Exhibit 197

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TOXICOLOGICAL REVIEW

OF

Tetrachloroethylene (Perchloroethylene)

(CAS No. 127-18-4)

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

February 2012

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

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GUIDE TO READERS OF THIS DOCUMENT

For ease of reading, it is recommended that the Executive Summary, Section 1, and Section 6 be read prior to Sections 2–5.

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

Section 2 summarizes information about tetrachloroethylene uses, occurrence and exposure.

Section 3 describes the toxicokinetics and physiologically based pharmacokinetic (PBPK) modeling of tetrachloroethylene and metabolites.

Section 4 is the hazard characterization of tetrachloroethylene. This section discusses tetrachloroethylene toxicity on an organ-specific basis. 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. The order of presentation is as follows: neurotoxicity (refer to Section 4.1); kidney and bladder toxicity and cancer (refer to Section 4.2); liver toxicity and cancer (refer to Section 4.3); esophageal cancer (refer to Section 4.4); lung and respiratory cancer (refer to Section 4.5); immunotoxicity, hematologic toxicity, and cancers of the immune system (refer to Section 4.6); developmental and reproductive toxicity, and reproductive cancers (refer to Section 4.7); genotoxicity (refer to Section 4.8); and susceptible populations (refer to Section 4.9). Section 4.10 provides a summary of the hazard identification.

Section 5 is the dose-response assessment of tetrachloroethylene. Section 5.1 and 5.2 describes the dose-response analyses for noncancer effects and Section 5.3 describes the dose-response analyses for cancer. Appendix D provides details of the cancer dose-response modeling analyses.

Section 6 is the summary of the major conclusions in the characterization of tetrachloroethylene hazard and dose response.

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

Appendix B documents the essential design features, exposure assessment approaches, statistical analyses (including assessment of exposure- or concentration-response), and potential

sources of confounding and bias for epidemiologic studies on cancer and tetrachloroethylene. This analysis supports the discussion of site-specific cancer observations in Sections 4.2–4.7. Appendix C presents a comparative quantitative analysis of the carcinogenicity of trichloroacetic acid (including that predicted using PBPK modeling to be produced from tetrachloroethylene) with the carcinogenicity of tetrachloroethylene.

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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	lower bound benchmark concentration
BMD	benchmark dose
BMDL	lower bound 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 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
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
IUGR	intrauterine growth restriction

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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
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 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
NYSDOH	New York State Department of Health
NYSOAG	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
SES	socio-economic status
SGA	small for gestational age
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SSB	single-strand break
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 nonspecific 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	visual 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.

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

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

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EXECUTIVE SUMMARY

Tetrachloroethylene is a widespread contaminant that is present in ambient air, indoor air, soil, and groundwater. Once exposed, humans, as well as laboratory animal species, rapidly absorb tetrachloroethylene, which is then distributed to tissues via systemic circulation, metabolized, and then excreted primarily in breath as unchanged tetrachloroethylene or CO_2, or in urine as metabolites. Based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that tetrachloroethylene poses a potential human health hazard for noncancer toxicity to the central nervous system, kidney, liver, immune and hematologic system, and on development and reproduction. Neurotoxicity is identified as a sensitive endpoint following either oral or inhalation exposure to tetrachloroethylene. Neurotoxic effects have been characterized in human controlled exposure, occupational and residential studies, as well as in experimental animal studies, providing evidence that tetrachloroethylene exposure results in visual changes, increased reaction time, and decrements in cognition.

Following EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, tetrachloroethylene is *"Likely to be Carcinogenic to Humans"* by all routes of exposure. This characterization is based on suggestive evidence of carcinogenicity in epidemiologic studies and conclusive evidence that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, increases tumor incidence (JISA, 1993; NTP, 1986; NCI, 1977). In the rodent bioassays, tetrachloroethylene increased the incidence of liver tumors (hepatocellular adenomas and carcinomas) in male and female mice and of mononuclear cell leukemia (MCL) in both sexes of rats. These findings were reproducible in multiple lifetime bioassays employing different rodent strains and, in the case of mouse liver tumors, by the inhalation and oral exposure routes. Additional tumor findings in rats included significant increases in the NTP bioassay of testicular interstitial cell tumors and kidney tumors in males, and brain gliomas in males and females. In mice, hemangiosarcomas in liver, spleen, fat, and subcutaneous skin were reported in males in the JISA study. The epidemiologic evidence provides a pattern associating tetrachloroethylene exposure and several types of cancer, including bladder cancer, non-Hodgkin lymphoma and multiple myeloma. Associations and exposure-response relationships were reported by studies using more precise exposure-assessments for tetrachloroethylene. For other sites, including esophageal, kidney, lung, cervical and breast cancer, more limited data suggestive of an effect are available.

As tetrachloroethylene toxicity and carcinogenicity are generally associated with tetrachloroethylene metabolism, susceptibility to tetrachloroethylene health effects may be

modulated by factors affecting toxicokinetics, including lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, lifestyle, and nutrition status. These and other factors (e.g., socioeconomic status and multiple exposures) may contribute to variation in response to tetrachloroethylene or its metabolites, once produced. In addition, it is not known how tetrachloroethylene interacts with known risk factors for human diseases.

Dose-response analyses of the noncancer database focused on the neurotoxicity data set as a basis for derivation of inhalation and oral reference values via the LOAEL/NOAEL approach. The two principal studies that were used demonstrated color vision changes (Cavalleri et al., 1994), and cognitive and reaction time changes (Echeverria et al., 1995). Candidate RfCs derived from these studies span a range from 0.015 to 0.056 mg/m^3. The midpoint of this range (**0.04 mg/m^3**) was chosen as the RfC for tetrachloroethylene.

The RfD for noncancer effects of **6×10^{-3} mg/kg-day** was derived through route-to-route extrapolation of the above inhalation studies. The RfD is equivalent to a drinking water concentration of 0.21 mg/L, assuming a body weight of 70 kg and a daily water consumption of 2 L. These noncancer reference values are supported by estimates from multiple human neurotoxicity studies. Additionally, quantitative dose-response analyses of the findings for other toxicity endpoints (i.e., kidney, liver, immunologic and hematologic, and reproductive and developmental toxicity), detailed in Section 5 and summarized in Sections 6.2.5 and 6.2.7, are considered to be supportive of these values.

For cancer, the majority of the NRC peer review panel recommended that the mouse hepatocellular tumors be used for cancer risk estimation. Therefore, the inhalation unit risk is **2×10^{-3} per ppm** or **3×10^{-7} per µg/m^3**, based on the male mouse hepatocellular tumor data from the JISA (1993) bioassay. The oral slope factor, developed by PBPK model-derived route-to-route extrapolation from the same data, is **2×10^{-3} per mg/kg-day**.

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 inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD but provides a continuous inhalation exposure estimate. 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 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

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

Development of these hazard identification and dose-response assessments for tetrachloroethylene has followed the general guidelines for risk assessment set forth by the National Research Council (NRC, 1994, 1983). EPA Guidelines and Risk Assessment Forum technical panel reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986c), *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, 1991b), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (Whalan and Redden, 1994), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996a), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998b), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000a), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006c), 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. Primary, peer-reviewed literature identified through August 2011 was included where that literature was determined to be critical to the assessment. The relevant literature included publications on tetrachloroethylene that were identified through Toxicology Literature Online (TOXLINE), PubMed, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate. It should be noted that references have been added to the Toxicological Review after the external peer review in response to peer reviewer‘s comments and for the sake of completeness. These references have not changed the overall qualitative and quantitative conclusions.

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, 1997a). Table 2-1 lists the physical and chemical properties of tetrachloroethylene (ATSDR, 1997a).

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 (Gilbert et al., 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 tetrachloroethylene can vary based on season and environmental conditions, it has been estimated at 96 days under typical conditions (ATSDR, 1997a).

Ambient tetrachloroethylene concentrations vary from source to source and with proximity to the source. Outdoors, the high volatility of tetrachloroethylene leads to increased ambient air concentrations near points of use (ATSDR, 1997a; U.S. EPA, 1996b). Specific to early lifestage exposure scenarios, elevated ambient air concentrations include measurements taken outside of a daycare center adjacent to a dry cleaner (NYSDOH, 2005c) and on a playground near a factory (Monster and Smolders, 1984). 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 (1997a) reported mean tetrachloroethylene concentrations of 8.8 $\mu g/m^3$ in areas close to points of release.

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Table 2-1. Physical and chemical properties of tetrachloroethylene

Property	Information	Reference
Molecular formula	C_2Cl_4	HSDB (2001)
Molecular weight	165.83	Lide (1990)
Color	Colorless	Sax and Lewis (1987)
Physical state	Liquid (at room temperature)	Sax and Lewis (1987)
Melting point	–19˚C	Lide (1990)
Boiling point	121˚C	Lide (1990)
Density at 20˚C	1.6227 g/mL	Lide (1990)
Density at 25˚C	No data	
Odor	Ethereal	HSDB (2001)
Odor threshold: water	0.3 ppm	U.S. EPA (1987)
Odor threshold: air	1 ppm	U.S. EPA (1987)
Solubility: water at 25˚C	150 mg/L	HSDB (2001)
Solubility: organic solvent(s)	Miscible with alcohol, ether, chloroform, benzene, solvent hexane, and most of the fixed and volatile oils	HSDB (2001)
Partition coefficients: Log K_{OW}	3.4	HSDB (2001)
Partition coefficients: Log K_{OC}	2.2–2.7	Seip et al. (1986) Zytner et al. (1989)
Vapor pressure at 25˚C	18.47 mm Hg	HSDB (2001)
Henry's law constant at 25˚C	1.8×10^{-2} atm-m^3/mol	Gossett (1987)
Autoignition temperature	No data	
Flashpoint	None	HSDB (2001)
Flammability limits	Nonflammable	HSDB (2001)
Conversion factors, air	1 mg/L = 141.4 ppm 1 ppm = 6.78 mg/m^3	HSDB (2001)
Explosive limits	No data	

Source: ATSDR (1997a).

EPA has carried out modeling to characterize the geographic distribution of tetrachloroethylene for its National-Scale Air Toxics Assessment database (U.S. EPA, 1996b). 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, 2001a). Adgate and colleagues measured tetrachloroethylene in outside and indoor air at school, indoor air at home, and using personal samplers on children, and demonstrated that tetrachloroethylene levels are lower in homes with greater ventilation and in homes in non-urban settings (Adgate et al., 2004b; Adgate et al., 2004a). Mean indoor air concentrations in apartments above dry cleaning shops of 4.9 mg/m^3 have been reported [Altmann et al. (1995); also refer to McDermott et al. (2005); Schreiber et al. (2002); Garetano and Gochfeld (2000); Schreiber et al. (1993)]. Measurements have also been made in a daycare center adjacent to a dry cleaners (NYSDOH, 2005a, b, c) and in a classroom exposed to tetrachloroethylene from an air "emission from a small chemical factory" (Monster and Smolders, 1984). Mean concentrations inside dry cleaning facilities were reported to be 454−1390 mg/m^3 in the United States and 164 mg/m^3 in Nordic countries during the 1960s and 1970s. Overall levels declined from 95−210 mg/m^3 in the 1980s to 20−70 mg/m^3 over the next decades in these countries (Lynge et al., 2011; Gold et al., 2008; Lynge et al., 2006).

The off-gassing of garments that have recently been dry-cleaned may be of concern [Tichenor et al. (1990); also refer to Thomas et al. (1991)]. 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 (1997a); also refer to Aggazzotti et al. (1994a; 1994b)]. 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; also refer to Gulyas and Hemmerling (1990). 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. The authors 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 (refer to 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, 2001a; ATSDR, 1997a; Environment Canada & Health Canada, 1993; Lagakos et al., 1986). Most of this contamination is probably due to release in water following industrial use or by public use of consumer products. Therefore, 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 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 (Environment Canada & Health Canada, 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) (Environment Canada & Health Canada, 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 μg/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. In addition, incidental water consumption may occur for children when swimming or bathing (U.S. EPA, 2008).

Although dermal exposure is possible via contaminated tap water during showering, bathing, or swimming, this is generally not considered a major route of exposure (Poet et al., 2002; Nakai et al., 1999; Stewart and Dodd, 1964). 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 (2001a); also refer to Daft (1988); Heikes and Hopper (1986); McConnell et al. (1975)]. 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 (U.S. EPA, 2001a; Schreiber, 1997). In 1988, elevated tetrachloroethylene levels were observed in margarine and butter samples obtained from grocery stores located near dry cleaning facilities [Entz and Diachenko (1988); also refer to Uhler and Miller (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 include the Food and Drug Administration (2003) and Fleming-Jones and Smith (2003).

2.2.4. Soil

Where contamination occurs, tetrachloroethylene can be measured in soil (U.S. EPA, 2001a). This pathway for ingestion of tetrachloroethylene has not been directly examined. A clear need exists to evaluate this pathway, particularly for children with pica, who can ingest high quantities of contaminated soil through hand-to-mouth activity, as has been shown for lead (U.S. EPA, 2008).

2.2.5. Breast Milk

Due to its lipid solubility, tetrachloroethylene can concentrate in human breast milk (Schreiber et al., 2002; U.S. EPA, 2001a; NYSDOH, 2000; Schreiber, 1997, 1993; Sheldon et al., 1985; Pellizzari et al., 1982; Bagnell and Ellenberger, 1977), as well as in milk from cows (Wanner et al., 1982), goats (Hamada and Tanaka, 1995), and rats (Byczkowski et al., 1994; Byczkowski and Fisher, 1994). 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, 2001a). In one case study, the breast milk of a woman was found to contain 10 mg/L of tetrachloroethylene 1 hour following a visit to her husband at his work in a dry cleaning establishment. This concentration dropped to 3 mg/L after 24 hours. Her child suffered from obstructive jaundice and hepatomegaly, but these conditions improved when breastfeeding was discontinued (Bagnell and Ellenberger, 1977).

Physiologically based pharmacokinetic (PBPK) models have been utilized to estimate tetrachloroethylene doses from milk to the human infant (Fisher et al., 1997; Byczkowski and Fisher, 1995; Schreiber, 1993), and rat (Byczkowski et al., 1994). Schreiber (1993) used a PBPK model to estimate the dose a nursing infant might receive from an exposed mother's breast milk. Using different exposure scenarios, Schreiber (1993) predicted that human breast milk concentrations could range from 1.5 mg/L for a typical residential scenario, 16–3,000 mg/L for a residential scenario near a dry cleaner, and to 857–8,440 mg/L for an occupational scenario. Assuming that a 7.2 kg infant ingests 700 mL of breast milk per day, Schreiber estimated the dose to the infant could range from 0.0001 to 0.82 mg/kg/day. 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, 45.8 mg/m^3), three located above facilities that used transfer machines (average concentration, 7.7 mg/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 and Fisher (1995) predicted lower doses to the infant (0.0009–0.202 mg/kg-day). Using milk production and suckling variables, Fisher et al. (1997) estimated the dose that a human infant might receive after maternal occupational exposure to be 1.4 mg/day.

Ingestion through breast milk and infant exposures is discussed further in Section 4.9. 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.

2.2.6. 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 (Poet et al., 2002; Nakai et al., 1999; Stewart and Dodd, 1964).

3.1.1. Inhalation

The major exposure route for tetrachloroethylene is considered to be inhalation. 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 the concentration in the inspired air (Monster et al., 1979; Hake and Stewart, 1977).

Chiu et al. (2007) reported that peak levels of tetrachloroethylene in venous blood and air occurred near the end of a 6-hour inhalation exposure to 1 ppm and declined thereafter. In the Monster et al. (1979) study, uptake after 4 hours 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 10–20 [e.g.,(Reitz et al., 1996; Byczkowski and Fisher, 1994; Gearhart et al., 1993; Hattis et al., 1990; Ward et al., 1988; Droz and Guillemin, 1986)], meaning that if tetrachloroethylene is in equilibrium, the concentration in blood will be 10−20 times higher than the concentration in the alveoli.

Opdam and Smolders (1986) determined concentrations of tetrachloroethylene in alveolar air for 1−60-second residence times (the time interval from the beginning of an inhalation to the end of the next inhalation) for six volunteers exposed to 0.5−9.8 ppm of chemical for 1−60 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 postexposure for residence times less than 10 seconds. Alveolar air tetrachloroethylene concentration correlated with the concentrations in pulmonary artery mixed venous blood.

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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 (Dallas et al., 1994b; Pegg et al., 1979).

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., 1995, 1994b; Frantz and Watanabe, 1983; Schumann et al., 1980; Pegg et al., 1979). 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 (Nakai et al., 1999; Riihimaki and Pfaffli, 1978). The amount of chemical absorbed during the immersion of one thumb in liquid tetrachloroethylene is equivalent to the uptake during inhalation of 10−15 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 (McDougal et al., 1990; Tsuruta, 1989), 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 (Savolainen et al. 1977; Monster et al. 1979; Dallas et al. 1994). 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 et al., 2002; NYSDOH, 2000; Schreiber, 1997, 1993). Higher concentrations occur in milk having higher fat content. For example, the milk:blood partition coefficient is equal to 12 for rats but 2.8 for humans (Byczkowski and Fisher, 1994). This noticeable difference reflects 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 (Byczkowski and Fisher, 1994; Dallas et al., 1994b; Gearhart et al., 1993; Ward et al., 1988). 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 (Skender et al., 1991; Altmann et al., 1990; Stewart et al., 1977). 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 hours (Monster, 1979; Fernandez et al., 1976). Therefore, during a single 8-hour 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 5-day-a-week work schedule, an equilibrium is eventually established, but it requires a time period of 3−4 weeks of exposure for adipose tissue to reach plateau concentrations.

Animal studies provide clear evidence that tetrachloroethylene distributes widely to all tissues of the body, readily crossing the blood:brain barrier and the placenta (Dallas et al., 1994a; Ghantous et al., 1986; Schumann et al., 1980; Savolainen et al., 1977a). Following exposure of rats to tetrachloroethylene, the compound has been measured in blood, fat, brain, lungs, liver, kidneys, heart, and skeletal muscle (Dallas et al., 1994a; Savolainen et al., 1977a). The highest tissue concentrations were found in adipose tissue (60 or more times blood level) and in the brain and liver (4 and 5 times that found in the blood, respectively), as can be calculated from the rat tissue-distribution data of (Dallas et al., 1994a; Savolainen et al., 1977a). Dallas et al. (1994a) found the concentration of tetrachloroethylene in fat to be 9−18 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 concentrations in the liver were 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. Refer to 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, refer to (Lash and Parker, 2001; Anders et al., 1988; Dekant et al., 1987)]. 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]) mixed-function oxidase system and glutathione (GSH) conjugation followed by subsequent further biotransformation and processing, either through the cysteine conjugate β-lyase pathway or by other enzymes including flavin-containing monooxygenase 3 (FMO3) and CYP3A (Lash and Parker, 2001; Lash et al., 1998; Völkel et al., 1998; Birner et al., 1996; Anders et al., 1988; Dekant et al., 1987; Costa and Ivanetich, 1980; Filser and Bolt, 1979; Pegg et al., 1979; Daniel, 1963). The conjugative pathway is toxicologically significant because it yields relatively potent toxic metabolites (Lash and Parker, 2001; Werner et al., 1996; Vamvakas et al., 1989a, c; Vamvakas et al., 1989d; Anders et al., 1988; Vamvakas et al., 1987; Dekant et al., 1986a; Dekant et al., 1986c). Figure 3-1 depicts the overall scheme of tetrachloroethylene metabolism. Known metabolites presented in this figure are identified by an asterisk.

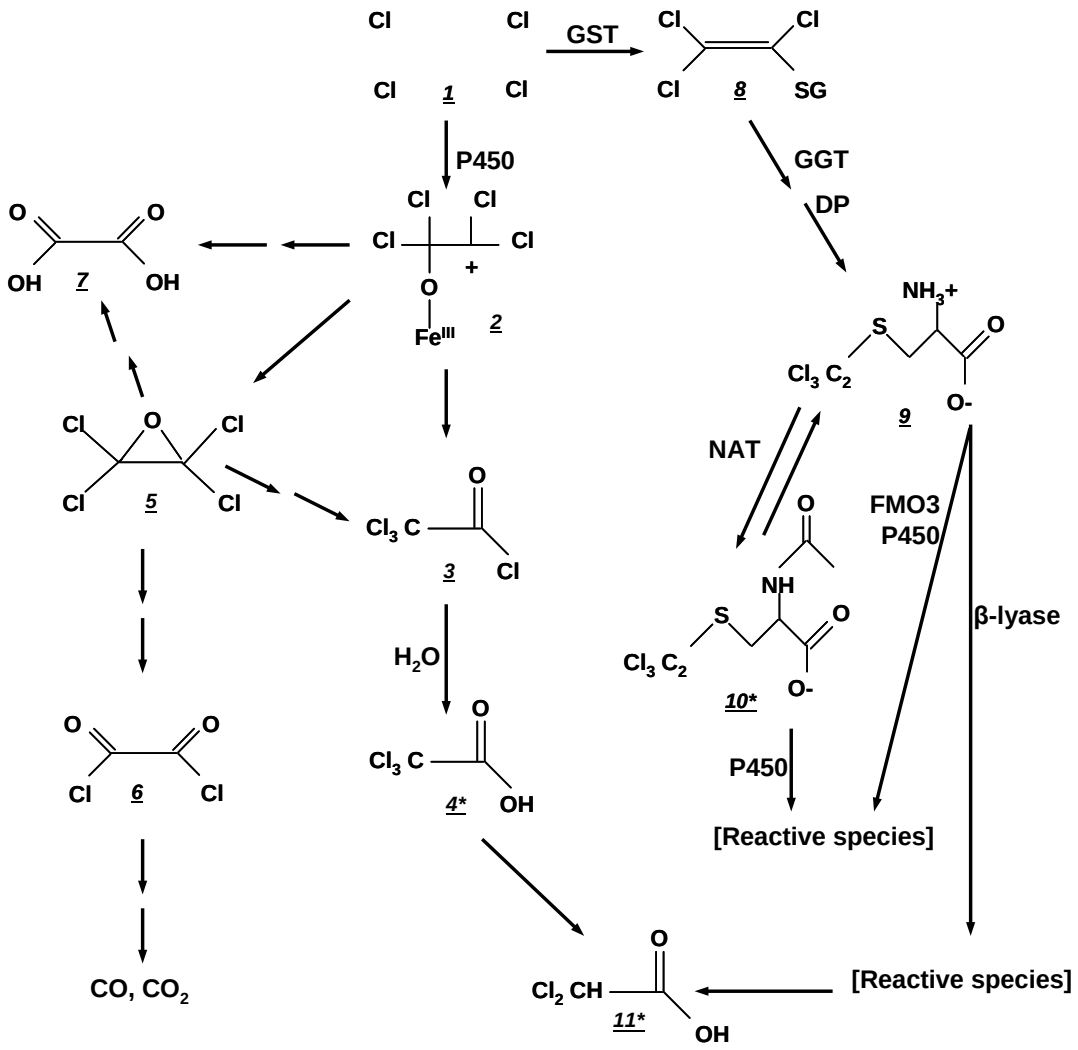

Figure 3-1. Postulated scheme for the metabolism of tetrachloroethylene by the cytochrome P450 (P450) oxidative pathway and glutathione *S*-transferase (GST)-mediated glutathione (GSH) conjugation pathway.

PCE and identified (*) urinary metabolites: (1) PCE, (2) PCE-Fe-O intermediate, (3) trichloroacetyl chloride, (4) trichloroacetic acid, (5) PCE oxide, (6) ethandioyl dichloride, (7) oxalic acid, (8) *S*-(1,2,2-trichlorovinyl) glutathione (TCVG), (9) *S*-(1,2,2-trichlorovinyl)-*L*-cysteine (TCVC), (10) *N*-acetyl trichlorovinyl cysteine (NAcTCVC), (11) dichloroacetic acid. Enzymes: cytochrome P450 (P450), GST, gamma-glutamyltransferase (GGT), dipeptidase (DP), β-lyase, flavin mono-oxygenase-3 (FMO3), *N*-acetyl transferase (NAT).

Sources: Adapted from Pegg et al. (1979), Costa and Ivanetich (1980), Dekant et al. (1986a), Lash and Parker (2001), Yoshioka et al. (2002), Chiu et al. (2007).

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3.3.2. Extent of Metabolism

Studies in both animals and humans indicate that overall metabolism of tetrachloroethylene is relatively limited—particularly at higher exposures [reviewed in (Lash and Parker, 2001)], as evidenced by the high percentage of absorbed dose excreted in the breath as the parent molecule (Chiu et al., 2007; Völkel et al., 1998; Buben and O'Flaherty, 1985; Frantz and Watanabe, 1983; Monster et al., 1983; Ohtsuki et al., 1983; Schumann et al., 1980; Filser and Bolt, 1979; Monster, 1979; Monster et al., 1979; Pegg et al., 1979; Fernandez et al., 1976; Essing et al., 1973; Ikeda and Ohtsuji, 1972; Stewart et al., 1970; Boettner and Muranko, 1969; Daniel, 1963; Stewart et al., 1961; Yllner, 1961). 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 (Monster et al., 1983; Monster and Houtkooper, 1979; Monster et al., 1979; Fernandez et al., 1976; May, 1976; Essing et al., 1973; Bolanowska and Golacka, 1972; Ikeda et al., 1972; Stewart et al., 1970; Boettner and Muranko, 1969; Stewart et al., 1961). Several studies reported only about 1–3% of the estimated amounts inhaled were metabolized to trichloroacetic acid (TCA) and other chlorinated oxidation products, although additional tetrachloroethylene—as much as 20% or more of the dose—may be metabolized over a longer period (Bois et al., 1996; Bogen et al., 1992; Monster et al., 1979). For example, Chiu et al. (2007) noted that although an average of 0.4% of tetrachloroethylene intake (1 ppm for 6 hours) was recovered in urine as TCA, total recovery in urine and exhaled air accounted for, on average, only 82% of intake. This would imply that 18% were metabolized, but Chiu et al. (2007) noted substantial uncertainty and variability in these calculations and concluded that 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. 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 2–86%. Based on data from Monster et al. (1979), Bois and colleagues (Chiu and Bois, 2006; Bois et al., 1996) used physiologically based pharmacokinetic (PBPK) modeling to predict that at exposure levels near 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 would be considerably higher (23−36%).

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 percentage metabolized. Rats metabolized a lower percentage of a given tetrachloroethylene body burden

than did mice (Frantz and Watanabe, 1983; Schumann et al., 1980; Filser and Bolt, 1979; Pegg et al., 1979; Daniel, 1963; Yllner, 1961). Urinary excretion data from studies by Filser and Bolt (1979) and Buben and O'Flaherty (1985) suggest that metabolism of tetrachloroethylene is greater in mice than in rats.

3.3.3. Pathways of Metabolism

The two known biotransformation pathways for tetrachloroethylene metabolism are (1) oxidation by CYP enzymes and (2) conjugation with GSH followed by further processing of the conjugate through various pathway bifurcation branches. As shown in Figure 3-1, the initial step in the metabolism of tetrachloroethylene is formation of an Fe-O intermediate for the oxidative pathway or conjugation with GSH for the secondary pathway (Yoshioka et al., 2002; Lash and Parker, 2001; Dekant et al., 1998; Lash et al., 1998; Dekant et al., 1987; Dekant et al., 1986c; Miller and Guengerich, 1983, 1982; Costa and Ivanetich, 1980). It is possible that other yet unrecognized pathways for tetrachloroethylene could exist in humans (Bois et al., 1996; Monster et al., 1979; Sakamoto, 1976).

3.3.3.1. Cytochrome P450-Dependent Oxidation

3.3.3.1.1. Oxidative metabolites

In vivo, the major excretory metabolite of the oxidative pathway, TCA, is excreted in the urine of all species tested (Völkel et al., 1998; Birner et al., 1996; Dekant et al., 1987; Ohtsuki et al., 1983; Leibman and Ortiz, 1977, 1970; Daniel, 1963; Yllner, 1961). Oxalic acid has been reported to be a relatively major urinary metabolite in rats (Pegg et al., 1979; Dmitrieva, 1967). Oxalic acid might either arise from action of microsomal epoxide hydrase on the epoxide intermediate or may be a separate product from the initial Fe-O intermediate. The oxalate metabolite excretory product may also be derived from dichloroacetic acid (DCA) or monochloroacetic acid (Tong et al., 1998a, b). Pulmonary excretion of carbon dioxide (CO_2) amounting to <10% of the administered dose has been identified in exhaled breath from rodents exposed to 14C-labeled tetrachloroethylene (Frantz and Watanabe, 1983; Schumann et al., 1980; Pegg et al., 1979), accounting for less than either exhaled tetrachloroethylene or urinary metabolites.

Trichloroethanol (TCOH) has been detected in the urine of subjects exposed to tetrachloroethylene in some studies (Schreiber et al., 2002; Birner et al., 1996; Monster et al., 1983; Weichardt and Lindner, 1975; Ikeda and Ohtsuji, 1972; Ikeda et al., 1972; Ogata et al., 1971; Tanaka and Ikeda, 1968; Ogata et al., 1962), but it could not be identified by others (Chiu et al., 2007; Völkel et al., 1998; Buben and O'Flaherty, 1985; Costa and Ivanetich, 1980; Monster et al., 1979; Hake and Stewart, 1977; Fernandez et al., 1976; Daniel, 1963; Yllner,

1961). Most of the studies reporting TCOH have involved occupational or environmental exposures in which there may be simultaneous exposure to trichloroethylene, for which TCOH is a major urinary metabolite. In vitro, TCA—and, to a lesser extent—oxalic acid, but not TCOH, are detected in incubations of tetrachloroethylene in rat microsomal protein (e.g., Yoshioka et al., 2002). Thus, it appears likely that the reports of TCOH following tetrachloroethylene exposure may have been artifacts of the analytical methodology used or of simultaneous trichloroethylene exposure. 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).

It was initially proposed that the first step in the oxidation of tetrachloroethylene is hypothesized to yield 1,1,2,2-tetrachloroethylene oxide, a relatively unstable epoxide (Miller and Guengerich, 1983, 1982; Costa and Ivanetich, 1980). Although an initial epoxide metabolite has not been unequivocally demonstrated for tetrachloroethylene, evidence for this epoxide does exist. The epoxide has been chemically synthesized (Kline et al., 1978; Bonse et al., 1975; Frankel et al., 1957). The potential fates of tetrachloroethylene epoxide include trichloroacetyl chloride, oxalate dichloride through tetrachloroethylene glycol, trichloroacetyl aminoethanol, and, possibly, chloral hydrate (in equilibrium with chloral) (Pegg et al., 1979; Henschler and Bonse, 1977; Bonse and Henschler, 1976).

