays. Van Wijngaarden and Hertz-Picciotto (2004) demonstrated a simple methodology using epidemiologic data for four chemical exposures including tetrachloroethylene. For tetrachloroethylene specifically, a linear dose-response model was fit to laryngeal cancer observations in the upper airway cancer case-control study of Vaughan et al. (1997). Van Wijngaarden and Hertz-Picciotto (2004) presented both an ED_{01} and LED_{01} (effective dose for a 1% additional lifetime risk over background and the lower confidence interval on this dose, called the TD1 and LCL1 in their paper) for humans exposed for 45 years, 240 days/year, a standard occupational exposure scenario. The ED_{01} was 228.40 mg/day and LED_{01} was 60.16 mg/day. In order to compare these results with those derived from the JISA (1993) study, we assumed a continuous lifetime exposure (70 years, 365 days/year, and 20 m^3/day breathing rate), resulting in an equivalent ED_{01} of 4.8 mg/m^3 and LED_{01} of 1.3 mg/m^3. Using the continuous lifetime equivalent LED_{01} as the POD and a low-dose linear approach, a risk per unit concentration based upon Vaughan et al. (1997) is 8×10^{-6} per μg/m^3 (or, $0.01/1.3 \times 10^3$ μg/m^3). This estimate falls in the lower end of the range of cancer risk estimates from male and female rat MCL tumors in JISA (1993). A cancer risk estimate from human data using the ED_{01} as the POD is 2×10^{-6} per μg/m^3 (or, $0.01/4.8 \times 10^3$ μg/m^3).

While the analysis of van Wijngaarden and Hertz-Picciotto (2004) can provide some insight on the rodent-based tetrachloroethylene cancer risk estimate, it is still quite limited due to the possible biases in Vaughan et al. (1997) and other factors. While individual bias in Vaughan et al. (1997) may influence observed risk estimates from this study in either a positive (overestimate) or null (underestimate) direction, the overall direction of all bias is likely toward the null. First, Vaughan et al. (1997) do not have exposure information on individual cases and controls and make an assumption that case and controls are exposed to tetrachloroethylene concentrations as described by industrial hygiene surveys in dry cleaning establishments. For this reason, bias related to exposure misclassification is likely great in this study. Second, as is common to many population case-control studies, exposure prevalence to tetrachloroethylene is low. Only 5 of 235 laryngeal cancer cases were identified as having exposure to tetrachloroethylene, and 4 of these 5 cases as more likely than not as being exposed. Low exposure prevalence may lead to reduced study power lead and imprecise estimates of the relative risk (OR) that are not statistically significant. Last, epidemiologic evidence is available to suggest an association between esophageal cancer and tetrachloroethylene, a site also

examined by Vaughan et al. (1997). The OR between esophageal cancer and tetrachloroethylene exposure was larger than that for laryngeal cancer, OR = 11.9 (95% CI = 1.1–124). Cancer risk estimates based on esophageal cancer observations would lead to a higher estimate than that identified by van Wijngaarden and Hertz-Picciotto (2004). Also, Vaughan et al. (1997) only considered respiratory cancers; given the epidemiological results discussed earlier these results may represent an underestimate of total risk. An examination of site concordance with animal observations, additionally, is not possible because the rat is a poor model for laryngeal cancer. Odds ratios in Vaughan et al. (1997) are adjusted for a number of possible confounders such as age, sex, education, study period, alcohol consumption, and cigarette smoking, and the use of adjusted odds ratios is a strength of the van Wijngaarden and Hertz-Picciotto (2004) analysis.

5.4.5.2. *Recommended Inhalation Unit Risk*

Human inhalation cancer risk has been assessed using several different gender-species animal tumor data sets and three different human PBPK models of total metabolism rate. These results have been discussed above and are summarized in Figure 5-14.

In choosing which species-sex combination is most relevant for extrapolating to humans, the MOA information does not provide a clear rationale. Although target organ concordance is not a prerequisite for evaluating the implications of animal study results for humans (U.S. EPA, 2005a), it is notable that the leukemias (in both sexes of rats) support the observation of lymphopoietic cancers in individuals employed as dry cleaners and degreasers, and the liver tumors (in both sexes of mice) support the observation of liver tumors in dry cleaners (see Section 4.10.1.1.2).

The male rat leukemia data provide the most sensitive response of the four species-sex combinations in the JISA study for deriving a unit risk, defined as the plausible upper-bound excess lifetime cancer risk estimated to result from continuous exposure to tetrachloroethylene per unit of concentration. From Table 5-10, the **recommended unit risk value range is 1.4×10^{-2} to 1.4×10^{-1} per ppm, or 2×10^{-6} to 2×10^{-5} per µg/m^3**. This range reflects uncertainty in the choice of pharmacokinetic model.

Comparison with previous EPA assessment: EPA (U.S. EPA, 1986, 1991) reported an overall unit risk of 5.8×10^{-7} per µg/m^3 (3.9×10^{-3} per ppm), which was a geometric mean of six risks per unit concentration from the 1986 NTP study: male and female rat leukemias, male and female mouse liver carcinomas, and male and female mouse liver adenomas and carcinomas. The highest risk per unit concentration in that range was 9.5×10^{-7} per µg/m^3, corresponding to the leukemias in male rats from the NTP study (using total metabolism as the dosimeter).

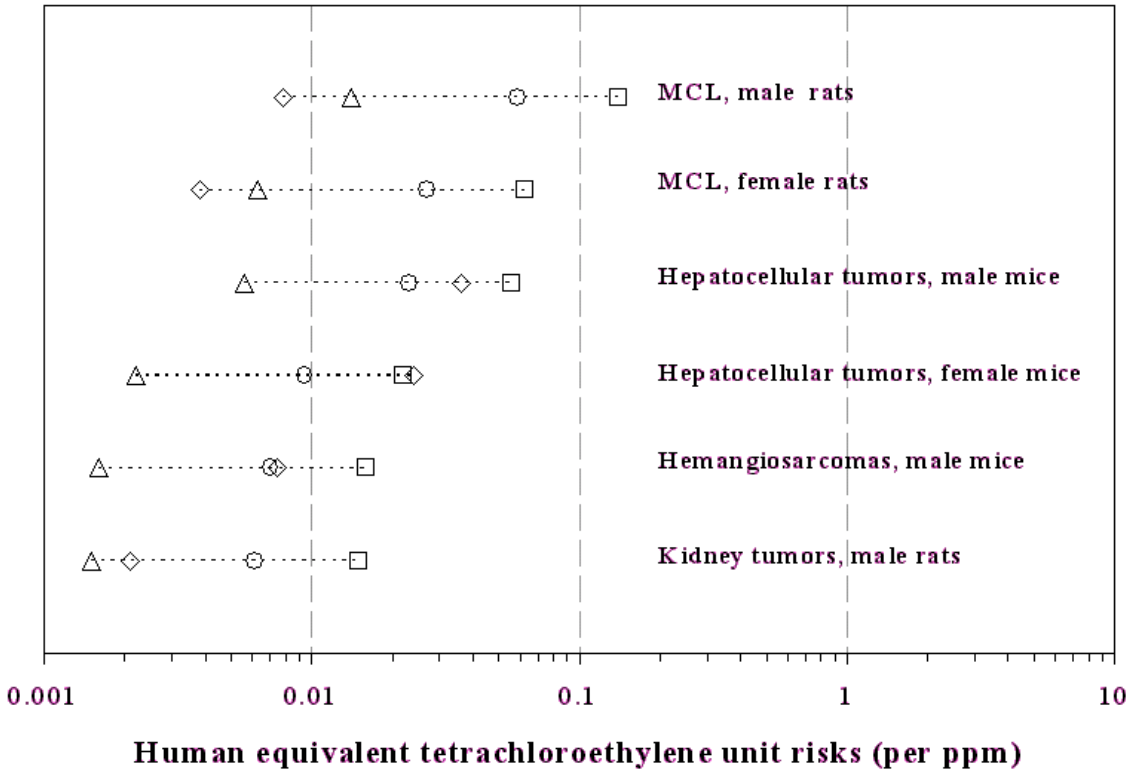


Figure 5-14. Comparison of inhalation risks per unit concentration for tetrachloroethylene derived from rodent bioassays using four different dose metrics—continuous equivalent inhalation concentration (◊), Bois et al. (1996) PBPK model (□), Reitz et al. (1996) PBPK model (○), and Rao and Brown (1993) PBPK model (Δ). See Table 5-9 for PBPK model-derived estimates and Table 5-10 for estimates relying on administered tetrachloroethylene.

This analysis supports a unit risk 14-fold higher than in EPA's 1991 assessment. This difference is attributable to number of considerations. A comparison of the results from the two bioassays, using the Reitz et al. (1996) model to characterize internal dose for both data sets but not extrapolating to humans, indicates that the JISA study leads to risks per unit concentration that are approximately twofold lower than those from the NTP study (not shown), if all else can be considered equal. The remaining differences between the human equivalent inhalation risks per unit concentration are attributable to differences in the particular PBPK models used, the change in cross-species scaling factor from $BW^{2/3}$ to $BW^{3/4}$ (U.S. EPA, 1992), and use of the most sensitive response rather than a (geometric) mean of the significant tumor responses. Concerning the latter decision, use of a mean response treats the observations as if all are equal likely alternatives (in the case of geometric means, the highest responses are disproportionately

discounted relative to the lower responses). Use of the most sensitive response acknowledges the weight contributed by all of the observed responses as independent indicators of human risk, and provides a plausible upper bound on potential human risk.

5.4.5.3. *Recommended Oral Slope Factor*

The oral slope factor was developed from inhalation data because the only available oral bioassay was less relevant for extrapolating to lifetime risk in humans, for several reasons. First, the study was conducted by gavage at relatively high doses. Human exposures are more likely not to occur in boluses, and high doses are associated at least with saturable metabolism processes which may involve a different profile of toxicological processes than those prevalent at more likely environmental exposure levels. Also, the animals were dosed for only approximately 75% of the more usual 2-year period (NCI, 1977), making the oral study less useful for estimating lifetime risk. Route-to-route extrapolation from the inhalation PODs developed from the JISA study (see Table 5-9) was carried out using the human pharmacokinetic models described in Section 3.5. Table 5-12 summarizes the resulting slope factors. Because the oral slope factors are linear conversions of the inhalation risks per unit concentration, no figure analogous to Figure 5-14 is provided; such a figure would be identical to Figure 5-14 with the exception that the x-axis would reflect mg/kg-day units rather than ppm units.

The same arguments that led to selecting the range based on male rat leukemias for the inhalation unit risk apply to the oral slope factor. In order to account for the uncertainty contributed by the human PBPK models, the oral slope factor is given by the range 1×10^{-2} to 1×10^{-1} per mg/kg-day. This range is equivalent to drinking water risks per unit concentration of 4×10^{-7} to 4×10^{-6} per μg/L of tetrachloroethylene in water (assuming 70 kg body weight and a daily water consumption of 2 L/day). The **recommended slope factor range is 1×10^{-2} to 1×10^{-1} per mg/kg-day**. This range reflects uncertainty in the choice of pharmacokinetic model.

Comparison with previous EPA assessment: EPA (U.S. EPA, 1985) reported a slope factor of 5.1×10^{-2} per mg/kg-day, based on the liver tumor incidence in female mice in the NCI (1977) oral gavage study, total metabolized dose, and $BW^{2/3}$ cross-species scaling. This value falls near the center of the range developed in the current assessment.

5.4.5.4. *Quantitative Adjustment for Sensitive Populations*

Although a mutagenic MOA would indicate increased early-life susceptibility, there are no data exploring whether there is differential sensitivity to tetrachloroethylene carcinogenicity across life stages. This lack of understanding about potential differences in metabolism and

Table 5-12. Summary of tetrachloroethylene oral slope factors, estimated from dose-response modeling of inhalation-exposed animals and by extrapolation to oral exposure using pharmacokinetic models

Tumor type	Group	POD (and extra risk), in terms of human equivalent metabolized dose (mg-eq/kg-day) [a]		Oral slope factor (mg/kg-day)$^{-1}$ [b]		
				Rao and Brown (1993) model [c]	Reitz et al. (1996) model [d]	Bois et al. (1996) model [e]
Hepatocellular adenomas or carcinomas, mice	Male mice	0.58	(10%)	5.6×10^{-3}	2.4×10^{-2}	5.3×10^{-2}
	Female Mice	1.5	(10%)	2.2×10^{-3}	9.4×10^{-3}	9.4×10^{-3}
Hemangiosarcomas, male mice	Male mice	1.9	(10%)	1.7×10^{-3}	7.0×10^{-3}	1.6×10^{-2}
Mononuclear cell leukemia, rats	Male rats	0.23	(10%)	1.4×10^{-2}	5.9×10^{-2}	1.4×10^{-1}
	Female rats	0.51	(10%)	6.3×10^{-3}	2.7×10^{-2}	6.3×10^{-2}
Kidney tumors	Male rats (NTP)	0.53	(5%)	1.5×10^{-3}	6.2×10^{-3}	1.5×10^{-2}
Concentrations above which these risks per unit concentration should not be used due to nonlinearity of metabolism and dose-response.				1 mg/kg-day	1 mg/kg-day	1 mg/kg-day

[a] See Table 5-9 for derivation of human equivalent metabolite estimates.

[b] Points of departure in the previous column were converted to human equivalent oral doses using the pharmacokinetic models detailed below (intermediate calculation not shown), then converted to risks per unit concentration by dividing extra risk by the corresponding risk-specific oral doses.

[c] At exposures below about 1 mg/kg-day (divided between nine equally spaced doses during waking hours), approximately 0.033 (mg-eq/kg-day)/(mg/kg-day) of ingested tetrachloroethylene was estimated to be metabolized at steady-state, using the Rao and Brown (1993) model modified for oral exposure (see Section 3.5 and Figure 3-13) The conversion factor was calculated by dividing 0.01 mg-eq/kg-day (the total metabolite production in 24 hrs) by the total oral dose of tetrachloroethylene estimated to produce that level of metabolites, 21 mg/70 kg.

[d] At exposures below about 1 mg/kg-day (divided between nine equally spaced doses during waking hours), approximately 0.14 (mg-eq/kg-day)/(mg/kg-day) of ingested tetrachloroethylene was estimated to be metabolized at steady-state, assuming that the proportional relationship observed between the Rao and Brown (1993) model and the Reitz et al. (1996) model for the inhalation route holds for oral exposure (see Section 3.5 and Figure 3-13). The conversion factor was calculated by dividing 0.01 mg-eq/kg-day (the total metabolite production in 24 hrs) by the total oral dose of tetrachloroethylene estimated to produce that level of metabolites, 5.1 mg/70 kg.

[e] At exposures below about 0.1 mg/kg-day (divided between nine equally spaced doses during waking hours), approximately 0.31 (mg-eq/kg-day)/(mg/kg-day) of ingested tetrachloroethylene was estimated to be metabolized at steady-state, assuming that the proportional relationship observed between the Rao and Brown (1993) model and the Bois et al. (1996) model for the inhalation route holds for oral exposure (see Section 3.5 and Figure 3-13). The conversion factor was calculated by dividing 0.01 mg-eq/kg-day (the total metabolite production in 24 hrs) by the total oral dose of tetrachloroethylene estimated to produce that level of metabolites, 2.25 mg/70 kg.

susceptibility across exposed human populations thus represents a source of uncertainty. Nevertheless, the existing data do support the possibility of a heterogenous response that may function additively to ongoing or background exposures, diseases, and biological processes. As noted in Section 4.9.5, there is some evidence that certain subpopulations may be more susceptible to exposure to tetrachloroethylene. These subpopulations include early and later life stages and groups defined by health and nutrition status, gender, race/ethnicity, genetics, and multiple exposures and cumulative risk. As discussed below, these considerations strengthen the scientific support for the choice of a linear non-threshold extrapolation approach. However, because the MOA for tetrachloroethylene has not been established, it is not appropriate to derive age-adjustment factors for early life exposures, as discussed in *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

5.4.6. Discussion of Uncertainties in Cancer Risk Values

A number of uncertainties underlie the cancer unit risk for tetrachloroethylene. These are discussed in the following paragraphs. Specifically addressed is the impact on the assessment of issues such as the use of models and extrapolation approaches, the reasonable alternatives and the choices made and the data gaps identified. In addition, the use of assumptions, particularly those underlying the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) is explained and the decision concerning the preferred approach is given and justified. Several of the uncertainties with the largest impact cannot be considered quantitatively. Thus an overall integrated quantitative uncertainty analysis is not presented. Section 5.4.6.1 and Table 5-13 summarize principal uncertainties.

5.4.6.1. *Sources of Uncertainty*

5.4.6.1.1. *Human population variability*. The extent of inter-individual variability in tetrachloroethylene metabolism has not been characterized. As noted above, several enzymes of the oxidative and GSH metabolism, notably CYP2E1, CYP3A4, GSTZ, GSTA, GSTM, and GSTT, show genetic polymorphisms with the potential for variation in production of specific metabolites. Tetrachloroethylene metabolism has been shown to increase by inducers of CYP450 enzymes such as toluene, phenobarbital, and pregnenolone-16 alpha-carbonitrile, whereas CYP enzyme inhibitors such as SKF 525A, metyrapone, and carbon monoxide have been shown to decrease tetrachloroethylene metabolism. Additionally, chronic exposure to tetrachloroethylene has been shown to cause self-induction of metabolism. Human population variability has also been discussed in Chapter 3.

Table 5-13. Summary of uncertainties in tetrachloroethylene cancer unit risk estimate

Consideration/ Approach	Impact on unit risk	Decision	Justification
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk ↑ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity, including whether children are more sensitive. Mutagenic MOA (if established) would indicate increased early-life susceptibility.
Low-dose extrapolation procedure	Departure from EPA's *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ risk per unit concentration an unknown extent	Multistage model to determine POD, linear low-dose extrapolation from POD (default approach)	Available MOA data do not inform selection of dose-response model but do not support non-linearity (mutagenicity is plausible contributor and cannot be ruled out); male rat MCL data are linear in observed range; linear approach in absence of clear support for an alternative is generally supported by scientific deliberations supporting EPA's *Guidelines for Carcinogen Risk Assessment*.
Dose metric	Alternatives could ↑ or ↓ risk per unit concentration by an unknown extent	Considered total metabolism and administered concentration	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not clearly identified.
Species /gender combination (see Table 5-14)	Human risk could ↓ or ↑, depending on relative sensitivity	Male rat MCL	MCL is the largest response and is reproducible across studies, despite high background response rate. There are no MOA data to guide extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across species. Generally, direct site concordance is not assumed; consistent with this view, some human tumor types are not found in rodents (*i.e.*, cervical, esophageal cancer deaths) and rat and mouse tumor types also differ.
PBPK model	10-fold range in risk per unit concentration among the three available models	All are considered	There is no scientific basis for choosing among pharmacokinetic results for estimating total metabolism of tetrachloroethylene given limitations in available data. The highest value provides a reasonable upper estimate of potential human cancer risk.
Cross-species scaling	Alternatives could ↓ or ↑ risk per unit concentration (e.g., 3.5-fold ↓ [scaling by BW) or ↑ 2-fold [scaling by $BW^{2/3}$])	$BW^{3/4}$ (default approach)	There are no data to support alternatives. Because the dose metric was not an AUC, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks.

Table 5-13. Summary of uncertainties in tetrachloroethylene cancer unit risk estimate (continued)

Consideration/ Approach	Impact on unit risk	Decision	Justification
Bioassay	↑ risk per unit concentration 2-fold if NTP study used	JISA study	JISA study used the lowest experimental exposures (reduces extrapolation uncertainty)
Statistical uncertainty at POD	↓ risk per unit concentration 1.6-fold if EC_{10} used rather than LEC_{10}	LEC (default approach for calculating plausible upper bound)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on concentration.

LEC10 = 95% lower confidence limits on the air concentrations associated with a 10% extra risk of cancer incidence

A separate issue is that the human variability in response to tetrachloroethylene is also poorly understood. The effect of metabolic variation, including potential implications for differential toxicity, has not been well studied.

Although a mutagenic MOA would indicate increased early-life susceptibility, there are no data exploring whether there is differential sensitivity to tetrachloroethylene carcinogenicity across life stages. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty. Nevertheless, the existing data do support the possibility of a heterogenous response that may function additively to ongoing or background exposures, diseases, and biological processes. As noted in Chapter 4 (see Section 4.9.5), there is some evidence that certain subpopulations may be more susceptible to exposure to tetrachloroethylene. These subpopulations include early and later life stages and groups defined by health and nutrition status, gender, race/ethnicity, genetics, and multiple exposures and cumulative risk. As discussed in the section on low-dose extrapolation below, these considerations strengthen the scientific support for the choice of a linear non-threshold extrapolation approach.

5.4.6.1.2. ***Choice of low-dose extrapolation approach.*** The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. MOA data are lacking or limited for all candidate cancer endpoints for tetrachloroethylene (i.e., rat MCL and kidney tumors, mouse hepatocellular tumors and hemangiosarcomas). When the MOA cannot be clearly defined, EPA uses a linear approach to estimate low-exposure risk, based on the following broad and long-term scientific assumptions, which supported the development of the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

- A chemical's carcinogenic effects may act additively to ongoing biological processes, given that diverse human populations are already exposed to other agents and have substantial background incidence of various tumors.

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

- The use of linear extrapolation provides consistency across assessments as well as plausible upper-bound risk estimates that are believed to be health-protective (U.S. EPA, 2005a).

The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the MOA for tetrachloroethylene were known with a high degree of confidence is of interest, but clear data on the MOA of tetrachloroethylene is not available, and even if it were, incorporation of MOA into dose-response modeling might not be straightforward and might not significantly reduce the uncertainty about low-dose extrapolation. This is because the MOA as well as other factors, especially human response variability, are determinants of the dose-response function in humans.

This chemical assessment also evaluates the extent to which a collection of mathematical functions, fit to one of the tetrachloroethylene bioassay data sets and extrapolated down to low doses, could inform uncertainty. There is not sufficient information regarding the MOA to support a chemical-specific inference about dose-response behavior at low dose for tetrachloroethylene. Thus, it is of interest to observe how different functions fit to the tumor data may diverge when extrapolated downward. Much previous experience has supported a general mathematical property that different curves, though fitting observed experimental data well, often diverge widely when extrapolated to doses well outside the observed range. Indeed, the inability of curve-fitting procedures to provide useful compound-specific information about low dose risks has been a principal motivation for the "model free" approach of straight line extrapolation from a POD within the observed range of the data (Krewski and van Ryzin, 1981; NRC, 1983).

Calculations here considered four alternative functional forms frequently used for noncancer dose-response assessment in the observable range of the experimental data (multistage, Weibull, log-logistic, and log-probit). These can accommodate a wide variety of dose-response shapes, including threshold-like behavior. These models were fit to the MCL data

in male rats using the EPA BMDS program without restricting the shape parameters of the latter three models (see Appendix 5B for more details). Parameters describing the risk distribution (mean, median and 95% upper and lower confidence bounds) were estimated by a bootstrap procedure because these parameters are not all readily available in the current BMDS software. In comparing these distributions from the bootstrap procedure, the mean (as a measure of central tendency) and the 95% upper and lower bounds calculated from the bootstrap procedure are of interest. The resulting risk distributions were compared at two exposure levels—at a generalized POD and at an environmental level approximately 10^5-fold lower than the POD.

At POD C, corresponding to a risk of approximately 0.1 using the mean estimate from the multistage model, the bootstrap procedure yielded similar risk distributions for the four models (see Figure 5-15a). The means and corresponding confidence bounds agree within an order of magnitude and the spreads in the distributions (the distance between the upper and lower confidence bounds) are within two orders of magnitude. Note that the probability calculations are in terms of metabolized dose in the male rat and do not directly characterize human risks.

EPA also examined the bootstrap results from those same models at a dose that is lower than the POD C by a factor of 10^5 (although EPA's actual low dose risk estimates are developed using a linear extrapolation from a POD to the origin rather than using low-dose estimates from a model). Figure 5-15 illustrates these results. In the region of extrapolated concentrations ($C \times 10^{-5}$), the mean risks of the latter three models (Weibull, log-logistic and log-probit) are about one to three orders of magnitude higher than the mean of the multistage model risks. The spreads of all the models are quite broad, with a six order of magnitude 95% confidence interval for the multistage and much greater spreads for the other three models. The upper bounds of risk for the other three models are higher than that for multistage model, within about three orders of magnitude, and their lower bounds of risk are much lower than that of multistage, by nine or more orders of magnitude. With such large spreads in confidence intervals, the extrapolated models in effect provide little information about low-dose risks. The extrapolation of the multistage model does result in estimates reasonably close to the low-dose estimates from the model-independent straight line extrapolation from the POD, in that the mean and upper-bound risks at the lower concentration are both within 10% of the estimates resulting from applying linear extrapolation to the results at the higher concentration.