However, recent data (Yoshioka et al., 2002) favor the hypothesis that the epoxide is not an obligatory intermediate to formation of trichloroacetyl chloride. In particular, the pattern of products of tetrachloroethylene oxide hydrolysis reported by Yoshioka et al. (2002) is dominated by carbon monoxide (CO) and carbon dioxide (CO_2), which differs markedly from the products of oxidation in vivo or in vitro. Because TCA is believed to be derived primarily from trichloroacetyl chloride (through hydrolysis or through reaction with amino groups of cellular proteins), this would favor the hypothesis that the epoxide is a minor product of tetrachloroethylene oxidation. Instead, the Fe-O intermediate is postulated to collapse via chlorine migration to yield predominantly trichloroacetyl chloride (Yoshioka et al., 2002).

DCA has been identified as a tetrachloroethylene urinary metabolite (Völkel et al., 1998; Dekant et al., 1987; Yllner, 1961) and may arise as a product of further metabolism of TCA or as a result of β-lyase bioactivation of GSH conjugation metabolites. 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. 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). However, data indicate that this does not contribute to DCA detected systemically after trichloroethylene exposure, and a similar conclusion is

reasonable for tetrachloroethylene, given the lower rate of formation of TCA from tetrachloroethylene as compared to trichloroethylene. In addition, data from trichloroethylene suggest that for that compound, DCA formation is likely dominated by hydrolysis of dichloroacetyl chloride—rather than dechlorination of TCA. As compared to tetrachloroethylene exposure, trichloroethylene exposure leads to higher amounts of TCA, in conjunction with the lower amounts of DCA, detectable in blood or urine. This is inconsistent with dechlorination of TCA being the origin of DCA detected in urine after tetrachloroethylene exposure and supports the hypothesis that DCA is derived predominantly from GSH conjugation of tetrachloroethylene.

3.3.3.1.2. Species differences

Although thought to be qualitatively similar, there are clear differences among species in the quantitative aspects of tetrachloroethylene metabolism (Lash and Parker, 2001; Völkel et al., 1998; Schumann et al., 1980; Ikeda and Ohtsuji, 1972). These differences are in the relative yields and kinetic behavior of metabolites (Völkel et al., 1998; Green et al., 1990; Ohtsuki et al., 1983). 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 metabolite concentrations in blood are obtained in rodents as compared with 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-life in the body of these metabolites is, however, noticeably longer for humans than for rodents (144 hours in humans vs. approximately 10 hours or less in rodents). 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 (AUC).

A study of species differences in tetrachloroethylene metabolism conducted by Dekant and colleagues is presented in Völkel et al. (1998). These investigators compared both oxidative and GSH-dependent metabolism in rats and humans exposed for 6 hours 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−eightfold, 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 GST enzymes, which, in turn, limit its detectability in urine.

3.3.3.1.3. Cytochrome P450 (CYP) isoforms and genetic polymorphisms

Oxidative metabolism of tetrachloroethylene, irrespective of the route of administration, occurs predominantly in the liver but also at other sites. For example, the kidneys exhibit cytochrome P450 enzyme activities, mostly in the proximal tubules, although total activity is markedly less than in the liver (Lash and Parker, 2001; Lash et al., 2001). The rat kidney expresses CYP2E1 (Cummings et al., 1999; Speerschneider and Dekant, 1995), although the human kidney has not been shown to do so (Cummings et al., 2000a; Amet et al., 1997). However, the human kidney expresses other CYP enzymes. CYP enzymes occurring in other extrahepatic tissues—brain and lungs, for example—may also contribute to oxidative metabolism of tetrachloroethylene.

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 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 (refer to text 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 the 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 (Hu et al., 1999; McCarver et al., 1998) and CYP3A4 (Sata et al., 2000; Westlind et al., 1999).

3.3.3.2. Glutathione (GSH) Conjugation Pathway

The GSH conjugation pathway was recognized much later than was the oxidative pathway, yet it may be toxicologically influential (Lash and Parker, 2001). Similar to trichloroethylene, GSH conjugation of tetrachloroethylene is associated with renal toxicity (Lash and Parker, 2001; Lash et al., 2000; Dekant et al., 1989; Anders et al., 1988).

3.3.3.2.1. Glutathione (GSH) conjugation metabolites

The initial conjugation of tetrachloroethylene with GSH occurs mainly in the liver (Green et al., 1990; Vamvakas et al., 1989c; Dekant et al., 1987; Vamvakas et al., 1987), 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 3-1, tetrachloroethylene is initially conjugated with GSH to form *S*-(1,2,2-trichlorovinyl) glutathione (TCVG). This reaction, which is catalyzed by the GSH-*S*-transferase (GSTs) enzymes, 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. TCVG is then processed through the cysteinylglycine conjugate *S*-(1,2,2 trichlorovinyl)-*L*-cysteinylglycine to *S*-(1,2,2-trichlorovinyl)-*L*-cysteine (trichlorovinyl cysteine, or TCVC) by the enzymatic removal of glutamyl and glycine residues by gamma-glutamyltransferase (GGT) and various membrane-bound dipeptidases known as cysteinylglycine dipeptidase [reviewed by (Lash and Parker, 2001; Dekant et al., 1989; Anders et al., 1988)]. 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 β-lyases [reviewed by (Lash and Parker, 2001; Lash et al., 2000; Dekant et al., 1989; Anders et al., 1988; Dekant et al., 1988)]. Renal β-lyases are known to cleave TCVC to yield an unstable thiol, 1,2,2-trichlorovinylthiol, that may give rise to cytotoxic and mutagenic reactive chemical species that can form covalent adducts with cellular nucleophiles, including DNA and proteins (Volkel et al., 1999; Dekant et al., 1990; Dekant et al., 1986a). In addition, DCA is a downstream urinary excretion product of β-lyase bioactivation of TCVC and has been detected in urine of rats exposed to tetrachloroethylene (Völkel et al., 1998). β-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 β-lyase is distinct from renal β-lyase and has not been found to have a role in TCVC metabolism. β-lyase activity is higher in the rat kidney than in the 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 β-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). TCVCSO is a more potent nephrotoxicant than TCVC (Elfarra and Krause, 2007). These TCVC sulfoxide and β-lyase cleavage products rearrange, forming a

thioketene (Ripp et al., 1997; Dekant et al., 1988), which is a potent acylating agent capable of binding to cellular macromolecules, including DNA (Pahler et al., 1999a; Pahler et al., 1999b; Volkel et al., 1999; Birner et al., 1996). Interestingly, the thioketene can degrade to form DCA, potentially making this metabolite a product of both tetrachloroethylene metabolism pathways (Völkel et al., 1998; Dekant et al., 1987).

In addition to β-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 (Birner et al., 1996; Bartels, 1994; 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 deacetylated 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).

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). More recent data clearly demonstrate the existence of the pathway in humans (Schreiber et al., 2002; Völkel et al., 1998; Birner et al., 1996). Quantitatively, 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; rather, it reflects only the portion that is excreted. In particular, 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. However, there are discrepancies regarding reported rates of tetrachloroethylene GSH metabolism (Lash et al., 2007; Lash and Parker, 2001; Dekant et al., 1998; Lash et al., 1998; Green et al., 1990). 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 in vitro findings concerning TCVG production would predict similar susceptibility for humans than for rodents with regard to renal toxicity (Lash et al., 1998), while others appear to predict much less susceptibility (Dekant et al., 1998; Green et al., 1990).

3.3.3.2.2. Species differences in gamma-glutamyltransferase (GGT) and β-lyase

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 the human liver than in the 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.

The β-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 β-lyase-dependent metabolism is greater in rats than in mice or in humans and greater in male than in female rats (Völkel et al., 1998; Green et al., 1990; Lash et al., 1990). This is not entirely in keeping with metabolic rates, in general, which are higher in mice than in rats, which, in turn, have higher metabolic rates than do larger animals, including humans. Studies that measured only cytoplasmic β-lyase activity did not consider the importance of mitochondrial β-lyase activity, which may be key to tetrachloroethylene metabolite toxicity (Lash et al., 2001).

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 relatively higher β-lyase enzyme activity and higher thioketene production in rats if the DCA is indeed largely the product of the GST pathway rather than the oxidative pathway (Völkel et al., 1998). It is not known whether sex-dependent variation of β-lyase activity exists in humans as it does in rats (Völkel 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 (Alberati-Giani et al., 1995; Malherbe et al., 1995; Larsen and Stevens, 1986; Tomisawa et al., 1986; Larsen, 1985; Stevens, 1985; Tomisawa et al., 1984; Stevens and Jakoby, 1983; Dohn and Anders, 1982; Tateishi et al., 1978), 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 β-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.

3.3.3.2.2.1. 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. GSTα, designated as GSTA in humans, is the predominate isoenzyme expressed in normal kidney from rodents and humans (Cummings et al., 2000b; Rodilla et al., 1998; Mitchell et al., 1997; Overby et al., 1994; Campbell et al., 1991). Available data thus far do not indicate that variability in activity of this isoenzyme is important to differences in individual susceptibility to toxicity. GSTζ (GSTZ) catalyzes the oxidative metabolism of DCA to glyoxylate (Tong et al., 1998a, b; Board et al., 1997); 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). 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 (Campbell et al., 1991; Terrier et al., 1990). GSTπ (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 the rabbit kidney (Cummings et al., 1999).

Two homodimeric GSTθ (GSTT) isoenzymes have been identified in the human kidney (Cummings et al., 2000a; Veitch et al., 1997). GSTT has been detected in rat and mouse liver and in mouse but not rat kidney (Cummings et al., 1999; Quondamatteo et al., 1998). GSTμ (GSTM) has been detected in rat kidney distal tubule cells (Cummings et al., 2000b) and in mouse and rabbit liver and kidney (Mitchell et al., 1997; Overby et al., 1994)—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).

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

Although it is clear that in mice and rats, the oxidative CYP pathway is quantitatively more important than the GSH conjugation pathway, the relative roles of these pathways in humans has not been determined directly. Moreover, 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 β-lyase and other enzymes is associated with tetrachloroethylene-induced renal toxicity. There is a possibility that β-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 effects.

Data from experiments designed to assess the effects of enzyme modulation suggest competition between the two pathways (Lash et al., 2001; Lash et al., 1999; Völkel et al., 1998; Dekant et al., 1987). Other data show relatively low urinary excretion of mercapturates as compared to CYP-derived products. On the basis of these findings, some researchers 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 (Völkel et al., 1998; Green et al., 1997; Green et al., 1990). 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 20−50-fold have been reported (Lieber, 1997; Stephens et al., 1994; Yoo et al., 1988), 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 (Raunio et al., 1995; Goldstein et al., 1969). No chemical-specific data regarding the manner in which CYP enzyme isoforms might affect susceptibility to adverse effects are available for tetrachloroethylene.

Diagnoses of polymorphisms in carcinogen-activating and -inactivating enzymes and cancer susceptibility have been noted (Raucy, 1995; Stephens et al., 1994; Yoo et al., 1988). 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 3.3.3.2. 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 (Moore et al, 2011). There are no direct, chemical-distinctive data with regard to the specific isoenzyme family responsible for TCVG formation in the 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 the 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 (Lash and Parker, 2001; IARC, 1995). 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-16α-carbonitrile, whereas CYP inhibitors such as SKF 525A, metyrapone, and carbon monoxide decrease tetrachloroethylene metabolism (Costa and Ivanetich, 1980; Moslen et al., 1977; Ikeda and Imamura, 1973). Chronic exposure to tetrachloroethylene has been shown to cause self-induction of metabolism (Kaemmerer et al., 1982; Savolainen et al., 1977a; 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 coexposures and cumulative risk in greater detail.

3.3.5. Comparison of Tetrachloroethylene Metabolism with Trichloroethylene Metabolism

3.3.5.1. 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 (Lash and Parker, 2001; Völkel et al., 1998; Ohtsuki et al., 1983). 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 (refer above 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.

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, the data from Schumann et al. (1980) and Pegg et al. (1979) indicate that, in rats exposed to 10 and 600 ppm of tetrachloroethylene for 6 hours, the

percentage of tetrachloroethylene body burdens excreted as unchanged parent compound are 68 and 99%, respectively. 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 inhaled dose and rats metabolizing 98 and 79% of the low and high inhaled 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 are relatively greater than at other dose levels. Tetrachloroethylene appears to be a lower-affinity substrate for CYP enzymes than trichloroethylene (Völkel et al., 1998; Ohtsuki et al., 1983). In vitro, the Michaelis-Menten constant (K_m) value for tetrachloroethylene is reported to be 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. Comparisons of potencies for kidney cancer are more difficult because there is a lack of studies on these compounds using comparable species/strains and routes of exposure.

3.3.5.2. Cytochrome P450 (CYP)-Mediated Oxidation

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 it is also an excretion product of trichloroethylene, whereas TCOH is the major trichloroethylene urinary excretion product. As discussed previously in Section 3.3.3.1, the formation of chloral and TCOH in metabolism of tetrachloroethylene is not likely to be significant. Therefore, 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, but more likely directly from chlorine migration of the Fe-O intermediate. 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.

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/decreased hepatic metabolism following treatment with agents now known to selectively induce/inhibit CYP3A and/or CY2B specifically.

3.3.5.3. Glutathione (GSH) Conjugation Pathway

The GSH-dependent pathway for tetrachloroethylene exists in both rodents and humans, and the pathway is also operative for trichloroethylene in these species (Völkel et al., 1998; Birner et al., 1996). The flux through this pathway at experimental or occupational exposures is thought to be quantitatively less than that through the P450 pathway, although direct evidence is lacking, particularly in humans. 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 (Lash and Parker, 2001; Lash et al., 2000; Dekant et al., 1989; Anders et al., 1988). For both compounds, recovery of urinary mercapturates, the stable end-products of the GSH pathway, comprises 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 dichlorovinyl cysteine and their respective *N*-acetylated forms derived from trichloroethylene might also act as substrates for renal β-lyases and other enzymes such as FMO3 and CYP3A [(Lash and Parker, 2001; Lash et al., 2000; Anders et al., 1988; Dekant et al., 1988) reviewed by (Dekant et al., 1989)] (refer to Section 3.3.3). 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).

3.3.5.4. 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. Interestingly, although tetrachloroethylene is not as extensively oxidized

as trichloroethylene, they have similar potency for liver tumors, with which oxidative metabolism is associated. TCA, DCA, chloral, and TCOH are reported P450 biotransformation products of both tetrachloroethylene and trichloroethylene; however, only TCA predominates for tetrachloroethylene, whereas TCOH predominates for trichloroethylene. In addition, DCA is likely formed via GSH conjugation for tetrachloroethylene and via oxidation for trichloroethylene. The fact that the two compounds produce different reactive intermediate P450 metabolites is also important to consider. Excretion of urinary mercapturates suggests 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 because 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 tumorigenesis, 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.

3.4. EXCRETION

Tetrachloroethylene is eliminated from the body by pulmonary excretion of the parent compound and urinary excretion of metabolism products, with a small amount of pulmonary excretion of metabolism products. Tetrachloroethylene that is not metabolized is exhaled unchanged, and this process is the primary pathway of tetrachloroethylene excretion in humans for all routes of administration (Opdam and Smolders, 1986; Koppel et al., 1985; Monster et al., 1979; Stewart et al., 1977; Guberan and Fernandez, 1974; Stewart et al., 1974; Stewart et al., 1970; Stewart and Dodd, 1964; Stewart et al., 1961). Pulmonary excretion of (unchanged) parent compound is also important in animals (Bogen et al., 1992; Frantz and Watanabe, 1983; Schumann et al., 1980; Pegg et al., 1979; Yllner, 1961). A very small amount of tetrachloroethylene has been shown to be excreted through the skin (Bolanowska and Golacka, 1972); however, it represents an insignificant percentage of total tetrachloroethylene disposition.

Pulmonary excretion 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/minute initially and decreased to 3.8 L/minute after 4 hours (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/minute at rest. Pulmonary excretion 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 12−16, 30−40, and 55−65 hours, 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 EI, 1963), is independent of the body burden of tetrachloroethylene, indicated by parallel blood and exhaled air concentration decay curves. 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 the time curve of the exhaled chemical, varies from 5−20 minutes for the first phase of elimination up to approximately 50 hours 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 hours.

Urinary and pulmonary clearances of metabolism products of tetrachloroethylene provide other means of excretion. The mean half-time of urinary excretion for total trichloro-compounds for 13 subjects exposed to tetrachloroethylene was determined to be 144 hours (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 percentage of the total absorbed dose of tetrachloroethylene in humans (Völkel et al., 1998; ATSDR, 1997a). Urinary excretion of TCA (or total trichloro-compounds) was estimated to be only 1−3% in balance studies conducted in humans (Chiu et al., 2007; Monster et al., 1983; Monster and Houtkooper, 1979; Monster et al., 1979; Fernandez et al., 1976; Essing et al., 1973; Ikeda et al., 1972; Stewart et al., 1970; Boettner and Muranko, 1969; Stewart et al., 1961), with urinary excretion of GSH-derived metabolism products representing an even smaller fraction (Völkel et al., 1998). However, these studies did

not follow urinary excretion for more than 3−7 days, and it is possible that a larger percentage of the tetrachloroethylene dose was eventually excreted in urine. In studies that also measured pulmonary excretion, the entire dose was not always accounted for in the sum of exhaled tetrachloroethylene and urinary excretion of TCA (Chiu et al., 2007; Monster et al., 1979). Part of the dose may be metabolized to biotransformation products that were not measured, including oxidative products such as carbon monoxide, carbon dioxide, or oxalic acid, and GSH conjugation products such as sulfoxides and reactive thiols (refer to Section 3.3). In addition, the lowest exposures in these studies were around 1 ppm in air (Chiu et al., 2007), which is several orders of magnitude higher than ambient environmental exposures.

In laboratory animals, there is both a species- and dose-dependence to the amount of pulmonary excretion of unchanged tetrachloroethylene that reflects the degree of metabolism (Dallas et al., 1994b; Bogen et al., 1992; Schumann et al., 1980; Pegg et al., 1979). 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 (Schumann et al., 1980; Pegg et al., 1979). 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 metabolism of tetrachloroethylene is greater in mice than in rats, so conversely, the amount of pulmonary excretion is greater in rats than in mice.

3.5. TOXICOKINETIC MODELING

Understanding tetrachloroethylene toxicokinetics is critical to both the qualitative and quantitative assessment of human health risks from environmental exposures. A number of the neurotoxic effects of tetrachloroethylene appear well correlated with parent compound concentrations at the target site (Bushnell et al., 2005), so characterizing tetrachloroethylene blood or tissue concentrations can aid in performing risk assessment-related extrapolations, such as between rodents and humans or between exposure routes. In addition, understanding tetrachloroethylene metabolism is especially important toxicologically because specific metabolites or metabolic pathways are associated with a number of endpoints of observed toxicity. A more detailed discussion of the evidence for these associations, the specific metabolites involved, and identification of the most appropriate dose metric are provided in Section 5.

3.5.1. Choice of Physiologically Based Pharmacokinetic (PBPK) Model for Use in Dose-Response Modeling

3.5.1.1. Limitations of Previously Developed Physiologically Based Pharmacokinetic (PBPK) Models

A large number of PBPK models have been developed for tetrachloroethylene toxicokinetics in both rodents and humans for various purposes. PBPK models can provide estimates of tissue concentration as well as total metabolism of tetrachloroethylene. An overview of the models in literature is provided below—the aim of which is not to exhaustively cover all of the models in the literature—but rather to capture the different assumptions made, the range of data that has been used, and to indicate that these assumptions limit the ability of the models to predict relevant tetrachloroethylene metabolite levels in humans.

Chen and Blancato (1987) developed a PBPK model for rats, mice, and humans. The metabolic parameters maximum velocity (V_{max}) and 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.

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. 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-hour 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 postexposure radioactive metabolites recovered from all excreta and carcass homogenates. The metabolic parameters for humans were estimated 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. 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

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scaled to humans. The values obtained by Reitz et al. (1996) through both these independent methods were comparable.

Rao and Brown (1993) developed a human PBPK model for the purpose of investigating neurotoxicological endpoints. 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 V_{max} (allometrically scaled by body-weight$^{3/4}$) and K_m (considered invariant across species). Other parameters 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).

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 tetrachloroethylene chamber concentration 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.[2] 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. (1986c). 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 0.49−0.59 in rats and from 0.56−0.66 in mice for exposure concentrations that varied by two orders of magnitude.

Clewell et al. (2005) evaluated and extended the Gearhart et al. (1993) model further, using tetrachloroethylene blood concentrations and urinary and blood TCA data gathered by Volkel et al. (1998) on human subjects exposed to tetrachloroethylene concentrations of 10−40 ppm for 6 hours. They included metabolism of tetrachloroethylene in the kidney, allowing for excretion directly into urine. By assuming metabolism in this organ to be at 10% of the capacity of the liver, they obtained substantial improvement in the agreement with experimental data on urinary excretion of TCA. An advantage in using the Volkel et al. (1998)

[2]Details pertaining to the derivation of parameters for metabolism in humans are not provided in the original paper but are available in a review by Clewell et al. (2005).

data is that they pertain to exposure concentrations that are lower than those in other studies [e.g., 72−144 ppm in Monster (1979)].

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 (personal communication 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).

In addition, a number of PBPK models were developed only in humans, primarily to characterize uncertainty and/or human variability. To assess intraindividual variability in uptake and elimination over multiple exposure levels and scenarios, Chien (1997) collected exhaled breath measurements on a single individual following four different exposure scenarios in a controlled environmental facility (twelve, 30 or 90 minute exposures ranging from 0.5−3 ppm in concentration) and following tetrachloroethylene exposure in 22 dry-cleaning facilities, where ambient levels of tetrachloroethylene were recorded and exposures were carefully timed.

Bois et al. (1996), which was updated by Chiu and Bois (2006), used a Bayesian analysis in conjunction with a PBPK model that was structurally similar to that used by Reitz et al. (1996) and that was only calibrated to the parent compound data (blood and exhaled breath) of the individuals in Monster et al. (1979). The shape of the prior distribution was observed 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 observed to fall within the 95th percentile envelope of predictions. The exposure concentrations in this study were 5−100 times lower than those used in the Monster et al. (1979) study; thus, this comparison provides further weight to the strength of the model.

Covington et al. (2007) applied the same methodology to the Clewell et al. (2005) human PBPK model, using additional data on the parent compound tetrachloroethylene and urinary excretion data of its metabolite TCA (Monster et al., 1979; Fernandez et al., 1976), with a range of exposure concentrations from 10−150 ppm. However, TCA blood concentrations from Monster et al. (1979) were dropped from the analysis because the authors, in preliminary calculations using a one-compartment PBPK model for TCE from Clewell et al. (2000), were unable to reproduce the urinary excretion of TCA using the blood concentration data on TCA from the same study. In addition, Covington et al. (2007) used only grouped data from both these studies because the individual data were not available to them.

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The Covington et al. (2007) analysis was revisited by Qiu et al. (2010), with the following modifications:

1. A brain compartment was added.

2. Human kinetic data from Chiu et al. (2007) and Chien (1997) were used in addition to the data used by Covington et al. (2007): namely Fernandez et al. (1976), Monster et al. (1979), and Volkel et al. (1998). Thus, the human exposures used in the Qiu et al. (2010) modeling range from 0.5−150 ppm.

3. Individual human data were used. However, blood TCA measurements from Volkel et al. (1998) were not used, which the authors stated was because blood samples could not be matched with individuals' urine samples and because there were not enough data points to inform the time course for blood TCA. In addition, none of the TCA data from Monster et al. (1979) were used.

4. Correlation between parameters (such as between cardiac output and alveolar ventilation or between V_{max} and K_m) was addressed by reparameterization.

5. Adjustment factors were added to maintain mass balance among fractional blood flows and fractional tissue volumes.

These models provide substantially similar estimates of the tetrachloroethylene concentration in the tissue. For example, as illustrated in Figures 3-2 and 3-3, estimated venous blood concentration and alveolar concentration of tetrachloroethylene were in agreement to within a factor of 2.0 among various models and experiment. However, the same models have different approaches to estimating the metabolic parameters, thereby differing hugely in their prediction of the amount metabolized at low dose—as shown in Figure 3-4 [adapted from (Chiu et al., 2007)]. These differences have major implications for the quantitative risk assessment and represent the key controversy surrounding the application of PBPK models to tetrachloroethylene toxicokinetics.

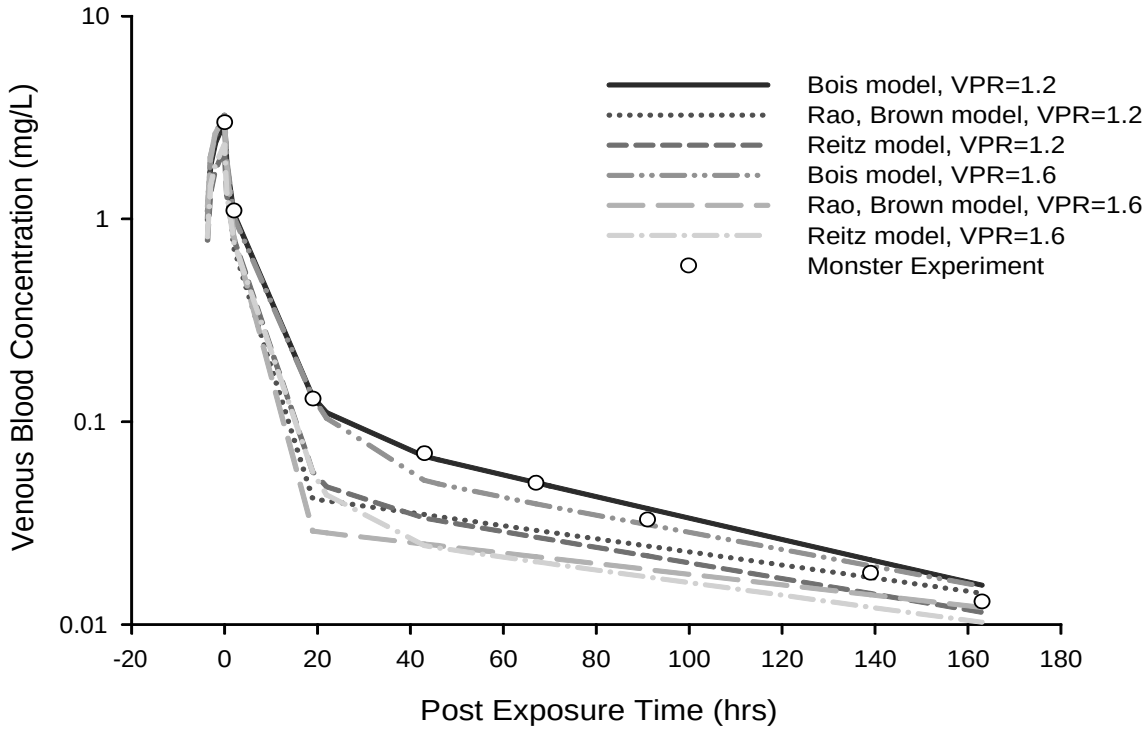

Figure 3-2. Comparison of model predictions for blood concentration with experiment.

PCE inhaled concentration was 72 ppm. Predictions are at different ventilation-to-perfusion ratios and at an alveolar ventilation rate of 7 L/minute (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 hours, respectively). Experimental data adapted from Monster et al. (1979).

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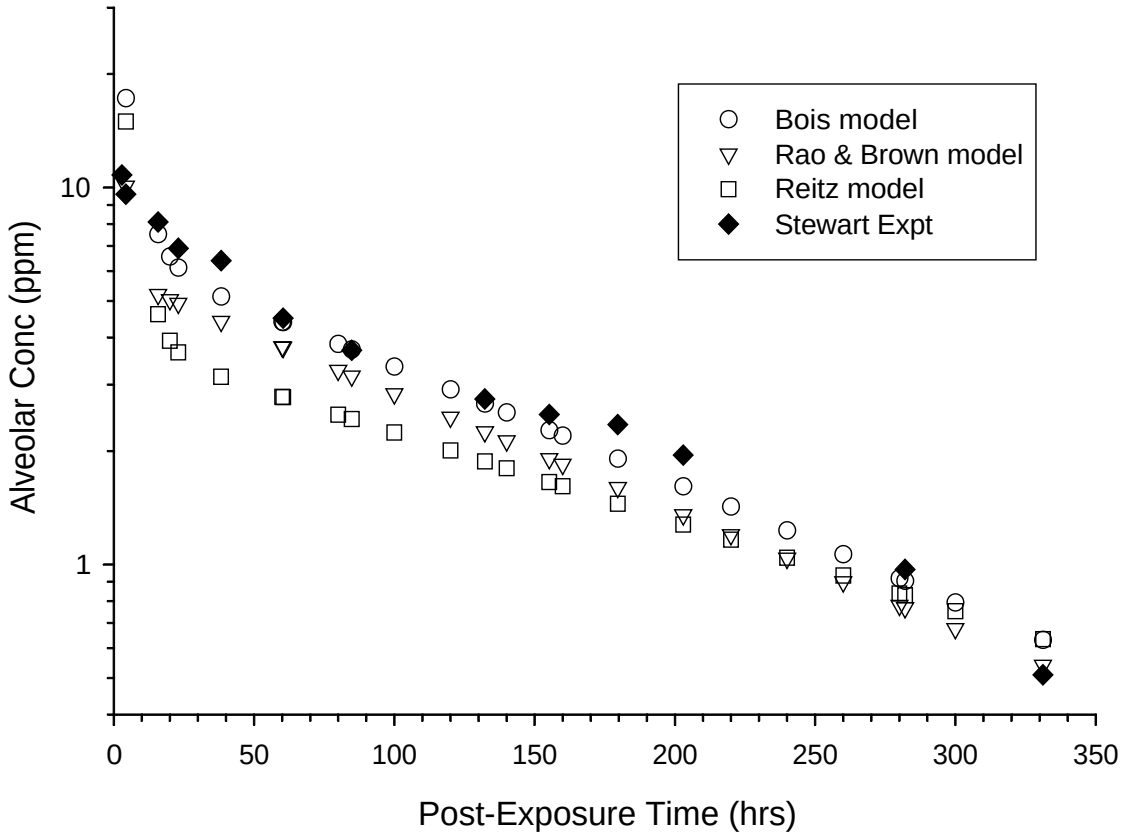

Figure 3-3. Comparison of model predictions for alveolar concentration of tetrachloroethylene with experimental data on humans.