This comparison of risk distributions has several limitations. First, the selected models do not represent all possible models one might fit, serving primarily to illustrate a range of possibilities. That is, other models could be selected to yield more extreme results, both higher and lower than those shown here. Further, the results apply only to the prediction of MCL in male rats. For reasons discussed above concerning expected additivity to background processes

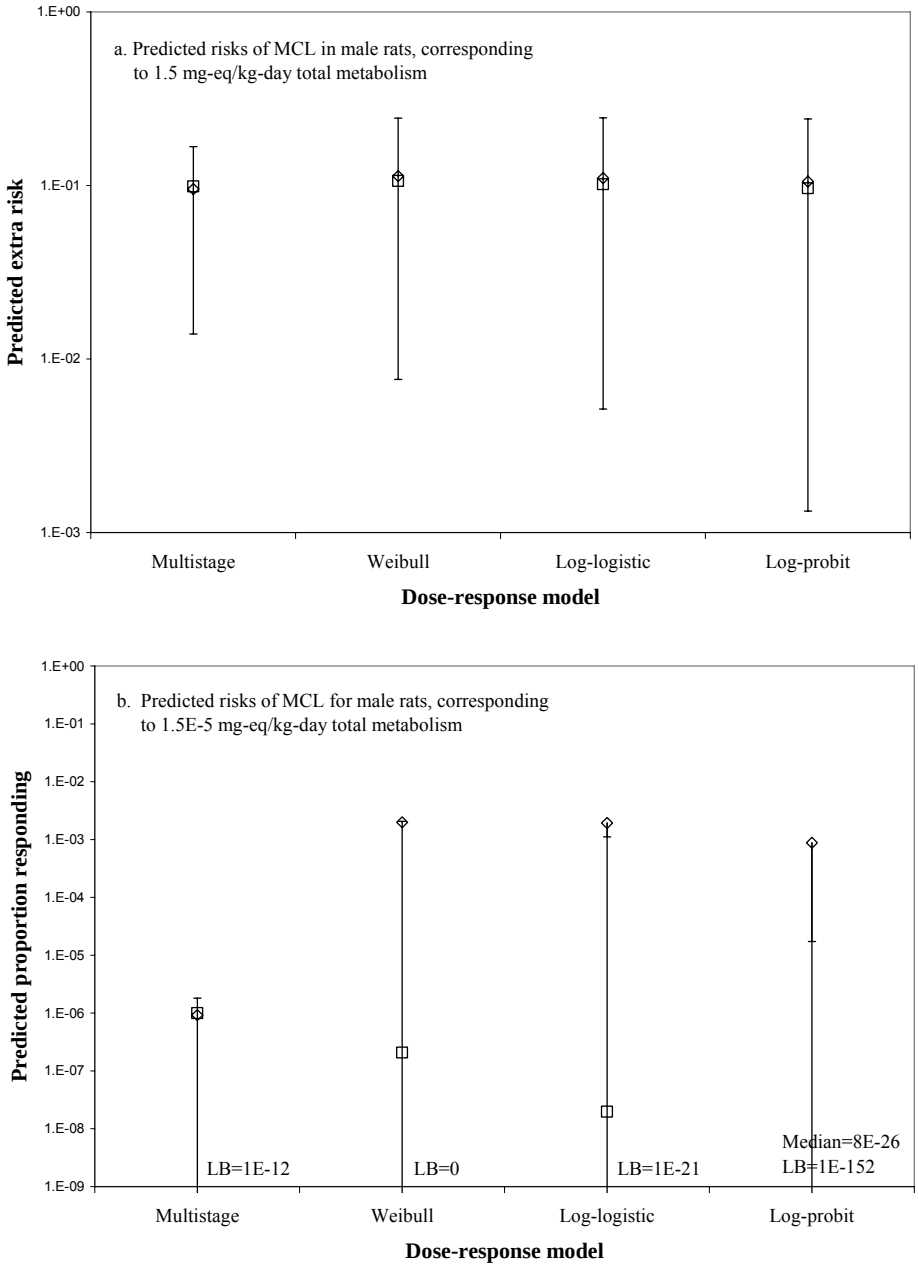


Figure 5-15. Illustration of sensitivity to model selection for low-dose extrapolation. The risk distributions associated with four dose-response models which adequately fit the tetrachloroethylene dose-response data for MCL in male rats (JISA, 1993) were compared. The mean (◊) and median (□) risks for each model are indicated with symbols, and the 5th and 95th percentiles are indicated by bars. Risks are in terms of metabolized tetrachloroethylene in male rats. Figure a shows the comparison at a generalized POD, selected as the mean exposure estimate from the multistage model corresponding to a risk of approximately 0.1—that is, 1.5 mg-eq/kg-day, equivalent to about 50 ppm as administered in the bioassay. Figure b compares risk distributions at an exposure corresponding to an environmental concentration of tetrachloroethylene approximately 10^5-fold lower than the POD, or 1.5×10^{-5} mg-eq/kg-day, which is equivalent to about 50×10^{-5} ppm if administered as in the bioassay. Note that three lower bounds (Weibull, log-logistic, and log-probit) and one median (log-probit) could not be plotted on the graph. See Appendix 5B for more details.

in humans and greater heterogeneity of human populations, more linear fits are expected to better capture the anticipated response in a human population. The low-dose extrapolation to humans from threshold-like models (*i.e.*, log-logistic and log-probit) carries a relatively greater degree of uncertainty than extrapolation from the multistage and Weibull fits. These calculations illustrate the expected finding that alternative functional forms fit to the tetrachloroethylene tumor data yield a wide range of numerical values for probability of response when extrapolated down to low dose and are uninformative of the actual risk.

Given the current state of scientific knowledge about tetrachloroethylene carcinogenicity, the straight line based risk estimates presented above form the preferred recommendation for estimating a plausible upper-bound estimate of potential human risks from tetrachloroethylene. This approach is supported by both general scientific considerations, including those supporting the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), as well as chemical-specific findings. The former include the scientific principles articulated above (the expectation that a chemical functions additively to background exposures, diseases, and processes, that variability within the human population would broaden the dose-response curve and eliminate individual thresholds if present, and that the approach provides consistency across assessments facilitating direct comparison of the derived risk values). The latter includes evidence that, within the dose range of the cancer bioassays, the observable tumor response data are consistent with a linear model and do not suggest occurrence of a threshold, and that variability in the human response across the population is expected (see Human population variability, above).

5.4.6.1.3. ***Dose metric*****.** Tetrachloroethylene is metabolized to several intermediates with carcinogenic potential. Although much data exist for TCA, several analyses indicate that TCA alone is not able to explain the toxicity associated with tetrachloroethylene exposure; therefore, at least one other toxic agent appears to be involved. Whether total metabolism, either as a measure of a precursor or intermediate or as a surrogate directly proportional to the toxic agent(s), is an adequate indicator of potential risk is unclear. Use of administered dose (without use of a PBPK model) yields risk estimates intermediate between those based on the higher and lower PBPK models. Consequently, a role for the parent compound has not been ruled out, nor is it clear that the toxic agent(s) are not proportional to administered concentration.

5.4.6.1.4. ***Choice of species/gender.*** The factors influencing the choice of rodent tumor data set for human risk characterization are summarized in Table 5-14. The carcinogenic response occurs in rodents as well as in humans. There is no information on tetrachloroethylene to indicate that the observed rodent tumors are not relevant to humans, and there are no non-rodent

Table 5-14. Summary of considerations for each rodent tumor type

	Mononuclear cell leukemia	Liver	Kidney	Hemangiosarcoma
Magnitude of response	+++	++	++	+
Specificity of response	Rats, both genders	Mice, both genders	Male rats only	Male mice only
Replication of findings in multiple bioassays	4/4 study datasets	6/6 study datasets	1/2 study datasets	1/3 study datasets
Other considerations contributing to uncertainty in rodent data	High background rate	High background rate in male mice	Rare tumor (unlikely to be due to chance, but low incidence)	Rare tumor (unlikely to be due to chance, but low incidence)
Relevance to humans: a) *qualitative (biologic) site concordance*	Yes	Yes	Yes	Yes
b) occurrence in human studies	Yes, but exact match of tumor classification is not found/may not be possible	Yes, but association is weak	Yes, but association is weak	No, but tumor type is rare
c) confidence in MOA	No data	PPAR-α activation may contribute, but is not sole MOA	Multiple MOAs may play a role	No data
Overall considerations for choice of tumor type	Rodent response of highest magnitude, reproducible; no MOA data	Rodent response of considerable magnitude, reproducible, some MOA data	Rare tumor in rodents and humans; MOA data are strongest	Rare tumor in rodents and humans; no MOA data

cancer bioassay data. Further, no tetrachloroethylene data exist to guide quantitative adjustment for differences in sensitivity among rodents and humans. Human-rodent site concordance generally is not assumed, e.g., due to potential differences in pharmacokinetics, DNA repair, other protective systems across species and tissues (U.S. EPA, 2005a). In keeping with this view, certain tumors associated with tetrachloroethylene exposure in human mortality studies (e.g., cervix and esophagus) were not observed in rodents; cancer of the lymphoid system was associated with tetrachloroethylene exposure in humans, with some evidence for an association with bladder, kidney, and lung cancer. In addition, rat and mouse tumor types also differ from each other. Finally, conclusive MOA data are lacking for the observed rodent and human tumors.

MCL is the cancer response of highest magnitude, and it is reproducible in two bioassays and both genders. Although MCL has a high and variable incidence in unexposed F344 rats, a biologically and statistically significant increase over background was observed. The qualitative similarities among MCL to certain lymphoid cancers, and the implications regarding human relevance, are addressed in Section 4.8.2.4.1.2; also addressed is that elevated lymphoma mortality has been associated with tetrachloroethylene exposure in humans. The MOA for MCL remains unexplored.

Occasionally, if the multistage model does not adequately fit a data set, an alternate model can be used to determine the POD. In the case of female rat MCL data, the best-fitting model (Weibull) allowed for a plateau and yielded estimates of risk per unit concentration 10-fold higher than those from the multistage model fit of the male rat MCL data. While the female rat MCL data suggest a plateau (also apparent for female rat MCL data from the NTP bioassay), the multistage model fit was technically adequate ($p = 0.48$).

The mouse liver tumor is a robust finding in several studies, including in both sexes. As is the case with MCL, the background for this tumor type is high especially in males. A biologically and statistically significant increase over background was observed in males and females. There is evidence that activation of the PPAR-α receptor by the tetrachloroethylene metabolite TCA contributes in part to the induction of mouse liver tumors. However, it is not the only operative MOA involved in hepatocellular tumorigenesis. Thus, the MOA remains unresolved.

Two tumor types were observed in only one bioassay. Kidney tumors rarely occur in unexposed rodents and were significantly elevated with tetrachloroethylene exposure in the male rat NTP bioassay. The MOA is better understood for kidney tumors than for the other sites. Hemangiosarcoma is another rare tumor associated with tetrachloroethylene exposure in the male mouse JISA study. There are no MOA data for hemangiosarcomas.

5.4.6.1.5. ***Physiologically based pharmacokinetic (PBPK) model.*** Toxicokinetic models are used in this assessment for deriving dose metrics to support dose-response analyses. The evidence suggests that by-products of tetrachloroethylene metabolism are responsible for liver and kidney toxicity and for carcinogenicity. Inhaled concentration of the parent compound is, therefore, not an appropriate dosimeter for these effects, and pharmacokinetic modeling of daily overall metabolized dose is expected to be an improvement in spite of the many attendant uncertainties in the modeling. Of the available toxicokinetic models on tetrachloroethylene, the assessment considers three recently developed models that describe parent tetrachloroethylene and overall metabolism of the parent compound in humans. These models do not describe the

kinetics and transformation of total metabolic products or any individual metabolite. All three models provide reasonably good predictions of exhaled breath and blood tetrachloroethylene concentrations, so there is no particular basis for preferring one model over another. A 10-fold difference is shown in model predictions of the rate of metabolism in humans, a reflection of model differences in the values for the metabolic parameters. Because the accuracy of the models has been evaluated only against blood and breath concentrations of the parent compound—quantities that are insensitive to these parameters—the reliability of these models for predicting the rate of total metabolism in humans is unknown. Data on total metabolite levels are not available in humans, and the use of available urinary and blood TCA data is problematic. The overall difference in risk estimates using these three models is approximately 10-fold.

5.4.6.1.6. ***Cross-species scaling.*** An adjustment for cross-species scaling ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between each rodent species and humans, consistent with the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a); the approach is detailed in Section 5.4.4.2.1. It is assumed that, without data to the contrary, equal risks result from equivalent constant exposures. While the true correspondence of equipotent tetrachloroethylene exposures across species is unknown, the use of $BW^{3/4}$ scaling is expected neither to over- or underestimate human risk (U.S. EPA, 1992).

5.4.6.1.7. ***Choice of bioassay.*** The JISA inhalation bioassay provides data on the lowest experimental exposures, and its use, therefore, reduces extrapolation uncertainty slightly. For mice, the lowest-exposure concentration of 10 ppm was 10-fold lower than the lowest-exposure concentration in the NTP inhalation study (NTP, 1986). For rats, the low-exposure concentration of 50 ppm was fourfold lower than in the NTP study. Although the JISA and NTP inhalation bioassays used similar rodent strains, it is possible that differences in the animals used (in addition to other unidentified factors) may have contributed to the twofold higher incidence of hepatocellular tumors and MCL in the NTP study. The estimated risks for these sites are consequently twofold lower than in previous EPA assessments which relied on the NTP bioassay (U.S. EPA, 1991).

5.4.6.1.8. ***Statistical uncertainty at the Point of Departure (POD).*** Parameter uncertainty within the chosen model reflects the limited sample size of the cancer bioassay. For the multistage model applied to this data set, there is a reasonably small degree of uncertainty at the 10% extra risk level (the POD for linear low-dose extrapolation).

5.4.6.2. *Summary and Conclusions*

The uncertainties presented in Table 5-13 have a varied impact on risk estimates. Some suggest risks could be higher than was estimated, while others would decrease risk estimates or have an impact of an uncertain direction. Several uncertainties are quantitatively characterized—the range of uncertainty in the PBPK models considered, together with the statistical uncertainty in the multistage modeling estimate, for the significantly increased rodent tumors. Sensitivity to model selection is quantitatively explored in Figure 5-15, with a focus on thresholded, non-linear alternatives, illustrating the expected finding that such alternatives yield a wide range of estimates that are uninformative of the actual risk. Alternatives that would yield higher risk estimates (e.g., supralinear models), which are equally scientifically valid, are not presented. In addition, the results apply only to the prediction of MCL in male rats, not in humans. Due to limitations in the data, particularly regarding the MOA and relative human sensitivity and variability, the quantitative impact of other uncertainties of potentially equal or greater impact has not been explored. As a result, an integrated quantitative analysis that considers all of these factors independently was not undertaken.

APPENDIX 5A:
BENCHMARK DOSE MODEL RESULTS

ABBREVIATIONS

AIC = Akaike Information Criterion (see, e.g., U.S. EPA, 2000)

BMC_{xx} = Effective concentration at a specified level of extra risk; e.g., BMC_{10} is the concentration corresponding to 10% extra risk, BMC_{05} corresponds to 5% extra risk

BMC_S = Effective concentration corresponding to a one standard deviation difference in the mean response from the control mean response (for continuous data). This is approximately equivalent to 10% of the responses at the effective concentration being more extreme than 98% of the controls if the adverse response is an increase relative to the controls, or 10% of the responses being more extreme than 2% of the controls if the adverse response is a decrease relative to controls.

$BMCL_{xx}$ = Lower 95% confidence bound on the estimated BMC_{xx}

$BMCL_S$ = Lower 95% confidence bound on the estimated BMC_S

NA = Not applicable

POD = Point of departure

Table 5A-1. Benchmark modeling summary: hepatic parenchymal changes in humans with occupational exposure to tetrachloroethylene, data from Brodkin et al. (1995)

8-hr TWA exposure (ppm)	Equivalent continuous exposure[a] (ppm)	Number examined		Number (%) with parenchymal changes in hepatic ultrasonograph	
0.0007 4.6 19.8	0.0003 1.6 7.1	26 9 18		10 (38) 5 (55) 13 (72)	
Quantal models	**Goodness of fit *p*-value**	**AIC**	**Maximum χ^2 residual near POD**	**BMC_{10} (ppm)**	**$BMCL_{10}$ (ppm)**
Multistage[b]	0.71	72.4	0.2	0.9	0.5
Gamma, Weibull,	0.71	72.4	0.3	0.9	0.5
Logistic	0.63	72.5	0.4	1.2	0.7
Probit	0.62	72.5	0.4	3.5	2.2
Probit, log-transformed dose	0.53	72.7	0.6	1.7	0.8

[a] Exposures adjusted by 10/20 (m^3/day) × 5/7 (days) to estimate equivalent continuous exposure levels. Measurements were taken from personal samplers for a subset of the individuals in the two higher-exposure groups. The background level of 0.0007 ppm is the high end of a range from Hartwell et al. (1985).

[b] Multistage model selected as best fitting-model. Models had similar fits, multistage had lowest AIC and closest fit near the EC_{10}. Multistage model given by:

$$P(d) = 1 - \exp(-q_0 - q_1 d)$$

where: d = continuous exposure level (ppm)
$q_0 = 0.40$
$q_1 = 0.11$

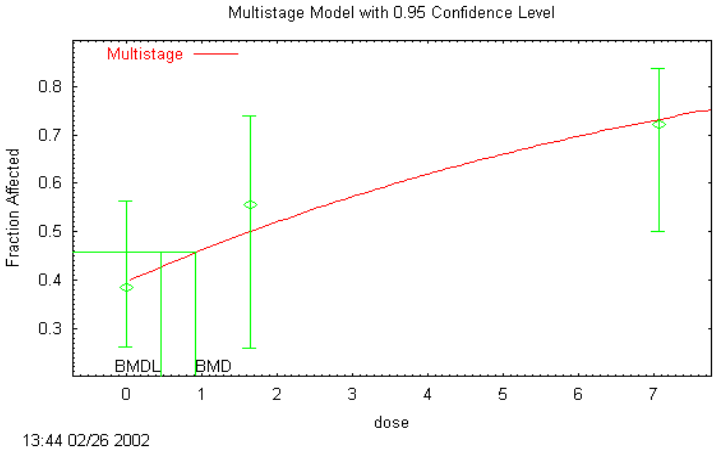


Table 5A-2. Benchmark modeling summary: increased liver weight in female mice exposed to tetrachloroethylene, data from Kjellstrand et al. (1984)

Administered exposure, 24 hrs/day, 30 days (ppm)	Human equivalent continuous exposure (ppm)	N	Female Mice Liver weight (g) mean ∀ s.d.
0	0	42	108 ± 13.5
9	9	11	142 ± 25.6
37	37	10	210 ± 24.2
75	75	10	241 ± 41.4
150	150	10	230 ± 31.1

Continuous models[a]	Goodness of fit p-value	AIC	Maximum χ^2 residual near POD	BMC_S (ppm)	$BMCL_S$ (ppm)
Hill[b]	0.56	510	0.3	3.2	0.6
Power	0.001	524	0.6	6.6	5.1
Linear, Polynomial	0.003	522	0.6	6.6	5.1

[a] Nonconstant variance models fitted.
[b] Hill model was the only adequately fitting model:

$$P(d) = \text{intercept} + v * \text{dose}^n/(k^n + \text{dose}^n),$$

where: intercept = 108.0
v = 193.6
n = 1.08
k = 35.3

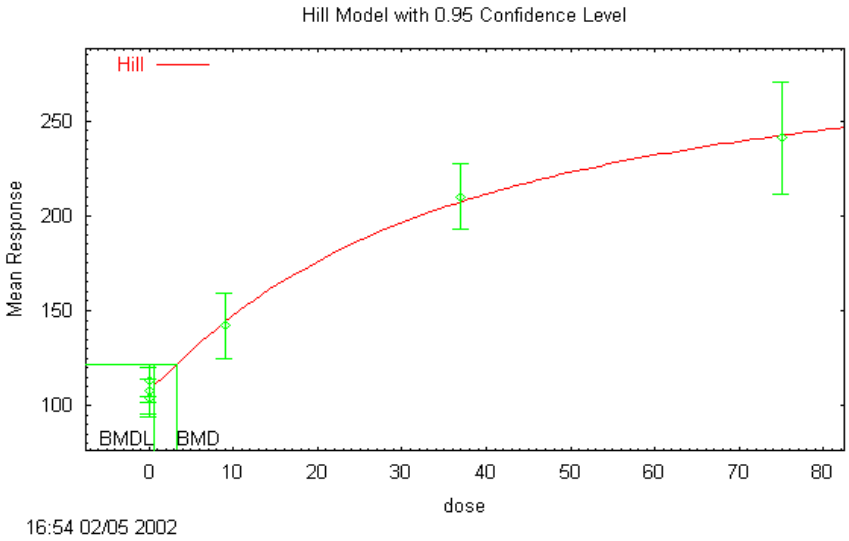


Table 5A-3. Benchmark modeling summary: increased liver weight in female mice exposed to tetrachloroethylene, using response data from Kjellstrand et al. (1984) and human equivalent metabolized dose as dose metric

Administered exposure, 24 hr/day, 30 days (ppm)	Human equivalent metabolized dose[a] (mg-eq/kg-day)	N	Female Mice Liver weights (g) mean ∀ s.d.
0	0.0	42	108 ± 13.5
9	3.2	11	142 ± 25.6
37	12	10	210 ± 24.2
75	19	10	241 ± 41.4
150	28	10	230 ± 31.1

Continuous models[b]	Goodness of fit p-value	AIC	Maximum χ^2 residual near POD	BMC_S (mg-eq/kg-day)	$BMCL_S$ (mg-eq/kg-day)
Hill[c]	0.58	510	0.4	1.1	0.3
Power[c]	0.78	515	0.3	0.7	0.2
Polynomial (linear)	0.03	513	0.4	1.8	1.4

[a] Metabolite levels were estimated using the Reitz et al. (1996) PBPK model, and adjusted to equivalent human doses using surface area scaling by multiplying by $[0.03 \text{ kg}/70 \text{ kg}]^{0.25} = 0.144$.

[b] Nonconstant variance models fitted. Highest-dose group omitted due to poor fits for all models.

[c] Among the models with adequate fits ($p > 0.1$), the Hill and Power models had very similar $BMCL_S$s. The average of these $BMCL_S$s was 0.3 mg-eq/kg-day. The Hill model fit is shown as a representative of the two fits. Using the three human pharmacokinetic models, the human equivalent inhalation exposures ranged from 1.4 to 10 ppm (see Figure 3-9 and Chapter 3).

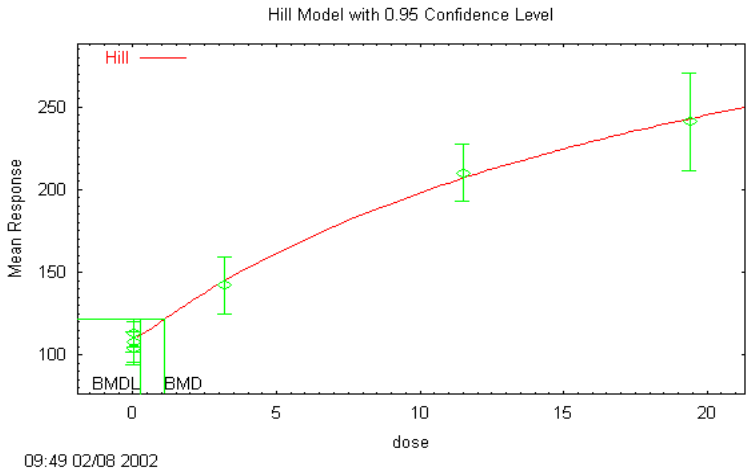


Table 5A-4. Benchmark modeling summary: hepatic angiectasis in male mice exposed to tetrachloroethylene, data from JISA (1993)

Administered exposure, 6 hrs/day, 5 days/wk, 2 years (ppm)	Human equivalent continuous exposure[a] (ppm)	N		Incidence (%) of hepatic angiectasis in male mice	
0 10 50 250	0.0 1.8 8.9 45.0	50 50 50 50		1 3 12 30	(2) (6) (24) (60)
Quantal models	**Goodness of fit p-value**	**AIC**	**Maximum χ^2 residual near POD**	**BMC_{10} (ppm)**	**$BMCL_{10}$ (ppm)**
Gamma[b]	0.57	161	0.4	3.6	1.4
Log-logistic[b]	0.78	161	0.2	3.8	1.7
Multistage[b]	0.66	160	0.3	4.9	3.7
Weibull[b]	0.59	161	0.4	3.6	1.5
Log-Probit[b]	1	161	0	3.8	1.8
Probit	0.02	166	2.2	12.2	10
Logistic	0.02	167	1.5	13.3	11

[a] Exposure adjusted by 6/24 (hrs/day) × 5/7 (days/wk) to estimate equivalent continuous exposure levels.
[b] All models except logistic and probit, had acceptable fits ($p > 0.1$), similar AICs, and $BMCL_{10}$s within a factor of 3 of each other. Average of these 5 $BMCL_{10}$s is 2 ppm. The log-probit model fit is shown as a representative fit.