Inhaled concentration is 100 ppm, 7 hours/day, for 5 days, and predictions assume alveolar ventilation rate of 5.02 L/minute and a ventilation-to-perfusion ratio of 1.0. Experimental data show mean alveolar concentration in subjects in Stewart et al. (1970). Some points early in the time course were deleted because of difficulty in obtaining numerical values from the author's plot.

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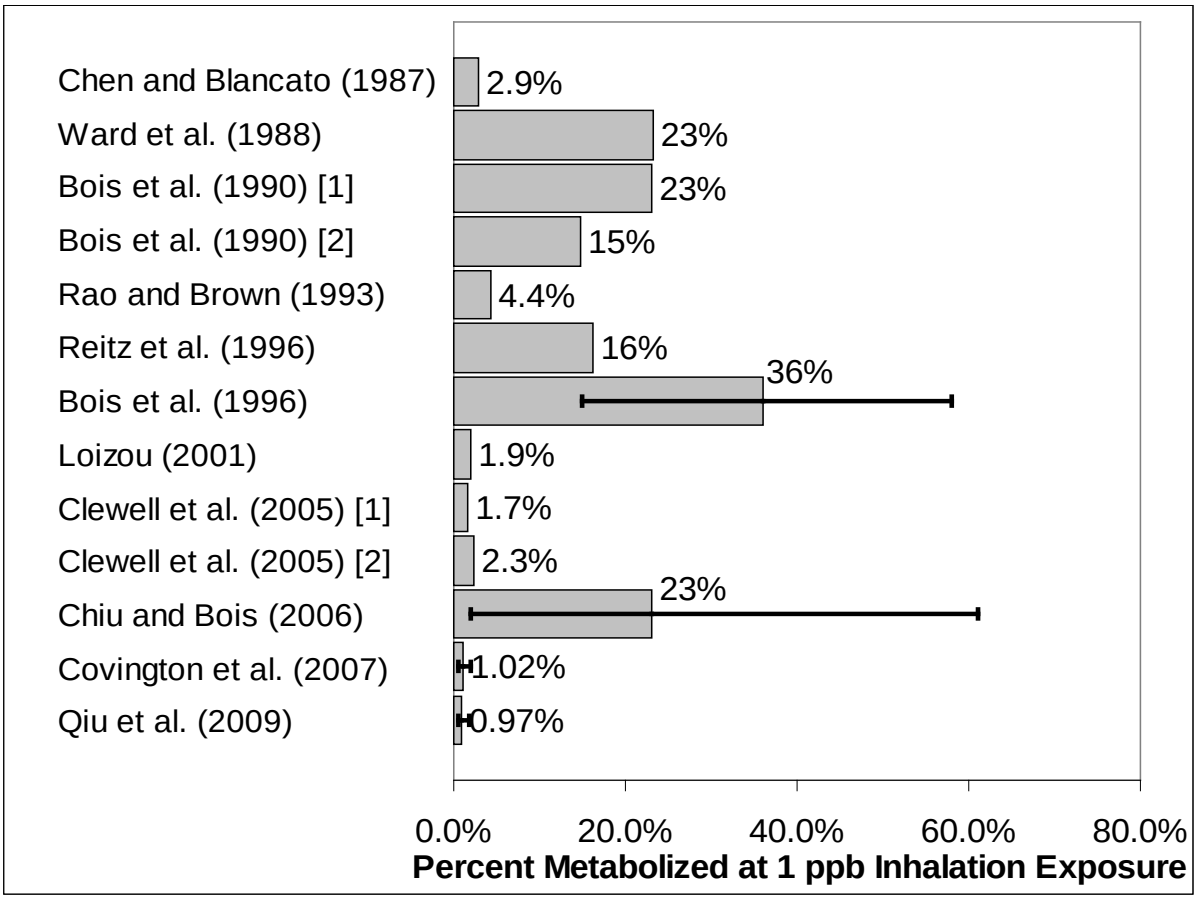

Figure 3-4. Previously published estimates for the total amount of tetrachloroethylene metabolized at 0.001 ppm (1 ppb) continuous inhalation exposure.

All estimates are point estimates except for Bois et al. (1996) and Chiu and Bois (2006), which are estimates of combined uncertainty and population variability (95% confidence intervals [CIs]), and Covington et al. (2007) and Qiu et al. (2010), which are estimates of uncertainty in the population mean (90% CIs).

The various analyses in Figure 3-4 for tetrachloroethylene have a number of key limitations. First, in no case have all the available data in mice, rats, and humans been considered together in a single analysis. Thus, the extent to which different results reflect use of different data sets and model structures is unclear. Moreover, while all the models estimate total metabolism, those estimates are based on different types of data—in some cases, disappearance of the parent compound, and in other cases, TCA and, therefore, oxidation—none of which address GSH conjugation. These limitations and the above-mentioned controversy were also noted in the National Research Council (NRC) report *Review of the Environmental Protection Agency's Draft IRIS Assessment of Tetrachloroethylene* (NRC, 2010). In particular, NRC concluded that, while a number of PBPK models have been developed for tetrachloroethylene, they all have some key limitations that reduce the confidence with which they can be used for

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risk assessment. NRC (2010) recommended the development of a "harmonized" PBPK model that would integrate previous models and data. They pointed to the availability of in vitro and in vivo data relevant to the GSH conjugation pathway, and they recommended exploring the possibility of adding the GSH pathway to a harmonized PBPK model. This is important because tetrachloroethylene causes tubular toxicity in mice and rats in the kidney, and is associated with small increases in the incidences of kidney tumors reported in multiple strains of tetrachloroethylene-exposed rats (JISA, 1993; NTP, 1986). These effects are thought to be associated with the tetrachloroethylene metabolism by GSH conjugation based on the production of nephrotoxic and genotoxic metabolites in the kidney from this pathway (Lash and Parker, 2001).

3.5.1.2. The Chiu and Ginsberg (2011) Model

In response to this advice from the NRC, another PBPK model was developed by Chiu and Ginsberg (2011). This model was developed to address many of the limitations of the existing models for tetrachloroethylene, discussed above. Among the most important improvements are (1) the utilization of all the available toxicokinetic data for tetrachloroethylene and its metabolites in mice, rats, and humans; (2) the incorporation of available information on the internal toxicokinetics of TCA derived from the most current PBPK modeling of trichloroethylene and TCA; and (3) the separate estimation of oxidative and conjugation metabolism pathways. Therefore, this assessment utilizes the Chiu and Ginsberg (2011) model to calculate relevant dose metrics to be used in dose-response modeling. An overview of this model follows below.

In developing this model, first, a comprehensive literature search was made of relevant toxicokinetic studies, and the available toxicokinetic data were digitized. These data were further separated into "calibration" and "validation" data sets utilizing a wider range of data than any previous analysis alone. Second, a harmonized PBPK model structure was developed that separately tracked tetrachloroethylene oxidation and GSH conjugation. The Chiu and Ginsberg (2011) model includes a comprehensive analysis of TCA dosimetry originally developed by the primary author for TCE, and it includes the urinary excretion kinetics of the metabolites NAcTCVC and DCA. The Chiu and Ginsberg (2011) model is described by the schematic below. The reader is referred to Chiu and Ginsberg (2011) for further details of the model structure.

The model structure and parameters (shown in Figure 3-5) used in the Chiu and Ginsberg (2011) harmonized model differed from other human models along the following lines:

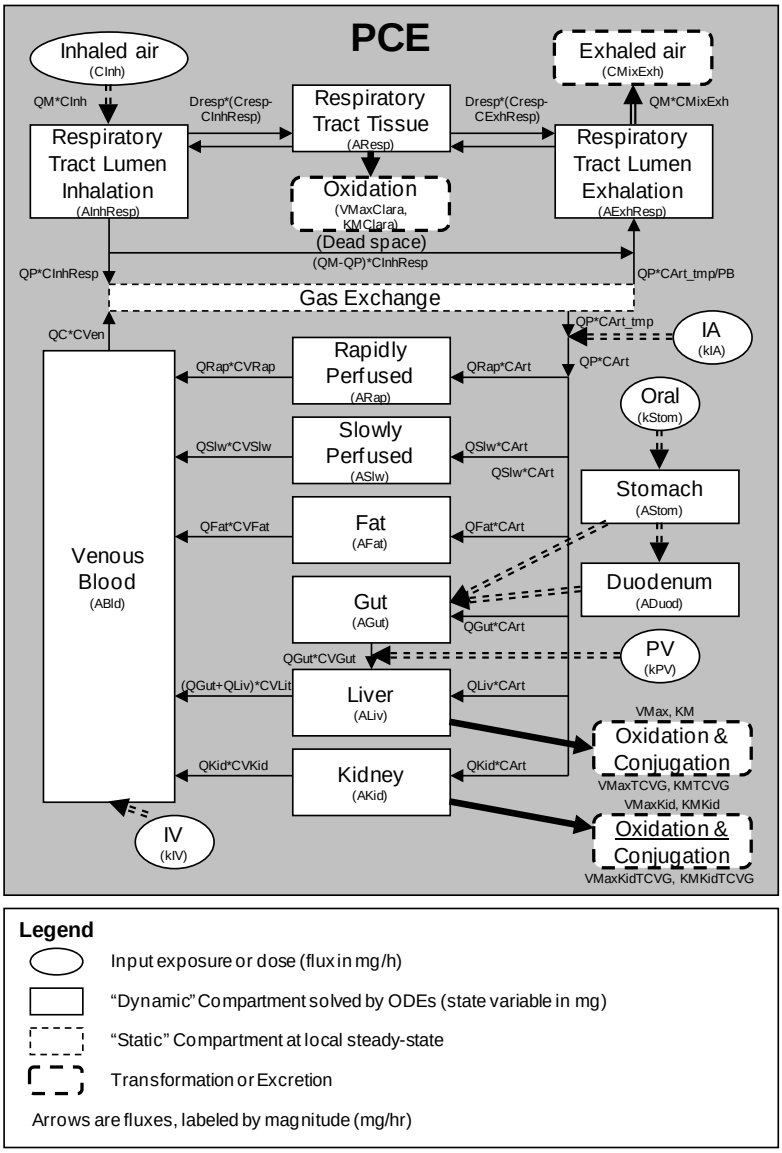

Figure 3-5. Overall structure of updated physiologically based pharmacokinetic (PBPK) model for tetrachloroethylene and metabolites.
Boxes with underlined labels are additions or modifications of the Chiu et al. (2009) model for trichloroethylene, and are described in the Toxicological Review of Trichloroethylene (U.S. EPA, 2011b). "Inputs" are exposures/doses by which tetrachloroethylene enters the body, and lead to a flux of the chemical into various body compartments. Dynamic compartments are those solved by ordinary differential equations (ODEs). "Static" compartments are those solved by algebraic equations, and lead to instantaneous changes in the amount of tetracholoroethylene in the compartment. Transformation or excretion represents fluxes of compounds leaving a compartment for another compartment (if any) via metabolism or excretion.

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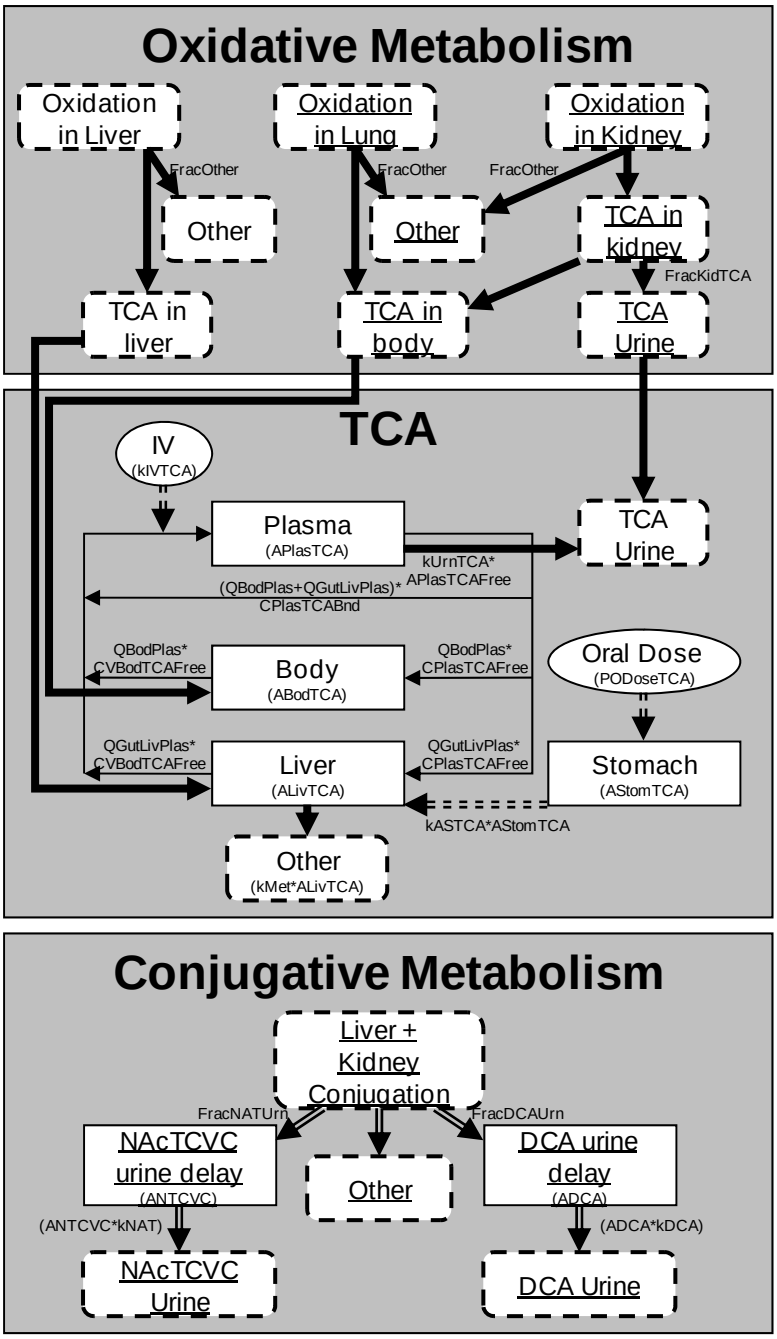

Figure 3-5. (Continued) Overall structure of updated physiologically based pharmacokinetic (PBPK) model for tetrachloroethylene and metabolites.

- All the available data on mice, rats, and humans were considered together in a single analysis.

- The wash-in–wash-out process in the lung was included.

- Oxidative metabolism in the lung was included.

- The model explicitly addressed GSH conjugation of tetrachloroethylene in the liver and kidney.

- The urinary data on DCA (Völkel et al., 1998) were included so as to be able to consider separate β-lyase-dependent and β-lyase-independent pathways for the bioactivation of TCVC in the GSH conjugation pathway.

- An empirical "delay" parameter (whose value was "fitted") was added for urinary excretion of DCA and NAcTCVC and represented a "lumped" delay in the time course due to the processes of formation, urinary excretion, and other clearance pathways.

- For tetrachloroethylene oxidation, metabolic parameters were obtained from four in vitro studies. These consisted of data from microsomes or cells from the liver and microsomes from the kidney (Lash et al., 2007; Reitz et al., 1996; Costa and Ivanetich, 1984, 1980).

- A fraction of tetrachloroethylene oxidation was assumed to form compounds other than TCA. A baseline value of 10% was used for the fraction not forming TCA.[3]

- GSH conjugation metabolic parameters were obtained from four studies that measured tetrachloroethylene GSH conjugation in vitro (Lash et al., 2007; Dekant et al., 1998; Lash et al., 1998; Green et al., 1990). These studies were utilized to select a baseline value for metabolic clearance along this pathway in all species.

- The model incorporated all in vivo data considered in the literature for the PBPK modeling of tetrachloroethylene and metabolites, dividing these data into two groups, one for model calibration, and the other for model validation. These data included short and long dosing periods.

- A full Bayesian uncertainty/variability analysis was not performed. The limited Bayesian analysis involving flat priors and making inferences only using posterior modes was used for the estimation of a limited number of metabolism parameters for which there was significant discrepancy between baseline predictions (using baseline values of these parameters) and in vivo data related to metabolism [refer to Table A-1 of Chiu and Ginsberg (2011) and associated text for rationale]. The Markov Chain Monte Carlo (MCMC) approach was used for optimization.[4]

[3]In vitro data measuring TCA alone; TCA along with chloral hydrate, TCOH, and DCA; and total water soluble metabolites are all generally found to be consistent with each other.
[4]The Markov Chain Monte Carlo (MCMC) method provides an algorithm to sample from a desired probability distribution—in this case, the likelihood function—the output of which is a sequence of samples—the "Markov

- The model structure allowed it to be used to calculate internal dose metrics for inhaled and oral exposure to tetrachloroethylene for mice, rats, and humans. Thus, the analysis could be used for route-to-route extrapolation or interspecies extrapolation, comparison of parent and metabolite toxicity based on a common internal dose metric, and investigation of the shape of the dose-response curve. The following dose metrics could be determined using this model, and the confidence with which it can make predictions for internal dose metrics of interest was further evaluated by the authors:
    - Daily area-under-the-curve of tetrachloroethylene in blood
    - Fraction of tetrachloroethylene intake metabolized by oxidation
    - Fraction of tetrachloroethylene intake metabolized by GSH conjugation
    - Equivalent daily production of TCA per kg body weight.[5]

3.5.1.2.1. Estimated human parameter values for oxidation and conjugation in Chiu and Ginsberg (2011)

The results for all estimated parameters are shown in Table 3-1. The estimated metabolism parameters for oxidation and conjugation, which are of particular interest, are briefly addressed here, and the reader is referred to the original paper for further details on these and other parameter estimations. Figure 3-6 compares the in vivo predictions for hepatic metabolism with available in vitro data. For oxidation, in mice and rats, the optimized values are about an order of magnitude higher than baseline values, whereas in humans, the optimized values are quite similar to baseline values. However, they do not appear unreasonable compared to the limited data available from other related compounds (TCE and some halomethanes), as shown in Figure 3-6. For example, the linear rates are lower than those for TCE, which is known to be more extensively oxidized by P450s than tetrachloroethylene. At higher substrate concentrations, the predicted rate of oxidation of tetrachloroethylene in mice and humans is greater than that for TCE, but this is an artifact of the assumption of a linear rate necessitated by KM being unidentifiable. For GSH conjugation, the range of the in vitro data is quite wide, especially when also taking into considering data from other compounds (refer to Figure 3-6). In mice and rats, the in vitro data on tetrachloroethylene GSH conjugation (filled symbols in Figure 3-6) span the range of estimates from optimization to in vivo data. For humans, the in vitro data

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chain," or "chain" for short. Each "chain" has a random starting point. In order to capture the potential uncertainty due to different starting points, 24 chains with different starting points were run for mice and rats, and 48 chains were run for humans. The posterior mode from each chain was determined—i.e., the "chain-specific posterior modes." Then, the highest posterior model among the 24 (or 48) chains was determined—i.e., the "overall posterior model," or simply the "posterior mode." GNU MCSim version 5.0.0 was used for computations. A PDF of the model code is available as Supplementary Materials (Chiu and Ginsberg, 2011), and simulation files are available in a WinZIP archive (U.S. EPA, 2011a).

[5] TCA produced in the kidney and excreted directly to urine was not included, because it does not reach any target organ (i.e., the liver) or enter systemic circulation.

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only consist of nondetects from Dekant et al. (1998), which, if assumed to be half the detection limit, are more consistent with the alternative posterior modes. Overall, however, the ranges of predicted rates for tetrachloroethylene are consistent with the range inferred from halomethanes, and the in vivo optimized values do not appear to be substantially outside the bounds suggested by available in vitro data.

Chiu and Ginsberg (2011) observe that, overall, the fits to the data and validation were generally within threefold of the observed data, and more consistently so for rats and humans, given the inter- and intraindividual variability [refer to Supplementary Materials (Chiu and Ginsberg, 2011) in their paper]. In a few cases, the discrepancies were larger, but did not exceed an order of magnitude. To a large extent, the discrepancies in model fits reflected variability. There was, for instance, difficulty in fitting the time course of TCA in mice and the fraction of retained tetrachloroethylene exhaled.

3.5.1.2.2. Dose metric predictions based on posterior modes

Tables 3-2−3-5 summarize the PBPK model dose metric predictions (listed in the previous subsection) based on the baseline, overall posterior mode, and chain-specific posterior mode parameters. The uncertainty due to the distribution of chain-specific posterior modes contributes to the overall uncertainty in the predicted dose metric. The blood tetrachloroethylene dose metric has by far the least amount of this "sampling" uncertainty. This appears to be true across all species, routes of exposure, and exposure levels. The dose metrics with the next lower amount of sampling uncertainty are tetrachloroethylene oxidation and TCA formation. The predictions for GSH conjugation are more uncertain. In the rat, the ranges of chain-specific posterior modes span up to twofold, and in mice, up to 10-fold. However, in humans, the ranges span about 3,000-fold, discussed above.

3.5.1.2.3. Overall pertinent conclusions on tetrachloroethylene dosimetry

Chiu and Ginsberg (2011) also presented detailed sensitivity analyses that enable determination of the confidence with which a particular dose metric can be estimated [refer to Table 9 and Supplementary Materials (Chiu and Ginsberg, 2011) in their paper]. These have to be analyzed together with the residuals for error in calibration and validation (refer to Table 8 of their paper) and the ranges in the values of the predicted dose metrics (presented above in Tables 3-2−3-5) to obtain perspective on the overall uncertainty in the PBPK model predictions. Table 3-6 summarizes the various measures that may contribute to this overall uncertainty.

Table 3-1. Log-likelihood and parameters after calibration

Parameter	Baseline	Postcalibration (posterior mode)	GSD of posterior modes across chains	Range of posterior modes across chains
Mouse				
Ln (Likelihood)	-	−1,780	-	−1808–1780
QP (L/hr)	2.09	2.89	1.03	2.86–3.22
V_{max} (mg/hr) (saturable oxidation pathway)	0.23	0.026	1.16	0.022–0.0369
K_m (L/hr) (saturable oxidation pathway)	88.6	0.417	1.28	0.338–0.892
V_{max2}/K_{m2} (L/hr) (linear oxidation pathway)	–	0.0188	1.05	0.0165–0.0207
V_{max}TCVG/K_mTCVG (L/hr) (linear conjugation pathway)	0.656	6.83E-05	3.83	3.05e-05–0.00179
kMetTCA (/hr)	1.48	0.638	1.05	0.56–0.695
kUrnTCA (/hr)	2.93	1.26	1.05	1.11–1.38
Rat				
Ln (Likelihood)	-	−1314	-	−1321–−1314
QP (L/hr)	10.2	6.31	1.02	6.28–6.68
V_{max} (mg/hr) (saturable oxidation pathway)	0.256	0.87	1.37	0.415–1.93
K_m (L/hr) (saturable oxidation pathway)	69.7	31.1	1.39	14.8–71.9
V_{max}TCVG/K_mTCVG (L/hr) (linear conjugation pathway)	2.22	0.00204	1.27	0.00131–0.00355
kDCA (/hr)	-	0.129	1.65	0.0758–0.451
FracNATUrn	-	0.0143	1.29	0.00919–0.0253
FracDCAUrn	-	0.702	1.26	0.43–0.98

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Table 3-1. Log-likelihood and parameters after calibration (continued)

Parameter	Baseline	Postcalibration (posterior mode)	GSD of posterior modes across chains	Range of posterior modes across chains
Human				
Ln (Likelihood)	-	1,828	-	1,790–1,828
QP (L/hr)	372	476	1.1	450–640
VMax/KM (L/hr) (linear oxidation pathway)	0.353	0.454	1.08	0.346–0.468
VMaxKid/KMKid (L/hr) (linear oxidation pathway)	0.00076	0.0947	1.09	0.0702–0.105
VMaxTCVG/KMTCVG (L/hr) (linear conjugation pathway)	0.0196	5.26	17.1	0.00194–5.48
kNAT (/hr)	-	0.28	1.07	0.228–0.293
FracNATUrn	-	0.000482	15.8	0.000472–1.00
FracDCAUrn	-	0.00022	18.5	1.12e-05–0.442

GSD = geometric standard deviation.

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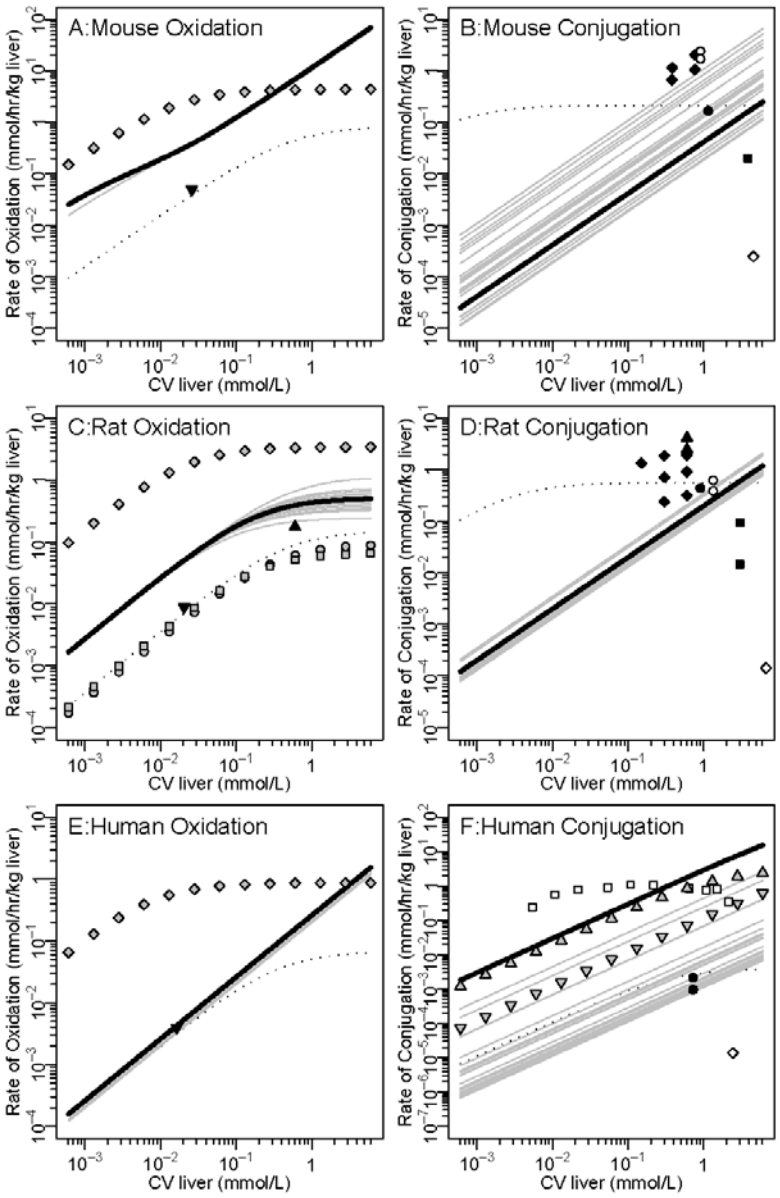

Figure 3-6. Comparison of mouse (A-B), rat (C-D), and human (E-F) rates of hepatic oxidation (A, C, and E) or conjugation (B, D, and F) measured in vitro (symbols) and predicted by the model (lines).

Data shown consist of measurements of tetrachloroethylene in vitro oxidation and conjugation [solid circle: Dekant et al. (1998); solid square: Green et al. (1990); solid diamond: Lash et al. (1998); solid triangle: Lash et al. (2007); solid upside-down triangle: Reitz et al. (1996) reported fits of in vitro tetrachloroethylene V_{max} and K_m for oxidation [grey-filled circle: Costa and Ivanetich (1980); grey-filled square: Costa and Ivanetich (1984); grey-filled diamond: Lipscomb et al. (1998), TCE; grey-filled triangle: Wheeler et al. (2001) CH_2I_2; grey-filled upside-down triangle: Wheeler et al. (2001), CH_2Cl_2]; and measurements of TCE in vitro conjugation [open circle: Lash et al. (1998); open square: Lash et al. (1999); open diamond: Green et al. (1997)]. Model predictions are using baseline parameters (dotted line), overall posterior mode parameters (solid thick line), and alternative posterior mode parameters (grey lines).

Table 3-2. Predictions for area-under-the-curve of tetrachloroethylene in blood (mg-hr/L-day per ppm in air or mg-hr/L-day per mg/kg-day oral intake) using posterior mode parameters

Species/continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Mouse				
0.01 ppm	1.2	2.13	1.03	2.11–2.42
0.1 ppm	1.2	2.13	1.03	2.12–2.42
1 ppm	1.26	2.18	1.02	2.16–2.44
10 ppm	1.73	2.43	1.01	2.39–2.53
100 ppm	2.8	2.64	1	2.64–2.68
1,000 ppm	2.98	2.68	1	2.67–2.72
0.01 mg/kg-day	0.0217	0.104	1.06	0.0957–0.126
0.1 mg/kg-day	0.0218	0.104	1.06	0.0958–0.126
1 mg/kg-day	0.0221	0.105	1.06	0.0965–0.127
10 mg/kg-day	0.0265	0.112	1.05	0.103–0.129
100 mg/kg-day	0.168	0.152	1.03	0.138–0.152
1,000 mg/kg-day	0.296	0.178	1.03	0.159–0.18
Rat				
0.01 ppm	1.03	2.25	1	2.25–2.27
0.1 ppm	1.03	2.25	1	2.25–2.27
1 ppm	1.04	2.25	1	2.25–2.27
10 ppm	1.11	2.25	1	2.25–2.27
100 ppm	2	2.29	1	2.28–2.32
1,000 ppm	2.4	2.39	1.01	2.36–2.42
0.01 mg/kg-day	0.0737	0.852	1.02	0.807–0.86
0.1 mg/kg-day	0.0738	0.852	1.02	0.807–0.86
1 mg/kg-day	0.0744	0.852	1.02	0.807–0.86
10 mg/kg-day	0.0816	0.854	1.02	0.809–0.861
100 mg/kg-day	0.23	0.864	1.02	0.821–0.869
1,000 mg/kg-day	0.543	0.912	1.02	0.869–0.919

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Table 3-2. Predictions for area-under-the-curve of tetrachloroethylene in blood (mg-hour/L-day per ppm in air or mg-hour/L-day per mg/kg-day oral intake) using posterior mode parameters (continued)

Species/continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Human				
0.01 ppm	2.35	2.03	1.05	2.01–2.36
0.1 ppm	2.35	2.03	1.05	2.01–2.36
1 ppm	2.35	2.03	1.05	2.01–2.36
100 ppm	2.35	2.03	1.05	2.01–2.36
1,000 ppm	2.35	2.03	1.05	2.01–2.36
0.01 mg/kg-day	2.37	2.04	1.05	2.01–2.36
0.1 mg/kg-day	2.71	1.74	1.03	1.58–1.82
1 mg/kg-day	2.71	1.74	1.03	1.58–1.82
10 mg/kg-day	2.71	1.74	1.03	1.58–1.82
100 mg/kg-day	2.71	1.74	1.03	1.58–1.82
1,000 mg/kg-day	2.72	1.74	1.03	1.58–1.82

GSD = geometric standard deviation.
Dose metrics calculated based on daily average given continuous exposure for 100 weeks. Baseline parameters are based on in vitro data for metabolic parameters, and literature values for other parameters. Posterior modes are based on the updated parameters listed in Table 3-1.