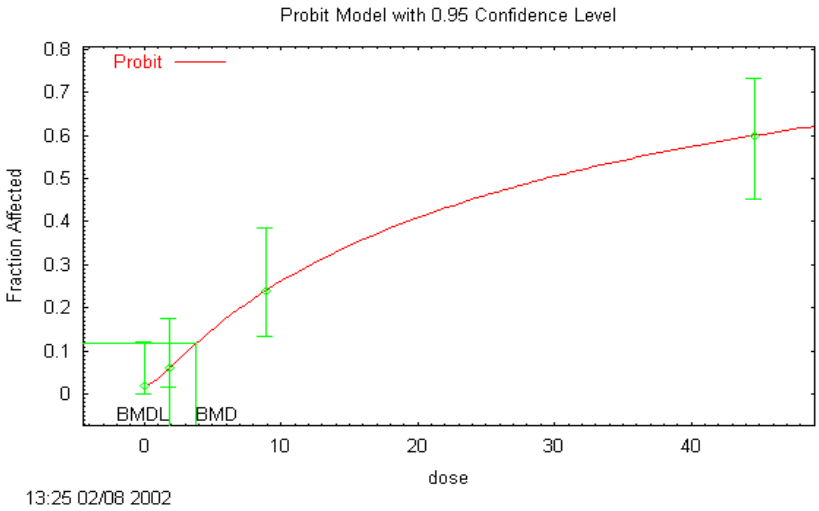


Table 5A-5. Benchmark modeling summary: hepatic angiectasis in male mice exposed to tetrachloroethylene, using data from JISA (1993) and human equivalent metabolized dose as dose metric

Administered exposure, 6 hr/day, 5 days/wk, 2 years (ppm)	Human equivalent metabolized dose[a] (mg-eq/kg-day)	N		Incidence (%) of hepatic angiectasis in male mice	
0 10 50 250	0.0 0.6 2.3 5.8	50 50 50 50		1 3 12 30	(2) (6) (24) (60)
Quantal models	**Goodness of fit p-value**	**AIC**	**Maximum χ^2 residual near POD**	**BMC_{10} (mg-eq/kg-day)**	**$BMCL_{10}$ (mg-eq/kg-day)**
Gamma[b]	0.87	161	0.1	1.2	0.6
Logistic[b]	0.23	162	1.3	2	1.6
Log-logistic[b]	0.73	161	0.2	1.2	0.6
Multistage[b]	0.85	161	0.1	1.1	0.6
Probit[b]	0.35	161	1.1	1.8	1.5
Log-Probit[b]	0.51	161	0.4	1.2	0.6
Weibull[b]	0.95	161	0.04	1.2	0.6

[a] Metabolite levels were estimated using the Reitz et al. (1996) PBPK model, were estimated adjusted to equivalent human doses using surface area scaling by multiplying by $[0.048 \text{ kg}/70 \text{ kg}]^{0.25} = 0.162$.

[b] All models achieved satisfactory fits ($p > 0.1$, similar AICs), except the logistic and probit models were least consistent with the data, having acceptable but relatively large χ^2 residuals near the $BMCL_{10}$. The $BMCL_{10}$s from the remaining models were all 0.6 mg-eq/kg/day. The multistage model fit is shown as a representative fit. Using the three human pharmacokinetic models with the POD of 0.6 mg-eq/kg-day, the human equivalent inhalation exposures ranged from 4.3–23 ppm.

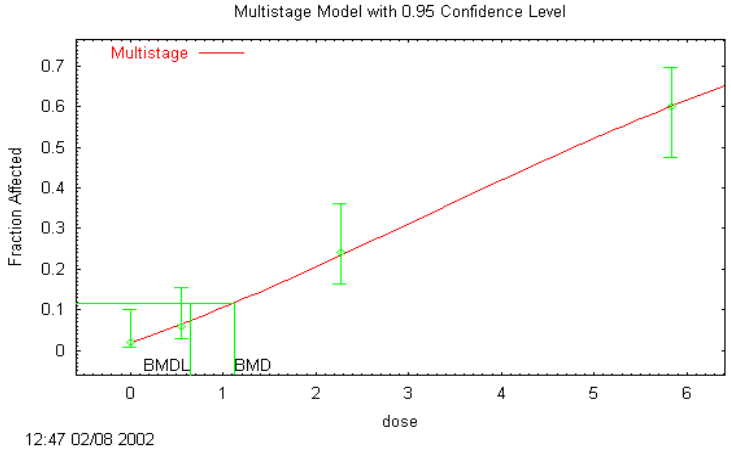


Table 5A-6. Benchmark modeling summary: incidence of karyomegaly in male rats exposed to tetrachloroethylene, data from NTP (1986)

Administered exposure, 6 hrs/day, 5 days/wk, 2 years (ppm)	Human equivalent administered dose (ppm)[a]	N		Incidence (and %) of Karyomegaly in male rats	
0 200 400	0 36 71	49 49 50		1 37 47	(2) (76) (96)
Quantal models	**Goodness of fit p-value**	**AIC**	**Maximum χ^2 residual near POD**	**BMC_{10} (ppm)**	**$BMCL_{10}$ (ppm)**
Gamma, Log-logistic, Log-Probit, Weibull	NA				
Logistic	<0.01	98.3	1.3	10.6	7.8
Multistage[b]	0.98	91	0.01	2.7	2.2
Probit	<0.01	101	1.9	9.8	7.5

[a] Exposure adjusted by 6/24 (hr/day) × 5/7 (days/wk) to estimate equivalent continuous exposure levels.

[b] With only two nonzero exposure groups, options for fitting these data were limited. Among the models with two or less parameters to estimate, the multistage model was the only one to fit adequately ($p > 0.1$).

$$P(d) = 1 - \exp(-q_0 - q_1 d),$$

where: d = continuous exposure level (ppm)
$q_0 = 0.02$
$q_1 = 0.04$

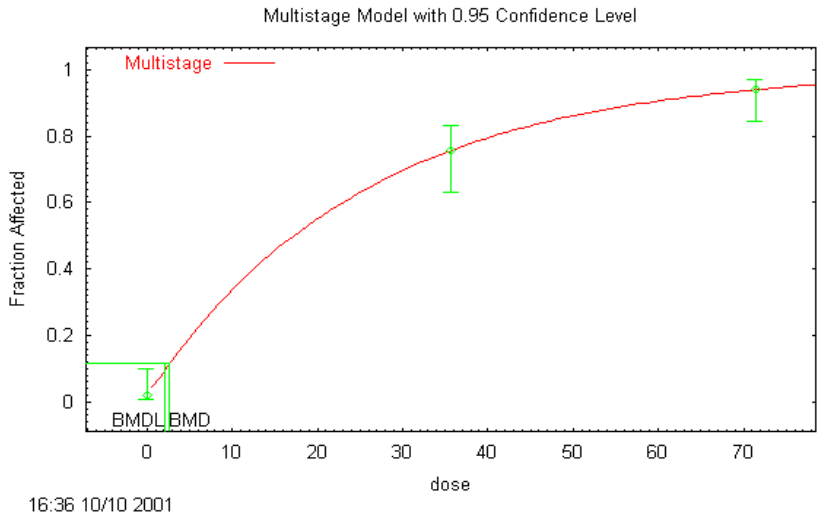


Table 5A-7. Benchmark modeling summary: deaths by Day 29 in offspring of rats exposed to tetrachloroethylene, data from Tinston (1994)

Administered exposure, 6 hrs/day, 5 days/wk, 11 weeks; every day during gestation (ppm)	Human equivalent administered exposure[a] (ppm)	Number of F2A Litters	Average per-litter percent of deaths by Day 29
0	0	23	8.4
100	18	22	9.5
300	54	21	11.4
1000	180	19	33.5

Nested models[b]	Goodness of fit p-value	AIC	BMC_{01}[c] (ppm)	$BMCL_{01}$[c] (ppm)
Nested logistic	0.072	656.9	24.4	2.1
NCTR[d]	0.12	656.9	23	1.8
Rai and vanRyzin[d]	0.12	656.9	23	1.8

[a] Exposures adjusted to equivalent continuous exposures by multiplying by 6/24 (hrs/day) × 5/7 (days/wk).
[b] All nested models fit best when including intralitter correlations. Litter size was not used as a litter-specific covariate.
[c] $BMCL_{01}$ selected as relevant response level because of the severity of the response (pup death) and because the response level occurred within the range of the data set.
[d] The NCTR and the Rai and van Ryzin model fits were identical for these data, whereas the nested Logistic did not provide an adequate fit.

$$P(d) = 1 - \exp[-(\alpha + \beta \times d^{\rho})],$$

where: d = continuous exposure level (ppm),
$\alpha = 0.089$
$\beta = 4.38 \times 10^{-5}$
$\rho = 1.73$

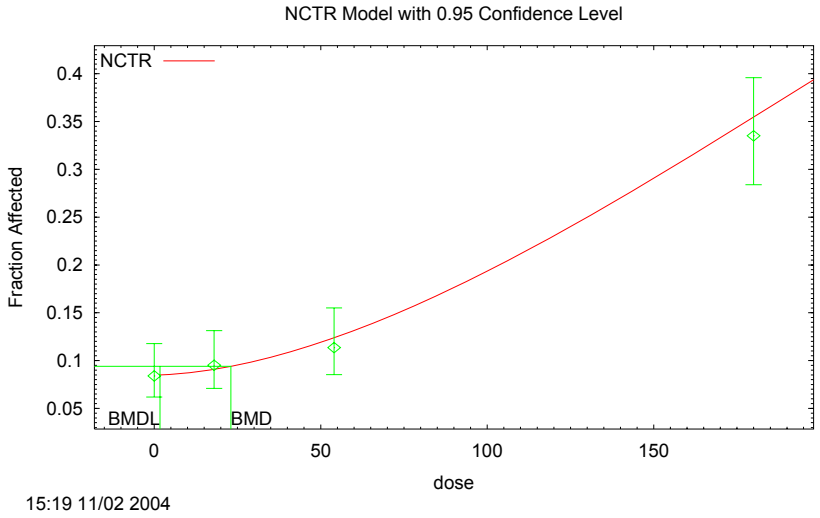


Table 5A-8. Benchmark modeling summary: liver to body weight ratio in rats exposed to tetrachloroethylene, data from Buben and O'Flaherty (1985)

Administered doses (5 days/wk, 6 weeks) (mg/kg-day)	N	Liver weight/ body weight, mean % ± s.d.
0	26	5.21 ± 0.46
20	13	5.51 ± 0.40
100	13	5.97 ± 0.40
200	15	6.45 ± 0.46
500	15	7.35 ± 0.62
1000	19	7.89 ± 0.70
1500	6	8.10 ± 0.66
2000	6	9.00 ± 0.27

Continuous models[b]	Goodness of fit p-value	AIC	Maximum χ^2 residual near POD	BMD_S (mg/kg-day)	$BMDL_S$ (mg/kg-day)
Hill[c]	0.11	-24.1	0.8	8.3	6.4
Power	<0.001	28	1	38	33
Polynomial, Linear	<0.001	24	1	38	33

[a] Gavage doses were adjusted for daily exposure (× 5/7) and were adjusted to equivalent human doses using surface area scaling by multiplying by $[0.048 \text{ kg}/70 \text{ kg}]^{0.25} = 0.162$.
[b] Constant variance models used.
[c] Hill model was the only model to fit adequately.

$$P(d) = \text{intercept} + v * \text{dose}^n/(k^n + \text{dose}^n),$$

where: intercept = 5.27
v = 4.18
n = 1
k = 58.2

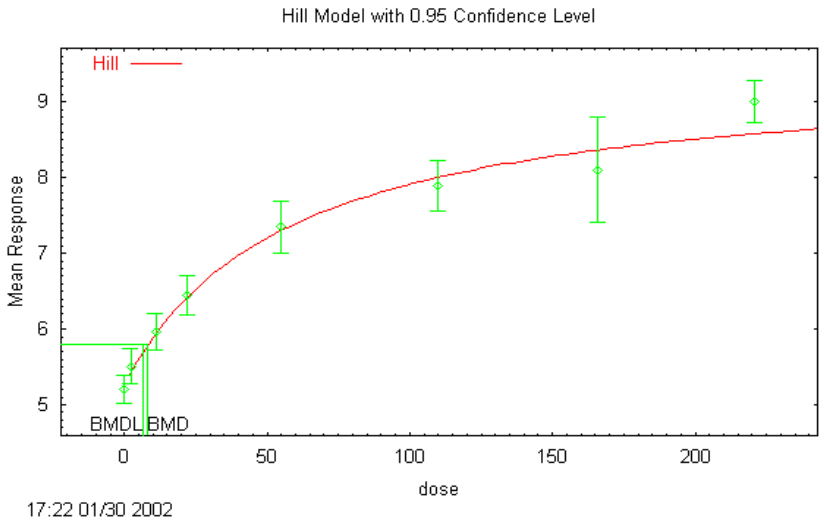


Table 5A-9. Benchmark modeling summary: liver to body weight ratio in rats exposed to tetrachloroethylene, using data from Buben and O'Flaherty (1985) and human equivalent metabolized dose as dose metric

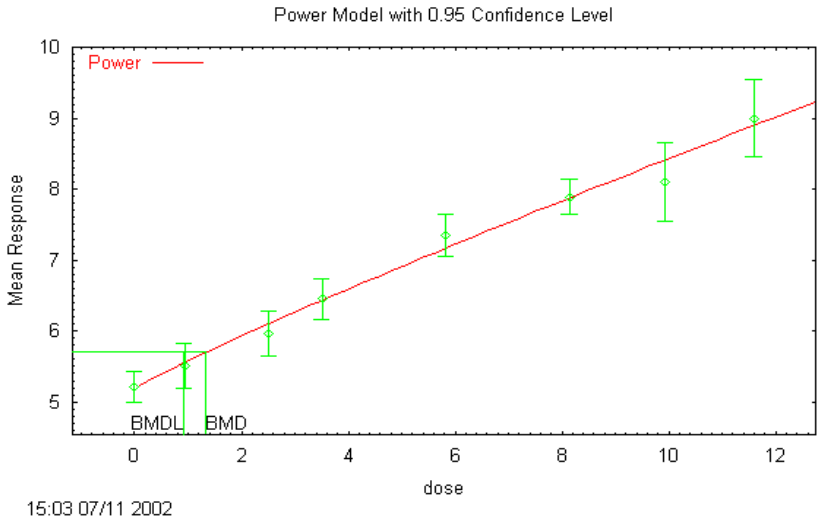
Administered doses (5 days/wk, 6 wks) (mg/kg-day)	Continuous equivalent metabolized dose[a] (mg-eq/kg-day)	N	Liver weight/ body weight, mean % ± s.d.
0	0.0	26	5.21 ± 0.46
20	0.58	13	5.51 ± 0.40
100	2.52	13	5.97 ± 0.40
200	3.51	15	6.45 ± 0.46
500	5.83	15	7.35 ± 0.62
1000	8.15	19	7.89 ± 0.70
1500	9.94	6	8.10 ± 0.66
2000	11.6	6	9.00 ± 0.27

Continuous models[b]	Goodness of fit *p*-value	AIC	Maximum χ^2 residual near POD	BMD_S (mg-eq/kg-day)	$BMDL_S$ (mg-eq/kg-day)
Hill[c]	0.51	-30.9	0.2	8.8	NA
Power[c]	0.35	-30.5	0.2	7.9	6.5
Linear, Polynomial[c]	0.35	-32.5	0.6	7.9	6.5

[a] Metabolites for mice were estimated using the Reitz et al. (1996) model and were adjusted for continuous daily exposure (× 5/7).

[b] Nonhomogeneous variance models.

[c] All continuous models fit reasonably well, except the Hill model could not provide a BMDL. The power model fit is shown as a representative of the other two fits. The $BMDL_S$ was converted to an equivalent human dose using surface area scaling by multiplying by $[0.048 \text{ kg}/70 \text{ kg}]^{0.25} = 0.162$, or 1.1 mg-eq/kg-day. Using the three human pharmacokinetic models, the human equivalent oral exposures ranged from 3.4 to 32 mg/kg-day (see Table 5-10 for conversion factors).



APPENDIX 5B:
PROBABILITY DISTRIBUTIONS OF CANCER RISK ESTIMATES

Given the importance of characterizing central tendency estimates of risk when feasible (U.S. EPA, 2005a), and the observation that MLEs resulting from typical dose-response analyses can be unstable, an analysis of the distributions underlying the range of site-specific tetrachloroethylene estimates of risk per unit concentration was undertaken. In addition, the distributions underlying estimates of risk per unit concentration based only mononuclear cell leukemias was explored for three dose-response models frequently used for noncancer dose-response assessment in the observable range of the experimental data: the Weibull, log-logistic, and log-probit models.

The bootstrap analysis (Efron and Tibshirani, 1993) was used to characterize the distributions of risk per unit concentration for the six tumor/sex types identified for tetrachloroethylene. For each of the six data sets in Figure 5-14 (see Tables 5-4 and 5-6 for group data), for each exposure group a simulated incidence level was generated using binomial distribution with probability of success equal to the observed incidence. This was repeated until there were 10,000 simulated experiments for each tumor type. Then each simulated data set was used to obtain estimates of BMDs using BMDS (U.S. EPA, 2000) in the same manner as for the tetrachloroethylene assessment, including using the multistage model. The BMDs were estimated at a benchmark response (BMR) of 10% extra risk for all sites except kidney tumors, which were evaluated at 5% extra risk because 10% fell above the observed data. Distributions of cancer slope values were obtained by calculating the distributions of the ratios BMR/BMDs. Upper and lower bounds on the linear extrapolation were determined by the 95^{th} and 5^{th} percentiles of the resulting distributions.

In the same manner as in the preceding paragraph, the bootstrap analysis was used to characterize the distributions of risks per unit concentration resulting from fitting the male rat leukemia data with the multistage, Weibull, log-logistic, and log-probit models. These models were fit to the mononuclear cell leukemia data in male rats using the EPA BMDS program without restricting the shape parameters of the latter three models. Parameters describing the risk distribution (mean, median and 95% upper and lower confidence bounds) were estimated by a bootstrap procedure, because these parameters are not all readily available in the current BMDS software. The resulting risk distributions were compared at two exposure levels—at a generalized POD selected near the 10% response level estimated by the multistage model fit, at 1.5 mg-eq/kg-day, and at an environmental level 10^5-fold lower than the generalized POD, at 1.5×10^{-5} mg-eq/kg-day. In comparing these distributions from the bootstrap procedure, the mean

(as a measure of central tendency) and the 95% upper and lower bounds calculated from the bootstrap procedure are of interest.

5.B.1. MULTISTAGE MODEL RESULTS

Table 5B-1 compares cancer risk values calculated based on BMDS output (left half of table) with those calculated based on the bootstrap distribution of BMDs (right half of table). BMDS estimates the BMD as an MLE, and derives the 95% lower bound (BMDL) and the 95% upper bound (BMDU) on the BMD using the asymptotic distribution of the profile likelihood. Dividing the BMR by these values, one obtains estimates of the slopes of linear extrapolation to background responses from the BMDLs and BMDUs.

One can observe that there is generally a very good correspondence between asymptotic (BMDS) results and re-sampling (bootstrap- based) results. This is in agreement with analysis of other models in BMDS (Moerbeek et al., 2004), but differs from the conclusions of Bailer and Smith (1994). However, the latter paper's conclusions were based on 1,000 runs, and Moerbeek et al. (2004) demonstrated that at least 2,000 runs are needed to stabilize confidence interval estimates. Additionally, risk estimates corresponding to the BMD were derived using the average of the inverse distribution of BMDs. While agreement with risk estimates calculated using BMDS is generally good, for one data set (male mice liver tumors) the discrepancy is noticeable, with the MLE (BMD) and bootstrap estimates differing by about 50% (8.07×10^{-3} vs. 1.16×10^{-2}). The difference is due to asymmetry of the distribution of BMDs, so that the MLE may be different from the average of the distribution in such situations. The estimate based on the bootstrap average is therefore a preferred estimate of central tendency in such a case.

5.B.2. RESULTS USING ALTERNATE MODELS

At 1.5 mg-eq/kg-day, corresponding to a risk of approximately 0.1 using the mean estimate of risk from the multistage model, the bootstrap procedure yielded similar risk distributions for the four models (see Table 5B-2 and Figure 5B-1a). The means and corresponding confidence bounds agree within an order of magnitude and the spreads in the distributions (the distance between the upper and lower confidence bounds) are within two orders of magnitude. Note that the probability calculations are in terms of metabolized dose in the male rat, and do not directly characterize human risks.

EPA also examined the bootstrap results from those same models at a dose 10^5 –fold lower (although EPA's actual low dose risk estimates are developed using a linear extrapolation from a POD to the origin rather than using low-dose estimates from a model). These results are shown in Table 5B-2 and Figure 5B-1b. In the region of extrapolated concentrations (1.5×10^{-5}

mg-eq/kg-day), the mean risks of the latter three models (Weibull, log-logistic and log-probit) are about one to three orders of magnitude higher than the mean of the multistage model risks. The spreads of all the models are quite broad, with a six order of magnitude 95% confidence interval for the multistage and much greater spreads for the other three models. The upper bounds of risk for the other three models are higher than that for multistage model, within about three orders of magnitude, and their lower bounds of risk are much lower than that of multistage, by nine or more orders of magnitude. With such large spreads in confidence intervals, the extrapolated models in effect provide little information about low-dose risks. The extrapolation of the multistage model does result in estimates reasonably close to the low-dose estimates from the model-independent straight line extrapolation from the POD, in that the mean and upper-bound risks at the lower concentration are both within 10% of the estimates resulting from applying linear extrapolation to the results at the higher concentration.

Table 5B-1. Comparison of BMDS and bootstrap-based cancer risk values derived using the multistage model to fit rodent bioassay data for tumor types associated with tetrachloroethylene exposure, and using total metabolites as the dose metric

Tumor type	Model order[a]	BMR	Slopes, (mg-eq/kg-day)$^{-1}$, of linear extrapolation using BMDS estimates of:			Bootstrap-based slopes, (mg-eq/kg-day)$^{-1}$		
			BMD	BMDL	BMDU	Mean	Upper Bound	Lower Bound
Male rat kidney tumors[b]	2	0.05	1.22×10^{-2}	2.63×10^{-2}	ND[c]	1.20×10^{-2}	2.32×10^{-2}	ND
	1	0.05	1.22×10^{-2}	2.63×10^{-2}	ND	1.24×10^{-2}	2.52×10^{-2}	ND
Male mice liver hemangio-sarcomas	3	0.1	4.82×10^{-3}	8.04×10^{-3}	2.06×10^{-3}	4.61×10^{-3}	7.66×10^{-3}	2.26×10^{-3}
	1	0.1	4.82×10^{-3}	8.04×10^{-3}	2.06×10^{-3}	4.88×10^{-3}	8.01×10^{-3}	1.97×10^{-3}
Male mice liver tumors	3	0.1	8.07×10^{-3}	2.77×10^{-2}	5.46×10^{-3}	1.16×10^{-2}	2.77×10^{-2}	5.76×10^{-3}
Female mice liver tumors	3	0.1	5.33×10^{-3}	1.04×10^{-2}	4.14×10^{-3}	6.02×10^{-3}	9.69×10^{-3}	4.30×10^{-3}
Male rat leukemias	3	0.1	7.64×10^{-2}	1.24×10^{-1}	2.67×10^{-2}	6.75×10^{-2}	1.16×10^{-1}	2.92×10^{-2}
	2	0.1	7.64×10^{-2}	1.24×10^{-1}	2.93×10^{-2}	6.89×10^{-2}	1.16×10^{-1}	3.30×10^{-2}
Female rat leukemias	3	0.1	2.46×10^{-2}	4.93×10^{-2}	ND	2.36×10^{-2}	4.81×10^{-2}	ND
	1	0.1	2.46×10^{-2}	4.93×10^{-2}	ND	2.49×10^{-2}	5.03×10^{-2}	ND

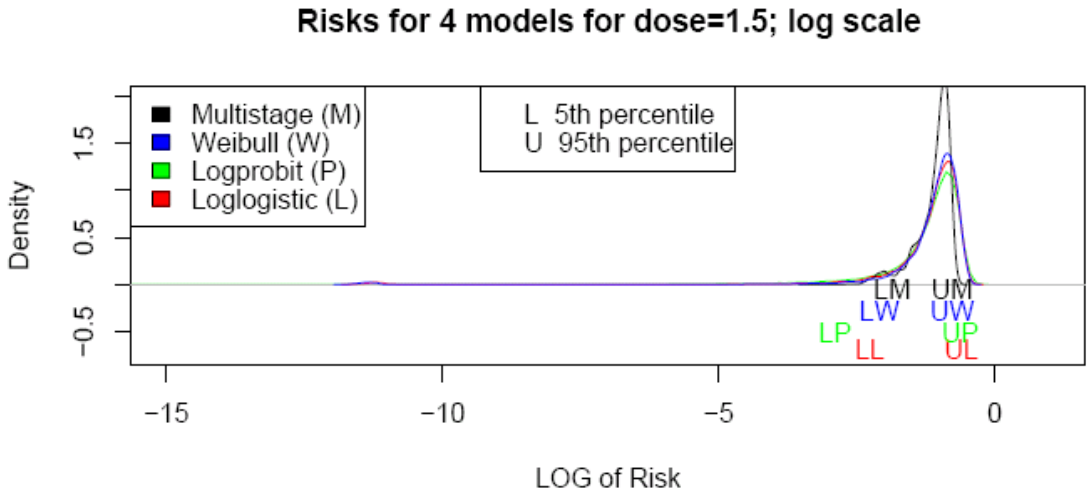
[a] Order of polynomial in multistage model. Different order models were compared where various order models fit equally well.