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Table 3-3. Predictions for fraction of tetrachloroethylene oxidized by cytochrome P450 (P450s) (mg/kg-day oxidized per mg/kg-day intake) using posterior mode parameters

Species/continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Mouse				
0.01 ppm	0.00252	0.188	1.1	0.12–0.192
0.1 ppm	0.00254	0.187	1.09	0.12–0.191
1 ppm	0.00269	0.174	1.08	0.115–0.179
10 ppm	0.0062	0.118	1.06	0.0934–0.124
100 ppm	0.0141	0.0732	1.04	0.0632–0.075
1,000 ppm	0.00716	0.0664	1.05	0.0574–0.0688
0.01 mg/kg-day	0.00367	0.561	1.08	0.395–0.574
0.1 mg/kg-day	0.00368	0.561	1.08	0.395–0.574
1 mg/kg-day	0.00374	0.557	1.07	0.394–0.57
10 mg/kg-day	0.00445	0.524	1.07	0.38–0.535
100 mg/kg-day	0.0253	0.35	1.04	0.308–0.367
1,000 mg/kg-day	0.0361	0.239	1.03	0.216–0.25
Rat				
0.01 ppm	0.000501	0.0419	1.02	0.0387–0.042
0.1 ppm	0.000502	0.0419	1.02	0.0387–0.042
1 ppm	0.000514	0.0418	1.02	0.0386–0.0419
10 ppm	0.000662	0.0409	1.02	0.0379–0.0409
100 ppm	0.0025	0.0331	1.07	0.0263–0.0358
1,000 ppm	0.00153	0.011	1.27	0.00587–0.0181
0.01 mg/kg-day	0.00143	0.106	1.02	0.0988–0.107
0.1 mg/kg-day	0.00144	0.106	1.02	0.0988–0.107
1 mg/kg-day	0.00145	0.106	1.02	0.0987–0.107
10 mg/kg-day	0.00158	0.105	1.02	0.0977–0.105
100 mg/kg-day	0.00431	0.0934	1.04	0.0817–0.096
1,000 mg/kg-day	0.00686	0.0434	1.2	0.026–0.0631

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Table 3-3. Predictions for fraction of tetrachloroethylene in oxidized by cytochrome P450 (P450s) (mg/kg-day oxidized per mg/kg-day intake) using posterior mode parameters (continued)

Species/continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Human				
0.01 ppm	0.00971	0.0098	1.12	0.00694–0.0104
0.1 ppm	0.00971	0.0098	1.12	0.00694–0.0104
1 ppm	0.00969	0.0098	1.12	0.00694–0.0104
10 ppm	0.00955	0.0098	1.12	0.00694–0.0104
100 ppm	0.00828	0.0098	1.12	0.00694–0.0104
1,000 ppm	0.00355	0.0098	1.12	0.00693–0.0104
0.01 mg/kg-day	0.0173	0.0175	1.09	0.0134–0.0184
0.1 mg/kg-day	0.0173	0.0175	1.09	0.0134–0.0184
1 mg/kg-day	0.0173	0.0175	1.09	0.0134–0.0184
10 mg/kg-day	0.0169	0.0175	1.09	0.0134–0.0184
100 mg/kg-day	0.0138	0.0175	1.09	0.0134–0.0184
1,000 mg/kg-day	0.00492	0.0175	1.09	0.0133–0.0184

GSD = geometric standard deviation.
Dose metrics calculated based on daily average given continuous exposure for 100 weeks. Baseline parameters are based on in vitro data for metabolic parameters, and literature values for other parameters. Posterior modes are based on the updated parameters listed in Table 3-1.

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Table 3-4. Predictions for fraction of tetrachloroethylene conjugated with glutathione (GSH) (mg/kg-day conjugated per mg/kg-day intake) using posterior mode parameters

Species/continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Mouse				
0.01 ppm	0.348	0.000151	3.87	6.39e-05–0.00415
0.1 ppm	0.347	0.000152	3.87	6.43e-05–0.00417
1 ppm	0.337	0.000159	3.86	6.83e-05–0.0043
10 ppm	0.244	0.000207	3.81	8.95e-05–0.00523
100 ppm	0.0299	0.000251	3.79	0.000109–0.00642
1,000 ppm	0.00301	0.000258	3.79	0.000111–0.00663
0.01 mg/kg-day	0.929	0.000481	3.89	0.000208–0.0134
0.1 mg/kg-day	0.929	0.000481	3.89	0.000208–0.0134
1 mg/kg-day	0.928	0.000485	3.89	0.00021–0.0135
10 mg/kg-day	0.914	0.000521	3.87	0.000229–0.0141
100 mg/kg-day	0.454	0.000706	3.82	0.00031–0.0181
1,000 mg/kg-day	0.0485	0.000821	3.81	0.000362–0.0212
Rat				
0.01 ppm	0.303	0.00308	1.27	0.00195–0.00519
0.1 ppm	0.303	0.00308	1.27	0.00195–0.00519
1 ppm	0.301	0.00309	1.27	0.00195–0.0052
10 ppm	0.286	0.00309	1.27	0.00196–0.00521
100 ppm	0.0939	0.00316	1.27	0.002–0.00529
1,000 ppm	0.0099	0.00335	1.27	0.00213–0.00559
0.01 mg/kg-day	0.874	0.00783	1.27	0.00498–0.0133
0.1 mg/kg-day	0.874	0.00783	1.27	0.00498–0.0133
1 mg/kg-day	0.873	0.00783	1.27	0.00498–0.0133
10 mg/kg-day	0.861	0.00785	1.27	0.00499–0.0133
100 mg/kg-day	0.608	0.00795	1.27	0.00506–0.0134
1,000 mg/kg-day	0.078	0.00838	1.27	0.00535–0.0141

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Table 3-4. Predictions for fraction of tetrachloroethylene in conjugated with glutathione (GSH) (mg/kg-day conjugated per mg/kg-day intake) using posterior mode parameters (continued)

Species/continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Human				
0.01 ppm	0.000544	0.0936	17.5	3.16e-05–0.1
0.1 ppm	0.000543	0.0936	17.5	3.16e-05–0.1
1 ppm	0.000543	0.0936	17.5	3.16e-05–0.1
10 ppm	0.000535	0.0936	17.5	3.16e-05–0.1
100 ppm	0.000468	0.0935	17.5	3.16e-05–0.1
1,000 ppm	0.000207	0.0926	17.4	3.16e-05–0.0991
0.01 mg/kg-day	0.000972	0.177	17.1	6.47e-05–0.188
0.1 mg/kg-day	0.000972	0.177	17.1	6.47e-05–0.188
1 mg/kg-day	0.00097	0.177	17.1	6.47e-05–0.188
10 mg/kg-day	0.00095	0.177	17.1	6.47e-05–0.188
100 mg/kg-day	0.000788	0.177	17.1	6.47e-05–0.187
1,000 mg/kg-day	0.000289	0.175	17	6.47e-05–0.185

GSD = geometric standard deviation.
Dose metrics calculated based on daily average given continuous exposure for 100 weeks. Baseline parameters are based on in vitro data for metabolic parameters, and literature values for other parameters. Posterior modes are based on the updated parameters listed in Table 3-1.

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Table 3-5. Predictions for Trichloroacetic acid (TCA) produced systemically (mg/kg-day systemic TCA per ppm in air or mg/kg-day systemic TCA per mg/kg-day oral intake) using posterior mode parameters

Species/ continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Mouse				
0.01 ppm	0.0361	3.74	1.08	2.63–3.94
0.1 ppm	0.0363	3.71	1.08	2.62–3.9
1 ppm	0.0384	3.45	1.07	2.53–3.59
10 ppm	0.0886	2.34	1.04	2.05–2.47
100 ppm	0.202	1.46	1.03	1.36–1.55
1,000 ppm	0.103	1.32	1.04	1.18–1.43
0.01 mg/kg-day	0.00325	0.497	1.08	0.35–0.509
0.1 mg/kg-day	0.00326	0.496	1.08	0.35–0.508
1 mg/kg-day	0.00331	0.493	1.07	0.349–0.505
10 mg/kg-day	0.00394	0.464	1.07	0.337–0.473
100 mg/kg-day	0.0224	0.31	1.04	0.273–0.325
1,000 mg/kg-day	0.032	0.212	1.03	0.191–0.222
Rat				
0.01 ppm	0.00352	0.182	1.02	0.173–0.189
0.1 ppm	0.00353	0.182	1.02	0.173–0.189
1 ppm	0.00361	0.181	1.02	0.173–0.189
10 ppm	0.00465	0.177	1.02	0.169–0.183
100 ppm	0.0176	0.144	1.07	0.117–0.158
1,000 ppm	0.0108	0.0476	1.26	0.0261–0.0798
0.01 mg/kg-day	0.00127	0.0941	1.02	0.0875–0.0952
0.1 mg/kg-day	0.00127	0.0941	1.02	0.0875–0.0951
1 mg/kg-day	0.00128	0.094	1.02	0.0874–0.095
10 mg/kg-day	0.0014	0.0929	1.02	0.0866–0.0934
100 mg/kg-day	0.00382	0.0828	1.04	0.0724–0.0851
1,000 mg/kg-day	0.00607	0.0385	1.2	0.023–0.0559

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Table 3-5. Predictions for Trichloroacetic acid (TCA) produced systemically (mg/kg-day systemic TCA per ppm in air or mg/kg-day systemic TCA per mg/kg-day oral intake) using posterior mode parameters (continued)

Species/continuous exposure	Baseline	Posterior mode	GSD of posterior modes across chains	Range of posterior modes across chains
Human				
0.01 ppm	0.0106	0.0125	1.02	0.0117–0.0128
0.1 ppm	0.0106	0.0125	1.02	0.0117–0.0128
1 ppm	0.0106	0.0125	1.02	0.0117–0.0128
10 ppm	0.0104	0.0125	1.02	0.0117–0.0128
100 ppm	0.00906	0.0125	1.02	0.0117–0.0128
1,000 ppm	0.00388	0.0125	1.02	0.0117–0.0128
0.01 mg/kg-day	0.0153	0.0145	1.09	0.0111–0.0152
0.1 mg/kg-day	0.0153	0.0145	1.09	0.0111–0.0152
1 mg/kg-day	0.0153	0.0145	1.09	0.0111–0.0152
10 mg/kg-day	0.015	0.0145	1.09	0.0111–0.0152
100 mg/kg-day	0.0123	0.0145	1.09	0.0111–0.0152
1,000 mg/kg-day	0.00436	0.0145	1.09	0.011–0.0152

GSD = geometric standard deviation.
Dose metrics calculated based on daily average given continuous exposure for 100 weeks. Baseline parameters are based on in vitro data for metabolic parameters, and literature values for other parameters. Posterior modes are based on the updated parameters listed in Table 3-1.

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Table 3-6. Summary evaluation of the reliability of tetrachloroethylene dose metrics

Dose metric species	Calibration error or variability (GSD)[a]	Validation error or variability (GSD)[a]	Optimization runs range[a]	Additional potential concerns from sensitivity analysis
AUCCBld				
Mouse	~2-fold	~2-fold	<10%	None
Rat	~2-fold	~2-fold	<10%	None
Human	~2-fold	~2-fold	<20%	None
FracOx				
Mouse	~2-fold	~2-fold	<40%	Some sensitivity to lung metabolism
Rat	~2-fold	~2-fold	<20%	None
Human	~2-fold	~3-fold	<1.5-fold	Some sensitivity to fraction of oxidation to TCA
FracGSH				
Mouse	NA	NA	~60-fold	None
Rat	~2-fold	NA	<30%	None
Human	~2-fold	NA	~3,000-fold	Calibration data cannot distinguish between modes
TCASys				
Mouse	~2-fold	~2-fold	<30%	Some sensitivity to fraction of oxidation to TCA
Rat	~2-fold	~2-fold	<20%	Some sensitivity to fraction of oxidation to TCA
Human	~2-fold	~3-fold	<40%	Some sensitivity to fraction of oxidation to TCA

[a]Evaluated in rodents at 10 ppm in air by inhalation and 100 mg/kg-day orally, and in humans at 0.01 ppm in air by inhalation and 0.01 mg/kg-day orally.

GSD = geometric standard deviation.

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The highest confidence dose metric in the Chiu and Ginsberg (2011) analysis is the AUC of tetrachloroethylene in blood (refer to Table 3-2). The main source of uncertainty in this case is the residual difference between the model predictions and the calibration and validation data—a factor of about twofold for each species. Therefore, this dose metric should be considered reliable for use in risk assessment with the acknowledgement of a possible twofold residual error.

The next highest confidence as seen from Table 3-6 is in the estimates of tetrachloroethylene oxidation and TCA formation. Here, the estimates of tetrachloroethylene oxidation in mice and rats have similar uncertainty to that for AUC of tetrachloroethylene in blood—predominantly twofold in the residual difference between model predictions and the calibration and validation data. The range of estimates of tetrachloroethylene oxidation in humans is largely dominated by interindividual variability—i.e., the differences in urinary excretion of TCA across individuals. Thus, the central tendencies for the population are well estimated—even if particular individuals may vary to a fair degree. Thus, at the population level, these dose metrics should be considered reliable for use in risk assessment with the acknowledgement of a residual error of about twofold or less.

In terms of predicted interspecies differences, the PBPK model generally predicts the greatest oxidative metabolism in mice, followed by rats, and then humans. Humans would be predicted to receive a *smaller* internal dose of oxidative metabolites for the same applied dose, whether scaled by body weight or allometrically by body weight to the ¾ power.

On the other hand, estimates of GSH conjugation appear more uncertain—especially for humans. In rats, both the calibration data and the range of different optimization runs suggest about a twofold uncertainty. In mice, there are no data on this pathway other than as a "mass balance" from total metabolism (e.g., closed-chamber studies). Nonetheless, the range of estimates based on the different optimization runs is about 60-fold. It is in the human predictions that the range of estimates becomes extraordinarily large. In particular, there are evidently two local maxima, each of which gives similar model fits, but for which model predictions differ by 3,000-fold. This is a reflection not of the calibration data, which are fit quite well regardless, but of the results of different optimization runs. Therefore, overall, the predictions for rat GSH conjugation are considered reliable to about twofold, those for the mouse to about 60-fold, and those for humans vary by about 3,000-fold. At this point, it is not possible to disentangle the contributions of uncertainty and variability to the very large range of estimates of tetrachloroethylene GSH conjugation in humans.

Interestingly, the predictions appear to support the default assumption of equivalent concentrations in air leading to equivalent internal doses, as the estimates of AUC of tetrachloroethylene in blood are within twofold of each other across species. In addition, at the

higher oral doses (e.g., 100 mg/kg-day), rescaling the AUC in blood by body weight to the ¾ power leads to estimates across species within threefold of each other. These can be explained by the sensitivity analysis, which showed AUC in blood to be most sensitive to cardiac output, alveolar ventilation, and the partition coefficient, all of which either are similar across species or scale approximately allometrically by body weight to the ¾ power across species.

The implications of these results are quite substantial—particularly for interspecies extrapolation between rats and humans. In rats, all the evidence appears to support a low amount (<1% of dose) of GSH metabolism. At environmental exposures, the overall posterior mode predicts about 15− to 30−fold *more* GSH conjugation as a fraction of dose in humans relative to rats, but the uncertainty range in humans overlaps with the rat estimates, so the data are also consistent with humans having either equal or greater GSH conjugation.

The analysis in Chiu and Ginsberg (2011) appears to have resolved a conflict between PBPK model-based analyses that predicted high versus low amounts of tetrachloroethylene metabolized in humans in two key aspects. This makes it particularly suited for use in this assessment. First, there is now fairly high confidence in the predictions of *oxidative* metabolism across species. Second, these results make it clear that the previously debated uncertainties in total metabolism can be essentially attributed to uncertainty in GSH conjugation, which is substantial. Those analyses that concluded low total tetrachloroethylene metabolism all restricted the fraction of *total* (not oxidative) metabolism that was TCA to a fairly significant percentage—30−100% [e.g.,(Qiu et al., 2010; Covington et al., 2007; Clewell et al., 2005; Chen and Blancato, 1989)]. Thus, as was noted by the NRC (2010), total metabolism was essentially only measuring oxidative metabolism. On the other hand, those analyses that concluded high total tetrachloroethylene metabolism essentially lumped oxidative and GSH conjugation metabolism together without restrictions as to the fraction producing TCA and/or made inferences based on disappearance of the parent compound [e.g., (Chiu and Bois, 2006; Bois et al., 1996; Reitz et al., 1996; Bois et al., 1990; Ward et al., 1988)]. Thus, the analysis in Chiu and Ginsberg (2011) essentially reconciles the disparate conclusions as to human tetrachloroethylene metabolism from previously published PBPK models. First, the conclusion of "low metabolism" is certainly true for oxidation. Second, the conclusion of "high metabolism" *may* be true for GSH conjugation but is highly uncertain. In essence, both conclusions are consistent with the data if augmented by some additional qualifications: *oxidative* metabolism is low in humans, while *GSH conjugation* metabolism may be high *or* low in humans*, with high uncertainty and/or variability* (Chiu and Ginsberg, 2011).

Results obtained by applying the Chiu and Ginsberg (2011) model for the dose-response modeling in this assessment are presented in Section 5.

3.5.2. Age and Gender-Related Differences in Tetrachloroethylene Pharmacokinetics

Age and gender-specific differences in pharmacokinetics can have a substantial 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 were 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 3-7 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 3-7.

Considerable gender differences in blood concentrations of TCA and tetrachloroethylene were observed 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.

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Table 3-7. Ratio of average daily dose at various life-stages to the average daily dose for a 25-year-old adult: physiologically based pharmacokinetic (PBPK) simulations

Dose metric	Life-stage			
	0–6 months	0.5–5 years	5–25 years	25–75 years
PCE 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).

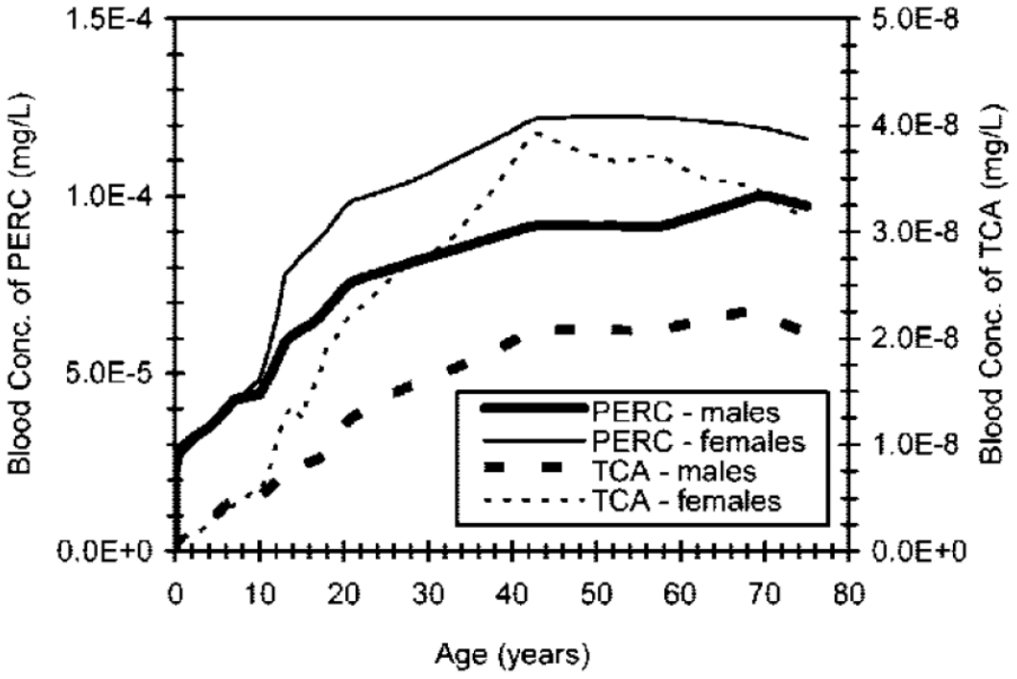

Figure 3-7. Physiologically based pharmacokinetic (PBPK) simulations of variations with age and gender in blood concentrations of tetrachloroethylene and its main metabolite trichloroacetic acid (TCA).
Simulations are for continuous lifetime oral exposure at a constant daily intake of 1 µg/kg-day.

The same group of authors [i.e., (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 nursing 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

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blood concentrations were not pronounced because changes in tissue composition occurred in both the mother and the 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. Metabolic Interactions with Other Chemicals

Fisher et al. (2004) used PBPK modeling and complementary studies in mice to investigate the effect of coexposures 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 hours after administration of CT and 1 hour after single dose 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 coexposure to mixtures of 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 coexposure to TCE.

4. HAZARD IDENTIFICATION

This section discusses tetrachloroethylene toxicity on an organ-specific basis. 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. The order of presentation is as follows: neurotoxicity (refer to Section 4.1); kidney and bladder toxicity and cancer (refer to Section 4.2); liver toxicity and cancer (refer to Section 4.3); esophageal cancer (refer to Section 4.4); lung and respiratory cancer (refer to Section 4.5); immunotoxicity, hematologic toxicity, and cancers of the immune system (refer to Section 4.6); developmental and reproductive toxicity, and reproductive cancers (refer to Section 4.7); genotoxicity (refer to Section 4.8); and susceptible populations (refer to Section 4.9). Section 4.10 provides a summary of the hazard identification.

The database of published epidemiologic studies on cancer and tetrachloroethylene exposure was examined to assess its ability to inform the cancer hazard from tetrachloroethylene exposure. The analysis of epidemiologic studies on cancer and tetrachloroethylene presented in Appendix B documents each study's essential design features, exposure assessment approaches, statistical analyses (including assessment of exposure- or concentration-response), and potential sources of confounding and bias. This analysis supports the discussion of site-specific cancer observations in Sections 4.2–4.7. In those sections, study findings for site-specific cancers are presented with an assessment and discussion of their overall weight of evidence. The key considerations in the weight of evidence are: study design, exposure assessment methodologies, exposure- or concentration-response, and the potential for alternative explanations, including bias and confounding. Greater weight is given to studies that (i) employ a cohort or case-control design, (ii) use exposure assessment methodologies with a relatively high level of sensitivity and specificity, and a low likelihood of exposure misclassification (iii) show a exposure- or concentration-response gradient, and (iv) have less potential for alternative explanations. Sample size (number of cases in a cohort study; number of exposed in a case-control study) was also considered, but a larger sample size in itself did not outweigh the considerations based on type of exposure assessment methodology. Studies that are more limited in one or more of these characteristics are accorded lesser relative weight, but are not necessarily excluded from the overall weight of evidence.

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4.1. NEUROTOXICITY

4.1.1. Human Studies

A wide range of effects on neurologic function have been observed for both acute and chronic-duration exposure to tetrachloroethylene in humans, as summarized below. Most of the reports evaluating neurological function in humans were inhalation chamber or chronic exposure studies. Study designs, exposure-assessment methods, and results of individual studies are presented with a discussion of chamber studies in Section 4.1.1.1 and chronic exposure studies in Section 4.1.1.2. Within the latter section, the studies are further divided by type of exposure setting (occupational; residential). In residential settings, exposure is more likely to be continuous and of lower concentrations compared with the more intermittent, higher concentration, more variable exposure experienced in work settings. Section 4.1.1.3 presents a summary of neuropsychological and neurobehavioral effects in low- and moderate-exposure studies with observations across studies discussed by neurological domain, categorized by visual function, cognitive function, motor function, and neurological and neurobehavioral disorders.

Acute controlled inhalation exposures of 100 ppm and higher induced symptoms consistent with depression of the central nervous system (CNS), such as dizziness and drowsiness. Changes in visual function as measured by electroencephalograms (EEGs) have also been noted with controlled inhalation exposures at this level (Stewart et al., 1977). Acute exposure to lower levels of tetrachloroethylene (50 ppm for 4 hours/day for 4 days) induced alterations in neurobehavioral function, with changes indicative of visual system dysfunction including delayed neuronal processing time (Altmann et al., 1992; Altmann et al., 1990). A wide range in susceptibility to neurological effects among the participants in these studies was observed.

Epidemiologic studies of workers or residents with chronic exposure to tetrachloroethylene show that the nervous system is a target, with most of these studies reporting decrements in one or more nervous system domains. The visual and cognitive domains are most commonly affected (NYSDOH, 2010; McDermott et al., 2005; NYSDOH, 2005a, b; Sharanjeet-Kaur et al., 2004; Schreiber et al., 2002; Gobba et al., 1998; Spinatonda et al., 1997; Altmann et al., 1995; Echeverria et al., 1995; Cavalleri et al., 1994; Echeverria et al., 1994; Ferroni et al., 1992; Nakatsuka et al., 1992; Seeber, 1989; Lauwerys et al., 1983). Other reports (Till et al., 2005; Laslo-Baker et al., 2004; Till et al., 2001a; Till et al., 2001b) 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 compared with children of unexposed mothers were observed (Till et al., 2005; Till et al., 2001a; Till et al., 2001b). These reports are not discussed further in this

section because they do not provide specific data pertaining to tetrachloroethylene exposure. 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. The influence of tetrachloroethylene exposure on risk of these neurological diseases is not addressed in this Toxicological Review.

4.1.1.1. Chamber Studies

Several controlled experiments were conducted in the 1970s examining neurological effects from short-term exposures (5−7.5 hours per day for 4 or 5 consecutive days) to tetrachloroethylene at levels up to 100 ppm. There is no description in the published reports of the informed consent and other human subjects research ethics procedures undertaken in these studies, but there is no evidence that the conduct of the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

In a study by Stewart et al. (1970), 12 healthy adults were exposed to 100 ppm for 7 hours; neurological symptoms including eye and nose irritation was reported by 60% of the subjects, a slight frontal headache by 26%, mild lightheadedness by 26%, drowsiness by 40%, and difficulty speaking by 25%. Of five healthy men exposed to 100 ppm, for 7 hours/day, on 5 consecutive days, one reported a mild frontal headache during each exposure, and two consistently reported mild eye and throat irritation. Individual responses during exposures to 0 ppm were not assessed. Three tests of equilibrium (a modified Romberg test, 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 hours, 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 exhibited impairments to equilibrium within the first 3 hours of exposure but were able to perform the test normally when given a second chance. Stewart et al. (1970) 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.

In the 6-week study by Hake and Stewart (1977), four healthy men were exposed 7.5 hours/day to 0 ppm (2 days in Week 1, 1 day in Week 3, and 2 days in Week 6), 21 ppm (4 consecutive days in Week 3), 100 ppm (5 consecutive days in Week 2), and a time-weighted average (TWA) of 100 ppm (5 consecutive days in Week 4) when exposure levels were more than 53, 100, or 155 ppm (5 consecutive days in Week 5). In addition, four healthy women were exposed to 100 ppm for 7.5 hours/day on 5 consecutive days and to 0 ppm on 2 days. The subjects were told that they would be exposed to various concentrations of tetrachloroethylene,

but they were not told their sequence of exposures (a single-blind protocol). Reports of symptoms (e.g., headache) varied among individuals, but overall, complaints during exposures were similar to those during control conditions—exposures to 0 ppm tetrachloroethylene. The evaluation of visual function through 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 hours of exposure. Recordings of visual evoked potentials (a measure of visual function) 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 was not significantly affected by any exposure. The performance of men on a second test of motor function (Flanagan coordination) was 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 Hake and Stewart (1977) 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. Hake and Stewart (1977) concluded that (1) there is considerable interindividual variation in response to tetrachloroethylene vapors, (2) visual function changes through EEG analysis indicates preliminary signs of narcosis in most subjects exposed to 100 ppm for 7.5 hours, (3) impairment of coordination may occur in subjects exposed to 155 ppm for 7.5 hours, 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.

Altmann et al. (1992; 1990) examined neurological effects of tetrachloroethylene on healthy adults exposed to 10 ppm or 50 ppm for 4 hours on 4 consecutive days. 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. However, neurophysiological measurements were made on only 22 subjects (12 at the low-exposure level and 10 at the high-exposure level). Three neurophysiological measurements evaluating visual and auditory function were taken on the day before exposure started and on each of the four exposure days: (1) visual evoked potentials in response to black-and-white checkerboard

patterns; (2) a visual contrast sensitivity (VCS) test; and (3) recordings of brainstem auditory-evoked potentials (neurophysiological measurements of the electrical signals generated by the hearing system in response to auditory stimuli) to evaluate peripheral hearing loss. All measurements were started 2 hours after a subject entered the chamber and were completed within 1 hour. 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 preexposure baseline assessment and at the end of the study. Subsets of the battery covering motor function and mood were given at the beginning and end of each 4-hour 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 1, 2, 3, and 4 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 1, 2, 3, and 4 of exposure, respectively.

The visual evoked potential latencies of subjects during the 3rd hour of exposure to 50 ppm on Days 1, 2, 3, and 4 of exposure were significantly longer ($p < 0.05$) compared with those measured on the control day, and the differences became progressively longer on successive exposure days. One set of visual evoked potential latencies on the day after the end of the exposure period remained longer than the control day values (statistical significance not reported). Visual evoked potential 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 visual evoked potential latencies of subjects exposed to 10 ppm and those exposed to 50 ppm. Data on visual 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. There were no indications of peripheral hearing loss at either exposure level. Neurobehavioral tests results were reported only for those tests given repeatedly on 4 consecutive days (finger tapping, eye-hand coordination test, simple reaction time, continuous performance, and moods). There were postexposure 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

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were no exposure-related effects on the finger-tapping or moods test. Altmann et al. (1990) concluded that visual function in healthy, young, adult males is mildly affected by tetrachloroethylene exposures to 50 ppm maintained for 4 hours on each of 4 days and stated that the impaired performance on tests of motor/cognitive and motor function suggests that 50 ppm cannot be considered a NOAEL for neurobehavioral endpoints indicative of CNS depression (Altmann et al., 1992).

4.1.1.2. Chronic Exposure Studies

Table 4-1 summarizes details of the chronic-duration tetrachloroethylene exposure studies evaluating neurological function using tests of specific neurological domains in humans. Most of these are studies of dry-cleaning and laundry workers, but some studies examined neurobehavioral or visual system effects among residents living in close proximity to a dry-cleaning establishment (NYSDOH, 2005a, b; Schreiber et al., 2002; Altmann et al., 1995) or in other workers employed in the same building as a dry-cleaning business (Schreiber et al., 2002). Exposure levels were approximately an order of magnitude higher in occupational settings compared with residential exposure. Tetrachloroethylene concentrations reported in the dry-cleaning and laundry worker studies ranged from an 8-hour TWA mean of 6 ppm for dry-cleaner and ironing workers in Cavalleri et al. (1994) to an 8-hour TWA of 41 ppm for operators of a wet-transfer dry-cleaning machine in Echeverria et al. (1995). Mean tetrachloroethylene concentrations in residences near a dry-cleaning business were 0.4 ppm and 0.7 ppm, respectively, in studies in New York City (Schreiber et al., 2002) and Germany (Altmann et al., 1995). Two additional studies examining color vision in solvent-exposed workers (Muttray et al., 1997) and peripheral neuropathy among patients with solvent-induced encephalopathy (Albers et al., 1999) were identified but are not presented because they involved solvent mixtures.

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Table 4-1. Summary of human neurotoxicity studies of occupational or residential exposures to dry-cleaning facilities using tetrachloroethylene

Subjects, methods	Exposure levels	Results	Reference(s)
Occupational exposures: dry-cleaning settings			
Belgium, 26 dry cleaners, 33 unexposed workers (controls), B, EA, PA, U; not blinded to exposure status	Mean TWA = 21 ppm, mean duration = 6.4 yr	Statistically significant differences for simple reaction time (before work) and critical flicker fusion (before and after work); better scores in exposed workers.	Lauwerys et al. (1983)
Germany, 101 dry cleaners (both sexes), 84 unexposed workers (controls). PA, AA; blinded to exposure status	Low-exposure group (n = 57): mean TWA = 12 ppm, mean duration = 11.8 yr; high-exposure group (n = 44): mean TWA = 53 ppm, mean duration = 10.6 yr	Decrease in information-processing speed (perceptual threshold, choice reaction time), visual scanning (cancellation d2 test), visuospatial memory (digit reproduction) in dry cleaners compared with controls; no statistically significant difference between high- and low-exposure groups. No fine motor function deficits.	Seeber (1989)
China, 64 dry cleaners, 120 controls (clerical workers in factories). PA; not blinded to exposure status	Geometric mean TWA = 15 ppm (males), 11 ppm (females), duration not reported	No effect on color vision loss (using less sensitive Lanthony test).	Nakatsuka et al. (1992)
Italy, 60 dry cleaners, 30 controls (hospital launderers, no solvent use). B, A; blinded to exposure level but not status	Mean TWA = 15 ppm, mean duration = 10.1 yr	Impaired performance on simple reaction time, vigilance, stress. No fine motor function deficit. No effects on digit symbol test. No dose-response patterns observed.	Ferroni et al. (1992)
Italy, 22 dry cleaners and 13 ironers, 35 controls. PA, EA; blinded to exposure level	Mean TWA = 6 ppm (7.3 ppm, dry-cleaning workers; 4.8 ppm, ironers), mean duration = 8.8 yr	CCI elevated among all workers (p = 0.025) and dry cleaners (p = 0.007); statistically significant exposure (TWA)-response relationship. No effect observed in ironers.	Cavalleri et al. (1994)
Italy, 33 dry cleaners and ironers, self controls [baseline measurements in Cavalleri et al. (1994)]. PA; not clear if blinded	Geometric mean TWA ppm: Group A (n = 19) Group B (n = 14) Baseline 1.67 2.95 Follow-up 4.35 0.66	Increased CCI in Group A ($p < 0.01$); no change in Group B. CCI correlated with exposure levels (r = 0.38, $p < 0.05$).	Gobba et al. (1998) [follow up of Cavalleri et al. (1994)]
Michigan, 65 dry cleaners, pressers, clerks; no unexposed group, PA; blinded to exposure level	Chronic exposure score based on work history: low (n = 24; 2.1 yr), moderate (n = 18; 3.9 yr), high (n = 23; 14.6 yr)	Statistically significant decrease in high compared with low exposure on three tests of visuospatial memory. No effect on digit span.	Echeverria et al. (1995)

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Table 4-1. Summary of human neurotoxicity studies of occupational or residential exposures to dry-cleaning facilities using tetrachloroethylene (continued)

Subjects, methods[a]	Exposure levels	Results	Reference(s)
Washington, 45 dry cleaners matched to 69 laundry workers, 59 pressers, or counter clerks from the same shop as the dry cleaner operator. PA; blinded to exposure level	Chronic exposure score groups based on detailed work history and estimated measures: mean = 0, 68, and 1,150 with corresponding 8-h TWAs of <0.2, 3, and 9 ppm. Mean duration = 2.6 to 11 yr for low- and high-exposure groups, respectively	Evidence of associations between chronic exposure and reduced test performance on three tests of visuospatial memory: switching (p = 0.10), pattern memory (p = 0.03), and pattern recognition (p = 0.09).	Echeverria et al. (1994)
Italy, 35 dry cleaners, 39 age- and education-matched controls. AA; not blinded to exposure status	Median = 8 ppm, grab sample. Mean duration of employment = 10.6 yr (from Figure 2)	Increase in vocal reaction time to visual stimuli (reading task); dose-response relationship.	Spinatonda et al. (1997)
Malaysia, 14 dry cleaners, 29 controls (support staff of Universiti Kebangsaan Malaysia, control Group 2); not blinded to exposure status	No exposure information presented in paper other than PCE was used for dry cleaning	43 and 93% of dry cleaners compared to 0 controls had errors on the color vision D-15 test and FM 100 Hue test, respectively. Number of errors on FM 100 Hue test also increased in dry cleaners (p < 0.05).	Sharanjeet-Kaur et al. (2004)
Israel, 88,820 births, 1964−1976, identified in Jerusalem Perinatal Study, linked to national Psychiatric Registry for hospitalization with a schizophrenia-related diagnosis through 1997	Occupation of mother and father listed as dry cleaner on birth certificate	Four cases were identified in 144 offspring of dry cleaners. RR of 3.4 (95% CI: 1.3−9.2) for schizophrenia in the offspring of dry cleaners using proportional hazard modeling.	Perrin et al. (2007)
Occupational exposures: other settings			
New York, 9 employees of day-care center located in a building with a dry-cleaning business, 9 age- and gender-matched unexposed controls. PA, EA, B, U; not blinded to exposure status	Mean = 0.32 ppm (monitoring before closure of dry cleaners). No information on duration of employment	Decreased color discrimination among exposed but not statistically significant. Lower (worse) scores on tests of visual contrast sensitivity.	Schreiber et al. (2002)
New York, 4-yr follow-up of 13 children who had attended a day care located in a building with a dry-cleaning business, 13 children matched to exposed children on age, gender, and daycare experience; not blinded to exposure status	Exposure had ceased 4 yr earlier	No difference in visual function (VCS, CCI) or neurobehavioral function between exposed children and controls.	NYSDOH (2005b)

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Table 4-1. Summary of human neurotoxicity studies of occupational or residential exposures to dry-cleaning facilities using tetrachloroethylene (continued)

Subjects, methods[a]	Exposure levels	Results	Reference(s)
Residential exposures			
Germany, residents near dry-cleaning business, 14 exposed and 23 age- and gender-matched nonexposed controls. AA, B; not clear if blinded to exposure status	Mean = 7 d monitoring period, 0.7 ppm, mean duration = 10.6 yr	Statistically significant increase in simple reaction time and decrease in continuous performance and visuospatial function. No fine motor function deficits.	Altmann et al. (1995)
New York, 17 exposed (apartment residents living above dry-cleaning business) and 17 age- and gender-matched controls. AA, PA, EA, B, U; not blinded to exposure status	Mean = 0.4 ppm (monitoring before closure of dry cleaners). Mean duration of residence = 6 yr	Decreased color discrimination among exposed, but not statistically significant. Lower (worse) scores on tests of visual contrast sensitivity.	Schreiber et al. (2002)
New York, 65 households (67 adults and 68 children) in residential buildings with colocated dry cleaners, 61 households (61 adults and 71 children) in residential buildings without dry cleaners. AA; not blinded to exposure status	Geometric mean = 5 ppb (0.005 ppm). Mean duration of residence = 10 yr	Association ($p < 0.05$) between PCE (indoor air and blood) and performance on test of visual contrast 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.	NYSDOH (2005a); Storm et al. (2011) McDermott et al. (2005)

A = air sample, not specified area or personal sample, AA = area air samples, B = biological monitoring of blood, CCI = color confusion index, CI = confidence interval, EA = exhaled air samples, PA = Personal air samples, PCE = tetrachloroethylene, RR = relative risk, U = biological monitoring of urine for trichloroacetic acid, VCS = visual contrast sensitivity.

Vision testing in the four studies included tests of acuity, tests of spatial vision based on contrast sensitivity, and tests of color vision. 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. Contrast sensitivity measures the least amount of luminance difference between dark and light bars needed to detect a given pattern (e.g., a bar pattern). Impairments in color vision, beginning as blue-yellow confusion errors, have been reported in populations exposed to organic solvents (Campagna et al., 1996; Campagna et al., 1995; Mergler et al., 1991; Mergler et al., 1988a; Mergler et al., 1988b; Mergler and Blain, 1987; Mergler, 1987). The tetrachloroethylene exposure studies that assessed color

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vision relied on various versions of the Lanthony color vision test. This type of test consists of a series of small round "caps" that the subject is asked to arrange 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. Test scores are based on the subject's ability to arrange a set of 15 caps 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]) based on Total Color Distance Scores can be used for scoring (Geller, 2001; Bowman, 1982). The Lanthony D-15 desaturated test is more sensitive to mild and moderate changes in color vision compared with other versions of the test that use more contrasting hues (Lanthony, 1978). The vision tests are not recommended for epidemiological studies of children under 5 years of age.

Other types of neurobehavioral effects were assessed in these studies using standardized tests of cognitive or motor function, such as the digit symbol, digit span, Benton visual memory, and simple reaction time tests. The standardized neurobehavioral battery has a high rate of reliability and has been used to assess normal neurological function (Anger et al., 2000).

As with most conditions, age is an important factor that needs to be considered in interpreting measures of neurological function. Generally, the comparison group within these studies was age-matched (individually or frequency-matched) to the exposed subjects. Measures of cognitive function can also be influenced by education (or more broadly, socioeconomic status variables), by other intelligence measures, and by alcohol use. Thus, these attributes would also need to be considered in studies using cognitive tests such as visuospatial memory, vigilance, and information processing. Alcohol use, smoking, certain medications, chronic neurological conditions, and solvents other than tetrachloroethylene may affect visual contrast sensitivity and color vision measures (Paramei et al., 2004; Swinker and Burke, 2002). In contrast, color vision and spatial vision have not been shown to be related to education or socioeconomic status, so potential confounding by these factors is unlikely.

4.1.1.2.1. Occupational exposure studies: dry-cleaning settings

Lauwerys et al. (1983) studied 26[6] workers (24 women and 2 men) occupationally exposed to tetrachloroethylene in six dry-cleaning shops in Belgium for a mean of 6.4 years (range: 0.1 to 25 years) and 33 controls (31 women and 2 men) working in a chocolate factory (n = 20) or an occupational health service (n = 13) without occupational exposure to organic

[6] Abstract of paper reports 22 subjects were exposed to tetrachloroethylene, but the full text of the paper includes 26 subjects.

solvents. No information is provided in the paper on the methods used to identify subjects or their reasons for participating in the study. The level of education was similar in the exposed and control groups, but the prevalence of smokers was higher among dry-cleaning workers (50%) compared with the controls (27%). 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 workweek. Individuals also were questioned about chronic neurological symptoms (e.g., fatigue, depression, sleep disturbances). Blood samples were collected both before and after work. The mean tetrachloroethylene air concentration (8-hour 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). Trichloroacetic acid, a metabolite of tetrachloroethylene, was not detected (level of detection [LOD] not identified in published paper) in urine specimens from exposed subjects. An evaluation of the subjects was performed at each worksite, so examiners were not blinded to exposure status. The score of the critical flicker fusion test (a test of sensory function) was significantly increased (better performance) in the exposed workers compared with controls when given both before and after work. Decreased simple reaction time was observed among the exposed workers in the tests performed before work (mean ± standard deviation [SD]: 0.374 ± 0.120 and 0.448 ± 0.155 seconds in exposed and nonexposed workers, respectively) but not in the tests performed after work (mean ± SD: 0.341 ± 0.116 and 0.356 ± 0.128 seconds in exposed and nonexposed workers, respectively). The dry-cleaning workers did not differ from controls on the other three neurobehavioral tests. The prevalence of abnormal scores (those beyond the 5th or 95th percentile of the control group) did not vary significantly between the two groups.

Seeber (1989)[7] evaluated the neurobehavioral effects of tetrachloroethylene in 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) based on both activities and room air measurements. 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. Predominant characteristics of both groups included primarily

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

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standing work, contact with customers, and moderate physical exercise. 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, which focused the investigation on workers with relatively constant exposure levels. Mean tetrachloroethylene concentrations (8-hour TWA) for the low- and high-exposure groups were 12 (±8) ppm and 53 (±17) ppm, respectively, using results from active sampling of room air and passive sampling of personal air. The mean durations of occupational exposure for the low- and high-exposure groups were 11.8 and 10.6 years, respectively.

Several tests of neuropsychological functioning were administered using a German standardized neurobehavorial battery (Psychologisch-Neurologischer Fragebogen or PNF), including overall neurological signs, standardized personality tests (emotional lability), tests of sensorimotor function (including finger tapping and aiming), tests of attention (digit reproduction and digit symbol), test of visual scanning (cancellations) and tests of information processing speed (threshold of perceptual speed and choice reaction time) (Seeber, 1989). Some details of the testing procedures were not provided, and 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. In addition, subtests of the Wechsler Intelligence Test (digit span, digit symbol, and cancellations) were used, as was recognition of words, faces, and digits. Intelligence was assessed using the logical thinking subtest of the German Performance Test System. The neurobehavioral tests were given by two specialized clinic staff members who did not question the subjects regarding exposure status. Test outcomes were reported as means ± SDs.

The control group was younger than the dry-cleaning workers (mean ages: 38.2, 38.4, and 31.8 years, respectively, in the low-exposure, high-exposure, and control groups, respectively), and alcohol consumption also differed by group (mean: 8.2, 10.4, and 12.6 g/day in the low-exposure, high-exposure, and control groups, respectively) (Seeber, 1989). Higher scores on the intelligence test were observed among the control group (mean ± SD: 21.9 ± 5.8) compared with the dry-cleaning workers (mean ± SD: 18.3 ± 5.0 and 19.2 ± 5.2 in the low- and high-exposure groups, respectively). Age, gender, and intelligence scores were included in the regression models analyzing the relation between exposure and neurobehavioral test scores; additional control for group differences in alcohol consumption did not alter the observed results.

Dose-response relationships for several outcomes reported by Seeber (1989) were suggested by statistical analysis. Performance of both the low-exposure and high-exposure groups differed significantly from that of the unexposed control group on the threshold of perceptual speed test, digit reproduction, digit symbol, and cancellations; all pairwise comparisons had p-values < 0.01. "Delayed reactions" differed statistically significantly from

controls in the high-exposure group ($p = 0.03$), but not in the low-exposure group ($p = 0.08$). For other outcomes reported, personality test (emotional lability) and overall performance (neurological signs), dose-response relationships were less clear. Performance of the low-exposure group, but not the high-exposure group, differed statistically significantly from that of the unexposed control group on the personality test (emotional lability) and for neurological signs ($p \leq 0.05$ and $p > 0.10$ for low-exposure and high-exposure groups, respectively, on both tests), although there was no statistically significant difference between the exposed groups for both tests ($p > 0.10$). Last, the mean scores on "correct reactions" on a choice reaction time task were not statistically significantly different from control for either exposure group ($p \geq 0.10$ for both).

Characterization of a dose-response in this study is complicated by nonmonotonic response patterns in the mean scores for several outcomes. Specifically, mean scores of the low-exposure group for the outcomes of digit symbol and cancellations, as well as for emotional lability and for overall neurological signs, were worse compared with control than the high-exposure group scores. However, the wide confidence intervals around the individual means for each of these outcomes in the exposed groups indicates no statistical differences in outcomes between the two exposure groups. Further, there is substantial overlap between the exposure ranges of the two sets of dry cleaning workers, reflecting assignment to groups based partly on job activities, which also supports the inference that the treated groups are similar. Thus, exposure misclassification is a possible contributor to the observed pattern. Without individual test outcomes and exposure measurements, however, it is not possible to evaluate the dose-response relationships more thoroughly. Seeber (1989) concluded that there was a significant difference in outcomes between the control and exposed subjects. For some outcomes, NRC (2010) characterized the study as having discrepant results based on worse mean test scores (for neurologic signs, emotional lability, choice reaction time, cancellation d2 and digit symbol) in the low- compared with high-exposure group.

Nakatsuka et al. (1992) evaluated the effects of tetrachloroethylene exposure on visual function by examining the color vision of 64 dry-cleaning workers (34 women and 30 men) in China. 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 (34.2 years for men, 35.3 years for women) were similar to those of the male controls (34.0 years) but slightly higher than the female controls (32.6 years). The Lanthony new color test, used for screening color vision, and the Ishihara's color vision 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). (This color vision test is not as sensitive as the Lanthony D-15 test used in the other studies discussed in this section.) The geometric mean air concentrations of tetrachloroethylene (averaging time not reported) were 15.3 and 10.7 ppm for the men and women, respectively, using results from passive sampling of personal air. The overall geometric mean was 13 ppm. The authors reported no significant difference in the performance of the dry-cleaning workers (or other solvent-exposed groups included in the study) and unexposed controls on the Lanthony new color vision test, with 60% of the male dry-cleaning workers and 63% of male controls classified as "normal" color vision. Corresponding figures for females were 91 and 74% in the dry-cleaning workers and controls, respectively. Results for the males were not appreciably different when individuals with red-green vision loss were excluded.[8] Nakatsuka et al. (1992) concluded, overall, that they found no distinct color vision loss among the dry-cleaning workers.

Ferroni et al. (1992)[9] evaluated neurobehavioral effects and prolactin levels among 60 female dry cleaners and 30 unexposed female controls. Prolactin secretion by the pituitary is controlled by hypothalamic dopamine; dopamine is also important to neurotransmitter systems, and serum prolactin, as a biochemical signal and marker of nervous system function, is a proposed alternative for assessment of nervous system toxicity (Manzo et al., 1996). The workers at every dry-cleaning shop in a small town outside of Parma, Italy, were invited to participate in the study. 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 dry cleaners and controls were comparable in age (mean ages: 39.7 and 37.6 years, respectively), vocabulary level, height, weight, body mass index, smoking habits, and use of medication. Workplace air samples were randomly collected throughout the workweek during summer and winter to account for variability related to either the work cycle or seasonal environmental fluctuations. Blood samples were collected during the workday during summer

[8] A statistical analysis of the dry cleaners data using a Fisher's exact test (for differences in proportions with at least one sparse cell) indicated that tetrachloroethylene-exposed women were more likely to have normal color vision as compared with unexposed women ($p = 0.0423$), but no difference was observed among the males (0.83, based on Chi-squared test); reported in public comments of the Halogenated Solvents Industry Alliance to EPA (Halogenated Solvents Industry Alliance, 2004) on the Neurotoxicity of Tetrachloroethylene Discussion Paper (U.S. EPA, 2003).

[9] Dr. Mutti provided details on the selection process of exposed and control subjects and also clarified reported results to Dr. Ken Bodgen, NYSDOH, in written correspondence dated July 29 and September 5, 1995 [refer to NYSDOH (1997)].

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and winter. The median tetrachloroethylene air concentration (4-hour TWA) was 15 ppm (range: 1 to 67 ppm). The subjects' range of tetrachloroethylene blood levels was 0.012−0.864 mg/L [median = 0.145 mg/L; incorrectly expressed in Ferroni et al. (1992) as 12,864 and 145 mg/L; NYSDOH (1997)]. The mean duration of occupational exposure was 10 years.

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) (Ferroni et al., 1992). All subjects were examined in the morning before their work shift in the same room by the same examiners, using a standardized testing protocol (NYSDOH, 1997). 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 (NYSDOH, 1997). Serum prolactin levels were measured in all subjects using a blood sample taken at the time of the neurobehavioral testing; analysis was limited to those samples obtained during the proliferative (follicular) phase of the menstrual cycle (41 dry cleaners and 23 controls). Ferroni et al. (1992) did not describe the protocol for determining menstrual cycle phase, however. Serum samples from dry cleaners and controls were alternated and analyzed in the same experimental runs (NYSDOH, 1997).

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, $p < 0.005$) (Ferroni et al., 1992). Performance on the finger-tapping test (both hands) and digit symbol test was not affected (NYSDOH, 1997). Additionally, the mean serum level of prolactin was significantly higher in the workers than in the matched controls (mean: 12.1 compared with 7.4 μg/L, $p < 0.001$). Among the dry cleaners, 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. Ferroni et al. (1992) concluded that tetrachloroethylene exposure in dry-cleaning shops may impair performance.

Cavalleri et al. (1994) evaluated the effects of tetrachloroethylene exposure on the color vision of dry cleaners and a comparison group of matched controls. 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. 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 employed at a dry-cleaning facility for 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 (±3 years), alcohol consumption (±10 g/day), and cigarette use (±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. 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 the Lanthony D-15 desaturated panel test. Exposed and control subjects were tested in random order (NYSDOH, 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-hour TWA) was 6 ppm, and the range of TWA values was 0.4−31 ppm, using results from passive sampling of personal air (Cavalleri et al., 1994). For operators (n = 22), the mean air concentration 8-hour TWA was 7.3 ppm (range 0.4−31 ppm). For ironers (n = 13), mean air concentration (8-hour TWA) was 4.8 ppm (range 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 8-hour 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 on the color vision test ($p < 0.01$). 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 error rate (mean CCI score: 1.143 and 1.108 in workers and controls, respectively, p = 0.03). The effect was observed in dry cleaners (mean CCI score: 1.192 and 1.089 in dry cleaners and their matched controls, respectively, p = 0.007) but not among the ironers (mean CCI score: 1.061 and 1.073 in ironers and their matched controls, respectively). 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 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. 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 (NYSDOH, 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 is a chronic effect.

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. 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). The decrease in exposures was due to new equipment or other changes to the working conditions. As found in the first survey, color vision was impaired primarily in the blue-yellow range of color, with few subjects presenting red-green errors. Color vision performance for the entire group was related significantly to age ($r = 0.45$) and tetrachloroethylene concentration ($r = 0.39$; $p < 0.05$). The mean CCI score for Group A subjects showed a statistically significant difference between the two surveys (arithmetic mean: 1.16 and 1.26 in the first and second surveys, respectively, $p < 0.01$). 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 (arithmetic mean: 1.15 and 1.15 in the first and second surveys, respectively). The findings in Groups A and B were also supported using analysis of variance methods to examine the relation between CCI score and exposure level (log TWA), adjusting for age, alcohol consumption, or cigarette smoking between the subgroups.

Echeverria et al. (1995) assessed the performance of 65 dry-cleaning workers on neurobehavioral tests. The testing was conducted in 1986. The owners of 125 shops in Detroit, MI, were contacted, and 23 agreed to allow their workers to participate in the study. Within each shop, operators were matched on education and age (±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 ages were 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-hour 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, respectively. The estimates were based on a relationship between breath and air concentrations derived from a larger independent study (Solet et al., 1990). These estimates were comparable to those found in other surveys of dry-cleaning facilities in the United States.

All subjects were tested in a minivan at the worksite in groups of two, in the afternoon after work on the first or second day of their workweek (Echeverria et al., 1995). 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). Multivariate analysis was used to evaluate the relationship between a chronic index of lifetime exposure and performance on neurobehavioral tests, accounting for the potential confounding variables of current 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 reduced (compared with the low chronic exposure group) by 4% for pattern recognition, 7% for pattern memory, and 14% for visual reproduction (all p-values <0.01). These impairments of visually mediated function were consistent with the impairment of visuospatial functions observed in four patients who were diagnosed with tetrachloroethylene encephalopathy who had been previously studied by Echeverria et al. (1995). Other effects observed 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. Echeverria et al. (1995) 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

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tetrachloroethylene 8-hour 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 workers who were probably clerks in wet-transfer shops where the mean current exposure level was 11 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.

Echeverria et al. (1994) builds on the results of Echeverria et al. (1995),[10] hypothesizing degradation in behavior (particularly attention, executive function, visuospatial memory, short-term memory, and mood) is an early indicator of neurotoxicity, leaving motor, language-based skills, and long-term memory intact. The study was conducted in the Seattle/Tacoma, WA 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. The most common reasons for nonparticipation were disinterest, time constraints, lack of English proficiency, and concerns about pending regulatory actions concerning tetrachloroethylene. Recruitment ended when a total of 45 operators were enrolled. 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 1 year in the trade. The final sample excluded three subjects because of limited English and reading skills and six subjects who did not wear their prescription glasses on the day of testing or who were missing covariate information such as vocabulary test scores.

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-hour 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

[10] Although published a year after this study (Echeverria et al., 1994), the study by Echeverria et al. (1995), discussed previously, was conducted in 1986, 3 years before this study.

exposure (pressers/clerks), and 1,150 for the dry-cleaning workers with high exposure (operators). Mean exposures (8-hour 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-hour mean concentrations of 0.5, 3, and 20 ppm, respectively. Dry-cleaning workers placed in the acute and subchronic low exposure and high exposure 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 in the high chronic-exposure category could be found in the low acute- and low subchronic-exposure categories because these latter two indices were based on air concentrations on the day of testing.

The test battery included tests of cognitive function, including visuospatial memory, 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 preshift scores. 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.

Multivariate analysis was used to evaluate the relationship between exposure indices and levels and performance on neurobehavioral tests after adjusting for the potential confounders of age, gender, race, vocabulary level (as a surrogate for education and test-taking), and alcohol consumption. Indications of 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. 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-hour 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. In summary, Echeverria et al. (1994) detected deficits in visuospatial function (reduced performance in tests of pattern memory and pattern recognition)

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in the dry-cleaning workers categorized as having high lifetime chronic exposure and whose current exposure level was 9 ppm, 8-hour TWA. However, the exposure level of 9 ppm is not representative of past chronic exposure levels because of changes occurring in the industry in the study area (i.e., switching from wet-transfer to dry-to-dry machine). The investigators attributed the reduced performance to exposures 3 to 5 years previously that were about two to four times higher, and they hypothesized that a few years of reduced exposure may not be long enough to eliminate the residual effects on visuospatial function caused by the exposures associated with wet-transfer machines.

Spinatonda et al. (1997) assessed the effect of tetrachloroethylene exposure on cognitive function by measuring vocal reaction times among 35 dry cleaners and 39 unexposed controls. Controls were matched to exposed individuals by age (mean age of 35 years for both groups) and education. The published paper did not identify the population from which exposed subjects and controls were drawn or the inclusion criteria for exposed subjects and controls. Exposure was assessed by a "grab sample" collected at the time of the neurological testing and is not a TWA. 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 each 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. 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.

Sharanjeet-Kaur et al. (2004) examined visual function effects by assessing color vision in 14 workers, ages 24−53 years, in three dry-cleaning facilities using tetrachloroethylene in Malaysia. This study was part of a larger study assessing color vision in two other occupationally exposed populations (39 workers in a factory producing polyethylene resin plastic storage containers and 40 workers manufacturing polystyrene plastic bags). The paper does not

report how facilities were identified or recruitment methods for study subjects. Furthermore, the paper does not present any information on tetrachloroethylene concentrations, tetrachloroethylene biomarkers, or exposure levels in this type of work setting in Malaysia, making it difficult to judge the degree of exposure. Controls (n = 29)[11] were recruited from the support staff of the Universiti Kebangsaan Malaysia and were age-matched to dry-cleaning workers (mean age: 33 ± 8.5 years and 33 ± 3.9 years in dry cleaners and controls, respectively). However, dry-cleaning workers differed from controls on several variables: work duration (mean: 6.7 and 12.6 years in dry cleaners and controls, respectively), hours worked per day (mean: 9.8 and 8.3 in dry cleaners and controls, respectively), cigarette smoking (36 and 7% in dry cleaners and controls, respectively), and race (50 and 90% Malays in dry cleaners and controls, respectively); no information is presented on possible differences between dry cleaners and controls in socioeconomic status. Consent was obtained from all study participants. 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. Thus, the investigators were not blinded to exposure status during the testing procedure. Distance visual acuity was measured using the Snellen chart, and near visual acuity was measured using a reading chart. Subjects with poor visual acuity or with systemic, ocular, or neurological diseases were excluded; the number of excluded subjects is not specified in the paper. Color vision was assessed binocularly using Ishihara plates, the Lanthony D-15 test, and the Farnsworth Munsell (FM) 100 Hue test under a light box at an illumination of 1,000 lux. None of the controls or dry cleaners had color vision errors with the Ishihara plates. In contrast, errors on the Lanthony D-15 test and FM 100 Hue test were reported for 6 dry cleaners (43%) and 13 dry cleaners (93%) compared with no errors reported among controls, respectively. Statistical testing of these differences was not presented. Total error scores for the FM 100 Hue test differed between dry cleaners and controls ($p < 0.05$). It is difficult to interpret these findings due to the lack of information on potential tetrachloroethylene exposure other than job title, and differences between dry cleaners and controls regarding test conditions and smoking history.

Perrin et al. (2007) evaluated the risk of schizophrenia among a cohort of 88,829 subjects born between 1964−1976 in the Jerusalem Perinatal Project, a population-based cohort. Births in this cohort are linked to the database of Israel's Psychiatric Registry, 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

[11] An additional control group, Control Group 1, was included in the paper; this group was age matched to the other factory workers included in the study.

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% confidence interval [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, and 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).