[b] Data set had 2 non-zero dose groups, all others had three. See Tables 5-4 and 5-6 for data and study details.

[c] ND = could not be determined.

Table 5B-2. Comparison of BMDS and bootstrap-based cancer risk values derived using several dose-response models to fit rodent bioassay data for mononuclear cell leukemia incidence in male rats associated with tetrachloroethylene exposure, and using total metabolites as the dose metric

Model	Dose, mg-eq/kg-day	MLE of risk	Bootstrap distribution of extra risk			
			Mean	Median	5th percentile	95th percentile
Multistage	1.5	1.143×10^{-1}	9.442×10^{-2}	9.853×10^{-2}	1.163×10^{-2}	1.672×10^{-1}
	$1.5x\ 10^{-5}$	1.168×10^{-6}	9.172×10^{-7}	9.933×10^{-7}	4.546×10^{-13}	1.819×10^{-6}
Log-probit	1.5	9.717×10^{-2}	1.037×10^{-1}	9.544×10^{-2}	8.366×10^{-4}	2.426×10^{-1}
	1.5×10^{-5}	4×10^{-25}	$8.172\ x10^{-4}$	1×10^{-26}	2×10^{-170}	1.008×10^{-5}
Log-logistic	1.5	1.018×10^{-1}	1.079×10^{-1}	9.937×10^{-2}	4.320×10^{-3}	2.429×10^{-1}
	1.5×10^{-5}	2.553×10^{-8}	1.078×10^{-3}	1.401×10^{-8}	2×10^{-21}	7.776×10^{-4}
Weibull	1.5	1.064×10^{-1}	1.124×10^{-1}	1.052×10^{-1}	5.594×10^{-3}	2.452×10^{-1}
	1.5×10^{-5}	2.41×10^{-7}	1.339×10^{-3}	1.643×10^{-7}	0	1.893×10^{-3}



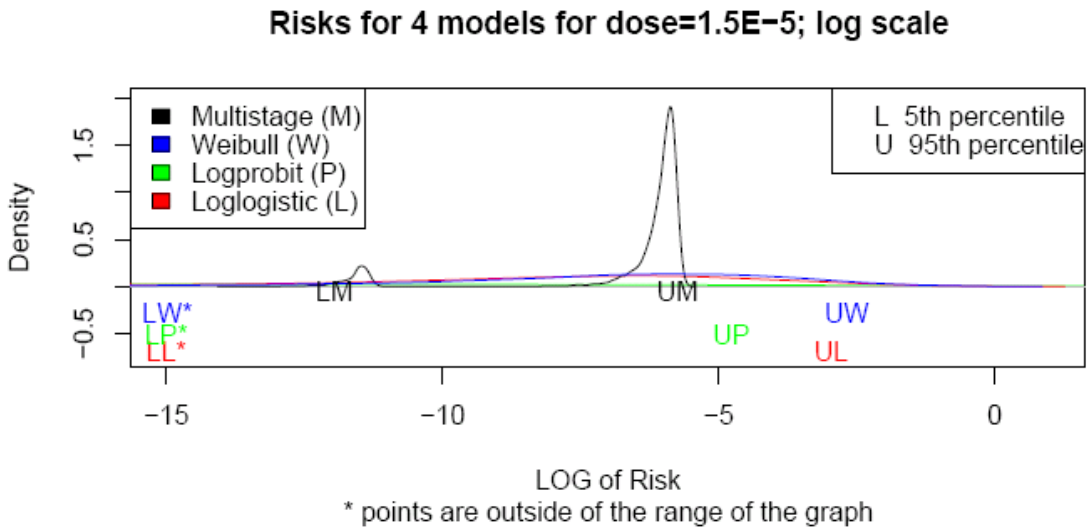


Figure 5B-1. Illustration of sensitivity to model selection for low-dose extrapolation. The risk distributions associated with four dose-response models which adequately fit the tetrachloroethylene dose-response data for MCL in male rats (NTP, 1986) were compared. Risks are in terms of metabolized tetrachloroethylene in male rats. Figure 5-2 shows the comparison at a generalized POD, selected as the mean exposure estimate, from the multistage model corresponding to a risk of approximately 0.1. Figure 5-3 compares risk distributions at an exposure corresponding to environmental concentrations of tetrachloroethylene, approximately 10^5-fold lower than the POD.

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6. CHARACTERIZATION OF HAZARD AND DOSE-RESPONSE

6.1. SUMMARY OF HUMAN HAZARD POTENTIAL

6.1.1. Exposure

Tetrachloroethylene (CASRN 127-18-4) is a solvent used for cleaning clothes and for metal cleaning and degreasing. It is a volatile liquid at room temperature. The largest human exposure occurs indoors to workers in dry cleaning, laundry, and metal finishing facilities. Release of tetrachloroethylene into the air from these facilities also results in measurable outdoor ambient air concentrations. Indoor residential exposure can also occur when dry cleaning facilities are located within residential areas. It has been detected in breast milk of women exposed to tetrachloroethylene in ambient air in or near these facilities. Tetrachloroethylene can enter water supplies, and it has been detected in drinking water. Exposure to airborne tetrachloroethylene can occur in homes via volatilization from tap water during showering as well as from water ingestion in homes with contaminated drinking water (see Chapter 2 for more information).

6.1.2. Absorption, Metabolism, Distribution, and Excretion

Tetrachloroethylene rapidly enters body tissues after inhalation, ingestion, and dermal exposure. Tetrachloroethylene metabolism is considered to be well characterized in rodents but not in humans. A significant portion of tetrachloroethylene inhaled by humans at ambient concentrations is not metabolized (about 64% according to the pharmacokinetic model of Bois, et al. 1996). The recovered metabolites in the urine represent only a fraction of what is actually metabolized (Bogen and McKone, 1988). Possible explanations for metabolites not reaching the urine are (a) binding to plasma proteins, (b) biliary excretion, (c) enterohepatic circulation of metabolites, (d) further metabolism of fat-sequestered parent compounds after the completion of the studies, and (e) metabolism to currently unidentified metabolites. However, data to support these hypotheses are sparse. The fraction of tetrachloroethylene metabolized appears to have a strong dependence on the exposure concentration. The PBPK model by Bois et al. (1996) predicts this fraction to be about 36% in humans at low environmental concentrations, whereas the human data indicate a very small fraction would be metabolized at higher concentrations (such as those corresponding to the laboratory animal bioassays; see Section 3.5 for more details.)

There are two major routes of metabolism: (1) the predominant oxidative pathway, which results in TCA and other urinary metabolites, as well as reactive intermediates and carbon dioxide; and (2) the GSH conjugation pathway, which results in TCVG and TCVC that are

further processed to chemically reactive products that can bind to tissue macromolecules. Metabolism occurs predominately in the liver. Further, metabolism of the GSH metabolites, including activation by beta lyase, occurs in the kidney. In addition, the CYP enzymes of the oxidative pathway as well as enzymes important to the GSH pathway are present in other tissues. Therefore, a potential exists for extrahepatic metabolism and formation of reactive metabolites at sites other than the liver and kidney (see Section 3.3.3.2).

Many steps in the oxidative metabolism of tetrachloroethylene are well characterized in both animals and humans; however, not all proposed intermediates have been identified or detected. Although an initial epoxide metabolite has not been unequivocally demonstrated for tetrachloroethylene, the epoxide intermediate is a reasonable[1] proposal. It has been chemically synthesized, and it is metabolized to TCA when injected into rodents. The tetrachloroethylene epoxide intermediate is considered to be unstable and short-lived in vivo and is thought to spontaneously rearrange and convert to other intermediate metabolites. Formation of trichloroacetyl chloride directly from tetrachloroethylene via the mechanism of CYP-mediated olefin oxidation without the obligatory formation of the epoxide intermediate has also been postulated. The formation of trichloroacylated protein adducts in liver and kidney of rats, liver of mice, and plasma of rats and humans following exposures to tetrachloroethylene provides evidence of a metabolic intermediate that can react with tissue proteins. TCA, a stable metabolite, is believed to result primarily from the oxidation of tetrachloroethylene to the potentially reactive trichloroacetyl choride. TCA has been detected in the blood and urine of humans and laboratory rodents, and excretion in urine is used as a biomarker for tetrachloroethylene exposure (see Section 3.3.3 for more details).

Other steps in tetrachloroethylene oxidative metabolism are not as well characterized. Both TCOH and TCOH-glucuronide have been detected in the urine of mice and humans following tetrachloroethylene exposure; however, there is uncertainty about formation of TCOH and its chloral hydrate precursor from tetrachloroethylene because not all studies have detected TCOH as a urinary excretion product. These different findings could be explained in several ways: (a) TCOH could be an artifact of the analytical methodology, (b) differences could exist in analytical methodologies, (c) contamination could have occurred by unknown exposures to another chemical, and (d) excretion of TCOH and its glucuronide might be dependent on dose. If the TCOH pathway exists in humans, the overall contribution to TCA from TCOH is expected to be relatively small when compared with the amount of TCA resulting from trichloroacetyl chloride.

[1] "Reasonable," as used is this chapter, is intended to imply the use of reasoned scientific judgment in data evaluation and decision-making, consistent with U.S. EPA practices and guidance (e.g., *Guidelines for Carcinogen Risk Assessment*, 2005a)

DCA is an intermediate metabolite that has been identified in the urine of rats, but not humans, exposed by inhalation to tetrachloroethylene. It is not clear whether DCA is a product of further metabolism of TCA or a product of another pathway originating with GSH conjugation, or both. The amount of DCA produced from tetrachloroethylene oxidative metabolism could vary across species and is likely to be relatively small when compared with the amount of TCA produced. Data on mutational changes in tetrachloroethylene-induced liver tumors in mice also support a limited role for DCA in tetrachloroethylene toxicity.

Quantitatively, the GSH conjugation pathway is relatively minor when compared with the P450 conjugation pathway. Urinary mercapturates comprise from 1% to as little as 0.03% of total recovered urinary metabolites (Green et al., 1990; Birner et al., 1996); however, this urinary excretion product does not reflect the amount of compound going through the GSH pathway, but, rather, it reflects only the portion that is excreted. The amount of the mercapturate product excreted in the urine also does not reflect the amount of the more important portion that is converted to toxic by-products through further metabolism. TCVG is the conjugation product of tetrachloroethylene, and its cleavage product, TCVC, reacts with a kidney enzyme, beta lyase, to produce metabolites that are mutagenic in bacteria and are also cytotoxic. These metabolites are believed to contribute to tetrachloroethylene-induced kidney toxicity. Uncertainty exists as to the relative contribution of GSH metabolism to toxicity in humans as compared with the rat due to study differences in reported amounts of human tetrachloroethylene GSH metabolism as measured by excreted mercapturate. These differences may be due, in part, to different chemical assay methodology or to problems resulting from the stability of the chemical product being measured, or both. In spite of these uncertainties, some of the published findings concerning TCVG production would not predict any less susceptibility for humans than for rodents with regard to renal toxicity. The higher percentage of mercapturate found in rat versus human urine does not indicate a higher level of production of toxic products in the rat, because excreted mercapturate allows no estimate of the amount of TCVC or N-acetyl TCVC being processed through alternate routes. Furthermore, it is not known whether sex-dependent variation of beta lyase activity exists in humans as it does in rats. Human variation might also explain study differences in reported excretion rates (see Section 3.3.3.2).

Several enzymes of the oxidative and GSH metabolism, notably CYP2E1, CYP3A4, GSTZ, GSTA, GSTM, and GSTT, show genetic polymorphisms with the potential for variation in production of specific metabolites. The effect of metabolic variation, including potential implications for differential toxicity, has not been well studied. The limited data available on tetrachloroethylene metabolites show DCA to be a potent, irreversible inhibitor of GSTZ activity, with greater inhibition of this enzyme in mice than in humans. Studies show that

inducers of CYP enzymes, such as toluene, phenobarbital, and pregnenolone-16 alpha-carbonitrile, *increase* tetrachloroethylene metabolism, whereas CYP enzyme inhibitors such as SKF 525A, metyrapone, and carbon monoxide *decrease* tetrachloroethylene metabolism. Additionally, chronic exposure to tetrachloroethylene has been shown to cause self-induction of metabolism (see Section 3.3.4).

6.1.3. Noncancer Toxicity in Humans and Laboratory Animals

Targets of toxicity observed in human and animal studies include the liver, kidney, CNS, reproductive system, and developing fetus. Both occupational and residential epidemiologic studies have examined the effects of tetrachloroethylene exposure via inhalation. Humans were found to be particularly sensitive for neurological effects, including decrements in vision or visuo-spatial function, and other neurobehavioral (cognitive) effects following inhalation exposure. These findings are supported by the consistency of the observations in a number of epidemiologic studies of different designs, populations, and statistical analyses, despite study flaws. Altmann et al. (1995) identified a pattern of neurobehavioral deficits in a study of residents living in buildings co-located with a dry cleaning establishment that is similar to the pattern observed in occupational populations with tetrachloroethylene exposures, thus providing evidence of an association with nonoccupational exposure. A second residential study (Schreiber et al., 2002) also suggests that children may be uniquely susceptible to visuo-spatial effects, but larger studies in humans and studies using animal models are needed to confirm this observation as well as reports of color vision discrimination and contrast sensitivity (black-white discrimination) changes. The large body of evidence assessing neurobehavioral effects and tetrachloroethylene does not permit a distinction between acute effects and effects of repeated exposure. Furthermore, no studies are available to evaluate chronic disabling neurological disease. Occupational studies have examined the effects of tetrachloroethylene on other endpoints as well, with the strongest evidence being for markers of liver and kidney damage and for reproductive/developmental effects such as spontaneous abortion. The few studies examining inhalation exposure to tetrachloroethylene and immune or endocrine system effects are inadequate for fully evaluating potential associations.

The measure of the extent of exposure in many of the epidemiologic studies is imprecise, and, in occupational situations, there are potential exposures to other solvents, although to a lesser extent than with tetrachloroethylene. Relationships between exposure to tetrachloroethylene and responses are not generally observed. Possible explanations for this are exposure misclassification due to use of current exposure measurements, an exposure or

response function that is above the increasing portion of the exposure-response curve, or, more unlikely, a response that does not increase with increasing tetrachloroethylene exposure.

Epidemiologic studies of oral exposures to tetrachloroethylene have only examined adverse pregnancy outcomes or postnatal effects (see Section 4.7.1). There is some evidence for growth retardation in infants born to mothers residing in housing with drinking water contaminated with tetrachloroethylene.

Tetrachloroethylene exposure to animals by the inhalation or oral route results in toxicity to the liver, kidney, and nervous system; by inhalation, it also causes developmental and reproductive effects. Specifically, several measures of toxicity have been observed in the liver, such as, increased liver weight, infiltration of fat, necrosis, peroxisome proliferation, polyploidy of hepatocytes, and increased triglycerides. In the kidney, increased weight, hyperplasia, hyaline droplets, and protein cast formation in tubules have been observed. In the CNS, alteration of brain neurotransmitter levels, increased motor activity, and delayed reaction times to visual stimuli have been observed. Animals exposed in utero to tetrachloroethylene by inhalation showed signs of fetal growth retardation, increased fetal mortality, and behavioral changes occurring after birth. There is little information on developmental or reproductive effects in animals by the oral route of exposure. There are very few studies of immune system toxicity, and none of these studies are in intact animals. No information is available on the effects of tetrachloroethylene on the endocrine system in animals.

Targets of toxicity are the same in animal and human studies (i.e., liver, kidney, CNS, reproductive system, and developing fetus). The effect domain in animals and humans indicates that both cognition and visual function are affected by tetrachloroethylene. Affected organs are all sites of high metabolic activity, and the CNS is also a lipid accumulation site, consistent with the absorption, distribution, metabolism, and elimination profile of tetrachloroethylene.

6.1.4. Carcinogenicity in Humans and Laboratory Animals

Overall, the epidemiologic evidence has associated tetrachloroethylene exposure with excess risks for a number of cancers, although a causal association has yet to be definitively established. Studies of tetrachloroethylene and cancer showed positive associations between exposure and cancer of the lymphoid system, esophagus, and cervix, with more limited evidence for cancer of the bladder, kidney, and lung. For both lymphoid and esophageal cancer, excess risk was observed in studies of human populations exposed to tetrachloroethylene and other solvents, including studies of exposures to dry cleaners or workers involved with degreasing metal parts. In these cases, average risks were doubled as compared with those of referents. Furthermore, studies of drinking water exposure also support an association between lymphoid

cancer and tetrachloroethylene and other solvents, as do case-control studies that assessed employment as dry cleaners or laundry workers. Chance and confounding by smoking are unlikely the sole explanations for the observed excesses in risks. Information is lacking on life style and socioeconomic factors, which are indirect surrogates for human papilloma virus infection, a known risk factor for cervical cancer (see Section 4.8.1.2 for more details).

The laboratory animal database includes 10 lifetime rodent bioassay data sets that demonstrate increased cancer incidence. (Two additional study data sets, in male and female rats exposed orally, were inconclusive due to excessive mortality caused by pneumonia or tetrachloroethylene-related toxic nephropathy). Hepatocellular adenomas and carcinomas in mice and MCL in rats occurred in multiple lifetime rodent bioassays, and hemangioendotheliomas in male mice (JISA, 1993) and cancers of the kidney and brain (glioma) in male rats (NTP, 1986) occurred in single lifetime bioassays. Also known as hemangiosarcomas, hemangioendotheliomas are rare tumors of the epithelial lining of blood vessels. These tumors have been observed in a limited number of bioassays, including vinyl chloride and 1,3-butadiene. Although the dose-response relationships for kidney and brain tumors observed in male rats were not as strong as for the preceding cancers, and the increasing dose-response trend for kidney tumors was not statistically significantly, both tumor types were considered tetrachloroethylene-related and biologically relevant (see Section 5.4.2.3).

The statistically significantly elevated incidences of hepatocellular carcinomas and adenomas in male and female mice and MCL in male and female rats are considered to be indicators of potential human health hazard, despite questions regarding high background incidences of these tumors in controls and MOA hypotheses (see Section 6.1.5.1). The finding of an increased incidence of hepatocellular carcinomas and adenomas in female mice with a low background incidence of these tumors suggests tetrachloroethylene is the etiological agent and supports an inference of tetrachloroethylene as a risk factor for liver tumors in male mice that have a higher background incidence. Moreover, kidney cancer and MCL in rats as indicators of a potential human cancer hazard appear reasonable, given the observations in the epidemiologic studies.

Although there are segments of the population who may be especially susceptible to the toxic effects of tetrachloroethylene, there are too few studies specifically on tetrachloroethylene to examine this hypothesis directly. A potential exists for infant exposures from several pathways, including breast or other milk containing tetrachloroethylene. Infants younger than 6 months of age have slower renal clearance and less active liver metabolizing enzymes. The nervous system in the developing fetus and in infants matures later than other systems. Elderly persons and those with liver and kidney diseases also have slower clearance of toxic

substances—especially lipophilic chemicals. Existing PBPK studies are not yet reliable for quantitative use for estimating pharmacokinetic susceptibility in infants or the elderly (see Section 4.9.1 for more details).

6.1.5. Mode-of-Action Information

Although a wealth of new data related to understanding the toxic effects caused by tetrachloroethylene exposure has been published over the past decade, the MOA is not yet sufficiently characterized, tested, or understood for any one of these adverse effects. A number of alternative hypotheses are identified and examined as possible MOAs for liver and kidney toxicity. Hypothesized MOAs for mononuclear cell leukemia, neurotoxicity, and developmental/reproductive effects are indirect and are based on experimental observations of exposures to agents other than tetrachloroethylene. The available evidence points to multiple hypothesized MOAs as being involved, and, in each case, no one MOA can be uniquely identified (see Section 4.10.3 for more details). The sections following immediately below summarize the MOA information available for liver, kidney, and other targets of tetrachloroethylene toxicity.

6.1.5.1. Liver Mode-of-Action Information

The MOA for tetrachloroethylene-induced liver toxicity, including tumor induction, is not known. Tetrachloroethylene-induced liver tumors in mice are believed to result from chloroacetic acid metabolites and other intermediate products of the oxidative pathway, with MOA hypotheses focused on the role of the major urinary metabolite TCA. Because both tetrachloroethylene and TCA have been shown to activate the PPAR-α, as evidenced by peroxisome proliferation, the ability of PPAR receptors to trigger a number of cellular events suggests a possible relationship with tumor induction. However, metabolism to TCA does not obviously explain tetrachloroethylene-induced liver tumors, suggesting that other metabolites or intermediates contribute to tetrachloroethylene liver toxicity. Key steps in one MOA hypothesis, namely that TCA alters cell signaling processes through activation of PPAR-α, have yet to be fully identified both in mice and in humans.

Experimental evidence does not support peroxisome proliferation, per se, as a proposed MOA. Specifically, peroxisome proliferation does not correlate well with tumor incidence. Peroxisomes are seen at exposure concentrations higher than those that induce liver tumors, and peroxisome proliferation is also seen in rat liver and mouse kidney, sites that do not show carcinogenicity (see Section 4.10.3). The ability of PPAR receptors to trigger nonperoxisomal events suggests that toxicity and tumor induction may not be causally related to peroxisome

proliferation, but that tumorigenesis may be only a concurrent happening with many other events. The relationship between these events and tetrachloroethylene tumor induction is not understood. At the current time, sufficient evidence does not exist to suggest that tetrachloroethylene or its oxidative metabolites could initiate hepatocarcinogenesis via a mutagenic MOA.

6.1.5.2. Kidney Mode-of-Action Information

Several MOAs for kidney toxicity are possible, although the supporting evidence is limited. Induction of alpha-2μ-globulin occurs only at doses higher than the doses that induce kidney cancer in male rat bioassays, and it is not likely to have an important role in toxicity or tumor induction. Peroxisome proliferation has been weakly detected in rat kidneys—which do show carcinogenicity—but peroxisome proliferation is more extensive in mouse kidneys, which, too, has not demonstrated cancer. Scientific evidence is more supportive of the possibility that reactive metabolites from the GSH conjugation pathway are in some way responsible for kidney toxicity. These metabolites are associated with cytotoxicity and are mutagenic in *Salmonella*.

6.1.5.3. Mode-of-Action Information for Other Targets of Toxicity

The MOA of tetrachloroethylene-induced leukemogenesis in rats is not well understood; specifically whether the parent compound, a metabolite, or several metabolites are involved. Metabolites from GSH metabolism may contribute to toxicity, as supported by the finding of aplastic anemia and DNA changes in lymphatic tissues in calves exposed to S-(1,2-dichlorovinyl)-L-cysteine DCVC, which is structurally similar to the TCVC that is produced through tetrachloroethylene GSH metabolism, although the study of TCVC in calves was negative.

For neurotoxicity, the parent compound, rather than the metabolites, might be exerting an anesthetic-like effect on the lipid membranes in the nervous system or interacting with several neurotransmitter receptors. However, this hypothesis is not supported by specific studies on tetrachloroethylene.

The MOAs hypothesized for developmental toxicity differ according to effect. The neurobehavioral effects during development may be mediated by the same MOA as the neurotoxic effects discussed above. For fetal toxicity, TCA, an organic acid, lowers the pH of the fetal compartment (see Section 4.7.4); this may be a contributing factor, given the finding of developmental toxicity with TCA exposure. These proposed hypotheses, however reasonable, lack experimental support.

The binding of reactive metabolites of tetrachloroethylene to proteins in liver, kidney and serum, has the potential to contribute to the pathogenesis of several diseases, including cancer and autoimmune disease.

6.1.5.4. Mode-of-Action Conclusions and Implications for Dose-Response Analyses

In summary, there is no obvious common MOA for the different toxicological effects of tetrachloroethylene, nor has a sequence of key events been identified for any of the individual adverse effects. MOA information does not indicate in any instance that toxicity endpoints in animals are not relevant to humans, nor does it provide a basis for non-default procedures for estimating risk or establishing reference values. Specifically, hypothesized rodent-only MOAs are not sufficiently established, and it is reasonable to use animal tumors as an indicator of a potential human cancer hazard. Rodent tumors, leukemia, and cancer of the liver and kidney have human analogues. For example, mononuclear cell leukemia in rats is also known as large granular lymphocytic leukemia; large granular lymphocytic leukemia represents a well-recognized group of lymphoid neoplasms in humans (Stromberg, 1985).

In the absence of a well characterized MOA that could explain dose-response relationships at doses lower than those leading to observed effects, the cancer dose-response modeling is carried out using a linear extrapolation performed in accordance with default recommendations in the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The available data on noncancer toxicity of tetrachloroethylene support using EPA's RfC/RfD methodologies to derive noncancer toxicity values. These approaches are detailed in Section 6.2.

6.1.6. Weight-of-Evidence Descriptor for Cancer Hazard

Tetrachloroethylene is "Likely to be carcinogenic to humans" by all routes of exposure, within the framework of the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). As specified in the guidelines, the descriptor "Likely to be carcinogenic to humans" expresses the conclusion regarding the weight of evidence for carcinogenic hazard potential and is presented only in the context of a weight-of-evidence narrative. Although the term "likely" can have a probabilistic connotation in other contexts, its use as a weight-of-evidence descriptor does not correspond to a quantifiable probability of whether the chemical is carcinogenic. The five recommended standard hazard descriptors are as follows:

- "Carcinogenic to humans"
- "Likely to be carcinogenic to humans"
- "Suggestive evidence of carcinogenic potential"

- "Inadequate information to assess carcinogenic potential"
- "Not likely to be carcinogenic to humans"

These descriptors are not unlike those used by the IARC, NTP, and other health agencies that weigh carcinogenicity evidence. If there are no or insufficient pertinent data, then the descriptors "Inadequate information to assess carcinogenic potential" or "Suggestive evidence of carcinogenic potential" are used. If the evidence is stronger, as is the case with tetrachloroethylene, the descriptor "Likely to be carcinogenic to humans" is used; convincing evidence, usually conclusive demonstration of causality in epidemiologic studies, would support "Carcinogenic to humans." On the other hand, if the conclusion is negative (i.e., strong, consistent and compelling information indicating the absence of human health hazard), the agent would be described as "Not likely to be carcinogenic to humans." Thus, going down the list of descriptors from "Carcinogenic to humans" to "Inadequate information to assess carcinogenic potential" indicates a decrease in the level of evidence of a human health hazard. In summary, use of the weight-of-evidence descriptor "Likely to be carcinogenic to humans" for tetrachloroethylene is intended to communicate that the available information indicates the presence of a human health hazard.

The weight-of-evidence conclusion represented by the top three levels of evidence is related to, but distinct from, the quantitative dose-response assessment/conclusions in that the judgment that an agent is a human carcinogen does not guarantee adequate data to quantitatively estimate human risk. Notably, evaluation of an agent that is judged a likely human carcinogen may offer data conducive to estimating human risk. Indeed, dose-response assessments are generally completed for agents considered "Carcinogenic to humans" and "Likely to be carcinogenic to humans." Section 6.2 provides the dose-response analyses for tetrachloroethylene.

Three lines of evidence in the hazard database support the weight-of-evidence descriptor for the cancer hazard for tetrachloroethylene: (1) tetrachloroethylene exposure is associated with excess risks for a number of cancers in human epidemiologic studies, although a causal association has yet to be sufficiently established; (2) tetrachloroethylene is a rodent carcinogen in 10 of 10 lifetime bioassay data sets, including by oral and inhalation routes; and (3) the available information indicates that the cancer bioassay data are relevant to use as indicators of potential human cancer hazard. Briefly, the epidemiologic evidence has associated tetrachloroethylene exposure with excess risks for a number of cancers including cancer of the lymphoid system, esophagus, and cervix, with more limited evidence for cancer of the bladder, kidney, and lung. For both lymphoid and esophageal cancer, excess risk was observed in studies of people who

work as dry cleaners or degreasers, populations who experience inhalation exposure to tetrachloroethylene and other solvents. In both cases, average risks were doubled as compared with those of referents. Furthermore, studies of drinking water exposure also support an association between lymphoid cancer and tetrachloroethylene and other solvents, as do case-control studies that assessed employment as a dry cleaner or laundry worker. Chance and confounding by smoking are unlikely explanations for the observed excesses in risks.

As summarized in Section 6.1.4, the laboratory animal database includes 10 lifetime rodent bioassay data sets demonstrating increased cancer incidence. The findings include liver cancer in both sexes of mice and mononuclear cell leukemia in both sexes of rats following either oral or inhalation exposures and, in single bioassays, male rat kidney and brain tumors (gliomas) and mouse hemangiosarcomas of the liver or spleen. In addition, although not all tetrachloroethylene metabolites have been tested for carcinogenicity in rodents, the oxidative metabolites TCA and DCA are hepatocarcinogens in one or more species. Taken together, these data support a weight-of-evidence descriptor of "Likely to be carcinogenic to humans" by all routes of exposure.

6.2. DOSE-RESPONSE CHARACTERIZATION

Quantitative estimates of risk to humans are derived separately for noncancer and cancer effects. RfD and RfC values are derived from epidemiologic studies of residential populations exposed to tetrachloroethylene from nearby dry cleaning facilities. Residents in these studies have shown an impaired ability to detect and respond to visual stimuli compared to responses of controls (see Section 5.1.1). Inhalation cancer risk has been estimated from animal data on malignant tumors induced in tests involving lifetime exposure to tetrachloroethylene at known concentrations.

6.2.1. Noncancer Toxicity (Reference Concentration [RfC]/Reference Dose [RfD])

A broad range of animal toxicology and human epidemiologic data are available for the hazard assessment of tetrachloroethylene. The nervous system appears to be a sensitive organ system, particularly in human studies (see Section 4.6.1). Nevertheless, critical data gaps have been identified and uncertainties associated with data deficiencies are more fully discussed in Chapter 5 and in the remainder of this section. Even with these uncertainties, the database of human and animal studies on inhalation and oral toxicity of tetrachloroethylene can support derivation of inhalation and oral reference values. A number of epidemiologic studies of neurological effects in either occupational workers or residential subjects with tetrachloroethylene exposure or toxicological studies in rodents are considered for developing an

RfC and RfD. No single epidemiologic study is considered to be without flaws and uncertainties, although these are different among studies and studied populations. Among the epidemiologic studies, seven studies were considered for supporting an inhalation reference value, and a study of neurobehavioral deficits in people residing near dry cleaning facilities (Altmann et al., 1995) was identified as the principal study using a weight-of-evidence approach (see Sections 5.1. and 5.2. for more details). The small number of subjects (14 exposed of 37 subjects studied) can introduce uncertainties particularly regarding stability of statistical inferences. However, statistically significant group differences between the adjusted mean scores of exposed and control subjects on three neurobehavioral tests (simple reaction time, $p < 0.05$ for the first test and $p < 0.01$ for the second test; continuous performance, $p < 0.05$; and visual memory, $p < 0.05$) were observed after adjusting for covariates and possible confounders of age, gender, and education. In all cases, the exposed subjects had slower response times or more errors than did the unexposed controls. Other factors were also considered in the overall weight-of-evidence analysis. Table 6-1 summarizes the rationale for selection of the principal study (the rationale is also addressed in Sections 5.1 and 5.2).

6.2.1.1. Assessment Approach Employed

Noncancer toxicity RfC and RfD are developed using EPA's RfC and RfD methodologies (U.S. EPA, 1993, 1994). The RfC for tetrachloroethylene is derived through a process of (1) considering all studies and selecting the critical effects that occur at the lowest exposure concentration, (2) selecting the point of departure (POD) at which critical effects either are not observed or would occur at a relatively low prevalence (e.g., 10%), (3) deriving the POD in terms of the HEC, and (4) reducing this exposure concentration by UFs to account for uncertainties in the extrapolation from the study conditions to an estimate of human environmental exposure.

The RfC is developed from the point of departure (POD) of 4.8 mg/m^3 (0.7 ppm), which was associated with impaired cognitive function and visual information processing in a study of people residing near dry cleaning facilities (Altmann et al., 1995). The assumption that the residents were continuously exposed to tetrachloroethylene eliminated the need for a duration adjustment to the POD. There is sufficient evidence from occupational studies of higher tetrachloroethylene concentrations to confirm that the nervous system is the primary target for the effects of tetrachloroethylene, with several studies showing a similar pattern of effects in the residential study (Seeber, 1989; Ferroni et al., 1992; Cavalleri et al., 1994, Echeverria et al., 1994, 1995). The median concentration in Altmann et al. (1995) is similar to the concentration in a pilot residential study reporting deficits in visual contrast sensitivity (Schreiber et al., 2002),

Table 6-1. Summary of rationale for principal study selection

Consideration/ approach	Type of data	Decision
Quality of study	Animal neurotoxicity studies.	Animal neurotoxicity studies are considered as supporting studies. An RfC/RfD from human data, if available and of adequate quality, is from the species of interest to EPA, reduces interspecies extrapolation uncertainties and is preferred.
Quality of study	Human neurotoxicity studies.	Both occupational and residential studies on tetrachloroethylene exposure contain uncertainties regarding their use for quantitative analysis. Some of these epidemiology studies carry greater weight for quantitative analysis than the less informative studies of Ferroni et al. (1992), Echeverria et al. (1994, 1995), and Spinatonda et al. (1997) with reporting or exposure assessment deficiencies.
Measurement tool	Standardized neurobehavioral battery.	Both occupational and residential epidemiology studies assessed neurobehavioral function using a standardized neurobehavioral battery. The battery has been widely administered to occupational populations in different settings with a reasonably high degree of validity. WHO and ATSDR recommend these test methods to evaluate nervous system deficits in adults and children.
Endpoint	Deficits in neurological domains such as attention, motor function, vigilance, or visuo-spatial function.	There is congruence of neurological effects observed in studies of both residential and occupational populations. These domains are also sensitive to acute tetrachloroethylene exposure in controlled human studies. The consistency of observed effects between occupational and residential populations and their persistence with lower tetrachloroethylene concentration, as experienced by residential populations, provide a strong rationale for a study of lower-level residential exposures as the basis for the RfC.
Relevance of Exposure Scenario	Epidemiology studies of residential populations.	Tetrachloroethylene exposure to residential populations is of lower concentration and of chronic duration compared to acute duration and higher concentration exposure to occupational populations. Additionally, potential to tetrachloroethylene peak or intensity concentrations is more common with occupational exposures. A study of residential exposure, if adequate and of similar quality as an occupational epidemiology study, is preferred for supporting the RfC because it represents exposure scenarios of interest to EPA.

and it is lower than the concentration associated with markers of kidney and liver damage and reproductive/developmental effects (spontaneous abortion and changes in sperm quality) in several epidemiologic studies of occupational exposure (Franchini et al., 1983; Gennari et al., 1984; Olsen et al., 1990; Eskenazi et al., 1991a, b; Mutti et al., 1992; Brodkin et al., 1995; Doyle et al., 1997; Verplanke et al., 1999; Trevisan et al, 2000). Effect levels in animal studies are, generally, similar to those in the occupational epidemiologic studies, with BMC modeling of liver toxicity showing effect concentrations only slightly higher than the POD from residents (Kjellstrand et al., 1984; JISA, 1993).

A composite UF of 300 is adopted to account for human variation (factor of 10), extrapolation from an observed-effect level to a no-effect level (factor of 10), and uncertainties in the database (factor of 3). Limited data on tetrachloroethylene blood concentration among human subjects indicated that a choice of 3 for the pharmacokinetic portion of the 10-fold human variation UF is reasonable. The rationale for a 3-fold database UF is based on critical data gaps and takes into account a lack of animal studies designed to clearly investigate the human findings in cognition and visual system dysfunction and a lack of cognitive testing in both developmentally exposed animals and adult animals exposed to tetrachloroethylene for longer than acute durations (see Sections 5.1, 5.2, and 5.3 for further discussion of these issues). These data are needed to allow for a fuller characterization of the exposure-response relationship. The RfC was calculated by dividing the POD by the composite UF = 4.8 $mg/m^3/300 = 1.6 \times 10^{-2}$ mg/m^3.

The database for oral exposure to tetrachloroethylene is limited to four subchronic gavage studies, one subchronic drinking water study, and no human studies. In addition to using the animal data on oral exposure, the assessment attempted to expand the database for derivation of an RfD using relevant inhalation data and route-to-route extrapolation with the aid of a pharmacokinetic model. Route extrapolation of human inhalation data is considered a reasonable alternative to using the limited oral data in animals because tetrachloroethylene has been shown to be rapidly and well absorbed by the oral and inhalation routes of exposure, and the metabolic pathways and kinetics of excretion with oral exposure are similar to those of inhalation exposure. Furthermore, human data, when adequate, are preferred for supporting the RfD, and human data of inhalation exposure are available.

The residential inhalation study of Altmann et al. (1995) of neurobehavioral deficits and three acute and subchronic toxicological studies were examined for supporting an RfD. The RfD was derived from Altmann et al. (1995) with the aid of an extrapolation from the inhalation to the oral route using pharmacokinetic modeling. The daily oral ingestion dose that results in the same tetrachloroethylene blood concentration associated with the POD for inhalation, 4.8 mg/m^3,

is 1.1 mg/kg-day. This value is equivalent to an oral LOAEL. Blood tetrachloroethylene concentration is a well-validated dose metric, and the estimate varies little between models. The human LOAEL falls within the PODs from oral studies in animals. The UFs, as used for the inhalation RfC, are adopted for the RfD for oral exposure, namely, a composite factor of 300 (10 for human variation, 10 for extrapolation from a LOAEL to a NOAEL, and 3 for database uncertainties; see also Sections 5.1, 5.2, and 5.3). The oral RfD is, therefore, 1.1 mg/kg-day/300 = 4×10^{-3} mg/kg-day.

To show the range of tetrachloroethylene concentrations at which different neurotoxic effects and toxic effects in other organ systems have been observed, the points of departure and reference values that could have been derived from these other studies were compared with that of the principal study. These graphs allow a direct visualization of how the values compare to the data from which the principal conclusions have been derived. This has been done for both inhalation reference concentrations and oral reference doses in Figures 6-1, 6-2, and 6-3.

6.2.1.2. Impact of Assumptions, Uncertainties and Alternatives on Reference Concentration and Reference Dose

A number of uncertainties underlie the RfC and RfD for tetrachloroethylene and are discussed below in this section. A quantitative characterization of the uncertainty in the RfC and RfD for tetrachloroethylene is not feasible because of the varied nature of the available database and the limited data available for many of the studies. Most significantly, the available chronic toxicity studies of tetrachloroethylene exposure demonstrated varying degrees of support for a POD for the RfC and RfD. A weight-of-evidence approach was adopted to identify principal or critical studies, with the additional studies supporting the principal studies (see also Sections 5.1 and 5.2).

6.2.1.2.1. ***Point of departure.*** Most of the available studies did not provide enough data to support benchmark dose modeling; they only supported PODs based on LOAELs, especially for the human studies, or LOAELs and NOAELs. Such a POD has a number of shortcomings relative to a POD obtained from benchmark dose-response modeling (i.e., a benchmark concentration). First, LOAELs and NOAELs are a reflection of the particular exposure levels at which a study was conducted, contributing some inaccuracy to the POD determination. Second, LOAELs and NOAELs reflect the number of study subjects or test animals and typically are dissimilar in detection ability and statistical power, with smaller studies tending to identify higher exposure levels as PODs relative to larger but otherwise similarly designed studies. This is an important consideration for studies with multiple exposure groups and studies that did not identify LOAELs but has much less impact for the single-group studies that identified a LOAEL.

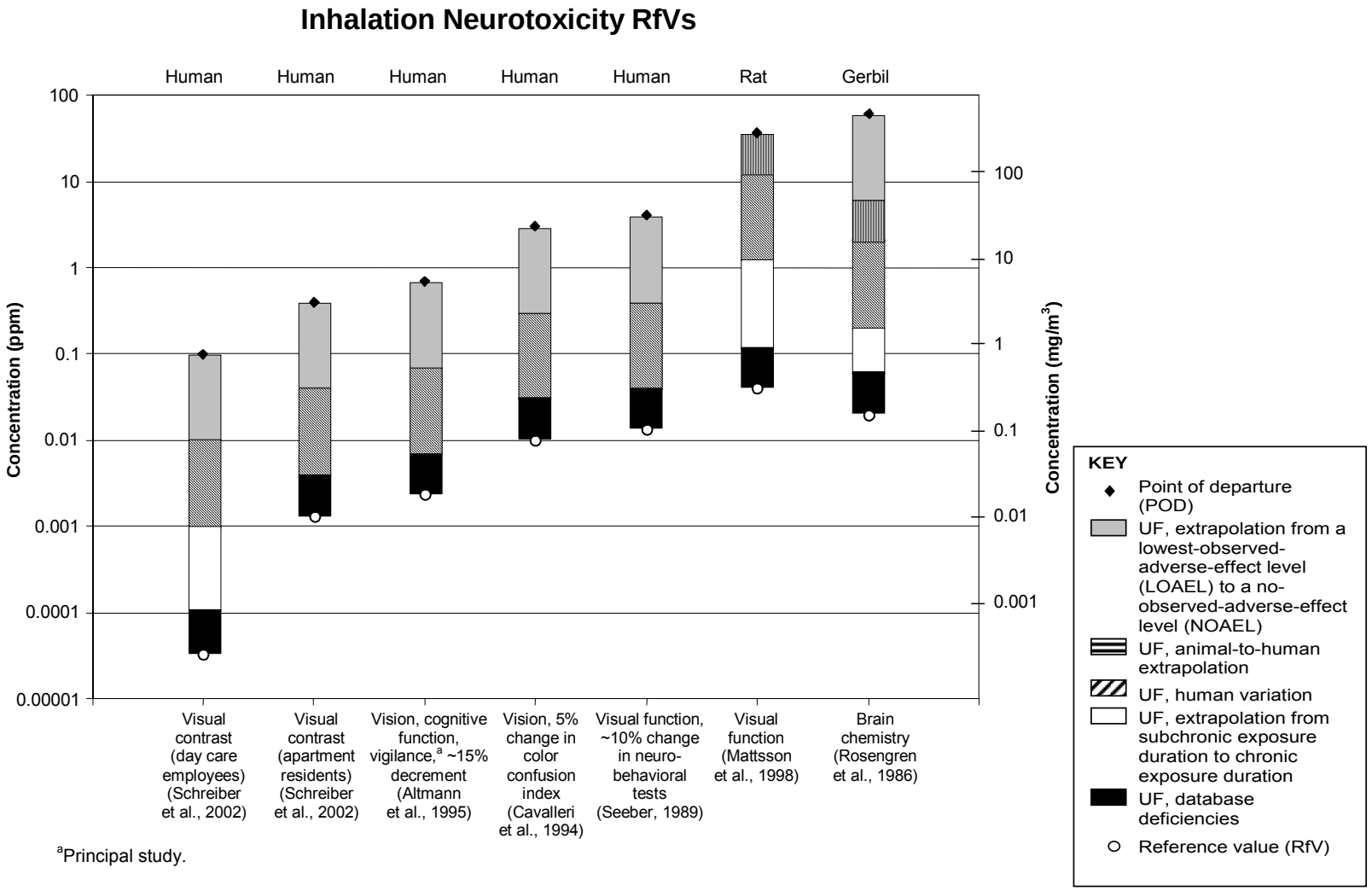
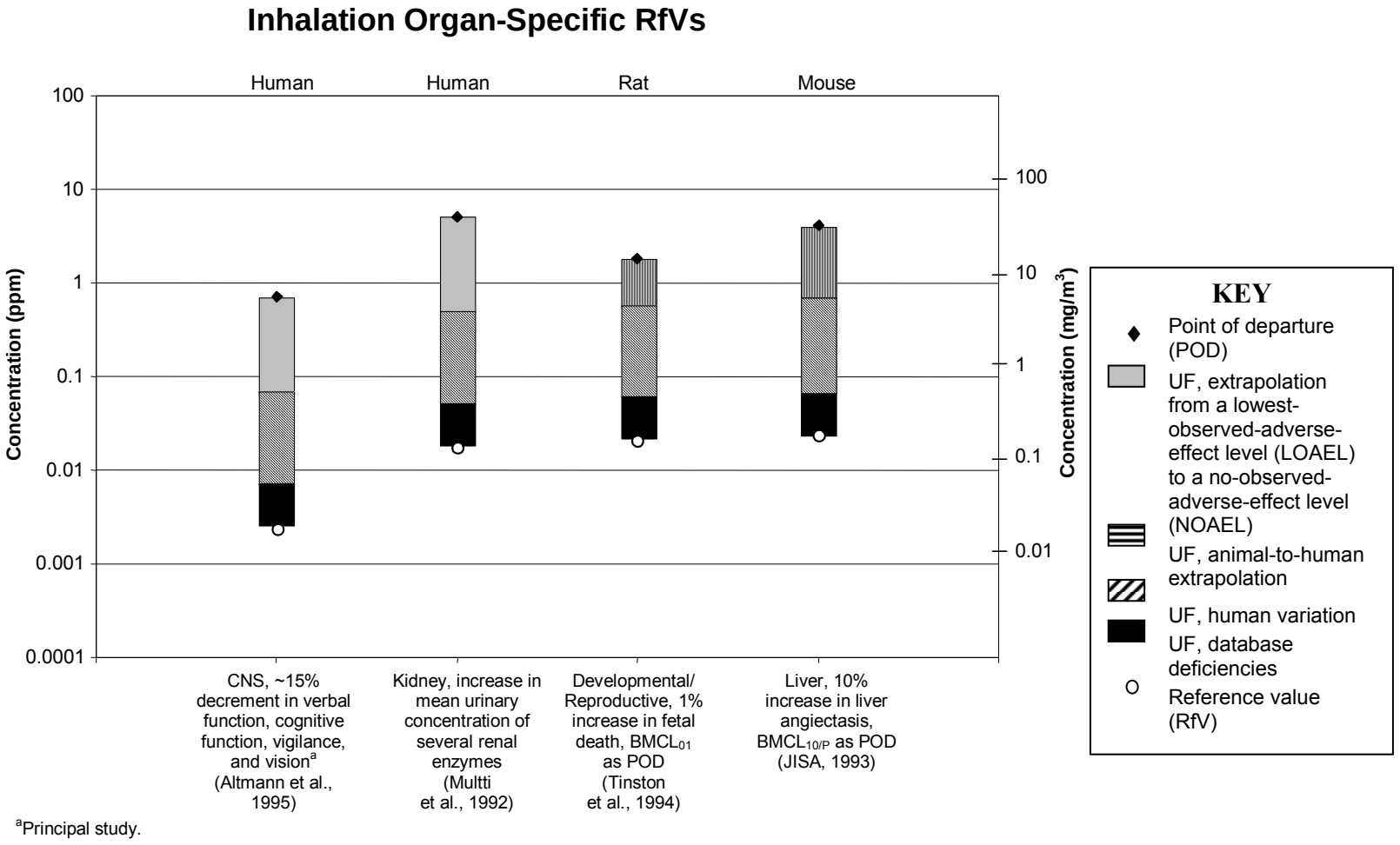
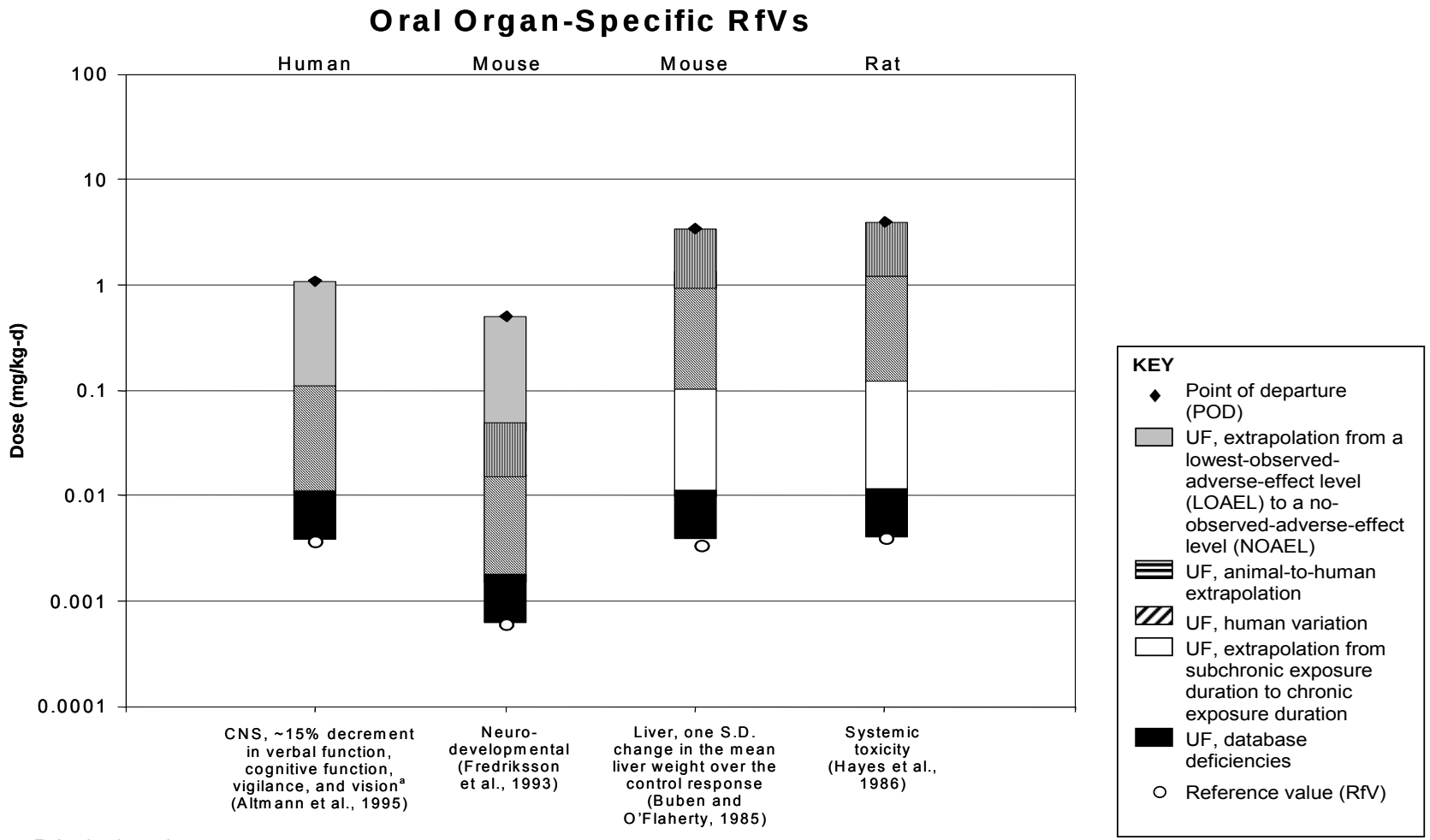


Figure 6-1. Array of PODs and reference values for a subset of neurotoxic effects in inhalation studies.