4.1.1.2.2. Occupational exposure studies: other settings

Schreiber et al. (2002) reported the findings from investigations using visual tests to assess neurologic function in two populations: apartment residents[12] and day-care employees who had potential environmental tetrachloroethylene exposure due to close proximity to dry-cleaning facilities. The study of day-care employees will be discussed in this section because their exposure would have been of a similar pattern to others in an occupational setting. The day-care facility, located near Albany, NY, was in a building that also housed a business that performed dry cleaning. 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, 5 weeks after removal of the dry-cleaning machines, approached background concentrations (range: 0.0012−0.0081 ppm).

Objectives of the investigations were to characterize tetrachloroethylene exposure and to screen for subclinical neurological effects using a battery of visual function tests (Schreiber et al., 2002). All participants signed consent forms. The study included all of the current staff members of the day-care center (n = 9, all adult females). Controls were age- and gender-matched acquaintances of the exposed participants, local retail shop employees, NYSDOH employees, or staff from other local day-care centers with no known tetrachloroethylene exposure. All subjects in the exposed and control groups were Caucasian (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 workers; mean duration of employment at the day-care center was 4 years. Sociodemographic data, lifestyle factors (e.g., personal and passive smoking, alcohol consumption, and exercise), medical history, and

[12] The results of the residential study are summarized in Residential Exposure Studies, following this section.

neurotoxicant exposure were obtained by questionnaire. Reported alcohol consumption was similar (low or moderate) in the exposed and control groups.

Visual function testing consisted of near visual acuity, near visual contrast sensitivity, and color vision (Schreiber et al., 2002). Examiners were not blinded as to a subject's exposure status. In the contrast sensitivity test, 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. 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 visual contrast sensitivity data. Color vision was assessed using the Lanthony D-15 test, with calculation of color confusion index (CCI) based on the accuracy of the chip placement. Group differences in the CCI were assessed using two-tailed Student's *t*-tests for matched-pair analyses.

The mean measure of visual acuity was 20:22.2 in the exposed day-care workers and 20:26.4 in controls ($p = 0.16$). There was a statistically significant lower group mean visual contrast sensitivity score across all spatial frequencies when day-care employees were compared with the control group (refer to Figure 4-1). The mean CCI scores were 1.22 and 1.18 in the exposed day-care workers and controls, respectively ($p = 0.39$).

Although it should be noted that the controls came from a different area (a rural area in upstate New York) compared to the exposed subjects from New York City, there is little evidence that degree of urbanity would be related to visual contrast sensitivity. Education has not been found to be related to performance on the visual contrast sensitivity test (NYSDOH, 2005b; U.S. EPA, 2004; Hudnell et al., 2001; Frenette et al., 1991; Mergler et al., 1991). Additionally, occupation is highly correlated with socioeconomic status (Deonandan et al., 2000) and is also not likely to confound the visual contrast sensitivity test.

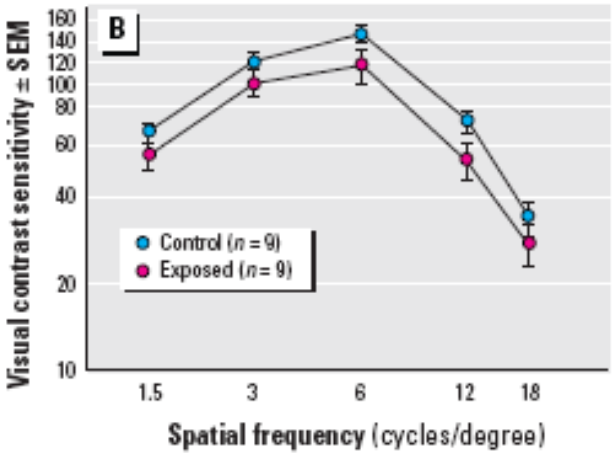

Figure 4-1. Visual contrast sensitivity functions for control and exposed participants in a study of workers in a day-care center located in a building with a dry-cleaning facility (Schreiber et al., 2002).

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). Blue circles (top line) = controls; red circles (bottom line) = exposed. For any frequency, a higher contrast sensitivity threshold represents better visual function. Visual contrast sensitivity was significantly lower across all spatial frequencies in exposed workers at a day-care center colocated with a dry-cleaning facility compared with their matched controls. Used with permission of the authors.

The Pumpkin Patch Day Care Center Follow-up Evaluation (NYSDOH, 2010, 2005a, b) examines the effect of tetrachloroethylene exposure on visual function in former students of the day-care center collocated in a building with a dry-cleaning facility that was studied by Schreiber et al. (2002). This study is discussed in this section because the children's exposure would have been of a similar pattern to others in an occupational setting, although exposure ceased 4 years prior to this study. Children eligible for testing in the current evaluation were enrolled in the New York State Volatile Organic Chemical (VOC) Registry and had attended the day-care center. Of the 115 who met this criterion, 27 children with the highest number of hours spent at the day-care center were invited to participate; 17 children completed vision testing, and 13 children completed some or all of the neurobehavioral assessment. Referents (controls) were children who attended other day-care centers and were matched to the exposed children by day-care experience, age, and gender. No information is provided on methods employed for referent participation. Overall, 17 Pumpkin Patch Day Care Center and 13 comparison children (13 matched pairs) completed vision testing, and 13 Pumpkin Patch Day Care Center and 13 comparison children (8 matched pairs) completed neurobehavioral testing, consisting 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. A parent or guardian completed the Child Behavioral Checklist and a

background history questionnaire. Neurobehavioral function of the 13 Pumpkin Patch Day Care Center children evaluated in this follow-up study did not differ from that of the 13 referent children, and Pumpkin Patch Day Care Center children performed better than referent children on several tests. Visual function testing consisted of visual acuity, far visual contrast sensitivity, and color vision. Visual contrast sensitivity was determined 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% CI) of visual contrast sensitivity at each spatial frequency. Color vision was assessed using both the Farnsworth D15 and Lanthony D-15 tests. Both color vision and contrast sensitivity tests were performed monocularly. Examiners were not specifically blinded to exposure status, but this information could have been revealed by the participant during the examination. Using the Wilcoxon matched-pairs signed-ranks test, Pumpkin Patch Day Care Center children performed better on the visual contrast sensitivity test compared to referent children. No significant difference in the proportions of children with abnormal color vision or with children making major errors, or with CCI scores were observed between Pumpkin Patch Day Care Center and referent children. Similar results on the vision tests were observed when excluding two pairs who were ≤6 years old.

4.1.1.2.3. Residential exposure studies

This section discusses studies of residential exposure scenarios. Residential exposure to tetrachloroethylene can result in nearly continuous exposure (NYSDOH, 2005b) and is distinct from the pattern of occupational tetrachloroethylene exposure.

Altmann et al. (1995) examined neurological effects of long-term exposure to tetrachloroethylene among residents of Mulheim, Germany, who lived near dry-cleaning shops. A total of 19 exposed subjects were chosen from a population of 92 individuals living in neighborhoods close to dry-cleaning facilities. Three criteria were used to select subjects: (1) a tetrachloroethylene blood level above 0.002 mg/L, (2) a period of living above or next to a dry-cleaning facility for at least 1 year, and (3) 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). Thirty potential controls (mean age: 37.2 years, range: 24−63 years) were recruited, mainly from the staff of a public health office or an institute for environmental hygiene. One or two controls, matched for age (±1 year, but ±3 years in one case and ±6 years in another case) and gender, were chosen for each exposed subject. Consent was obtained from all subjects prior to the initiation of testing. 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. All subjects met standards for visual acuity and vibration perception. The final exposed group included 14 subjects (5 men, 9 women), and the control group included 23 subjects (9 men, 14 women). The two groups did not differ with regard to consumption of alcoholic beverages, regular medication, smoking, or body mass index. Level of education was divided into three categories, "low," "medium," or "high" (definitions of these categories were not provided). The numbers of exposed subjects by education group (low, medium, and high) were 4, 8, and 2, respectively; the numbers of controls in these respective groups were 1, 12, and 10, indicating a considerable imbalance across these strata. The effect of tetrachloroethylene exposure on the neurophysiological and neurobehavioral measurements was evaluated using linear regression, adjusting for age, gender, and the three-level education variable.

Visual evoked potentials (a measure of visual function) in response to black-and-white checkerboard patterns were recorded for all individuals (Altmann et al., 1995). 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 joystick 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 testing was completed in a single 3-hour session; testing times were selected randomly for both exposed or control subjects.

Blood samples were taken in the exam room immediately before testing (all subjects) and, if possible, once when the exposed subjects were at home (Altmann et al., 1995). The mean blood level for exposed subjects at the examination 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 at the examination. 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, respectively. 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 ($r = 0.24$).

Altmann et al. (1995) observed statistically significant differences between the adjusted mean scores of exposed and control subjects on neurobehavioral tests of 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 as tested with the Benton visual retention test ($p < 0.05$). In all cases, the exposed subjects had slower response times or more errors than did the unexposed controls. The degree of change from control was approximately 15−20% for these tests. The potential for residual confounding by education should be considered; however, although education level was independently associated with these measures and its affect on performance adjusted in the statistical analysis, the use of three categories for education in the multivariate regression analyses may not fully account for all effects from this covariate. 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 visual evoked potentials, which may be less sensitive than direct measurement of visual function; or on vibration perception at the ankle using a tuning fork.

Schreiber et al. (2002) examined neurologic function as assessed by visual tests among apartment residents who had potential environmental tetrachloroethylene exposure due to close proximity to dry-cleaning facilities.[13] 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 exposed group consisted of 17 subjects (11 adults between the ages of 20 and 50, 2 adults over the age of 60, and 4 children, ages 6−18) from six families residing for a median of 6 years in two apartment buildings in New York City[14] (Schreiber et al., 2002). Preliminary monitoring of these buildings indicated tetrachloroethylene concentrations were elevated compared to eight other buildings also monitored by the NYSDOH. Exposed residents were from an affluent, English-speaking, Caucasian 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 noted tetrachloroethylene-like odors prior to the study. Controls were recruited from among NYSDOH Albany, NY employees and their families. All controls were Caucasian, 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

[13] Another study by Schreiber et al. (2002) of day-care staff from a center collocated with a dry-cleaning facility, using a similar testing protocol, was described in the Occupational Exposure Studies—Other Settings section.
[14] Study subjects were identified through several methods: (1) both families in the first building (Building A) had been referred to the NYSDOH for information about participating in the study by Consumer Union/Hunter College researchers, (2) one family in the second building (Building B) had previously contacted NYSDOH 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, 2004).

exposed subject. Mean age was 34.5 years for exposed apartment residents and 33.2 years for control subjects.

The assessment of tetrachloroethylene exposure of residents consisted of 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 1 month after closure of the facility for the remaining two families in the residential study. Adult residents also provided urine samples, which were analyzed for tetrachloroethylene as well as for three products of its metabolism: TCA, trichloroethanol, and the urinary acetyl metabolite. Ambient concentrations of tetrachloroethylene from 1 to 3 months before the date of visual testing, when active dry cleaning was occurring in both apartment buildings, were available for all subjects. Median concentrations in these samples 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.

All participants, or their guardians in the case of children, signed consent forms prior to study commencement. 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. Reported alcohol consumption (low to moderate) was similar in the adult exposed and control groups, and the Profile of Moods test scores of all residential exposed subjects were within normal limits. However, two of the four children had medically verified diagnoses of learning disabilities or developmental delays (NYSDOH, 2004).

As described in the previous discussion of Schreiber et al. (2002) (refer to Section 4.1.1.2.2 Occupational Exposure Studies—Other Settings), visual function testing consisted of near visual acuity, near visual contrast sensitivity, and color vision, and the investigators were not blinded as to a subject's status as either exposed or nonexposed. The mean measure of visual acuity was 20:27.7 in exposed residents and 20:22.8 in controls ($p = 0.12$). Group mean scores for visual contrast sensitivity across spatial frequencies were statistically significantly lower in exposed residents than in controls, indicating poorer visual function in the exposed groups (refer to Figure 4-2). An exposure-response analysis did not show an association between poorer performance and increasing tetrachloroethylene concentration. CCI scores (a measure of color vision) of the exposed group were lower than those of controls, but the difference was not

statistically significant (mean: 1.33 and 1.20 in exposed and control groups, respectively, $p = 0.26$).

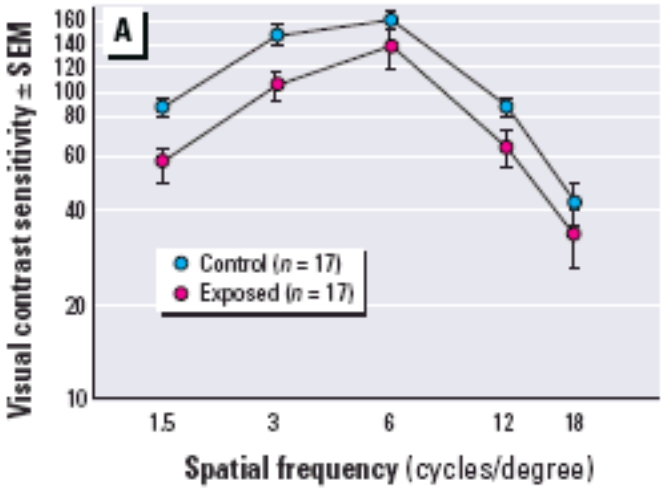

Figure 4-2. Visual contrast sensitivity functions for control and exposed participants in residential exposure study (Schreiber et al., 2002).
The X-axis represents the frequency of the stimulus bars, with finer bars toward the right. The Y-axis represents the inverse of the contrast at which the subject could no longer distinguish the orientation of the bars (threshold). Blue circles (top line) = controls; red circles (bottom line) = exposed. For any frequency, a higher contrast sensitivity threshold represents better visual function. Visual contrast sensitivity was significantly lower across all spatial frequencies in exposed residents of apartments in building with dry-cleaning facilities compared with their matched controls. Used with permission of the authors.

A larger study of the effect of tetrachloroethylene exposure on visual function in residents living in buildings colocated with a dry-cleaning establishment was also conducted (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a, b). This study, the New York City Perc Project, did not include the subjects in Schreiber et al. (2002) and employed different methods for testing visual contrast sensitivity and color vision. Study design and protocols were approved by Institutional Review Boards at the NYSDOH and other collaborating institutes (Mt. Sinai Medical Center and the Centers for Disease Control and Prevention). Sixty-five households in 24 residential buildings with dry cleaners using tetrachloroethylene on-site, and 61 households in 36 buildings without dry cleaners were recruited. 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 2001−2003. McDermott et al. (2005) presents exposure monitoring findings from the dry-cleaner households.

Subjects were identified in buildings from eleven contiguous zip code areas surrounding Central Park, New York City. Household eligibility criteria included the presence of 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 concentrations of tetrachloroethylene above the current NYSDOH residential air guideline of 0.015 ppm (0.1 mg/m^3). The study area was broadened to include buildings that had been the subject of a resident complaint and to include buildings in additional zip codes, primarily characterized by lower socioeconomic status or higher percentage of minority residents. 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. A total of 65 dry cleaner (67 adults, 68 children) and 61 referent households (61 adults, 71 children) participated in the study. The socioeconomic status characteristics, residence duration, education level, age, and smoking and alcohol use were similar in the adult residents of reference buildings and the residents of buildings with dry cleaners. 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. All participants or their guardians signed voluntary consent forms prior to study commencement.

NYSDOH staff visited participants in their residences to collect 24-hour indoor air samples and breath samples, and to give adult participants a questionnaire seeking information on residential, occupational, and medical history for themselves and their children. Indoor air tetrachloroethylene concentrations 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) in apartment buildings colocated with a dry cleaner. Monitoring was carried out using passive monitoring badges. In comparison, tetrachloroethylene concentrations in buildings 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 concentrations for adult and child subjects of dry-cleaning buildings. 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 nonminority subjects. Participants received financial compensation after completing the home visit ($50.00) and ophthalmology clinic visit ($50.00).

Ophthalmologic examinations and visual function tests were given to study participants at the Mt. Sinai Medical School of Medicine Department of Ophthalmology research clinic. The final report does not describe whether examiners were or were not blinded as to a subject's exposure status (NYSDOH, 2005a). The examination included determination of past ocular and medical history; measurement of visual acuity, pupil size, extraocular motility, and intraocular pressure; and anterior and posterior segment exams. Subjects with abnormalities or taking

medications that could influence visual contrast sensitivity 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. Visual contrast sensitivity was determined using the Functional Acuity Contrast Test (FACT) distance chart placed 10 feet from the participant under light conditions of 68−240 cd/m^2. These testing conditions differ from those employed by Schreiber et al. (2002) in their residential study where visual testing was carried out, assessing near-contrast sensitivity.

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

Analyses examining relationships between tetrachloroethylene and visual function were conducted using three categories of exposure: the referent exposure group (background exposure, living in a building without a dry cleaner, geometric mean: 2.9 µg/m^3 [0.0004 ppm], range: 1.5−4.2 µg/m^3 [0.0002−0.0006 ppm]); <100 µg/m^3 [geometric mean: 11.6 µg/m^3 {0.002 ppm}, range: 4.2−42.0 µg/m^3 {0.0002−0.006 ppm}]; and >100 µg/m^3 [geometric mean: 477.9 µg/m^3 {0.07 ppm}, range: 268.9−735.3 µg/m^3 {0.04−0.11 ppm}].[15] A decreasing trend ($p < 0.05$) was observed across these three exposure groups and the proportion of adults achieving the maximum contrast sensitivity score at 6 cpd (28.3, 14.3, and 8.3% in the referent, <100, and >100 µg/m^3 groups, respectively). This pattern was also observed in analyses stratified by race or ethnicity, or by income, although the smaller sample sizes resulted in larger p-values (from 0.09 to 0.30) for each of the individual strata. In children, decreasing scores were observed at 6 cpd (43.4, 33.3, and 18.2% in the referent, <100, and >100 µg/m^3 groups, respectively, trend: $p = 0.05$) and 12 cpd (37.7, 33.3, and 0.0% in the referent, <100, and >100 µg/m^3 groups, respectively, trend: $p = 0.02$). These effects were limited to minority and low income children in the ethnicity and income-stratified analyses.

Results from logistic regression analyses further support susceptibility of children—but not adults—to an adverse effect of tetrachloroethylene exposure on visual contrast sensitivity. Whereas adult visual contrast sensitivity in the worse eye at 6 or 12 cpd was not significantly influenced by any measure of tetrachloroethylene exposure, visual contrast sensitivity performance at 12 cpd among children was significantly influenced ($p < 0.05$) by

[15] 100 µg/m^3 = 0.015 ppm.

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tetrachloroethylene concentrations in either indoor air or in blood; i.e., a lower percentage of children achieved a maximum visual contrast sensitivity score with higher tetrachloroethylene exposure. Odds ratio estimates were 2.64 (95% CI: 1.41, 5.52), 3.37 (95% CI: 1.44, 9.29), and 3.54 (95% CI: 0.94, 17.79) for the association between visual contrast sensitivity performance in the worse eye at 12 cpd and indoor tetrachloroethylene, exhaled breath tetrachloroethylene at home, and blood tetrachloroethylene, respectively. The logistic regression models examining visual contrast sensitivity findings were adjusted for ethnicity or race and age, and, in adults, smoking and alcohol use.

Color vision was assessed biocularly using both the Farnsworth D-15 test (differentiates between strong/moderate and mild/normal CCI) and Lanthony D-15 test (differentiates between normal and mild CCI). Both tests were administered under light conditions specified by the manufacturer. 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 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 minority 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 visual contrast sensitivity performance, children appear to be a more susceptible population. There were no differences between exposure groups among adults or children in the percentage of subjects with major errors on both color vision tests. A comparison of mean CCI between exposure groups showed that children in the high-exposure category performed worse (mean: CCI of 1.3, range: 1.0−1.9) compared with children in the low-exposure category (mean: CCI of 1.1, range: 1.0−1.7) and compared with 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 NYSDOH (2005a). The higher mean difference in CCI between children and adults in the highest exposure category (>0.015 ppm or >100 $\mu g/m^3$) compared with referents was statistically significant. NYSDOH (2010) believed the decreased mean child-adult difference in CCI was likely influenced by the low adult CCIs in the highest

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exposure group. Children in the high-exposure group, furthermore, 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 limit 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 contrast vision, a number of analyses (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a) are suggestive of vulnerability among children. 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, nonminority groups, make 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 children studied.

4.1.1.2.4. Oral exposure studies

Risk of learning and behavioral disorders was evaluated in relation to prenatal and postnatal exposure to tetrachloroethylene in Cape Cod towns with a contaminated water distribution system during 1969−1983 (Janulewicz et al., 2008). Mothers reported developmental and educational histories and learning and behavioral disorders in self-administered questionnaires returned during 2002−2003. Developmental risks were evaluated in relation to the amount of tetrachloroethylene delivered to each subject's residence during the prenatal period (from the month and year of the last menstrual period through the month and year of the birth) and during the early postnatal period (from the month and year of the birth through the month and year of the 5th birthday). Prenatal and postnatal exposures were evaluated separately in generalized estimating equation regression models. After excluding 404 subjects because they had an attribute with a known association with the outcomes under study, there were 2,086 children in the final data set. Of these, 842 and 1,244 children had no and any prenatal exposure, respectively, and 760 and 1,326 children had no and any postnatal exposure, respectively. Exposed and unexposed children were similar with respect to demographic characteristics and behaviors. Low- and high-exposure categories were developed for the 9-month prenatal period and 5-year postnatal period using the number of grams of tetrachloroethylene that corresponded to an average drinking water concentration of 40 µg/L, the action level used in 1980, as a cutpoint. The authors reported that no meaningful associations were observed between prenatal exposure and receiving tutoring for reading or math, being placed on an Individualized Education Plan, or repeating a school grade. Increased odds ratios

were noted among subjects with low exposure compared to no exposure for receiving a diagnosis of attention deficit disorder or hyperactivity disorder, special class placement for academic or behavioral problems, or lower educational attainment (high school graduate or less). However, odds ratios were not markedly increased for subjects with high exposure (<1.1). For example, in generalized estimating equations models adjusted for maternal age, race, and education, child's sex, and prematurity and/or low birth weight, the odds ratio for attention deficit disorder was 1.4 (95% CI: 0.9−2.0) among subjects with low prenatal exposure and 1.0 (95% CI: 0.7−1.6) among subjects with high prenatal exposure. For postnatal exposure, no associations were observed for receiving tutoring for reading or math, special class placement for academic or behavioral problems, repeating a grade in school, or lower educational attainment. The same pattern of risk with exposure level also was observed for low and high postnatal exposure compared to no exposure. For example, the adjusted odds ratio for attention deficit disorder was 1.3 (95% CI: 0.9−1.9) among subjects with low postnatal exposure and 1.0 (95% CI: 0.6−1.7) among subjects with high postnatal exposure.

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

A summary of neuropsychological effects observed in chronic occupational or residential exposure studies of tetrachloroethylene is shown in Table 4-2 and discussed by domain below. Several studies (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a, b; Schreiber et al., 2002; Altmann et al., 1995; Echeverria et al., 1995) employed multiple measures of exposure (indoor air monitoring, personal monitoring, and in some cases, biological monitoring). Although some variation is expected and was observed in individual studies [Altmann et al. (1995) for example], the correlation between tetrachloroethylene concentration as assessed from indoor air monitoring or personal monitoring and biological metrics such as blood tetrachloroethylene concentration was quite strong, suggesting indoor air concentration as a reasonable exposure metric. Many studies did not include exposure monitoring of individual subjects, and the statistical analyses compare groups using *t*-tests or χ^2 tests (Spinatonda et al., 1997; Ferroni et al., 1992). Dose response and multiple logistic regression analyses are statistically more powerful, and five studies observed correlations or associations between various tetrachloroethylene exposure measures and specific neurobehavioral tests (Storm et al., 2011 [previously reported in NYSDOH, 2010]; Altmann et al., 1995; Echeverria et al., 1995; Cavalleri et al., 1994; Seeber, 1989).

Table 4-2. Summary of effects of chronic tetrachloroethylene exposure in humans observed in studies of neuropsychological function[a]

(Reference), *n* exposed, mean or median exposure(s)	Visual domain[a]			Cognitive domain (executive function, attention)[a]						Motor[a]	
	Spatial vision (VCS)	Color vision[b]	VEP	Visuo-spatial memory[c]	Vigilance	Trail-making	Digit span, symbol	Cancellation	Information processing[d]	Simple reaction time	Fine motor function
Occupational exposures—dry-cleaning settings											
Lauwerys et al. (1983), *n* = 26, 21 ppm										—*	
Seeber (1989), *n* = 101, 12 and 53 ppm				+			+	+	+		—
Nakatsuka et al. (1992), *n* = 64, 13 ppm		—									
Ferroni et al. (1992), *n* = 60, 15 ppm					+		—		—	+	—
Cavalleri et al. (1994), *n* = 35, 6 ppm		+									
Gobba et al. (1998); Cavalleri et al. (1994) follow-up, *n* = 33, 4 ppm		+									
Echeverria et al. (1995), *n* = 65, 11, 23, 41 ppm				+		—	—				
Echeverria et al. (1994), *n* = 173, <0.2, 3, 9 ppm				+		—	—				
Spinatonda et al. (1997), *n* = 35, 8 ppm									+		
Sharanjeet-Kaur et al. (2004), *n* = 14, not reported		+									

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Table 4-2. Summary of effects of chronic tetrachloroethylene exposure in humans observed in studies of neuropsychological function (continued)

(Reference), *n* exposed, mean or median exposure(s)	Visual domain[a]			Cognitive domain (executive function, attention)[a]						Motor[a]	
	Spatial vision (VCS)	Color vision[b]	VEP	Visuo-spatial memory[c]	Vigilance	Trail-making	Digit span, symbol	Cancellation	Information processing[d]	Simple reaction time	Fine motor function
Occupational exposures—other settings											
Schreiber et al. (2002), Day-care workers *n* = 9, 0.32 ppm	+										
Residential exposures											
Altmann et al. (1995), *n* = 19, 0.7 ppm			—	+	+					+	—
Schreiber et al. (2002), *n* = 17 (13 adults and 4 children), 0.4 ppm	+	+ (trend)									
McDermott et al. (2005); NYSDOH (Storm et al., 2011; NYSDOH, 2010), *n* = 68 children (C), *n* = 67 adults (A), 0.005 ppm	+ (C), — (A)	— (C), — (A)									

[a] + denotes effects observed (i.e., worse performance) in exposed group; — denotes no effect or better performance in exposed group; —* denotes better performance in the exposed group (before shift measure); blank cell denotes test not performed.
[b] Based on Lanthony D-15 test, except for Nakatsuka et al. (1992), who used a less sensitive version of this test.
[c] Tests include digit reproduction (Seeber, 1989); switching, pattern memory, and pattern recognition (Echeverria et al., 1995; Echeverria et al., 1994); and Benton test (Altmann et al., 1995).
[d] Tests include choice reaction time (Seeber, 1989), perceptual threshold (Seeber, 1989), finger tapping (Ferroni et al., 1992), and vocal reproduction to reading stimuli (Spinatonda et al., 1997).

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4.1.1.3.1. Visual function domain

Color vision and visual contrast sensitivity are the visual domains that have been observed to be affected by chronic exposure to tetrachloroethylene (refer to Table 4-2). Only Schreiber et al. (2002) and NYSDOH (2005a) assessed spatial vision (VCS, visual contrast sensitivity), an effect reported for exposure to other solvents (Schreiber et al., 2002; Hudnell et al., 1996a; Hudnell et al., 1996b; Broadwell et al., 1995; Campagna et al., 1995; Donoghue et al., 1995; Bowler et al., 1991; Frenette et al., 1991; Mergler et al., 1991). In Schreiber et al. (2002), visual contrast sensitivity deficits in subjects (mostly adults) with normal visual acuity were observed at low-exposure concentrations in residential populations, and in NYSDOH (2005a); evidence of these effects were observed in children but not in adults. Exposure levels were lower in the latter study [mean: 0.4 ppm and geometric mean: 0.005 ppm in Schreiber et al. (2002) and NYSDOH (2005a), respectively]. Potential bias and confounding could have been introduced, however, from a lack of blinding of testers and, in the latter study, the inability to control for socioeconomic and other factors that were highly correlated with higher tetrachloroethylene exposures.

Deficits in blue-yellow color vision, a well established effect of solvents, were observed in dry-cleaning workers in Italy in Cavalleri et al. (1994) and in a follow-up study (Gobba et al., 1998) of this population. Cavalleri et al. (1994) specifically noted that the color vision testing was conducted by examiners who were blinded to the exposure level of individual study participants, and the study participants were well-matched in terms of age, smoking, and alcohol use. Mean TWA exposure levels were approximately 6 ppm among all workers in Cavalleri et al. (1994). 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. This type of color vision deficit was not observed in the dry cleaners study by Nakatsuka et al. (1992), but the form of the color vision test used in the latter study, the Lanthony 15, is less sensitive to mild and moderate changes in color vision compared with the desaturated version of the test (Lanthony D-15) used in the other studies (Lanthony, 1978). Effects on color vision were also observed among 14 dry cleaners in the small study in Malaysia by Sharanjeet-Kaur et al. (2004), but the lack of exposure information (other than job title), and differences between dry cleaners and controls regarding test conditions and smoking habits indicate that this study should provide little weight in the overall conclusions regarding color vision. Two other small studies also reported lower scores on the Lanthony D-15 color vision test in exposed groups compared with controls, but the differences were not statistically significant: in a study of residents living above dry cleaners (mean

tetrachloroethylene exposure during active dry cleaning = 0.4 ppm), the mean CCI scores were 1.33 and 1.20 in 17 exposed and 17 control groups, respectively ($p = 0.26$); in a study of workers in a day-care center located in a building with a dry-cleaning business (mean tetrachloroethylene exposure: 0.32 ppm), the mean CCI scores were 1.22 and 1.18 in the exposed day-care workers and controls, respectively ($p = 0.39$) (Schreiber et al., 2002). The follow-up study of NYSDOH (2005a) further suggests tetrachloroethylene effects on color vision, particularly in children, although NYSDOH (2010) believed the decreased mean child-adult difference in CCI was likely influenced by the low adult CCIs in the highest exposure group.

Peer-consultation comments on EPA's earlier draft *Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper* (U.S. EPA, 2003) 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 relatively more sensitive than other vision tests or tests used for other domains (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 these studies is difficult, particularly in light of the paucity of data on this test in occupational populations with higher exposure concentrations and in animal studies.

Although Altmann et al. (1992; 1990) reported alterations in visual evoked potentials ($p \leq 0.05$) with 4-hour 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 response.