[a]Principal study.

Figure 6-2. Organ-specific RfVs for inhalation exposure to tetrachloroethylene.



[a]Principal study.

Figure 6-3. Oral organ-specific reference values for exposure to tetrachloroethylene.

Third, LOAELs and NOAELs represent different response rates, as noted on Figures 6-1, 6-2, and 6-3, and qualitative and quantitative comparisons are not possible lacking characterization of the underlying dose-response curve.

PODs identified from fitting benchmark dose models overcome some of the deficiencies associated with LOAELs and NOAELs. Benchmark dose models were fit to five data sets (Buben and O'Flaherty, 1985; JISA, 1993; NTP, 1986; Brodkin et al., 1995; Tinston, 1994) with sufficient information. The choice of benchmark dose model did not generally lead to significant uncertainty in estimating the POD since benchmark effect levels were within the range of experimental data. While this examination of a subset of chronic toxicity studies on tetrachloroethylene exposure provides some insight on study and endpoint differences in PODs, lacking characterization of dose-response curves for all studies, especially the more critical studies, uncertainty associated with the PODs cannot be adequately quantified in this database.

Effects in the CNS and in other organ systems in occupational populations and in animals are observed at higher average tetrachloroethylene concentrations than the Altmann et al. (1995) residential study. Uncertainties in other studies of neurotoxicity and of other organ systems differ from those of Altmann et al. (1995). For both occupational and residential populations, studies do not describe a NOAEL and human variation is not well characterized in study subjects. Uncertainties associated with the occupational studies include (1) potential for neurobehavioral effects at lower exposures and (2) exposure pattern differences between occupational and residential studies with peaks characterizing occupational exposures. Using an occupational study to support the RfC may not be fully protective of neurological effects as has been observed in populations co-located near dry cleaners (Altmann et al., 1995; Schreiber et al., 2002; and NYSDOH, 2005a, c). For animal studies, uncertainties are associated with extrapolating high concentration exposure, typically of subchronic duration to genetically inbred rodents, to infer a concentration of tetrachloroethylene that is likely to be without an appreciable risk of adverse health effects over a lifetime to a diverse human population.

6.2.1.2.2. ***Extrapolation from laboratory animal studies to humans*****.** Extrapolating from animals to humans embodies further issues and uncertainties. First, the effect and its magnitude associated with the concentration at the point of departure in rodents is extrapolated to human response. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing. This was possible for liver toxicity where limited MOA information suggests metabolism as important to toxicity. The ranges of BMCLs presented for liver effects (a 10-fold range of estimates of tetrachloroethylene metabolism) demonstrate the uncertainty in tetrachloroethylene pharmacokinetic models. The discrepancies among the models and

experimental data may point to large uncertainties in the parameters used in these models. Because the accuracy of the models has been evaluated only against blood and breath concentrations of the parent compound, their reliability for predicting total metabolites is unknown.

6.2.1.2.3. ***Human variation*****.** Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation needs consideration, also, in extrapolating dose from a subset or smaller sized population, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population.

In the absence of tetrachloroethylene-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation. Human variation may be larger or smaller; however, tetrachloroethylene-specific data for examining the potential magnitude of over- or under-estimation are few. The pharmacokinetic model of Clewell et al. (2004) of mean physiological parameters used to explore age-dependent pharmacokinetic differences suggests a 2-fold variation in blood tetrachloroethylene levels (Chapters 3 and 5). Bois et al. (1996) and Chiu and Bois (2006) have examined uncertainty and variation in a tetrachloroethylene pharmacokinetic model describing the amount of tetrachloroethylene metabolism. This analysis suggests large uncertainty is associated with estimating the quantity of tetrachloroethylene metabolism in humans.

6.2.1.2.4. ***Database uncertainties*****.** Critical data gaps have been identified with uncertainties associated with database deficiencies on developmental, immunologic, and neurotoxic effects, particularly data to characterize dose-response relationships and chronic visuo-spatial functional deficits and cognitive effects of tetrachloroethylene exposure under controlled laboratory conditions. Several halogenated organic solvents have been linked with altered immune system function in both animals and humans (e.g., toluene, trichloroethylene). Additional data from inhalation, oral, and dermal exposures at different durations are needed to assess the potential immunotoxicity of tetrachloroethylene. This lack of data, combined with the concern that other structurally related solvents, has been associated with immunotoxicity and contributes to uncertainty in the database for tetrachloroethylene.

Data from acute studies in animals (Warren et al., 1996; Umezu et al., 1997) suggest that cognitive function is affected by exposure to tetrachloroethylene. These studies do not address the exposure-response relationship for subchronic and chronic tetrachloroethylene exposures on cognitive functional deficits observed in humans (e.g., Seeber, 1989; Echeverria et al., 1994; and

Altmann et al., 1995). Even more importantly, cognitive testing is lacking in both developmentally exposed animals and adult animals following exposures to tetrachloroethylene that are longer than acute durations of exposure. Visual system dysfunction and processing of visuo-spatial information are sensitive endpoints in human studies. The exposure-response relationship of these functional deficits could be evaluated more definitively with studies using homologous methods that examine retinal and visual function in experimental animals. However, there has been a limited evaluation of visual function in rodents, with the exception of the evoked potential studies by Mattsson et al. (1998). These types of studies could help determine whether there are both peripheral and central nervous system effects of tetrachloroethylene exposure on visual perception, and they could be used as an animal model to better define the exposure-response relationships.

Subjects in the epidemiologic studies comprise adults, and some characterization of the response of children to tetrachloroethylene exposure was found in limited data for a similar neurological (visual system) parameter (Schreiber et al., 2002) and in a larger number of subjects (NYS DOH, 2005 a,c) using other visually based testing paradigms. Additionally, in a postnatal neurotoxicity study in mice (Fredriksson et al., 1993), persistent neurological effects (i.e., increased locomotion and decreased rearing behavior at 60 days of age, measured 43 days after exposure ceased) were observed at an oral dose of 5 ppm, with no NOAEL, although this study did not conform to traditional toxicity testing guidelines (see Section 4.6.2.2). These results suggest that if adequate, robust, dose-response data using the most appropriate neurophysiological and cognitive tests were available, the exposure eliciting an adverse response (and hence the POD for the reference value) could be lower than that established based on deficits in visuo-spatial and cognitive function following tetrachloroethylene exposure in healthy adults (Altmann et al., 1995).

6.2.2. Cancer Risk Estimates

Following the scientific principles and procedures outlined in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the cancer risk values are based on the 95% lower confidence limits on the air concentrations associated with a 10% extra risk of cancer incidence (LEC_{10}s). The LEC_{10} values were calculated from data on MCL in male rats, the most sensitive species/gender in the rodent cancer bioassay conducted at the lowest concentration range, using the multistage dose-response model. A linear low-dose extrapolation was then used, in accordance with default recommendations in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The approach and associated choices and assumptions are described in Sections 6.2.2.1.

A broad range of animal toxicology and human epidemiologic data are available for the identification of a carcinogenic hazard from exposure to tetrachloroethylene. Nevertheless, critical data gaps have been identified, and uncertainties associated with data deficiencies are more fully discussed in Chapter 5 and Section 6.2.2.2. Given the choices of tumor type, point of departure, and low-dose extrapolation approach necessary to provide a plausible upper bound risk estimate, there are additional considerations that contribute to uncertainty in the cancer risk values. These uncertainties have a varied impact on risk estimates. Some (i.e., the bioassay or cross-species scaling approach) suggest risks could be higher than estimated while others would decrease estimates or have an impact of uncertain direction (i.e., the human population variability, dose metric, and model-based uncertainty at the POD). While some uncertainties could be quantitatively characterized, it is likely that the residual uncertainties remain the largest, yet can only be qualitatively expressed: i.e., low dose extrapolation, MOA, and human sensitivity and variability. Even if experimental data could further elucidate these uncertainties, extrapolation of animal bioassay data to human (done here using allometric scaling) will remain a substantial and unknown uncertainty. The tetrachloroethylene unit risk estimate, calculated using three PBPK models, ranges from 2×10^{-6} to 2×10^{-5} per $\mu g/m^3$. From this range, the upper end unit risk of 2×10^{-5} per $\mu g/m^3$ is the most public health protective value for the upper bound risk estimate.

The tetrachloroethylene oral slope factor, using the three PBPK models for route-to-route extrapolation from the experimental data to humans, ranges from 1×10^{-2} to 1×10^{-1} per mg/kg-day. From this range, the upper end slope factor of 1×10^{-1} per mg/kg-day is the most public health protective value for the upper bound risk estimate. With the exception of the route-to-route extrapolation step, the uncertainties associated with the slope factor estimation are the same as for the unit risk estimation.

Section 6.2.2.2 describes the uncertainties outlined above, their impact on cancer risk estimation, the choices made and justification for each, and the associated data gaps. Section 6.2.2.3 provides a quantitative analysis of the potential numeric impact of three of these sources of uncertainty on the unit risk estimate (the statistical uncertainty, PBPK model, and tumor site) using the multistage model in the observed range and linear low-dose extrapolation. Section 6.2.2.4 and the table therein provide a summary of the cancer risk estimate.

6.2.2.1. *Assessment Approach Employed*

Animal bioassay data are used to derive quantitative cancer risk estimates for humans due to the lack of quantitative exposure information in the occupational epidemiology studies. The cancer dose-response analysis considers three bioassays but relies on the JISA (1993) study

results. This is primarily because the JISA (1993) study included lower exposures than did the two earlier bioassays for both species tested, and, therefore, its use reduces extrapolation uncertainty slightly. For mice, the lowest exposure concentration of 10 ppm was 10-fold lower than the lowest exposure concentration in the NTP inhalation study (NTP, 1986). For rats, the low-exposure concentration of 50 ppm was fourfold lower than in the NTP (1986) study.

Dose-response analyses for hepatocellular tumors in male and female mice, hemangiosarcomas in male mice, and mononuclear cell leukemia and kidney tumors in male and female rats are carried out using the rate of total metabolite production as estimated from the three recently developed toxicokinetic models as the dose metric. Dose-response analyses are also carried out using administered dose as the dose metric to allow comparison to the pharmacokinetic model-based risk estimates. EPA's methodology for cross-species scaling was applied for relevant tumor sites to address toxicological equivalence across species (U.S. EPA, 1992). This methodology is based on the observation that equal average lifetime concentrations or AUC of the toxic moiety has been associated with toxicological equivalence across species. This cross-species relationship has been shown to accommodate the general species variation in pharmacokinetics and the carcinogenic response to internal doses. Although the available pharmacokinetic data for tetrachloroethylene do not allow estimates of AUC, the use of metabolized tetrachloroethylene scaled to $mg/kg^{3/4}$-day in order to estimate the dose resulting in the same lifetime risk in animals and humans is consistent with the EPA methodology and further substantiated in the present document (see Section 5.4.4.2.1). This consideration of cross-species scaling and toxicological equivalence is consistent with EPA's other carcinogen assessments and its treatment of pharmacokinetic dose metrics.

The steps involved in generating the unit risk from the dose-response data are illustrated using the male rat MCL data, as follows:

(1) A fit of the tumor incidence versus total metabolite curve using a multistage model (BMDS, version 1.3.2) gave an LEC_{10}, or 95% lower confidence bound on the exposure associated with 10% extra risk, of 0.81 per mg-eq/kg-day (Figure 5-8a);

(2) The point of departure (LEC_{10}) was then transformed to a human equivalent value by dividing the animal value by (human body weight /animal body weight)$^{0.25}$ = (70/0.45)$^{0.25}$ = 3.53 to give a human equivalent value of 0.23 mg-eq/kg-day of metabolite formation;

(3) Three different models (see Section 3.5) of total human metabolite formation from tetrachloroethylene exposure were used to estimate the environmental exposure that would correspond to the human equivalent LEC_{10} (Bois et al., 1996; Rao and Brown, 1993; Reitz et al., 1996). The lowest human equivalent LEC_{10} resulting from the three models (Rao and Brown) is 47,000 $\mu g/m^3$. The highest human equivalent LEC_{10} resulting from the models is 4,700 $\mu g/m^3$ (Bois et al.);

(4) The unit risk calculated using three PBPK models ranges from 2×10^{-6} to 2×10^{-5} per $\mu g/m^3$. From this range, the upper-end unit risk of 2×10^{-5} per $\mu g/m^3$ is the most public health protective value for the upper bound risk estimate.

Age adjustment factors for early life exposures as discussed in the *Supplemental Guidance for Assessing Susceptibility for Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) are not recommended because little evidence exists to indicate that tetrachloroethylene or its oxidative metabolites directly damage DNA, information about genotoxicity of GSH metabolites in cell assays other than Salmonella or in in vitro experiments are lacking, and the MOA for tetrachloroethylene has not been established.

6.2.2.2. *Impact of Assumptions, Uncertainties and Alternatives on Unit Risk Estimates*

A number of uncertainties underlie the cancer unit risk for tetrachloroethylene. These are discussed in the following paragraphs. Specifically addressed is the impact on the assessment of issues such as the use of models and extrapolation approaches, the reasonable alternatives, the choices made, and the data gaps identified. In addition, the use of assumptions, particularly those underlying the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), is explained and the decision concerning the preferred approach is given and justified. Several of the uncertainties with the largest impact cannot be considered quantitatively, such as human population variability and the most relevant dose metric. Thus, an overall integrated quantitative uncertainty analysis is not presented.

6.2.2.2.1. *Human population variability*. The extent of inter-individual variability in tetrachloroethylene metabolism has not been characterized. As noted in Section 6.1.2, several enzymes of the oxidative and GSH metabolism, notably CYP2E1, CYP3A4, GSTZ, GSTA, GSTM, and GSTT show genetic polymorphisms with the potential for variation in metabolite production. The limited data available on tetrachloroethylene metabolites show DCA to be a potent, irreversible inhibitor of GSTZ activity, with greater inhibition of this enzyme in mice than in humans. Tetrachloroethylene metabolism has been shown to increase by inducers of CYP enzymes such as toluene, phenobarbital, and pregnenolone-16 alpha-carbonitrile, whereas CYP enzyme inhibitors such as SKF 525A, metyrapone, and carbon monoxide have been shown to decrease tetrachloroethylene metabolism. Additionally, chronic exposure to tetrachloroethylene has been shown to cause self-induction of metabolism. Human population variability is summarized above (see Section 6.2.1.2.3) and in covered in more detail in Chapter 3.

A separate issue is that the human variability in response to tetrachloroethylene is also poorly understood. The effect of metabolic variation, including potential implications for differential toxicity, has not been well studied. Although a mutagenic MOA would indicate increased early-life susceptibility, there are no data exploring whether there is differential sensitivity to tetrachloroethylene carcinogenicity across life stages. Thus, this lack of understanding about potential differences in metabolism and susceptibility across exposed human populations represents a source of uncertainty. Nevertheless, the existing data support the possibility of a heterogeneous response that may function additively to ongoing or background exposures, diseases, and biological processes. As noted in Section 4.9.5., some evidence shows certain subpopulations may be more susceptible to tetrachloroethylene exposure. As discussed under (2) below, these considerations strengthen the scientific support for the choice of a linear non-threshold extrapolation approach. In summary, the human equivalent risk estimates for tetrachloroethylene, therefore, do not reflect this source of uncertainty.

6.2.2.2.2. ***Choice of low-dose extrapolation approach*.** A key consideration in clarifying how risks should be estimated for low-dose exposure is the MOA. As noted above in Section 6.1.5, MOA data are lacking or limited for all of the candidate cancer endpoints for tetrachloroethylene (i.e., rat MCL and kidney tumors, mouse hepatocellular tumors and hemangiosarcomas). When the MOA cannot be clearly defined, EPA uses a linear approach to estimate low-dose exposure risk, based on the following broad and long-held scientific assumptions, which supported development of EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a):

- A chemical's carcinogenic effects may act additively to ongoing biological processes, given that diverse human populations are already exposed to other agents and have substantial background incidence of various tumors. Under these conditions, a nonzero slope of the response as a function of chemical exposure is expected.
- A broadening of the dose-response curve in the human population (less rapid fall-off with dose) and, accordingly, a greater potential for risks from low-dose exposures (see Zeise et al., 1987; Lutz et al., 2005) would result for two reasons. First, even if there is a threshold concentration at the cellular level, that threshold is likely to be different among different individuals. Second, greater variability is anticipated in response to exposures in the heterogeneous human population than in controlled laboratory species and conditions (due to, e.g., genetic variability, disease states, age).
- The general use of linear extrapolation provides plausible upper-bound risk estimates that are believed to be health-protective (U.S. EPA, 2005a) and also provides consistency across assessments.

The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the MOA for tetrachloroethylene were known with a high degree of confidence is of interest,

but clear data on the MOA of tetrachloroethylene is not available, and even if it were, incorporation of MOA into dose-response modeling might not be straightforward and might not significantly reduce the uncertainty about low-dose extrapolation. This is because the MOA, as well as other factors, especially human response variability, are determinants of the dose-response function in humans.

This chemical assessment also evaluates the extent to which a collection of mathematical functions fit to one of the tetrachloroethylene bioassay data sets and extrapolated down to low doses, could inform uncertainty. There is not sufficient information regarding the MOA to support a chemical-specific inference about dose-response behavior at low dose for tetrachloroethylene. Thus, it is of interest to observe how different functions fit to the tumor data may diverge when extrapolated downward. Much previous experience has supported a general mathematical property that different curves, though fitting observed experimental data well, often diverge widely when extrapolated to doses well outside the observed range. Indeed, the inability of curve-fitting procedures to provide useful compound-specific information about low-dose risks has been a principal motivation for the "model free" approach of straight line extrapolation from a point of departure within the observed range of the data (Krewski and van Ryzin, 1981; NRC, 1983).

Calculations here encompassed four alternative functional forms frequently used for noncancer dose-response assessment in the observable range of experimental data (multistage, Weibull, log-logistic, and log-probit) that can accommodate a wide variety of dose-response shapes, including threshold-like behavior. These models were fit to the mononuclear cell leukemia data in male rats using the EPA BMDS program, and distributions of model results were evaluated (see Appendix 5B for more details). These calculations confirm the expected finding that alternative functional forms fit to this tetrachloroethylene tumor data set are consistent with a wide range of numerical values for probability of response when extrapolated down to low dose, as illustrated in Table 6-2.

With such large spreads in confidence intervals, the extrapolated models, in effect, provide little information about actual low-dose risks. These results are not presented as the basis for alternative estimates of human risk, because they do not provide sound or useable scientific estimates for the compound-specific risks from tetrachloroethylene. As noted previously, such results serve to underscore the EPA Cancer Guidelines' rationale for the use of a consistent model-independent approach.

A number of different biological motivations have been put forward to support functional forms that might be used to estimate risks from low-dose exposure to carcinogens or other toxic substances. For cancer, the most prominent class of models treats tumorigenesis as a multi-event

Table 6-2. Summary of dose-specific extra risks (means and 95% confidence limits) for four dose-response models fit to incidence of leukemias in male rats exposed to tetrachloroethylene via inhalation (JISA, 1993)

Model	Estimated extra risk, $(mg\text{-}eq/kg\text{-}day)^{-1}$, corresponding to 1.5×10^{-5} mg-eq/kg-day internal dose in rats[a]		
	5^{th} percentile	Mean	95^{th} percentile
Log-probit	2×10^{-170}	8.172×10^{-4}	1.008×10^{-5}
Multistage	4.546×10^{-13}	9.172×10^{-7}	1.819×10^{-6}
Log-logistic	2×10^{-21}	1.078×10^{-3}	7.776×10^{-4}
Weibull	0	1.339×10^{-3}	1.893×10^{-3}

[a] From Appendix 5B.

process and characterizes the probability of accumulation of a series of changes (conceptualized as mutations or other events) that, together, will result in formation of a malignant tumor. In particular, EPA utilized the multistage model for low dose extrapolation of cancer risks in many assessments. Risk estimates utilizing EPA's application of the multistage model have been shown to be similar to the linear (straight line) risk estimation procedure now used by EPA (Subramaniam et al., 2006). More complex multi-event models allow for the modeling of formation and growth of populations of initiated and transformed cells and are still well recognized tools for investigating biologically based dose response modeling for carcinogenesis.

The concept of a distribution of individual thresholds is a second approach used to motivate functional forms for dose-response modeling. Such models assume that there is an "individual threshold" for each member of the human population, and interindividual variation in these thresholds determines the dose-response curve for a population. A recent National Research Council report on risk assessment issues for TCE (NRC, 2006) included a discussion of models based on distributions of thresholds. That report noted that if one assumes a normal or logit distribution for individual thresholds this leads to a probit or logistic dose-response function for the population and suggests that a variety of other distributions for thresholds would also lead to sigmoidal shaped dose-response functions. The NRC report expressed the view that, "Although linear extrapolation has been advocated as an intentionally conservative approach to protect public health, there are some theoretical reasons to think that sublinear nonthreshold dose-response models may be more relevant for human exposure to toxicants, regardless of the mode of action" (p. 319). On the other hand, the same report also noted that a very broad class

of dose-response functions can be obtained using distributions of thresholds models: "In fact any monotonic dose-response model, including the linearized multistage model, can be defined solely in terms of a tolerance distribution without resorting to mechanistic arguments. These considerations suggest that one must consider both the role of mode of action and the role of response variability among humans in determining the likely shape of the dose-response function" (p. 323).

The discussion from the NRC TCE document emphasizes some key points in risk assessment. Variability in the human population will have an important influence on the shapes of the dose-response relationships for that population. This is distinct from the amount of variability that may be observed in inbred animal strains. As noted in the NRC report, "One might expect these individual tolerances to vary extensively in humans depending on genetics, coincident exposures, nutritional status, and various other susceptibility factors..." (p. 320). Thus, if a distribution-of-thresholds approach is considered for a carcinogen risk assessment, application would depend on the ability of modeling to reflect the degree of variability in response in human populations. By design, most cancer bioassays are conducted in inbred rodent strains; accordingly, the parameters provided by curve fits of distribution-of-thresholds models to bioassay data would not be predicted to reflect the dose-response patterns in diverse human populations. It is important to note that the NRC text has no recommendation for an approach where a tolerance distribution model for humans is estimated by a statistical fit to rodent bioassay data.

The question of whether a tolerance distribution model is indeed an appropriate basis for a risk assessment also warrants consideration. Low-dose linearity can arise in other contexts distinct from effects of population variability and may be directly appropriate to a MOA. Low-dose linearity can also arise due to additivity of a chemical's effect on top of background chemical exposures and biological processes. In the case of chemicals such as tetrachloroethylene, basic biological data do not exist to support the appropriateness of an individual threshold model above models having inherent low-dose linearity. However, if distribution of thresholds modeling were supported, it would need to be developed based on an examination of predicted variability within in human population.

Given the current state of scientific knowledge about tetrachloroethylene carcinogenicity, the straight-line-based risk estimates presented above form the preferred recommendation for estimating a plausible upper-bound estimate of potential human risks from tetrachloroethylene. This approach is supported by both general scientific considerations, including those supporting the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), as well as chemical-specific findings. The former include the scientific principles articulated previously (ie, the expectation

that a chemical functions additively to background exposures, diseases, and processes; that variability within the human population would broaden the dose-response curve and eliminate individual thresholds if present; and that the approach provides consistency across assessments facilitating direct comparison of the derived risk values). The latter include evidence that, within the dose range of the cancer bioassays, the observable tumor response data are consistent with a linear model and do not suggest occurrence of a threshold, and that variability in the human response across the population is expected (eg, Bois et al., 1996; Clewell et al., 2004).

6.2.2.2.3. *Dose metric*. Tetrachloroethylene is metabolized to several intermediates with carcinogenic potential. Although much data exist for the metabolite TCA, several analyses indicate that TCA alone is not able to explain the toxicity associated with tetrachloroethylene exposure; therefore, at least one other toxic agent appears to be involved. It is unclear whether total metabolism—either as a measure of a precursor or intermediate, or as a surrogate directly proportional to the toxic agent(s)—is an adequate indicator of potential risk. Since the experimental evidence supports a role for metabolism in tetrachloroethylene's toxicity, use of total metabolism (the only measure of metabolism available) to estimate cancer risk is germane to this assessment. Use of administered dose (without use of a PBPK model) yields risk estimates intermediate between those based on the higher and lower PBPK models.

6.2.2.2.4. *Choice of species/gender*. Table 6-3 summarizes the factors influencing the choice of rodent tumor data set for human risk characterization. It is assumed that the observed rodent tumors are relevant to humans, an assumption supported by a number of factors. Primary among these factors is that a carcinogenic response is also observed in humans. Human-rodent site concordance is not generally assumed (e.g., due to potential differences in pharmacokinetics, DNA repair, other protective systems across species and tissues [U.S. EPA, 2005a]). In keeping with this view, certain tumors associated with tetrachloroethylene exposure in human mortality studies (e.g., cervix and esophagus) were not observed in rodents; cancer of the lymphoid system was associated with tetrachloroethylene exposure in humans, with some evidence for an association with bladder, kidney, and lung cancer. In addition, rat and mouse tumor types also differ from each other. Finally, conclusive MOA data are lacking for the observed rodent and human tumors.