4.1.1.3.2. Cognitive domain

Cognitive domains affected by tetrachloroethylene include visuospatial memory, attention, vigilance (continuous performance), and speed of information processing (refer to Table 4-2). Effects on visuospatial memory are of particular interest, given similar results in studies that examined this type of effect in occupational (Echeverria et al., 1995; Echeverria et al., 1994; Seeber, 1989) or residential (Altmann et al., 1995) settings, and given similar reports for other solvents (Daniell et al., 1999; Morrow et al., 1990). Echeverria et al. (1995) found effects among 23 dry cleaners classified as having a high chronic exposure (based on type of shop, job title, and years of employment) 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 categorized as having high lifetime chronic exposure and whose current exposure level was 9 ppm, 8-hour TWA; the exposure level

of 9 ppm is not representative of past chronic exposure levels because of changes occurring in the industry (i.e., switching from wet-transfer to dry-to-dry machine). Seeber (1989) also reported impaired visuospatial recognition in a low exposure (mean: TWA 12 ppm) and a high exposure group (mean: TWA 53 ppm), and Altmann et al. (1995) observed deficits on a test of visuospatial function in residents with much lower exposure concentrations (mean: 0.7 ppm) than those of the occupational studies. All of these studies except Altmann et al. (1995) reported that investigators were blinded to knowledge of the exposure level of the subject. These studies provide strong weight, given the numbers of subjects and their use of appropriate statistical methods, including adjustment for potentially confounding factors that may be relevant for measures of the cognitive domain. For example, Seeber (1989) adjusted for age, gender, and a measure of intelligence (alcohol was examined but not shown by these investigators as confounding the association between tetrachloroethylene and cognitive performance), and a variety of potential confounders were evaluated by Echeverria et al. (1995; 1994). It should be noted, however, that residual confounding from education level differences between exposed and referent subjects may still be present in Altmann et al. (1995).

The results pertaining to cognitive measures other than visuospatial memory are somewhat mixed. 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 (digit reproduction and digit symbol) that are subsets of the Wechsler 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 (cancellation d2) 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 NYSDOH (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). 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 (perceptual speed). In the choice reaction time task (correct reactions), effects were borderline in the lower-exposure group and negative in the higher-exposure group, with no exposure-response relationship. Spinatonda et al. (1997) observed longer mean reaction times and/or vocalization durations to vocal and visual stimuli.

Two 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. In both studies, lower performance [ranging from an increase in reaction time from 24 (11%, 102 mg/m^3) (Ferroni et al., 1992)] to 50 ms [20%, 4.99 mg/m^3 (Altmann et al., 1995)] was observed among the exposed workers compared with referents. A third study, Lauwerys et al. (1983), reported better performance on simple reaction time in exposed workers compared with referents when measured before a work shift but not when measured after work.

4.1.1.3.3. Motor function domain

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.

4.1.1.3.4. Other clinical tests and conditions

A clinical neurological examination that includes the Romberg test, neuroradiological examination, neurophysiological tests such as EEGs, and nerve conduction tests or other tests for peripheral neuropathy have observed limited use for assessing neurotoxicologic effects in tetrachloroethylene-exposed populations. Mental disease and behavioral disorders of neurologic origin have 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. A fourfold increased risk of schizophrenia was observed among offspring. However, in a small study, Janulewicz et al. (2007) did not observe an association between prenatal or early postnatal drinking water exposure to tetrachloroethylene and disorders of learning, attention, and behavior. Therefore, other studies are needed to understand the role of parental tetrachloroethylene exposure in the development of mental disease and behavioral disorders in children.

4.1.2. Animal Studies

Tetrachloroethylene exposure in experimental studies in animals results in general CNS-depressant activity (decreased activity, anxiolytic behavior, lethargy), impairment in balance and motor coordination, cognitive defects, sleep cycle changes, and changes in visual function and nerve conduction velocity. These changes have been observed following either an inhalation or oral/intraperitoneal (i.p.) exposure. In addition to these effects, several effects on brain pathology including DNA- and RNA-level changes, changes in neurotransmitter levels such as acetylcholine and glutamate, and changes in brain fatty acid composition, have been

observed. Some studies also document potential developmental neurotoxicity consequences following exposure to tetrachloroethylene during the gestation period.

4.1.2.1. Inhalation Studies

The animal inhalation neurotoxicity studies are summarized in Table 4-3 and described in more detail below. Neurobehavioral, neurophysiological, and developmental neurotoxicity effects have been reported following tetrachloroethylene exposure. Two neurobehavioral studies observed that there was an increase in motor activity following a 1-hour exposure in NMRI mice at 90 ppm and higher (Kjellstrand et al., 1985), and there was a decrease in immobility in Swiss OF1 mice at 649 ppm and higher during 4 hours of exposure (De Ceaurriz et al., 1983). A more recent neurobehavioral study examined effects of Long-Evans rats in a signal detection test and reported decreased sustained attention as a measurement of decreased trial completions and increased reaction time during an hour exposure to 500 ppm or higher (Oshiro et al., 2008). In F344 rats, significant changes in FEP latency and amplitude following a 12-week repeated exposure to 800 ppm or higher were reported by Mattsson et al. (1998), and in Long-Evans rats, changes in visual evoked potential amplitudes during an acute (60−120 minutes) exposure to 250 ppm or higher were reported by Boyes et al. (2009). Developmental neurotoxic effects were noted in three studies (Szakmáry et al., 1997; Tinston, 1994; Nelson et al., 1979) where changes such as decreases in muscular strength and exploratory behavior as well as other behavioral habits were significantly different from nonexposed litters. Finally, there were many changes in brain pathology as noted by decreased brain weight, brain DNA levels, and changes in neurotransmitter levels (Wang et al., 1993; Kyrklund and Haglid, 1991; Kyrklund et al., 1990, 1988; Karlsson et al., 1987; Kyrklund et al., 1987; Briving et al., 1986; Rosengren et al., 1986; Kjellstrand et al., 1984; Kyrklund et al., 1984; Honma et al., 1980a; Honma et al., 1980b; Savolainen et al., 1977a; Savolainen et al., 1977b).

4.1.2.1.1. Neurobehavior

De Ceaurriz et al. (1983) exposed male Swiss OF1 mice (n = 10 per exposure group) to 596, 649, 684, or 820 ppm tetrachloroethylene for 4 hours. Immediately following exposure, the mice 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.

Table 4-3. Summary of animal inhalation neurotoxicology studies

Subjects	Effect	NOAEL/LOAEL[a] (ppm)	Reference
Neurobehavioral studies			
Swiss OF1 mice, males 10/dose	Decreased duration of immobility	596, 649, 684, 820; 4 h	De Ceaurriz et al. (1983)
NMRI mice, males (n = 27 for 90, 320, 400, 600; n = 14 for 800, 1,200, 1,800, 3,600 ppm)	Increased motor activity	90, 3,600; 1 h	Kjellstrand et al. (1985)
Long-Evans rats, males (n = 12 total; animals served as own controls)	Increased number of false alarms, increased reaction time, and decreased trial completions in a signal detection task measuring sustained attention	0, 500, 1,000, 1,500; 60 min	Oshiro et al. (2008)
Neurophysiological studies			
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 d, 6 h/d 50, 200, 800; 13 wk, 6 h/d, 5 d/wk	Mattsson et al. (1998)
Long-Evans rats, males (n = 9−10/exposure)	Decreased frequency doubled amplitude in the steady state VEP	0, 250, 500, 1,000 for 1.5 h	Boyes et al. (2009)
Developmental neurotoxicity studies			
S-D 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 GDs 7−13 or on GDs 14−20, 7 h/d	Nelson et al. (1979)
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 d postnatally	0, 1,500 or 4,500 mg/m^3 GDs 1−20 for 8 h/d	Szakmáry et al. (1997)
S-D rats, multigeneration study 28 litters/dose	CNS depression in first 2 wk of F1 and F2 generations, which ceased 2 h after daily exposures	0, 100, 300, 1,000; 6 h/d, 5 d/wk, except during mating, 6 h/d-7 d/wk	Tinston (1994)

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Table 4-3. Summary of animal inhalation neurotoxicology studies (continued)

Subjects	Effect	NOAEL/LOAEL[a] (ppm)	Reference
Brain pathology			
S-D rats, males 8/dose	Decreased brain weight, DNA, protein	300, 600; 4 or 12 wk continuous (24 h/d)	Wang et al. (1993)
S-D rats, males 10/dose	Decreased brain RNA, increased brain cholinesterase and increased motor activity	200; 4 d	Savolainen et al. (1977a; 1977b)
S-D rats, males 5−6/dose	Change in fatty acid composition of cerebral cortex	320; 12 wk continuous (24 h/d), 30-d washout period; 320; 4 wk continuous (24 h/d)	Kyrklund et al. (1990, 1988)
S-D rats, males 5−6/dose	Neurotransmitter changes, brain regions	200, 400, 800; 4 wk continuous (24 h/d)	Honma et al. (1980a; 1980b)
Mongolian gerbils males and females 6/sex/dose	Decrease in DNA, frontal cortex Decrease in brain weight	60, 300; 12 wk, continuous (24 h/d); 16-wk washout period	Rosengren et al. (1986)
Mongolian gerbils males and females 4/sex/dose	Decrease in DNA, frontal cortex Decrease in brain weight	60;12 wk, continuous (24 h/d)	Karlsson et al. (1987)
Mongolian gerbils males and females 8/sex/dose	Taurine, glutamine changes in brain regions	120;12 mo continuous (24 h/d)	Briving et al. (1986)
Mongolian gerbils gender unspecified 6/dose	Decrease in brain weight, change in fatty acids	320; 12 wk continuous (24 h/d)	Kyrklund et al. (1987)
Mongolian gerbils males 6/dose	Decreased brain long-chain fatty acids	120;52 wk continuous (24 h/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; GDs 33 to 65 continuous (24 h/d)	Kyrklund and Hagid (1991)
NMRI mice, males and females 3−8/sex/dose	Increase in butyl cholinesterase	9[c], 37, 75, 150; 4 wk continuous (24 h/d)	Kjellstrand et al. (1984)
Males and females 10/sex/dose	Increased motor activity	150; 4 wk intermittent- (1, 2, 4, 8, or 16 h/d)	Kjellstrand et al. (1984)

[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.
EEG = electroencephalogram; FEP = Flash-evoked potential; GD = Gestational day; S-D = Sprague-Dawley; SEP = Somatosensory-evoked potential; VEP = Visual Evoked Potential

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The effects of exposure to 90−3,600 ppm tetrachloroethylene for 1 hour on motor activity were examined in male NMRI mice (n = 14−27 per exposure group) (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 performance was dose dependent.

Male Long-Evans rats (*n* = 12) previously trained to perform a visual signal detection task were exposed to 0, 500, 1,000, and 1,500 ppm tetrachloroethylene for 60 minutes (Boyes et al., 2009). In this learned task, rats are trained to respond to a light stimulus by pressing the stimulus lever and to press the blank lever when there is no stimulus. Food pellets are provided to the rat for each correct lever response. The parameters evaluated included measures of (1) correct responses (pressing stimulus lever with stimulus), (2) correct rejections (pressing blank lever when stimulus is not presented), (3) false alarms (pressing stimulus lever without stimulus), and (4) misses (pressing the blank lever when the stimulus is presented). Other endpoints measured included reaction times from presentation of stimulus to pressing of the lever and if the rat completed the signal detection task within the allotted period of time (2 minutes). Tetrachloroethylene (500−1,500 ppm) exposure significantly increased the number of false alarms, indicative of a decrement in sustained attention. Additionally, the authors reported that there was a dose-dependent increase in reaction time and decreased trial completions. Rats were also tested with different signal intensities to evaluate if the changes were partially due to visual deficits. The number of hits did not significantly change with the signal intensity of the stimulus, which strongly suggests that the observed effects of tetrachloroethylene in this study are due to cognitive changes rather than visual effects. The study authors reported a LOAEL of 500 ppm (60 minute exposure) for effects related to decrements in sustained attention.

4.1.2.1.2. Neurophysiology

Mattsson et al. (1998) studied the effects of acute exposure to tetrachloroethylene for 13 weeks on visual function (flash-evoked potentials [FEPs], EEGs, sensory function (somatosensory-evoked potentials [SEPs]), and rectal temperature in F344 rats. During the acute (pilot) study, male rats were exposed to 0 or 800 ppm tetrachloroethylene, for 6 hours/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 hours/day, for 13 weeks. Testing was performed during the week following cessation of exposure. A significant increase in the amplitude and in latency (~3.0 ms) for the mid-component peak of the FEP was 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.

Male Long-Evans rats (n = 9−10/group) were exposed to concentrations of tetrachloroethylene ranging from 0−4,000 ppm in two separate experiments evaluating visual function by measuring pattern-elicited steady state visual evoked potentials (Boyes et al., 2009). In the first experiment, rats were exposed to (mean ± SEM in parentheses) 0, 1,000 (1,006 ± 7.4), 2000 (1993 ± 8.3), 3,000 (3,018 ± 6.9), or 4,000 (4,016 ± 19) ppm for 2 hours (0, 1,000, 2,000 ppm), 1.3 hours (3,000 ppm), or 1 hour (4,000 ppm). In the second experiment, rats were exposed to 0, 250 (249 ± 1.1), 500 (488 ± 2.9), or 1,000 (1,053 ± 9.6) ppm for 1.5 hours. In both experiments, the visual evoked potentials were measured while the animal was exposed to tetrachloroethylene. The steady state visual evoked potential responses measured from the animals are sinusoidal in nature, and the potentials were transformed so that amplitudes were tabulated at the frequency of pattern presentation (F1) and at double the frequency of pattern presentation (F2). At all test conditions, tetrachloroethylene significantly decreased the F2 amplitude of the steady state visual evoked potential. The LOAEL for steady state visual evoked potentials for this study is 250 ppm tetrachloroethylene for 1.5 hours.

4.1.2.1.3. Developmental neurotoxicity

Developmental neurotoxicity is also discussed in Section 4.7.1.2. Nelson et al. (1979) investigated developmental neurotoxicity in Sprague-Dawley (S-D) rats by exposing pregnant dams to tetrachloroethylene at concentrations of 100 or 900 ppm during both early pregnancy (gestation days [GDs] 7 to 13) or late pregnancy (GDs 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 rotarod test (evaluation of neuromuscular function), whereas those in the late pregnancy group underperformed on the ascent test only at postnatal day (PND) 14. However, later in development (PNDs 21 and 25), their performance was higher than that of the controls on the rotarod test. These pups were markedly more active in the open field test at PNDs 31 and 32.

There were no effects on running in an activity wheel on PNDs 32 or 33 or avoidance conditioning on PND 34 and operant conditioning on PNDs 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 GDs 7−13. However, 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.

Szakmáry et al. (1997) exposed CFY rats to tetrachloroethylene via inhalation throughout gestation (i.e., GDs 1−20) for 8 hours/day at concentrations of 0, 1,500, or 4,500 mg/m^3 tetrachloroethylene. The primary focus of the study was prenatal developmental evaluations (refer to 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 PND 100 and evaluated for growth, development, and neurotoxic effects. The report did not specify whether the animals were exposed to tetrachloroethylene after birth. Preweaning 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) that normalized by PND 51, and significantly increased motor activity on PND 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 postweaning period or during adulthood.

Tinston (1994) performed a multigeneration 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 hours 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.

4.1.2.1.4. Brain pathology changes

Wang et al. (1993) exposed male S-D 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 the frontal cortex and the brain stem but not the 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, for 6 hours/day, for 4 days, in male S-D rats were examined for a number of endpoints (Savolainen et al., 1977a; Savolainen et al., 1977b). Rats were euthanized on the 5th day following a further 0−6 hours 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 hours 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 hours 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 S-D rats resulted in changes in the fatty acid composition of the cerebral cortex, which persisted after a 30 day recovery period (Kyrklund et al., 1990). Similar results were observed in the cerebral cortex and the hippocampus of Mongolian gerbils (sex unspecified) as well as reduced brain weight after exposure to 320 ppm (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 the cerebral cortex and the hippocampus (Kyrklund et al., 1984).

The effect of tetrachloroethylene on neurotransmitter levels in the brain was explored in male S-D rats exposed continuously to 200, 400, or 800 ppm tetrachloroethylene for a month (Honma et al., 1980a; Honma et al., 1980b). 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. γ-Aminobutyric acid (GABA), NE, 5-HT, and other amino acids were not affected.

In a study from the same laboratory, 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 the 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 to tetrachloroethylene at 120 ppm for 12 months. 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 the hippocampus. GABA levels were unaffected, as was uptake of GABA and glutamate.

Kyrklund and Haglid (1991) exposed pregnant guinea pigs to airborne tetrachloroethylene continuously from GD 33 through GD 65. 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. According to the study 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 study authors' earlier findings in rats. The statistical analysis, however, relied on pups as the experimental unit rather than the litters, so the *p*-values were likely underestimated. The results also suggested that tetrachloroethylene reduced the litter size, but a much larger study would be necessary to establish reduced litter size because the effect of tetrachloroethylene in this study was relatively small, and the reduction was not statistically significant.

Caucasian male and female NMRI mice were exposed to 9, 37, 75, or 150 ppm tetrachloroethylene continuously for 30 days, to 150 ppm tetrachloroethylene for one of several exposure periods ranging from 5−30 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, which resulted in a TWA of 150 ppm for 30 days. Motor activity was assessed following exposure. All concentrations of intermittent exposure increased motor activity. Results of motor activity following continuous exposure were not reported.

4.1.2.2. Oral and Intraperitoneal Studies

Table 4-4 presents a summary of the oral neurotoxicity animal studies, which are described in greater detail in the sections that follow. 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. 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

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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.

Table 4-4. Summary of oral neurotoxicity animal studies

Subjects	Effect	NOAEL/LOAEL[a] (mg/kg)	Reference
Neurobehavioral studies			
S-D rats, male 9/dose	Pain threshold, pain susceptibility, weight gain decrement Interpretation is unclear	Daily dose for 8 wk: 5, 50 mg/kg	Chen et al. (2002b)
S-D rats, male, 8-10/dose	Operant responses stopped immediately after 480 mg/kg dose, then 2/3 of animals recovered by 40 min Brain PCE concentrations were the same at both doses	Gavage single dose: 0, 160, 480 mg/kg	Warren et al. (1996)
ICR mice, male 8−10/dose	NOAEL/LOAEL: ↓ righting reflex, 2,000/4,000 Impaired balance, 1,000/2,000 Operant responses, 1,000/2,000 ↓ punishment responses, 500/1,000	Single i.p. doses: 0, 500, 1,000, 2,000, 4,000 mg/kg	Umezu et al. (1997)
F344 rats, female *n*/dose	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 d: 1,500 mg/kg is NOAEL	Moser et al. (1995)
Wistar rats, male *n*/dose	Transient delay in circadian activity, dose-related	i.p. doses: 0, 100, 500, 1,000 mg/kg-day for 3 d	Motohashi et al. (1993)
Developmental neurotoxicity study			
NMRI male mice, postnatal 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 PNDs 10−16	Fredriksson et al. (1993)

[a] Experimental/observational NOAEL is underlined; LOAEL is double-underlined.
n/dose = Number of animals per dose not clearly defined

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4.1.2.2.1. Neurobehavior

A study in male S-D rats assessed the acute or short-term effects of tetrachloroethylene by gavage on several screening tests (Chen et al., 2002a). A single dose of 500 mg/kg in adult rats produced changes on three different tests of pain threshold, locomotor activity, and seizure susceptibility threshold following pentylenetetrazol infusion, whereas 50 mg/kg resulted in statistically significant effects only on seizure threshold. In the short-term study, young, 45−50 g 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 required scoring by an observer. The study by Chen et al. (2002a) 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. Differences in body weight between control and treated rats add potential bias. 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 interobserver 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, response 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 hours following exposure. After acute exposure, a LOAEL of 150 mg/kg was identified for increased reactivity

to being handled 4 hours after dosing, with increased lacrimation, decreased motor activity, abnormal gait, decreased response to an auditory stimulus, decreased righting ability, and increased landing foot splay at higher doses at 4 and/or 24 hours postdosing. 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 acclimation 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. Locomotion, rearing, and total activity (vibration of the cage) were measured for 60 minutes at 17 and 60 days of age. A statistically significant increase in locomotor activity and total activity of treated mice in both dose groups was observed, and rearing behavior decreased as compared with controls for all three evaluations at 60 days of age, but not at 17 days of age when testing followed shortly after the last dose. Litter mates were used as independent observations in the statistical analysis, which tends to underestimate *p*-values and thereby overstate statistical significance [i.e., Holson (1992); Buelke-Sam (1985)]. However, the magnitude of the effects seen, more than a twofold increase in locomotion and total activity by the end of the Day 60 evaluation period, and the persistent effects of subacute developmental exposures in this study raise concern. 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 hours/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 S-D 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.

4.1.2.2.2. Developmental neurotoxicity

Evidence of potential developmental neurotoxicity was reported by Fredriksson et al. (1993). In this study (refer to Section 4.1.2.2), tetrachloroethylene was administered to male NMRI mice by gavage at dose levels of 0, 5, or 320 mg/kg-day on PNDs 10−16. At PNDs 17 and 60, spontaneous activity (locomotion, rearing, and total activity) was measured over three, 20 minute periods. No treatment-related alterations in activity were observed at 17 days of age; however, at 60 days of age, all three measures of spontaneous activity were altered.

4.1.3. Mode of Action (MOA) 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. The primary neurobehavioral changes that are observed following tetrachloroethylene exposure are visual changes, cognitive deficits, and increased reaction time. It is not clear if there are multiple mechanisms resulting in these outcomes. Additionally, there may be multiple mechanisms or MOAs, which may differ for adult and developmental exposure. The acute effects of tetrachloroethylene appear to share much in common with those of other chlorinated solvents such as trichloroethylene and dichloromethane as well as toluene, volatile anesthetics, and alcohols. It is unknown how these different neurological effects are induced, but there are data available to help elucidate what areas in the brain and specific molecular targets may be involved in the resulting neurotoxicological outcome.

Neuropathology and mechanistic studies have been conducted in animal models (rats, mice, gerbils) to determine how tetrachloroethylene may be producing the observed neurological effects. Changes in fatty acid composition of the brain following a 30 or 90 day exposure have been reported, and these changes persist for up to 30 days after the cessation of exposure (Kyrklund et al., 1990, 1988, 1987; Kyrklund et al., 1984). Studies that examined the entire brains of animals reported decreases in astroglial proteins (GFAP and S-100), decreased brain RNA content, and decreased levels of glutamine, threonine, and serine (Wang et al., 1993; Kyrklund et al., 1990, 1988, 1987; Rosengren et al., 1986; Kyrklund et al., 1984; Honma et al., 1980a; Honma et al., 1980b; Savolainen et al., 1977a). Brain regions examined following tetrachloroethylene exposure included the frontal cortex, the hippocampus, the striatum, and the cerebellum (Wang et al., 1993; Karlsson et al., 1987; Briving et al., 1986; Kyrklund et al., 1984; Honma et al., 1980a; Honma et al., 1980b). Notable changes include decreased DNA content in the frontal cortex following continuous exposure of 600 ppm for 4 weeks in rats (Wang et al., 1993) or a 60 ppm exposure for 3 months in Mongolian gerbils (Karlsson et al., 1987). Decreased taurine levels were noted in both the cerebellum and hippocampus following a 12-month exposure to 120 ppm tetrachloroethylene in Mongolian gerbils, but there were no

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changes in GABA levels or uptake (Briving et al., 1986). Decreased acetylcholine levels in the striatum were noted in male rats exposed to 800 ppm for 1 month (Honma et al., 1980a; Honma et al., 1980b).

Voltage and ligand-gated ion channels have been implicated in many neurological functions and have been studied as potential neurological targets for tetrachloroethylene and other structurally related chlorinated solvents (e.g., trichloroethylene, 1,1,1-trichloroethane, dichloromethane). Table 4-5 summarizes the available in vitro mechanistic studies with chlorinated solvents. Tetrachloroethylene has been demonstrated to inhibit calcium channel function (Shafer et al., 2005) and the neuronal nicotinic acetylcholine receptor (Bale et al., 2005). Based on the structural similarity of tetrachloroethylene to other chlorinated solvents as well as the similar neurobehavioral and mechanistic findings, it is likely that tetrachloroethylene also interacts with the other listed targets in Table 4-5. This solvent class has also been shown to interact with ion channels such as the $GABA_A$ and glycine receptors (Lopreato et al., 2003; Beckstead et al., 2000; Krasowski and Harrison, 2000). Overall, these solvents appear to potentiate the function of inhibitory receptors and inhibit the function of excitatory receptors [refer to Bushnell et al. (2007) and Bowen et al. (2006) for a review]. Additionally, this class of solvents blocks sodium channel (Haydon and Urban, 1983; Shrivastav et al., 1976) and voltage sensitive calcium channel function (Shafer et al., 2005) when the membrane is held at or near the resting membrane potential.

Based on these findings as well as other mechanistic studies conducted with tetrachloroethylene, some neurotransmitter systems may be more favorably involved in neurotoxicological outcomes than others. Also, based on the number of reported molecular targets, it is more likely that there are several plausible mechanisms responsible for the resultant neurotoxicological outcome, and those potential mechanisms (as well as a discussion of plausibility) are summarized below by the major observed outcomes (visual changes, cognitive deficits, increased reaction time).

Table 4-5. Summary of in vitro ion channel effects of tetrachloroethylene and other chlorinated solvents

Reference	Cellular system	Ion channel/receptor	Concentration	Effects
Tetrachloroethylene				
Shafer et al. (2005)	PC12 cells, primary cortical neurons	Voltage Sensitive Calcium Channels (VSCCs)	0−325 μM	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of −70 mV
Bale et al. (2005)	*Xenopus* oocytes	Human and rat α4β2, α3β2, and α7 nicotinic acetylcholine receptors	0−65 μM	Inhibition of nicotinic acetylcholine receptor function
Dichloromethane				
Haydon and Urban (1983)	Squid giant axon	Sodium channels	0, 15, 25 mM	Inhibition of inward sodium channel currents
Trichloroethylene				
Shafer et al. (2005)	PC12 cells, primary cortical neurons	VSCCs	0−2,100 μM	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of −70 mV
Beckstead et al. (2000)	*Xenopus* oocytes	Human recombinant glycine receptor α1, $GABA_A$ receptors, α1β1, α1β2γ2L	0, 390 μM	50% potentiation of the $GABA_A$ receptors; 100% potentiation of the glycine receptor
Lopreato et al. (2003)	*Xenopus* oocytes	Human recombinant serotonin 3A receptor	0, 390 μM	Potentiation of serotonin receptor function
Krasowski and Harrison (2000)	Human embryonic kidney, 293 cells	Human recombinant glycine receptor α1, $GABA_A$ receptors α2β1	Not provided	Potentiation of glycine receptor function with an EC_{50} of 0.65 ± 0.05 mM. Potentiation of $GABA_A$ receptor function with an EC_{50} of 0.85 ± 0.2 mM
Shrivastav et al. (1976)	Squid giant axon	Sodium channels	5−80% saturation	Shift of sodium channel activation to a more hyperpolarizing potential. Inhibition of inward sodium channel current at −70 mV
1,1,1-Trichloroethane				
Cruz et al. (2000)	*Xenopus* oocytes	NMDA-glutamate receptor NR1/2A, NR1/2B	0.1−10 mM	Inhibition of NMDA-glutamate receptor function
Beckstead et al. (2000)	*Xenopus* oocytes	Human recombinant glycine receptor α1, $GABA_A$ receptors, α1β1, α1β2γ2L	0.39 mM	Potentiation of $GABA_A$ and glycine receptor function
Beckstead et al. (2000)	Rat hippocampal slices	$GABA_A$ receptor	0.28 mM	Reversible increase in $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs)

4.1.3.1. Visual Function Domain

Although tetrachloroethylene produces changes in visual evoked potentials, there are no associated mechanistic studies to indicate what receptor systems may be involved. However, there is a characterization study evaluating the contribution of specific ligand-gated ion channels ($GABA_A$, NMDA-glutamate, nicotinic acetylcholine receptors) to the generation of the steady state visual evoked potential (Bale et al., 2005). The findings suggest that ion channels are involved in visual function and, specifically, the measured evoked potentials. The only administered drugs resulting in an effect similar to tetrachloroethylene were NMDA (NMDA-glutamate receptor agonist) and mecamylamine (nAChR antagonist). Therefore, the NMDA-glutamate and the nicotinic acetylcholine receptor systems may be more closely involved in the visual evoked potential changes resulting from solvent exposure.

With respect to the impact on color vision and visual contrast sensitivity following tetrachloroethylene exposure, the mechanisms behind these effects are unknown. These visual changes occur at exposures that are lower than the visual evoked potential changes. Cones at the level of the retina process color vision, and there may be a change in the function and/or signaling of the retina to the visual center in the CNS. In visual contrast sensitivity, retinal ganglion cells have been implicated as a sensitive target in processing changes in contrast (Beaudoin et al., 2008). The available literature suggests that NMDA-glutamate receptors (Manookin et al., 2010) and calcium channels (Hu et al., 2009) may be involved in visual contrast sensitivity changes. It is known that tetrachloroethylene exposure affects calcium channel function in vitro (Shafer et al., 2005), and a related chlorinated solvent, 1,1,1-trichloroethane, has been demonstrated to modulate NMDA-glutamate receptor function (Cruz et al., 2000).

4.1.3.2. Cognitive Domain

The hippocampus is involved in cognitive functions, but only changes in taurine levels were observed in this brain region following tetrachloroethylene exposure in gerbils (Briving et al., 1986). It was demonstrated that tetrachloroethylene inhibits both human and rat recombinant nicotinic acetylcholine receptors in vitro (Bale et al., 2005), and perhaps this finding may help explain why cognitive changes are observed with tetrachloroethylene exposure. However, more studies need to be conducted with tetrachloroethylene exposure and perhaps incorporating a challenge with nicotinic agonists and antagonists to determine the involvement of nicotinic acetylcholine receptors in cognitive function.

4.1.3.3. Motor Activity Domain - Reaction Time

Reaction time is a general measure of CNS function. With increased reaction time, it can be surmised that there is a general CNS decrease in movement. Currently, there are no available mechanistic studies with tetrachloroethylene that have evaluated neurological systems mediating reaction time activity. There is one study that has reported that decreased CNS function (anxiolytic profile) observed with tetrachloroethylene may be due to site-specific action on the $GABA_A$ receptors. Chen et al. (2002a) pretreated rats with tetrachloroethylene (50 or 500 mg/kg, oral gavage), and this pretreatment, following both an acute and a subchronic (5 or 50 mg/kg-day, 5 days/week, 8 weeks) schedule, significantly increased the seizure threshold when challenged with pentylenetetrazole (PTZ), a convulsant that blocks $GABA_A$ receptors. This study suggests that the GABAergic system may be involved in the anxiolytic and general CNS depressive behavior that is observed following tetrachloroethylene exposure and could be potentially related to observed increased reaction times in the various tasks.