MCL is the cancer response of highest magnitude and is reproducible in two bioassays and in both genders. Although MCL has a high and variable incidence in unexposed F344 rats, a biologically and statistically significant increase over background was observed (see Section 5.4.1). Section 4.8.2.2.1.4 addresses the qualitative similarities among MCL to certain

Table 6-3. Summary of considerations for each rodent tumor type

	Mononuclear cell leukemia	Liver	Kidney	Hemangiosarcoma
Magnitude of response	+++	++	++	+
Specificity of response	Rats, both genders	Mice, both genders	Male rats only	Male mice only
Replication of findings in multiple bioassays	4/4 study datasets	6/6 study datasets	1/2 study datasets	1/3 study datasets
Other considerations contributing to uncertainty in rodent data	High background rate	High background rate in male mice	Rare tumor (unlikely to be due to chance, but low incidence)	Rare tumor (unlikely to be due to chance, but low incidence)
Relevance to humans: a) *qualitative (biologic) site concordance*	Yes	Yes	Yes	Yes
b) occurrence in human studies	Yes, but exact match of tumor classification is not found/may not be possible	Yes, but association is weak	Yes, but association is weak	No, but tumor type is rare
c) confidence in MOA	No data	PPAR-α activation may contribute, but is not sole MOA	Multiple MOAs may play a role	No data
Overall considerations for choice of tumor type	Rodent response of highest magnitude, reproducible; no MOA data	Rodent response of considerable magnitude, reproducible, some MOA data	Rare tumor in rodents and humans; MOA data are strongest	Rare tumor in rodents and humans; no MOA data

lymphoid cancers, and the implications regarding human relevance. This section also addresses the association of elevated lymphoma mortality with tetrachloroethylene exposure in humans. The MOA for MCL remains unexplored.

Male rats had the higher response level of MCL as estimated using the multistage model. Occasionally, if the multistage model does not adequately fit a data set, an alternate model can be used to determine the POD. In the case of female rat MCL data, the best-fitting model (Weibull) yielded central tendency risk estimates 10-fold higher than those from the multistage model fit of the male rat MCL data. Consequently, there is some uncertainty in characterizing the magnitude of MCL response, with the use of the male rat MCL data possibly underestimating risk. While

the female rat MCL data suggest a supralinear fit extending into lower exposure levels (also apparent for female rat MCL data from the NTP bioassay), the multistage model fit was technically adequate ($p = 0.48$). In keeping with EPA's past practice of preferring the multistage model in order to provide some measure of consistency across different carcinogen assessments, the more linear multistage fit to the female rat MCL data supports the risk estimate derived from the male rat MCL data.

The mouse liver tumor is a robust (i.e., of significant magnitude) finding in several studies, including in both sexes. As is the case with MCL, the background for this tumor type is high—especially in males. A biologically and statistically significant increase over background was observed in males and females. There is evidence that activation of the PPAR-α receptor by the tetrachloroethylene metabolite TCA contributes to the induction of mouse liver tumors. However, it is not the only operative MOA involved in hepatocellular tumorigenesis. Thus, the MOA remains unresolved.

Two tumor types were observed in only one bioassay. Kidney tumors rarely occur in unexposed rodents but were significantly elevated with tetrachloroethylene exposure in the male rat NTP bioassay. The MOA is better understood for kidney tumors than for the other sites. Hemangiosarcoma is another rare tumor associated with tetrachloroethylene exposure in the male mouse JISA (1993) study. There are no MOA data for hemangiosarcomas.

6.2.2.2.5. *PBPK model*. Toxicokinetic models are used in this assessment for deriving dose metrics to support dose-response analyses. The evidence suggests that the by-products of tetrachloroethylene metabolism are responsible for liver and kidney toxicity and for carcinogenicity. Inhaled concentration of the parent compound is, therefore, not an appropriate dosimeter for these effects, and pharmacokinetic modeling of daily overall metabolized dose is expected to be an improvement in spite of the many attendant uncertainties in the modeling. Of the available toxicokinetic models on tetrachloroethylene, the assessment considers three recently developed models that describe parent tetrachloroethylene and overall metabolism of the parent compound in humans. These models do not describe the kinetics and transformation of total metabolic products or any individual metabolite. All three models provide reasonably good predictions of exhaled breath and blood tetrachloroethylene concentrations, so there is no particular basis for preferring one model over another. A 10-fold difference is shown in model predictions of the rate of metabolism in humans, a reflection of model differences in the values for the metabolic parameters. Because the accuracy of the models has been evaluated only against blood and breath concentrations of the parent compound—quantities that are insensitive to these parameters—the reliability of these models for predicting the rate of total metabolism in

humans is unknown. Data on total metabolite levels are not available in humans, and the use of available urinary and blood TCA data is problematic. The overall difference in risk estimates using these three models is approximately 10-fold.

6.2.2.2.6. ***Cross-species scaling.*** An adjustment for cross-species scaling ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between each rodent species and humans, consistent with the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a); the approach is detailed in Section 5.4.4.2.1. It is assumed that, without data to the contrary, equal risks result from equivalent constant exposures. While the true correspondence of equipotent tetrachloroethylene exposures across species is unknown, the use of $BW^{3/4}$ scaling is expected neither to over- or underestimate human risk (U.S. EPA, 1992).

6.2.2.2.7. ***Choice of bioassay.*** The JISA (1993) inhalation bioassay provides data on the lowest experimental exposures, and its use, therefore, reduces extrapolation uncertainty slightly. For mice, the lowest exposure concentration of 10 ppm was 10-fold lower than the lowest exposure concentration in the NTP inhalation study (NTP, 1986). For rats, the low-exposure concentration of 50 ppm was fourfold lower than in the NTP study. Although the JISA and NTP inhalation bioassays used similar rodent strains, differences in the animals used (in addition to other unidentified factors) may have contributed to the twofold higher incidence of hepatocellular tumors and MCL in the NTP study. Consequently, the estimated risks are twofold lower than previous EPA assessments which relied on the NTP bioassay (U.S. EPA, 1991).

6.2.2.2.8. ***Statistical uncertainty at the point of departure.*** Parameter uncertainty within the chosen model reflects the limited sample size of the cancer bioassay. For the multistage model applied to this data set, there is a relatively small degree of uncertainty at the 10% extra risk level (the point of departure for linear low-dose extrapolation).

6.2.2.3. *Quantitative Analysis of Multiple Uncertainties on Cancer Unit Risk*

Figure 6-4 and Table 6-4 show the central estimates and upper and lower confidence limits of the inhalation risk per unit concentration for the rodent data sets under consideration, as determined using BMDS (version 1.3.2). The upper bound inhalation risk per unit concentration has been calculated as the ratio of the benchmark response (10% extra risk for all data sets except the kidney tumors, which was 5%) to the 95% lower confidence limit of the benchmark dose (the LEC_{10}). These results show that the lower bound of risks ranges from 20% to 40% of the upper bound of risks. The values at the right end of each bar represent the unit risk estimates

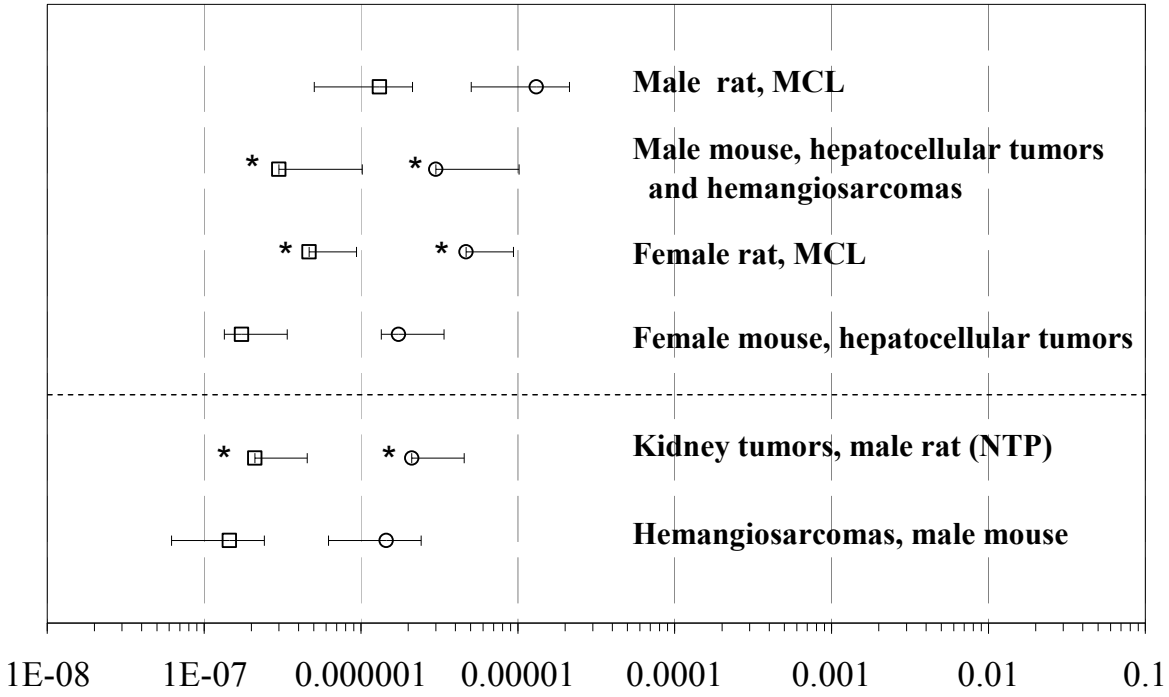


Figure 6-4. Cancer risk estimates for tumor sites associated with tetrachloroethylene exposure in rodent bioassays, using the multistage model. The four gender/species data sets are provided in the upper section of the graph while two tumor types observed in single bioassays are provided in the lower section. The symbols denote the slopes (to background risk) from the mean estimate of exposure corresponding to 10% extra risk, using the Rao and Brown (1993) PBPK model (□) and the Bois et al. (1996) model (○) to extrapolate to human equivalent exposures. The bars indicate the slopes from the lower and upper bounds on the mean estimates. * indicates lower bounds that could not be estimated.

Table 6-4. Combined impact on tetrachloroethylene cancer risk estimates (per µg/m^3) of statistical uncertainty,[a] PBPK model and tumor site(s), using multistage model in observed range and linear low-dose extrapolation

Gender/species (tumor type)		PBPK model	
		Rao and Brown (1993)	Bois et al. (1996)
Male rat (MCL)	LB	5×10^{-7}	5×10^{-6}
	C	1×10^{-6}	1×10^{-5}
	UB	2×10^{-6}	**2×10^{-5}**
Male mouse (hepatocellular tumor and hemangiosarcoma)	LB	*	*
	C	3×10^{-7}	3×10^{-6}
	UB	1×10^{-6}	1×10^{-5}
Female rat (MCL)	LB	*	*
	C	5×10^{-7}	5×10^{-6}
	UB	9×10^{-7}	9×10^{-6}
Female mouse (hepatocellular tumor)	LB	1×10^{-7}	1×10^{-6}
	C	2×10^{-7}	2×10^{-6}
	UB	3×10^{-7}	3×10^{-6}

[a] In some cases, the lower bounds on risk could not be estimated.

LB = Lower risk estimate derived from upper statistical confidence limit on the POD concentration (UEC_{10}).
C = Central risk estimate derived from the MLE estimate of EC_{10}, and from the mean of the bootstrap distribution of BMR/EC_{10} values (equal to each other in this case, see Appendix 5B).
UB = Upper bound risk estimate derived from the lower bound statistical confidence limit POD concentration (LEC_{10}).
Bolded value is used to derive assessment's unit risk estimate.

supported by each data set. Figure 6-4 and Table 6-4 also show the range of upper bound inhalation risks due to the highest and lowest metabolic rate pharmacokinetic models used to describe the rate of metabolism of tetrachloroethylene, as described in Sections 6.2.2.2 and 3.5 and Tables 5-8 and 5-9. A third model (Reitz et al., 1996), not shown in Figure 6-4, yields results between the other two. For each PBPK model, unit risk estimates based on the male mouse and female rat are similar, each about twofold lower than the male rat MCL unit risk estimate. The unit risk estimate based on the least sensitive species/gender (female mouse, hepatocellular tumor) is about eightfold less than that given by the male rat MCL estimate. Two tumor types, each seen in only one bioassay, would respectively give unit risk estimates eightfold lower (male mouse hemangiosarcoma in the JISA [1993] bioassay) and fivefold lower (male rat kidney tumor in the NTP bioassay) than the JISA male rat MCL unit risk estimate. Unit risk

estimates based on administered dose (without use of a PBPK model) do not correlate with those from any particular PBPK model, but they generally fall between the higher and lower unit risk estimates derived from the PBPK models.

Because rodent carcinogenicity is consistently evident in all data sets, with multiple types of tumors occurring, the concern for human carcinogenic risks is increased. This supports selection of the most sensitive observation as a basis for risk estimation. For tetrachloroethylene, MCL in male rats is the basis for risk estimation. While this tumor type tends to have a high background response in rats, it was not as high in the JISA (1993) rats, at about 20%, compared with the male NTP rats at about 56%. Consistent with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, it is still important to communicate the potential for increased cancer incidence over background. In addition, while there is no exact analogue of MCL in humans, as noted earlier, the EPA 2005 *Guidelines for Carcinogen Risk Assessment* notes that site concordance is not necessary for assessing potential carcinogenic risk to humans.

6.2.2.4. *Conclusions*

Tetrachloroethylene is "Likely to be carcinogenic to humans" by all routes of exposure, using the framework specified in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Three lines of evidence in the hazard database support this weight-of-evidence descriptor for the cancer hazard for tetrachloroethylene:

(1) Tetrachloroethylene is a carcinogen in rodents in 10 of 10 lifetime bioassay data sets—including by oral and inhalation routes

(2) It is reasonable to use these animal tumors as indicators of potential human cancer hazard

(3) Tetrachloroethylene exposure is consistently associated with excess risks for a number of cancers in human epidemiologic studies, although a causal association has yet to be definitively established.

The laboratory animal database includes 10 lifetime rodent bioassay data sets demonstrating increased cancer incidence (two more study data sets were inconclusive due to excessive mortality from pneumonia or tetrachloroethylene-related toxic nephropathy). The findings include liver cancers in both sexes of mice following either oral or inhalation exposures, and following inhalation exposures, mononuclear cell leukemias in both sexes of rats (multiple bioassays), as well as male rat kidney and brain tumors (gliomas) and male mouse hemangioendotheliomas of the liver or spleen (single bioassays). In addition, although not all tetrachloroethylene metabolites have been tested for carcinogenicity in rodents, the oxidative metabolites TCA and DCA are hepatocarcinogens in one or more species. Although insufficient to establish causality, the epidemiologic evidence has consistently shown a positive association

of inhalation and oral tetrachloroethylene exposure with excess risks for a number of neoplasms. These include cancer of the lymphoid system, esophagus, and cervix, with more limited evidence for cancer of the bladder, kidney, and lung. Taken together, these data support a weight-of-evidence descriptor of "Likely to be carcinogenic to humans" by all routes of exposure for tetrachloroethylene. Use of the weight-of-evidence descriptor "Likely to be carcinogenic to humans" for tetrachloroethylene is intended to communicate that the available information indicates the presence of a human health hazard.

Consistent with this view, dose-response assessments are generally completed for agents considered "Likely to be carcinogenic to humans." The unit risk is intended to be a plausible upper bound estimate of risk and, accordingly, all such estimates described below are based on the following: (1) the most sensitive tumor type in rodents, with regard to species, gender, and type of malignancy; (2) the POD based on the upper confidence bound on risk derived from statistical modeling of the observed dose-response data; and (3) a linear low-dose extrapolation approach. A linear extrapolation was performed in accordance with default recommendations in the *Guidelines of Carcinogen Risk Assessment* (U.S. EPA, 2005a) because of the lack of substantial biological basis for doing otherwise (particularly, the lack of knowledge about the MOA for any of the observed tumors), and other approaches to estimate upper bounds on risk were not considered informative for risk estimation. Table 6-5 gives a summary of the impact and justification of these choices. On the other hand, alternative choices for these approaches, while providing a perspective as to the overall uncertainty in human cancer risk, would not provide upper bounds on risk.

Given the choices of tumor type, point of departure, and low-dose extrapolation approach described in Table 6-5, there are additional considerations that contribute to uncertainty in the plausible upper bound unit risk, which are summarized in Table 6-6. These uncertainties have a varied impact on risk estimates. Some (i.e., the bioassay or cross-species scaling approach) suggest risks could be higher than estimated, while others would decrease estimates or have an impact of uncertain direction (i.e., the human population variability, dose metric, and model-based uncertainty at the POD). While some uncertainties could be quantitatively characterized, it is likely that the residual uncertainties represent the largest and can only be qualitatively expressed. Such uncertainties pertain to MOA and human sensitivity and variability. Even if these could be further elucidated by additional data, extrapolation of animal bioassay data to humans (done here using allometric scaling) will remain a substantial and unknown uncertainty. The PBPK model uncertainty is the only one for which there is no basis for preferring one alternative to another, so the tetrachloroethylene unit risk estimate, calculated using three PBPK models, ranges from 2×10^{-6} to 2×10^{-5} per $\mu g/m^3$.

Table 6-5. Considerations leading to the determination of a reasonable upper bound on risk

Consideration/ approach	Impact on estimated risk		Decision	Justification
Most sensitive tumor response	8-fold range from most to least sensitive rodent tumor type. For example, Bois PBPK model gives the following risk estimates using the LEC_{10} and linear low-dose extrapolation in $(\mu g/m^3)^{-1}$:		Male rats, MCL.	MCL had the greatest response and is reproducible across studies.
	Rats, MCL Males Female Mice, liver tumors Males Female	 2×10^{-5} 9×10^{-6} 1×10^{-5} 3×10^{-6}		
Statistical sampling uncertainty in observable range	↓ 1.6-fold if EC_{10} used rather than LEC_{10}, e.g. Bois PBPK model gives the following risk estimates in $(\mu g/m^3)^{-1}$ using linear low-dose extrapolation with male rat MCL data: Using UEC_{10}: 5×10^{-6} Using EC_{10}: 1×10^{-5} Using LEC_{10}: 2×10^{-5}		Lower bound on 10% risk concentration (LEC_{10})–the EPA's default approach for calculating a plausible upper bound.	Limited size of bioassay results in sampling variability; lower bound (LEC_{10}) is the lower 95% confidence interval on the concentration yielding a 10% risk and thus makes it less likely the estimate underestimates risk due to small sample size.
Dose-response relationship at low-dose	Could ↓ to a negligible value or ↑ to an unknown extent, and is among the largest uncertainties.		Linear low-dose extrapolation from POD (default approach).	Low-dose linear approach is supported by general considerations (additivity to background, population heterogeneity) and tetrachloroethylene-specific data (male rat MCL data are linear in observed range).

Table 6-6. Considerations that impact uncertainty in reasonable upper bound risk estimates

Consideration/ approach	Impact on estimated risk		Decision	Justification
Human population variability in metabolism and response/ sensitive subpopulations	There may be subpopulations at higher risk and there may be individuals for whom risk is negligible.		Considered qualitatively.	No data to support alternative estimates.
Dose metric (uncertainty about active moiety)	Alternatives could ↑ or ↓ risk estimate by an unknown extent.		Considered total metabolism and administered concentration.	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not clearly identified.
PBPK model	10-fold range among the three available models, e.g. see below estimates of risk per unit concentration $(\mu g/m^3)^{-1}$ using linear extrapolation from LEC_{10}.		The highest value provides a reasonable upper bound estimate of potential human cancer risk; a range of estimates is provided.	There is no scientific basis for choosing among pharmacokinetic results for estimating total metabolism of tetrachloroethylene given limitations in available data.
	Male Rats, MCL	Bois: 2×10^{-5} Reitz: 9×10^{-6} Rao: 2×10^{-6}		
Cross-species scaling	Alternative generic scaling approaches could ↓ or ↑ risk estimate (e.g., 3.5-fold ↓ [scaling by BW] or ↑ 2-fold [scaling by $BW^{2/3}$]). Residual uncertainty in scaling may ↑ or ↓ risk estimate by an unknown extent.		$BW^{3/4}$ (default approach).	$BW^{3/4}$ limits bias in estimate.
Bioassay	↑ 2-fold if NTP study used.		JISA study.	JISA study used the lowest experimental exposures (reduces extrapolation uncertainty).
Model-based uncertainty in POD	1.4-fold range in models that were explored.		Multistage model used.	Flexible model; limited additional uncertainty based on comparison with three other models.

As addressed in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), derivation of the central and lower bound risk estimate can be of value in some settings, such as screening analyses and for ranking agents as to their carcinogenic hazard. For such purposes, the cancer risk values based on the EC_{10} represent the central and lower bound estimates of risk, respectively, for a particular data set. For tetrachloroethylene, a range of central estimates (based on the EC_{10} using male rat MCL data from the JISA study and linear low-dose extrapolation and based on three PBPK model choices, as addressed in Table 6-4) is from 1×10^{-6} to 1×10^{-5} per $\mu g/m^3$. The corresponding range of lower bound estimates (derived from the UEC_{10} and based on the same choices of tumor type, low-dose extrapolation approach and using the three available PBPK model choices, as addressed above) is from 5×10^{-7} to 5×10^{-6} per $\mu g/m^3$.

To summarize, tetrachloroethylene is "Likely to be carcinogenic to humans" by all routes of exposure. A lack of human carcinogenicity, while not ruled out, is considered unlikely. Existing data indicate that (1) tetrachloroethylene is a rodent carcinogen in 10 of 10 lifetime bioassay datasets, including by oral and inhalation routes (2) the observed animal effects are relevant to use as indicators of human carcinogenic risk; and (3) tetrachloroethylene exposure is associated with excess risks for several cancers in human epidemiological studies, although a causal relationship has yet to be established. In addition, the carcinogenicity of tetrachloroethylene is also supported by other lines of evidence, including data on its metabolism and pharmacokinetics and the demonstrated hepatocarcinogenicity of the oxidative metabolites TCA and DCA in one or more species. In view of the likely carcinogenicity, a dose-response assessment was undertaken with the purpose of identifying a plausible upper bound estimate of risk. A range of unit risk estimates for tetrachloroethylene is from 2×10^{-6} to 2×10^{-5} per $\mu g/m^3$, with the upper-end unit risk of 2×10^{-5} per $\mu g/m^3$ being the most public health protective value for the upper bound risk estimate.

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APPENDIX A:

SUMMARY OF EARLIER ASSESSMENTS

APPENDIX A: SUMMARY OF EARLIER ASSESSMENTS

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A.1. ORAL INGESTION ASSESSMENTS

A.1.1. U.S. EPA Oral Ingestion Assessments

A.1.1.1. *IRIS Database, U.S. EPA, 1988*

In 1988, the U.S. Environmental Protection Agency (EPA) established a reference dose (RfD) for the ingestion of tetrachloroethylene (U.S. EPA, 2005). An RfD is 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 noncarcinogenic effects during a lifetime. For the oral RfD, EPA used the Buben and O'Flaherty (1985) gavage study. A no-observed-adverse-effect level (NOAEL) of 20mg/kg-day was determined, based on hepatotoxicity in mice. This value was duration adjusted and an uncertainty factor of 1,000 was applied (10 for intraspecies variability, 10 for interspecies variability, 10 for extrapolation from a subchronic study). EPA places medium confidence in the RfD derivation because the data set lacks information on reproductive and teratology endpoints. An RfD of 0.01 mg/kg-day was derived.

A.1.1.2. *Health Assessment Document for Tetrachloroethylene, U.S. EPA, 1985*

Dose response data for hepatocellular carcinomas observed in female mice in the National Cancer Institute gavage study (NCI, 1977) were used to derive the unit risk. The potency estimate for tetrachloroethylene was calculated using the linearized multistage model and the dose metabolized and eliminated in urine. The unit risk was derived by multiplying the assumed daily intake of 2 L of water contaminated with 1 μg/L tetrachloroethylene for a person (2.9×10^{-5} mg/kg-day) by the potency estimate for tetrachloroethylene (5.1×10^{-2}) to derive the unit risk. The upper-bound estimate of the incremental lifetime risk due to consuming water contaminated with 1 μg/L of tetrachloroethylene was calculated to be 1.5×10^{-6}.

A.1.2. Oral Ingestion Assessments Conducted by Non-EPA Agencies

A.1.2.1. World Health Organization, Concise International Chemical Assessment Document 68, 2006

"…The available information on oral exposure was inadequate for derivation of a TDI by the oral route. However, as tetrachloroethene is well absorbed after inhalation or ingestion and

there is little evidence of first-pass metabolism, a PBPK model was used to derive a TDI. The model predicted that tetrachloroethene consumed in drinking-water at a dose level of 0.047 mg/kg body weight per day would yield an AUC in plasma similar to that from continuous exposure to tetrachloroethene at 0.2 mg/m^3 in inhaled air. This oral figure was rounded to give a TDI of 50 μg/kg body weight."

An oral cancer risk value was not derived.

A.1.2.2. *California Environmental Protection Agency, 2001, Draft Public Health Goal for Tetrachloroethylene in Drinking Water*

The California Environmental Protection Agency (Cal EPA) developed a Public Health Goal (PHG) for tetrachloroethylene in drinking water on the basis of hepatocellular carcinomas observed in male and female mice orally exposed to tetrachloroethylene (Cal EPA, 2001). PHGs are based solely on health effects impacts and are set at levels that do not pose any significant health risk, as determined by the California Office of Environmental Health Hazard Assessment. For water-derived inhalation exposures, estimates were derived from studies showing hepatocellular adenoma or carcinoma in male mice and mononuclear cell leukemia in both male and female rats exposed by inhalation to tetrachloroethylene (NTP, 1986). The pharmacokinetic model described by Bogen et al. (1987) was used to estimate the "effective" dose for use in quantitative calculations. The Bogen et al. study was chosen over other studies (Bois et al., 1990; Chen and Blancato, 1987) because it provided dose estimates for mice and rats exposed orally and by inhalation.

Tetrachloroethylene was treated as a directly acting genotoxic carcinogen, and a linear low-dose extrapolation model was used. The PHG established by Cal EPA is 0.056 μg/L. This value corresponds to a unit risk estimate of 1.3×10^{-5} $(\mu g/L)^{-1}$. This health-protective concentration includes an estimate of inhalation exposure from showering in tetrachloroethylene-contaminated water, flushing toilets, and other household activities involving tap water.

Chronic toxicity, excluding cancer, was evaluated on the basis of neurobehavioral endpoints (delayed reaction time) observed in epidemiological studies of exposed humans. These studies evaluated persons who were exposed to inhaled tetrachloroethylene. Cal EPA concluded that no single study was sufficiently reliable to be used as the primary basis for a

health-protective standard; both the Altmann et al. (1995) and the Spinatonda et al. (1997) studies were quite small (14 and 35 subjects, respectively) and the Ferroni et al. (1992) study lacked details. Therefore, the geometric mean from the three studies was used to derive an estimated health-protective concentration in drinking water.

In calculating the mean, each study provided a lowest-observed-adverse-effect level (LOAEL) value, and a study-specific uncertainty factor was used (10 to account for the use of a LOAEL and 10 or 3 to account for potentially sensitive human subpopulations). A factor of 3% was applied for the relative source contribution because drinking water supplies only 3% of the total tetrachloroethylene exposure, and a water intake of 6.31 L/day was the calculated equivalent drinking water ingestion rate that would supply the total tetrachloroethylene dose from inhalation via showering and direct ingestion. The geometric mean of these safe concentrations calculated from the three studies is 1.1×10^{-2} mg/L (11 μg/L). The investigators concluded that this is the health-protective drinking water concentration for noncarcinogenic effects. With an assumption of 100% absorption from drinking water and an intake of 6.31 L/day, the equivalent dose corresponding to 11 μg/L is 1 μg/kg-day. This can be used to compare the California safe limits to the RfD of other organizations.

A.1.2.3. *Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Tetrachloroethylene (Update), September 1997*

ATSDR has established a minimal risk level (MRL) for the acute ingestion of tetrachloroethylene. MRLs are estimates of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. These values are based only on noncancer effects and are generally based on the most sensitive endpoint considered to be of relevance to humans. The acute oral MRL was derived from studies that showed hyperactivity in 60-day-old male mice that were treated with tetrachloroethylene for 7 days beginning at 10 days of age (Fredriksson et al., 1993). The MRL is based on a LOAEL (5 mg/kg-day) that was adjusted by an uncertainty factor of 100 to account for the use of the LOAEL (10) and extrapolation from animals to humans (10). For tetrachloroethylene, the acute oral MRL is 0.05 mg/kg-day.

A.1.3. Summary of Ingestion Risk Estimates

The following tables summarize the quantitative risk estimates that have been developed by EPA and other agencies. Table A-1 shows the cancer risk values. Table A-2 depicts the risk estimates developed for noncarcinogenic endpoints.

Table A-1. Estimates of ingestion unit risk using different methods

Agency guideline	Unit risk value $(\mu g/L)^{-1}$	Studies used	Critical target effect(s)
Public Health Goal (Cal EPA, 2000), based on metabolized dose	1.3×10^{-5}	NCI (1977), NTP (1986)	Liver adenomas and carcinomas in male and female mice, mononuclear cell leukemia in male and female rats
Dose metabolized (U.S. EPA, 1985)	1.5×10^{-6}	NCI (1977)	Liver carcinomas in female mice

Table A-2. Ingestion, noncancer endpoints

Agency guideline	Standard	Studies used	Critical target effect(s)
Cal EPA (1999)	11 μg/L (water concentration) 0.001 mg/kg-day equivalent dose	Altmann et al. (1995), Spinatonda et al. (1997), and Ferroni et al. (1992)	Delayed reaction times in humans.
Minimal risk level (ATSDR, 1997)	0.05 mg/kg-day	Fredriksson et al. (1993)	Hyperactivity in male mice
Reference dose (U.S. EPA, 1988)	0.01 mg/kg-day	Buben and O'Flaherty (1985)	Liver toxicity in mice

A.2. INHALATION ASSESSMENTS

A.2.1. U.S. EPA Inhalation Assessments

A.2.1.1. *Cleaner Technologies Substitutes Assessment: Professional Fabricare Processes, Office of Pollution Prevention and Toxics, U.S. EPA, 1998*

The Office of Pollution Prevention and Toxics (OPPT) developed the Cleaner Technologies Substitutes Assessment (CTSA) to provide comparative cost, risk, and performance information on professional fabricare processes. As part of this assessment, the health risks associated with the use of tetrachloroethylene in dry cleaning establishments was evaluated. For carcinogenic effects, the CTSA used human-equivalent metabolized doses using mouse and rat tumor data from the National Toxicology Program (NTP) (1986) study. The approach used was similar to that used by EPA (U.S. EPA, 1986), but the mouse carcinoma-only data set was omitted from the assessment to avoid double-counting of animals with adenomas and carcinomas. The analyses are based on taking the geometric mean of the unit risk from four data sets that evaluated the incidence of male and female mouse liver adenomas and/or carcinomas and male and female rat mononuclear cell leukemia. Using a linear-at-low-doses approach, the unit risk was estimated to be 7.1×10^{-7} per $\mu g/m^3$ of tetrachloroethylene in air. The CTSA report states that the unit risk should not be used for lifetime average daily exposures greater than 1.4×10^4 $\mu g/m^3$.

Noncarcinogenic effects were also evaluated in the CTSA report. A provisional RfC was derived on the basis of mild renal tubule damage seen in a cross-sectional occupational study (Franchini et al., 1983). The average level of tetrachloroethylene exposure was equivalent to 10 mg/m^3, and this value was used as the LOAEL. The LOAEL was adjusted to account for duration of exposure, and an uncertainty factor of 10 was applied to account for the use of a LOAEL. An uncertainty factor to account for sensitive individuals was not applied, because the derived RfC was to be used in the CTSA screening to evaluate occupational populations. The provisional RfC was established at 0.17 mg/m^3.

A.2.1.2. *Addendum to the Health Assessment Document for Tetrachloroethylene, U.S. EPA, 1986*

This assessment was conducted to reevaluate tetrachloroethylene carcinogenicity on the basis of the released NTP (1986) inhalation animal bioassay. On the basis of the evidence of carcinogenicity in rats and mice, together with the inconclusive epidemiologic evidence, tetrachloroethylene was recategorized as a Group B2 probable human carcinogen.

A new inhalation unit risk value was derived using the NTP (1986) inhalation study. The NTP bioassay doses for rats and mice were converted to metabolized doses using the previously

established dose-metabolism relationship (U.S. EPA, 1985). A linearized multistage model was used for the low-dose extrapolation. Six different data sets from the NTP study were used to derive unit risk estimates. These data included endpoints on leukemia in male and female rats, liver carcinoma in male and female mice, and liver carcinomas and adenomas in male and female mice. The revised upper-bound estimate of the incremental cancer risk due to lifetime exposure to 1 $\mu g/m^3$ of tetrachloroethylene in air was determined to range from 2.9×10^{-7} to 9.5×10^{-7}. This range includes the value determined using the NCI gavage study (NCI, 1977). The unit risk was stated to be applicable only for low-level exposures where the relationship between ambient air concentrations and metabolized dose is linear.

A.2.1.3. *Health Assessment Document for Tetrachloroethylene, U.S. EPA, 1985*

Tetrachloroethylene was categorized as a Group C possible human carcinogen on the basis of limited evidence of carcinogenicity in animals and inconclusive epidemiologic data. Dose response data for hepatocellular carcinomas observed in female mice in the NCI gavage study (NCI, 1977) were used to derive the unit risk. The potency estimate for tetrachloroethylene was calculated using the linearized multistage model and the dose metabolized and eliminated in the urine. Urinary metabolites were considered to account for 80% of total metabolites, as in Buben and O'Flaherty (1985). Unit risk was then calculated using human body burden data from Bolanowska and Golacka (1972). This study provided information on the relationship between the air concentration and the amount metabolized in urinary excretion in human subjects. The amount metabolized was assumed to be proportional to the air concentration and the duration of exposure. The upper-bound estimate of the incremental cancer risk due to 1 $\mu g/m^3$ of tetrachloroethylene in air was determined to be 4.8×10^{-7}.

A.2.2. Inhalation Assessments Conducted by Non-U.S. EPA Agencies

A.2.2.1. World Health Organization, Concise International Chemical Assessment Document 68, 2006

"In occupationally exposed cohorts, the most consistent adverse finding was neurotoxicity; therefore, the most informative study on neurotoxic effects in exposed workers was used to derive a TC. The mean exposure level (83 mg/m^3) was taken as a LOAEC. This was converted to an equivalent concentration for continuous exposure (20 mg/m^3), and two uncertainty factors of 10 were applied (one to account for interindividual differences, the other because the selected concentration was a LOAEC rather than a NOAEC), to derive a TC of 0.2 mg/m^3. For comparative purposes, a similar approach was used for studies reporting nephrotoxicity. The most informative study yielded a mean occupational exposure of 100

mg/m^3, which generated a TC of 0.24 mg/m^3, a value in good agreement with the TC protective against neurotoxic effects. Available data indicate that liver toxicity would occur only at exposures higher than those that affect the CNS and kidney. A TC for spontaneous abortions was not derived. However, the TC of 0.2 mg/m^3 is more than 3 orders of magnitude lower than the exposure concentration that induced mild adverse effects in laboratory animals, and so it was considered to be protective against reproductive toxicity in humans."

"Tetrachloroethene has induced several types of tumour in rats and mice. Currently, there is no convincing evidence that these tumours arise via modes of action that operate only in rodents, and hence their relevance to humans cannot be dismissed. Therefore, a BMC approach was used, and a BMC and its lower confidence limit (BMCL) were calculated for each animal tumour. Of the tumours observed in experimental animals, hepatocellular adenomas and carcinomas in male mice yield highest predicted risks. The TC derived above, 0.2 mg/m^3, corresponds to a cumulative lifetime risk of 0.4×10^{-3} when a linear extrapolation is applied to the BMC_{10} as the point of departure."

A.2.2.2. *California Environmental Protection Agency, 2002*

The California EPA Air Toxics Hot Spots program has derived an inhalation unit risk value for tetrachloroethylene (Cal EPA, 2002). The value was determined from data on hepatocellular adenomas and carcinomas in male mice reported in the NTP (1986) bioassay study. Two pharmacokinetic models were used to estimate the human inhaled concentrations equivalent to the bioassay concentrations. These two models were described only as (1) a steady-state model and (2) a physiologically based pharmacokinetic (PBPK) model. An assumption that 18.5% of the applied dose is metabolized in humans was incorporated. The cancer potency values expressed in terms of human dose rates and derived using the two different models and the rat and mouse studies ranged from 0.0025 to 0.093 per mg/kg-day. Considering the quality of the cancer bioassays and the uncertainty in human metabolism, Cal EPA decided that the best value for the inhalation unit risk was 5.9×10^{-6} per μg/m^3.

A.2.2.2. *Massachusetts' Derivation of Inhalation Unit Risk for Tetrachloroethylene, Massachusetts Department of Environmental Protection, 1998*

The Massachusetts Department of Environmental Protection (MA DEP) classifies tetrachloroethylene as a Group B2 carcinogen with suggestive evidence for mutagenicity. The unit risk was calculated on the basis of male and female liver tumors found in mice in the NCI gavage study (NCI, 1977). The dose calculations used were similar to those used by EPA (U.S. EPA, 1985), with two differences. MA DEP adjusted the lifetime average dose, which is based on urinary metabolites, to a dose of total metabolites. This adjustment was made on the assumption that the urinary metabolites are 80% of the total metabolism. To convert the carcinogenic potency value to an inhalation exposure, MA DEP assumed that the metabolized dose is equal to 70% of the inhaled dose. This differs from the 1985 EPA assessment, where 0.66% of the total inhaled dose in humans is assumed to be metabolized to urinary metabolites. MA DEP also calculated a unit risk using the NTP inhalation study, but did not consider this to provide a reasonable quantitative estimate due to uncertainty in the calculations of the metabolized dose. Using the NCI study, 5.5×10^{-5} per $\mu g/m^3$ is recommended as the unit risk.

A.2.2.3. *ATSDR Toxicological Profile for Tetrachloroethylene (Update), September 1997*

ATSDR has promulgated both acute and chronic MRLs for the inhalation of tetrachloroethylene. The acute inhalation MRL was derived from studies where male volunteers were exposed to 50 ppm tetrachloroethylene for 4 hrs/day for 4 days. The volunteers showed increased pattern reversal visually evoked potential (VEP) latencies and deficits for vigilance and eye-hand coordination (Altmann et al., 1992). Deficits were not seen at 10 ppm, and this value was used as the NOAEL. This value was duration adjusted to extrapolate from intermittent exposure, and an uncertainty factor of 10 was used to account for human variability. The acute inhalation MRL was established at 0.2 ppm (1.36 mg/m^3).

The chronic duration MRL for the inhalation of tetrachloroethylene was based on a study that showed increased reaction times in neurobehavioral tests given to female workers exposed to tetrachloroethylene in dry cleaning shops (Ferroni et al., 1992). Air exposures averaged 15 ppm tetrachloroethylene for an average of 10.1 years. The LOAEL in this study was 15 ppm. This value was adjusted from an occupational exposure to a continuous exposure; an uncertainty factor of 10 was used to account for the use of a LOAEL, and an additional factor of 10 was used to account for human variability. The chronic inhalation MRL was established at 0.04 ppm (0.27 mg/m^3).

A.2.2.4. ***Tetrachloroethane-Ambient Air Criteria Document, New York State Department of Health, 1997***

The New York State Department of Health agrees with the International Agency for Research on Cancer (IARC) classification of tetrachloroethylene as a Group 2A (known animal) carcinogen. A linearized multistage model was applied to metabolized dose data for liver tumors in mice and mononuclear cell leukemia in rats (NTP, 1986). Estimates of the metabolized dose were based on predictions of a physiologically based pharmacokinetic (PBPK) model (mice) and on experimental data on the production of urinary metabolites (mice and rats). For mononuclear cell leukemia in rats, estimates based on air concentrations were also derived. ED10 procedures were also used to calculate unit risk values. Due to uncertainty regarding which method provides a better estimate, the central tendency of all these estimates (linearized multistage model, ED10, metabolized dose, and air concentration) was used to derive an upper-bound unit risk value. The central tendency estimate for liver tumors in mice established an upper bound unit risk value of 0.88×10^{-6} $(\mu g/m^3)^{-1}$; for mononuclear cell leukemia in rats, the central tendency estimate was 1.3×10^{-6} $(\mu g/m^3)^{-1}$. An upper-bound risk estimate of 1×10^{-6} $(\mu g/m^3)^{-1}$ is the central tendency of the mouse- and rat-based unit risk estimates and is the recommended criteria for evaluating the excess human carcinogenic risk associated with chronic exposure to $1 \mu g/m^3$ tetrachloroethylene in ambient air.

New York State has determined that the strength of human evidence on the noncarcinogenic effects of tetrachloroethylene exposure support the use of human data for determining an ambient air criteria for noncarcinogenic effects. A weight-of-evidence approach was used, and multiple endpoints and epidemiologic studies were evaluated. Endpoints used in the derivation of the ambient air criterion included evidence of central nervous system (motor and cognitive effects) (Seeber, 1989), kidney (Mutti et al., 1992), and liver dysfunction (Gennari et al., 1992). The lack of reproductive and developmental studies was identified as a significant data gap because epidemiologic studies did not provide sufficient exposure data for criteria evaluation.

Lowest-observed-effect level (LOEL) data were provided in the epidemiologic studies listed above. LOELs were duration adjusted to account for continuous exposure using EPA inhalation guidelines (U.S. EPA, 1994). For adult criteria, an uncertainty factor of 100 was applied to each duration-adjusted LOEL (10 for variation in sensitivity among humans and 10 for the use of a LOEL from a subchronic study). For criteria protective of children, the appropriate scaling and uncertainty factors were used. Child-adjusted LOELs were derived using physical and physiological data for children. An uncertainty factor of 100 was applied to the child-

adjusted LOELs (10 for variation in sensitivity among humans, 3 for the use of a LOEL, and 3 for concerns about the increased sensitivity of children to tetrachloroethylene toxicity).

Listed below are the results of the safe ambient air level derivations:

Effects	Adults	Children
Central nervous system (Seeber, 1989)	0.30 mg/m^3	0.12 mg/m^3
Kidney (Mutti et al., 1992)	0.36 mg/m^3	0.14 mg/m^3
Liver (Gennari et al., 1992)	0.28 mg/m^3	0.10 mg/m^3

New York State estimated that an ambient air criterion of 0.1 mg/m^3 would provide the general population, including sensitive subpopulations of infants, children, the infirm and elderly, a sufficient margin of exposure over the air levels of tetrachloroethylene associated with noncarcinogenic effects in humans and animals. The ambient air criterion was established at 0.1 mg/m^3.

A.2.2.5. *Priority Substances List Assessment Report: Tetrachloroethylene, Canada Health and Welfare Agency, 1993*

Tetrachloroethylene has been classified in Group 3 (possibly carcinogenic to humans) of the classification scheme developed for use in the derivation for the guidelines for Canadian drinking water quality. A tolerable daily intake (TDI) was derived using data from the NTP (1986) study. It was assumed that 100% of the inhaled tetrachloroethylene was retained in the mice. A LOAEL of 100 ppm for reduced survival and hepatotoxic effects in male mice and lung congestion and nephrotoxic effects in male and female mice was used. An uncertainty factor of 5,000 was applied to account for intraspecies variation (10), use of a LOAEL (10), interspecies variation (10), and limited evidence of carcinogenicity (5). A TDI of 34 μg/kg bw/day was derived. Using standardized conversion assumptions (EPA), this value is equivalent to 0.018 ppm (0.12 mg/m^3).

A.2.3. Summary of Inhalation Risk Estimates

The following tables summarize the quantitative risk estimates that have been developed by EPA and other agencies. Table A-3 shows the cancer risk values. Table A-4 depicts the risk estimates developed for noncarcinogenic endpoints.

A.3. QUALITATIVE RISK ASSESSMENTS

This section contains a brief review of documents that included only a qualitative assessment of tetrachloroethylene toxicity and risk.

Table A-3. Estimates of cancer inhalation unit risk using different methods

Dose metric surrogate (agency)	Unit risk value $(\mu g/m^3)^{-1}$	Studies used	Critical target effect(s)
Dose metabolized (Cal EPA, 2002)	5.9×10^{-6}	NTP (1986)	Liver adenomas and carcinomas in male mice
Dose metabolized (U.S. EPA, 1998)	7.1×10^{-7}	NTP (1986)	Liver adenomas and carcinomas in male and female mice, mononuclear cell leukemia in male and female rats
Total metabolized dose (MA DEP, 1998)	5.5×10^{-5}	NCI (1977)	Liver tumors in male and female mice
Dose metabolized and administered (NYS DOH, 1997)	1×10^{-6}	NTP (1986)	Liver tumors in male and female mice, mononuclear cell leukemia in male and female rats
Dose metabolized (U.S. EPA, 1986)	2.9×10^{-7} to 9.5×10^{-7}	NTP (1986)	Liver adenomas in male and female mice, combined liver adenomas and carcinomas in male and female mice, mononuclear cell leukemia in male and female rats
Dose metabolized (U.S. EPA, 1985	4.8×10^{-7}	NCI (1977)	Liver carcinomas in female mice

Table A-4. Inhalation, noncancer endpoints

Agency guideline	Standard	Studies used	Critical target effect(s)
Provisional RfC (U.S. EPA, 1998)	0.025 ppm (0.17 mg/m^3)	Franchini et al. (1983)	Renal tubule damage in workers
Ambient air criterion NYS DOH, 1997)	0.015 ppm (0.1mg/m^3)	Seeber (1989), Mutti et al. (1992), Gennari et al. (1992)	Motor and cognitive effects, kidney dysfunction and liver dysfunction in workers
Acute inhalation minimal risk level (ATSDR, 1997)	0.2 ppm (1.36 mg/m^3)	Altmann et al. (1992)	Neurological effects (VEP, vigilance, and eye-hand coordination) in males
Chronic inhalation minimal risk level (ATSDR, 1997)	0.04 ppm (0.27 mg/m^3)	Ferroni et al. (1992)	Increased reaction times in female workers
Tolerable daily intake (CHWA, 1993)	0.018 ppm (0.12 mg/m^3)	NTP (1986)	Reduced survival and hepatotoxic effects in male mice, lung congestion and nephrotoxic effects in male and female mice

A.3.1. U.S. EPA Qualitative Risk Assessments

A.3.1.1. *Response to Issues and Data Submission on the Carcinogenicity of Tetrachloroethylene, U.S. EPA, 1991*

This document discusses issues relating to the classification of tetrachloroethylene as a B2 carcinogen. Lengthy deliberation is given to the specific mechanisms of action that may explain all the tumor endpoints observed after exposure to tetrachloroethylene. In conclusion, EPA stands behind the B2 classification and concludes that sufficient evidence of cancer in animals does exist.

A.3.2. Qualitative Risk Assessments Conducted by Non-U.S. EPA Agencies

A.3.2.1. *Organization for Economic Cooperation and Development (OECD), Screening Information Data Set (SIDS) Initial Assessment Report: Comprehensive Risk Assessment Report for Tetrachloroethylene, 1996*

The current European Union classification for tetrachloroethylene is Carcinogen Category 3. Category 3 indicates *"a substance which causes concern for man owing to possible carcinogenic effect but in respect of which the available information is not adequate for making a satisfactory assessment. There is some evidence from appropriate animal studies, but this is insufficient to place the substance in category 2."*

In the summary of carcinogenicity, the report (OECD, 1996) concludes that the liver tumors found in mice and the kidney tumors found in rats following repeated inhalation exposure are almost undoubtedly not of significance in relation to human health. This is based on believed differences in metabolic pathways and mechanisms of action. OECD does not believe that peroxisome proliferation in mice is relevant to human cancer. Similarly, it believes that the human renal beta lyase activity in humans is negligible compared to that in rats. In evaluating human carcinogenicity, OECD determined that the epidemiological studies do not show evidence supporting an increased risk of carcinogenicity in humans.

A.3.2.2. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 63, 1995*

IARC determined that tetrachloroethylene is probably carcinogenic to humans and has classified tetrachloroethylene as a Group 2A carcinogen. This judgment is based on limited evidence in humans and sufficient evidence of carcinogenicity in experimental animals. In evaluating tetrachloroethylene, the following evidence was considered: although tetrachloroethylene is known to induce peroxisome proliferation in mouse liver, poor quantitative correlation was seen between peroxisome proliferation and tumor formation in the liver after

administration of tetrachloroethylene by inhalation; the spectrum of mutations in proto-oncogenes in liver tumors from mice treated with tetrachloroethylene is different from that in liver tumors from mice treated with trichloroethylene; tetrachloroethylene induced leukemia in rats; and several epidemiological studies showed elevated risks for esophageal cancer, non-Hodgkin's lymphoma and cervical cancer.

Evidence of cancer in animal studies is supported by studies that included both oral and inhalation exposures. Cancer endpoints included increases in hepatocellular carcinoma in male and female rats after oral administration of tetrachloroethylene (NCI, 1977), increases in hepatocellular adenoma and carcinoma in male and female mice and increases in mononuclear-cell leukemia in male and female rats after inhalation exposure (NTP, 1986). IARC does not point to a single epidemiological study as being critical, but rather summarizes many studies that support a relationship between cancer and tetrachloroethylene exposures. IARC relies on the consistent positive associations between human exposures to tetrachloroethylene and the risks for esophageal cancer, non-Hodgkin's lymphoma, and cervical cancer.

A.3.2.3. *Report on Carcinogens, Eleventh Edition, NTP, 2005*

The NTP lists carcinogenic substances in one of two categories: (1) known to be a human carcinogen and (2) reasonably anticipated to be a human carcinogen. They present a brief two-page summary of the evidence for their classification. They classified tetrachloroethylene in Category 2, "reasonably anticipated to be a human carcinogen." It was first listed in the 5th Annual Report on Carcinogens (1989). They based their classification on sufficient evidence of carcinogenicity in experimental animals and limited evidence in humans.

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