4.1.4. Summary of Neurotoxic Effects in Humans and Animals

Human and animal studies provide complementary evidence regarding the association of neurobehavioral deficits and tetrachloroethylene exposure. Tetrachloroethylene exposure in humans has primarily been shown to affect visual function (including color vision) and visuospatial memory and other aspects of cognition. Brain-weight changes have been measured in animal studies. A more in-depth discussion of the human neurotoxicological studies can be found in Section 4.1.1.3, and the animal inhalation and oral or i.p. exposure studies are discussed in Sections 4.1.2.1 and 4.1.2.2, respectively.

Visual contrast sensitivity deficits as well as color discrimination deficits are commonly present prior to detectable pathology in the retina or optic nerve head, making them one of the earliest signs of disease and potentially more sensitive measures than evoked potentials from visual stimuli (Regan, 1989). Several independent lines of evidence can be found in the occupational and residential exposure studies to support an inference of visual deficits following chronic tetrachloroethylene exposure. The studies that observed effects on color vision using the Lanthony D-15 color vision test include cross-sectional and longitudinal designs in dry-cleaning settings (Gobba et al., 1998; Cavalleri et al., 1994) and residential studies (Schreiber et al., 2002). Decrements in color confusion were reported among all workers exposed to a mean TWA of 6 ppm for an average of 8.8 years (Cavalleri et al., 1994). A significant dose-response relationship between CCI value and tetrachloroethylene concentration ($r = 0.52$, $p < 0.01$) was also observed in Cavalleri et al. (1994). As noted previously, the color vision testing in this study was blinded to exposure level of the study participants, and the study participants were well matched in terms of age, smoking, and alcohol use. A follow-up of these workers 2 years

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later (Gobba et al., 1998) showed greater loss in color discrimination in those who were subsequently exposed to a higher concentration (increase in geometric mean from 1.7 to 4.3 ppm), with no change in those exposed to lower concentrations (decrease in geometric mean from 2.9 to 0.7 ppm). Although Gobba et al. (1998) demonstrates persistent color confusion effects in this follow-up evaluation, the study exposures are not clearly characterized over the course of the 2-year duration. Nakatsuka et al. (1992) did not observe an association with color vision among dry cleaners in China (n = 64, geometric mean TWA: 11 and 15 ppm in females and males, respectively), but the relative insensitivity of the specific type of color vision test used in this study (Lanthony, 1978) is a likely explanation for these results. Effects on color vision were also observed among 14 dry cleaners in the small study in Malaysia by Sharanjeet-Kaur et al. (2004), but this study provides little weight to the strength of the evidence because of the lack of exposure information (other than job title), and differences between dry cleaners and controls regarding test conditions and smoking habits. Two other small studies also reported lower scores on the Lanthony D-15 color vision test in much lower exposure settings, but the differences were not statistically significant: in a study of residents living above dry cleaners (mean tetrachloroethylene exposure during active dry cleaning = 0.4 ppm), the mean CCI scores were 1.33 and 1.20 in 17 exposed and 17 controls, respectively (p = 0.26); in a study of workers in a day-care center located in a building with a dry-cleaning business (mean tetrachloroethylene exposure 0.32 ppm), the mean CCI scores were 1.22 and 1.18 in the exposed day-care workers and controls, respectively (p = 0.39) (Schreiber et al., 2002). Overall, the evidence reveals a high degree of consistency in this aspect of visually mediated function.

Visual contrast sensitivity changes were reported in two NYSDOH residential studies. In a small pilot study (4 children and 13 adults), mean scores for visual contrast sensitivity (using a near vision visual contrast sensitivity test) across spatial frequencies were statistically significantly lower in exposed residents than in controls, indicating poorer visual function in the exposed groups (Schreiber et al., 2002). Controls were age- and sex-matched to the exposed group, and both groups were English speaking and predominately Caucasian ethnicity; however, they were drawn from different geographic areas. In addition, two of the four exposed children had diagnoses of learning disabilities or developmental delays, which could affect performance on this type of test. In the larger study (NYSDOH, 2010, 2005a, b), the test (Functional Acuity Contrast Test, FACT) assessed far vision visual contrast sensitivity, and the test had a low rate of detecting visual contrast changes. For contrast vision, a number of analyses in NYSDOH (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a) suggest a vulnerability among children. However, exposure to >0.015 ppm (>100 µg/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, nonminority groups, make it difficult to fully examine

possible effects of income, race, and age on vision. Therefore, while both studies report visual contrast sensitivity changes with exposed children being more sensitive, there are concerns with the methodological and analytic approaches in these studies.

Acute human exposure studies reported increased latencies of up to 3.0 ms in visual evoked potentials (Altmann et al., 1990) and changes in EEGs (magnitude of effect was not specified) (Hake and Stewart, 1977; Stewart et al., 1970) at higher exposures ranging from 340 to 680 mg/m^3.

In rats, acute inhalation exposure to tetrachloroethylene results in significant changes to the flash-evoked potential at 800 ppm (Mattsson et al., 1998), and a decrease in F2 amplitudes of the steady state visual evoked potential at 250 ppm (Boyes et al., 2009). In a subchronic exposure study (13 weeks, up to 800 ppm tetrachloroethylene), changes in flash-evoked potential responses were not observed at tetrachloroethylene exposures up to 200 ppm. In the 800 ppm group, there was a significant increase in the amplitude and a significant increase in latency (~3.0 ms) of the mid-flash-evoked potential waveform (N3), but histopathological lesions were not observed in the examination of the visual system brain structures [e.g., visual cortex; optic nerve; Mattsson et al. (1998)]

Effects on visuospatial memory in humans were also reported in each of the studies that examined this measure (Altmann et al., 1995; Echeverria et al., 1995; Echeverria et al., 1994; Seeber, 1989). These effects (increased response times or cognition errors) were observed in occupational and residential studies, and the occupational studies were quite large, involving 101, 65, and 173 dry-cleaning workers in Seeber (1989), Echeverria et al. (1995), and Echeverria et al. (1994), respectively. Several different types of tests were used including digit reproduction (Seeber, 1989), switching, pattern memory, and pattern recognition (Echeverria et al., 1995; Echeverria et al., 1994), and the Benton test (Altmann et al., 1995). Exposures for the increased reaction time observations (LOAELs) ranged from 4.99 to 102 mg/m^3 (Altmann et al., 1995; Echeverria et al., 1995; Ferroni et al., 1992). The changes in the cognitive tasks were observed at exposures (LOAELs) ranging from 53.9 to 364.22 mg/m^3 (Spinatonda et al., 1997; Echeverria et al., 1995; Seeber, 1989). All of these studies except Altmann et al. (1995) indicate that the neurobehavioral assessment was blinded to knowledge of the exposure level of the subject, and all of the studies adjusted for potentially confounding factors. It should be noted, however, that residual confounding from education level differences between exposed and referent subjects may still be present in Altmann et al. (1995).

Changes in the motor activity domain as measured by increased reaction time, increased number of false alarms, and decreased trial completions in a signal detection task (measures of decreased attention) were reported in an acute (60 minutes) exposure (6,782 mg/m^3 or higher) study in rats (Oshiro et al., 2008). Additionally, operant tasks that test cognitive performance

have demonstrated deficits in rats and mice following acute tetrachloroethylene oral (Warren et al., 1996) and i.p. (Umezu et al., 1997) exposures. These findings 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.

An occupational exposure study (n = 60) (Ferroni et al., 1992) and a residential exposure study (n = 14) (Altmann et al., 1995), with mean exposure levels of 15 ppm and 0.7 ppm, respectively, reported significant increases in simple reaction time of 24 ms (11%) (Ferroni et al., 1992) and 40 and 51.1 ms (15 and 20% increases, respectively, for two separate measurements) (Altmann et al., 1995) for the exposed subjects. A third study, Lauwerys et al. (1983), reported better performance on simple reaction time in 21 exposed workers (mean TWA: 21 ppm) compared with controls measured before a work shift but not after.

The changes in brain weight, DNA/RNA, and neurotransmitter levels that were observed in the animal studies are highly supportive of the neurobehavioral changes observed with tetrachloroethylene exposure. Changes in brain DNA, RNA, or protein levels and lipid composition were altered following inhalation, with changes observed in the cerebellum, the hippocampus, and the frontal cortex (Wang et al., 1993; Rosengren et al., 1986; Savolainen et al., 1977a; Savolainen et al., 1977b). The replication of these changes in biochemical parameters and effects in brain weight in both rats and gerbils is pathognomonic. Changes in neurotransmitters systems (Briving et al., 1986; Honma et al., 1980a; Honma et al., 1980b) and circadian rhythm (Motohashi et al., 1993) in animal studies are consistent with neuroendocrine alterations observed in humans (Ferroni et al., 1992).

In conclusion, the weight of evidence across the available studies of humans and animals exposed to tetrachloroethylene indicates that chronic exposure to tetrachloroethylene can result in decrements in color vision, visuospatial memory, and possibly other aspects of cognition and neuropsychological function, including reaction time.

4.2. KIDNEY AND BLADDER TOXICITY AND CANCER

4.2.1. Human Studies

4.2.1.1. Kidney Toxicity in Humans

High concentrations of inhaled tetrachloroethylene given acutely as an anesthetic are associated with symptoms of renal dysfunction, including proteinuria and hematuria (ATSDR, 1997a; Hake and Stewart, 1977). 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 blood urea nitrogen (BUN) (a measure of kidney function) in 12 healthy individuals [Stewart et

al. (1977), as reported in ATSDR (1997b)]. 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 to studies of urinary renal proteins as indicator of kidney function. One study has become available on end stage renal disease (ESRD) incidence in a cohort of dry cleaners (Calvert et al., 2011). The ATSDR (Lybarger et al., 1999; ATSDR, 1998a) recommends a standard battery of kidney function tests including 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, although it should be noted that NAG is not a sensitive and specific marker of tubular dysfunction (Lybarger et al., 1999). The proximal tubules house β-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 (refer to Section 3). For this reason, altered urinary indicators of proximal tubule function are consistent with knowledge of metabolic processing.

The epidemiologic studies are suggestive of subtle damage to the renal tubules. Table 4-6 summarizes the human kidney function studies. 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.[16]

[16] 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, respectively, in 118 subjects.

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Table 4-6. Summary of human kidney toxicity marker studies of occupational exposures to dry-cleaning facilities using tetrachloroethylene

Subjects, methods	Exposure levels	Results	Reference(s)
Occupational exposures: dry-cleaning settings			
Belgium, 26 dry cleaners, 33 unexposed workers (controls), B, EA, PA, U [before and after shift]	Mean TWA = 21 ppm, U-TCA = ND, mean duration = 6.4 yr	No differences in creatinine-adjusted urinary β2μ-globulin, retinol-binding protein and albumin.	Lauwerys et al. (1983)
Italy, 57 dry cleaners (mostly females) (Group 1), 188 painters (mostly males) (Group 2), 51 glass-fiber reinforced boat workers (Group 3), 212 workers exposed to C_5-C_7 alkanes (Group 4), 30 unexposed workers (mostly females) (Control Group 1) and 81 unexposed workers (mostly males) (Control Group 2). U [before and after shift]	Dry cleaners (Group 1): mean TWA = 10 ppm (extrapolated from postshift U-TCA according to Ikeda et al. (1972), mean duration = 13.9 yr	50% increase in creatinine-adjusted geometric mean concentration of urinary β2-glucuronidase and 100% increase in geometric mean urinary lysozyme in dry cleaners compared to either control group. No difference in total protein or albumin.	Franchini et al. (1983)
Czech Republic, 22 female dry cleaners, 15 female controls (clerical workers). PA, U [end of shift]	3 shops with mean TWA <12 ppm, 2 shops with mean TWA 42 ppm and 47 ppm, mean duration = 11 yr	Fourfold elevation in geometric mean creatinine-adjusted urinary concentration of lysozyme. No difference in albumin, β2μ-globulin and total protein, or prevalence of subjects whose urinary proteins above 95th percentile.	Vyskocil et al. (1990)
United State, 192 dry cleaners (mostly females), no controls. PA, U [collection time varied by subject]	Mean TWA = 7 ppm, mean duration = 11.6 yr	No correlation of exposure and creatinine-adjusted urinary protein, albumin, or *N*-acetyl-β-glucuronidase.	Solet and Robins (1991)
Italy, 50 dry cleaners and ironers (mostly females), 50 controls (blood donors). B, PA, U [before shift]	Mean TWA = 8.8 ppm, mean duration = 10 yr	1.5- to 4-fold increase in creatinine-adjusted mean concentration of 8 urinary renal proteins (albumin, transferrin, 3 brush border antigens, tissue nonspecific alkaline phosphatase, $p < 0.05$; glycosaminoglycans, Tamm-Horsfall glycoprotein, $p = 0.06$) and 2 serum proteins (anti-glomerular basement membrane, laminin fragments, $p < 0.05$) in dry cleaners; discriminated between dry cleaners and matched controls ($p < 0.05$). No difference in 12 other urinary renal proteins (includes total protein and *N*-acetyl-β-glucuronidase).	Mutti et al. (1992)

Table 4-6. Summary of human kidney toxicity marker studies of occupational exposures to dry-cleaning facilities using tetrachloroethylene (continued)

Subjects, methods	Exposure levels	Results	Reference(s)
Italy, 40 female dry cleaners, 45 female controls (ironers). PA, B, U [before and after shift]	Mean TWA = 14.8 ppm, mean duration = 15 yr	Positive correlation between preshift urinary PCE and total solutes and total proteins ($p <$ 0.01) and postshift urinary PCE and glutamine synthetase ($p < 0.001$). No difference in creatinine-adjusted mean urinary concentration of total solutes, total protein angiotensin converting enzyme, *N*-acetyl-β-glucuronidase, or glutamine synthetase.	Trevisan et al. (2000)
The Netherlands, 101 dry cleaners (mostly males), 19 controls (seamstresses, sorters or folders in dry-cleaning shops or laundry workers) (mostly females). PA, U [before shift]	Mean TWA = 8 ppm (dry cleaners), <2.2 ppm (controls), mean duration = 3.9 yr	Retinol binding protein (creatinine-adjusted mean concentration) elevated twofold among dry cleaners ($p = 0.01$). No difference in creatinine-adjusted mean of β-galactosidase, *N*-acetyl-β-glucuronidase, or alanine aminopeptidase. No difference in geometric mean albumin or total protein.	Verplanke et al. (1999)

A = air sample, not specified area or personal sample; AA = area air samples, B = biological monitoring of blood, BTX = benzene, toluene, xylene, EA = exhaled air samples, ND = not detectable, PA = personal air samples, PCE = tetrachloroethylene, U = biological monitoring of urine for trichloroacetic acid.

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 a 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 (Trevisan et al., 2000; Verplanke et al., 1999; Mutti et al., 1992; Solet and Robins, 1991) 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, given the limitations in terms of sensitivity and specificity of NAG as a 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: β2μ-globulin, intestinal alkaline phosphatase (IAP), tissue nonspecific alkaline phosphatase (TNAP), lysozyme, β2-glucuronidase, and glutamine synthetase. Both IAP and TNAP are indicators of proximal tubule brush border integrity (Price et al., 1996), whereas lysozyme and β2μ-globulin indicate a failure of the tubule to reabsorb protein (Lybarger et al., 1999; Kok et al., 1998; Bernard and Lauwerys, 1995). 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 β2μ-globulin 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 analysis. β2μ-Globulin, however, was not elevated among exposed subjects as compared with controls in the other two studies that examined this protein (Vyskocil et al., 1990; Lauwerys et al., 1983), although the mean concentration of β2μ-globulin appeared higher in subjects studied by Vyskocil et al. (1990) 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 β2-glucuronidase observed a statistically significant elevated mean concentration of these proteins among dry cleaners as compared with controls (Vyskocil et al., 1990; Franchini et al., 1983).

It is not clear whether tetrachloroethylene exposure affects 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. Because some albumin is normally filtered, small increases in the amount of albumin in the urine may result from tubular damage due to failure to reabsorb the small amount filtered (NRC, 2010).

Calvert et al. (2011) examined the incidence of end stage renal disease (ESRD) in a cohort of 1,704 dry cleaners assembled by the National Institute of Occupational Safety and Health (NIOSH), 618 who had worked only in a shop where tetrachloroethylene was the primary cleaning solvent (tetrachloroethylene-only subcohort) and 1,086 who worked in a shop that used tetrachloroethylene but who also had a history of employment in shops where the primary

solvent could not be identified (tetrachloroethylene-plus subcohort) (Ruder et al., 2001, 1994). All subjects alive as of 1977 were linked to the Renal Management Information System (RMIS), a database of individuals receiving Medicare benefits for ESRD, and followed to 2004. Thirty cases of ESRD were identified over the 27-year period (standardized incidence ratio [SIR]: 1.34, 95% CI: 0.90, 1.91), with 12 ESRD cases in the tetrachloroethylene-only subcohort (SIR: 1.30, 95% CI: 0.67, 2.26). Of these cases, eight were due to hypertensive ESRD (SIR: 2.66, 95% CI: 1.15, 5.23), of whom six cases were female subjects (SIR: 2.86, 95% CI: 1.05, 6.23). The observed risk estimate for hypertensive ESRD among tetrachloroethylene-only subjects appears larger than that for the tetrachloroethylene-plus subcohort (SIR: 1.53, 95% CI: 0.62, 3.16). An exposure-response pattern was further suggested because hypertensive ESRD risk was highest among those in the tetrachloroethylene-only subcohort employed for ≥5 years (SIR: 3.39, 95% CI: 1.10, 7.92). These findings support an association between tetrachloroethylene exposure and ESRD, particularly hypertensive ESRD. ESRD-observed risk is likely underestimated using RMIS records. An examination of cause of death among cohort subjects who had died by 2004 found five additional workers with chronic renal failure listed as an underlying cause of death. Medical records for three of these five deaths indicated two subjects with ESRD. Calvert et al. (2011), moreover, found substantial underreporting of chronic renal disease on death certificates, suggesting incidence as superior to mortality for assessing tetrachloroethylene exposure and kidney disease. Of the 23 deaths among the 30 ESRD subjects, cause of death on death certificates for 11 of these subjects was due to chronic renal disease and 3 due to "renal disease not otherwise specified."

Taken together, the epidemiologic studies support an association between tetrachloroethylene and chronic kidney disease, as measured by urinary excretion of renal proteins and ESRD incidence. The elevated urinary RBP levels observed in two studies (Verplanke et al., 1999; Mutti et al., 1992) and lysozyme or β2-glucuronidase in Franchini et al. (1983) provide some evidence for effects to the proximal tubules from tetrachloroethylene exposure. Effects are observed in populations of both males and females, and potential differences in susceptibility due to sex-related differences in rates of metabolism (refer to Section 3) 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 glutamine synthetase ($p < 0.001$). 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 4-6 summarizes the human kidney toxicity studies. Calvert et al. (2011) supports an association between inhalation tetrachloroethylene exposure and ESRD, particularly hypertensive ESRD. They observed a twofold elevated incidence (SIR: 2.66, 95% CI: 1.15, 5.23) among subjects who worked only in a shop where tetrachloroethylene was the primary cleaning solvent compared to that expected based on U.S. population rates. An exposure-response pattern was further suggested because hypertensive ESRD risk was highest among those employed for ≥5 years (SIR: 3.39, 95% CI: 1.10, 7.92).

4.2.1.2. Kidney Cancer in Humans

Twenty-seven epidemiologic studies reporting data on kidney cancer and tetrachloroethylene exposure were identified. This set of studies includes 13 cohort or nested case-control studies within a cohort (Seldén and Ahlborg, 2011; Calvert et al., 2011; Pukkala et al., 2009; Wilson et al., 2008; Sung et al., 2007; Ji et al., 2005b; Blair et al., 2003; Chang et al., 2003; Travier et al., 2002; Anderson et al., 1999; Boice et al., 1999; Anttila et al., 1995; Lynge and Thygesen, 1990); 11 case-control studies of occupational exposures (Lynge et al., 2006; Parent et al., 2000; Pesch et al., 2000a; Dosemeci et al., 1999; Delahunt et al., 1995; Mandel et al., 1995; Schlehofer et al., 1995; Auperin et al., 1994; Mellemgaard et al., 1994; McCredie and Stewart, 1993; Asal et al., 1988), and 3 studies of residential exposure through contaminated drinking water (Ma et al., 2009; Vieira et al., 2005; Aschengrau et al., 1993). Some sets of these studies represent overlapping study populations. For example, three papers examined cancer risk among occupational groups defined by census data in Sweden (Wilson et al., 2008; Ji and Hemminki, 2005a; Travier et al., 2002), one paper used a similar design in Denmark (Lynge and Thygesen, 1990), two papers were based on census data from Sweden, Denmark, Finland, and Norway (Lynge et al., 2006; Andersen et al., 1999), and a third paper added data from Iceland (Pukkala et al., 2009). Cases and controls in another four studies (Dosemeci et al., 1999; Schlehofer et al., 1995; Mellemgaard et al., 1994; McCredie and Stewart, 1993) were included in the National Cancer Institute's (NCI's) multicenter international renal cell study (Mandel et al., 1995).

Generally, cohort studies presented risk estimates for "kidney and other and unspecified urinary organs," and case-control studies presented risk estimates for renal cell carcinoma, a histological type included in the broader kidney and other and unspecified urinary organs category. The exceptions were two studies that presented risk estimates for cancer of the renal pelvis (Wilson et al., 2008; McCredie and Stewart, 1993) and two studies of the same cohort that presented risk estimates for kidney and urinary (bladder) organs (Sung et al., 2007; Chang et al., 2003). These 27 studies represent the core studies evaluated by EPA, as described in more detail

below. One other cohort study included information on tetrachloroethylene but did not report risk estimates for kidney cancer (Radican et al., 2008), and one case-control study identified only one exposed case (as a dry-cleaning operator) and did not provide an estimate of the association (Partanen et al., 1991), and so were not evaluated further. Appendix B reviews the design, exposure-assessment approach, and statistical methodology for each study. Most studies were of the inhalation route of exposure, of occupational exposure, and unable to quantify tetrachloroethylene exposure.

4.2.1.2.1. Consideration of exposure-assessment methodology

Many studies examine occupational title as dry cleaner, launderer, and presser as surrogate for tetrachloroethylene, given its widespread use from 1960 onward in the United States and Europe (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Wilson et al., 2008; Lynge et al., 2006; Ji et al., 2005b; Blair et al., 2003; Travier et al., 2002; Parent et al., 2000; Andersen et al., 1999; Dosemeci et al., 1999; Delahunt et al., 1995; Mandel et al., 1995; Auperin et al., 1994; Mellemgaard et al., 1994; McCredie and Stewart, 1993; Lynge and Thygesen, 1990; Asal et al., 1988). Seven studies conducted in Nordic countries are based on either the entire Swedish population or combined populations of several Nordic countries; strengths of these studies are their use of job title as recorded in census databases and ascertainment of cancer incidence using national cancer registries (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Wilson et al., 2008; Lynge et al., 2006; Ji et al., 2005b; Travier et al., 2002; Andersen et al., 1999). Some variation can be expected within an occupational group among countries; however, as Lynge et al. (2006) reported, average tetrachloroethylene usage in 1960−1970 in Sweden was higher than in Finland or Norway. Studies examining mortality among U.S. dry-cleaner and laundry workers (Calvert et al., 2011; Blair et al., 2003) are of smaller cohorts than the Nordic studies, with fewer observed kidney cancer events.

The exposure surrogate in studies of dry-cleaners and laundry workers is a broad category containing jobs of differing potential for tetrachloroethylene exposure. Thus, these studies have a greater potential for exposure misclassification bias compared to studies with exposure potential to tetrachloroethylene assigned by exposure matrix approaches applied to individual subjects. Three studies used additional information pertaining to work environment to refine the exposure classification (Calvert et al., 2011; Seldén and Ahlborg, 2011; Lynge et al., 2006). Seldén and Ahlborg (2011) obtained information about the dry-cleaning establishment (e.g., washing techniques, chemicals used, number of employees, and work history of individual employees) in a questionnaire sent to businesses in Sweden in the 1980s. Lynge et al. (2006), using job titles reported in the 1970 Census, identified subjects based on the occupational code of "Laundry and dry-cleaning worker" or industry code of "Laundry and dry cleaning." Additional

information used to refine this classification was sought for incident kidney cancer cases (and cases of cancer of the esophagus, gastric cardia, liver, pancreas, cervix, bladder, and non-Hodgkin lymphoma) within this defined cohort. Five controls, matched to the cases by country-, sex- age-, and calendar period, were also included in this study. The additional information sought by Lynge et al. (2006) included handwritten task information from the census form from Denmark and Norway, pension databases in Denmark and Finland, and next-of-kin interviews in Norway and Sweden. Exposure classification categories were dry cleaner (defined as dry cleaners and supporting staff if employed at a business with <10 workers), other job titles in dry cleaning (launderers and pressers), unexposed (job title reported on 1970 Census was other than in dry cleaning), or unclassifiable (information was lacking to identify job title of subject). The unclassifiable category represented 43 of 210 identified kidney cancer cases (20%) and 241 of the 1,060 controls (22%). Another dry-cleaning study of unionized dry cleaners in the United States included an analysis of subjects who worked for 1 or more years before 1960 in a shop known to use tetrachloroethylene as the primary solvent (Calvert et al., 2011; Ruder et al., 2001, 1994). The cohort was stratified into two groups based on the level of certainty that the worker was employed only in facilities using tetrachloroethylene as the primary solvent exposure; tetrachloroethylene-only and tetrachloroethylene-plus. Two of the five observed kidney cancer deaths were among the tetrachloroethylene-only subset (n = 618) of study subjects.

Only Blair et al. (2003; 1993) used an exposure metric for semiquantitative cumulative exposure within a dry-cleaning setting. Four other studies presented risk estimates by employment duration (Lynge et al., 2006; Ji et al., 2005b; Travier et al., 2002; Mandel et al., 1995). Because employment duration does not account for variation in exposure levels, it is a weaker exposure measure (i.e., more subject to misclassification) compared with one defined as a semiquantitative measure.

One case-control study used a job exposure matrix (JEM) or one with information on specific tasks, a job-task exposure matrix (JTEM), with semiquantitative exposure assessment across a variety of jobs (Pesch et al., 2000a), and two study centers (Dosemeci et al., 1999; Schlehofer et al., 1995) of the large NCI international renal cell carcinoma study used a JEM and occupations to assign overall tetrachloroethylene exposure potential to individual subjects. In Pesch et al. (2000a), the use of the German JEM identified approximately three times as many cases with any potential tetrachloroethylene exposure (38%) compared to the JTEM (12%), and, in both approaches, few cases were identified with substantial exposure (6% by JEM and 2% by JTEM). Pesch et al. (2000a) noted, "exposure indices derived from an expert rating of job tasks can have a higher agent-specificity than indices derived from job titles." For this reason, the JTEM approach, with consideration of job tasks, is considered a more robust exposure metric for

examining tetrachloroethylene exposure and kidney carcinoma due to likely reduced potential for exposure misclassification compared to exposure assignment using only job history and title.

Four other cohorts with potential tetrachloroethylene exposure in manufacturing settings have been examined. These studies include aerospace workers in the United States (Boice et al., 1999), workers primarily in the metal industry, workers in Finland (Anttila et al., 1995), and electronic factory workers in Taiwan (Sung et al., 2007; Chang et al., 2005). Boice et al. (1999) used an exposure assessment based on a job-exposure matrix, and Anttila et al. (1995) used biological monitoring of tetrachloroethylene in blood to assign potential tetrachloroethylene exposure to individual subjects. In contrast, the exposures in the Taiwan studies included multiple solvents, and tetrachloroethylene exposure was not linked to individual workers. These cohorts also included white-collar workers, who had an expected lower potential for exposure (Sung et al., 2007; Chang et al., 2003).

Three geographic studies focused on residential proximity to drinking water sources contaminated with tetrachloroethylene and other solvents (Ma et al., 2009; Vieira et al., 2005; Aschengrau et al., 1993). Two other studies in Cape Cod, MA, used either an exposure model incorporating leaching and characteristics of the community water distribution system to assign a household-relative dose of tetrachloroethylene (Aschengrau et al., 1993) or residential proximity to Superfund sites without identifying specific exposures and a generalized additive model that incorporates smoothing approaches and adjusts for covariates (Vieira et al., 2005). Ma et al. (2009) is an ecological-designed study examining the rate of hospital discharges with a diagnosis of kidney cancer and the average number of dry cleaners per square kilometer within New York City zip codes as an exposure surrogate.

In summary, with respect to exposure-assessment methodologies, nine studies with kidney cancer data assigned tetrachloroethylene exposure to individuals within the study using a job exposure matrix (Pesch et al., 2000a; Boice et al., 1999; Dosemeci et al., 1999; Schlehofer et al., 1995), or semiquantitative metric (Blair et al., 2003), biological samples (Anttila et al., 1995), an exposure model (Aschengrau et al., 1993), information about working conditions obtained through a questionnaire (Seldén and Ahlborg, 2011), or classifying the cohort by certainty of tetrachloroethylene exposure (Calvert et al., 2011). One other study based on occupational census data sought additional information for use in refining potential exposure within dry-cleaning settings (Lynge et al., 2006). The relative specificity of these exposure-assessment approaches strengthens their ability to identify cancer hazards compared to studies with broader and less sensitive exposure-assessment approaches. The least sensitive exposure assessments are those using very broad definitions such as working in a plant or factory (Sung et al., 2007; Chang et al., 2003) or density of dry-cleaning establishments by zip code (Ma et al., 2009).

4.2.1.2.2. Summary of results

Seven of the 27 studies evaluated by EPA reported estimated relative risks based on a large number of observed events: 50 or more deaths/incident cases in cohort studies (Pukkala et al., 2009; Ji and Hemminki, 2005a; Travier et al., 2002; Andersen et al., 1999), or 50 or more exposed cases in case-control studies (Pesch et al., 2000a; Dosemeci et al., 1999; Mandel et al., 1995). Two of these studies adopted a relatively high quality exposure-assessment approach to assign tetrachloroethylene exposure potential to individual subjects (Pesch et al., 2000a; Dosemeci et al., 1999). Pukkala et al. (2009) updates the analysis of Andersen et al. (1999), adding data from a 5th country, Iceland, and extending follow-up to 2005, and is preferred over Andersen et al. (1999) for these reasons.

The three[17] cohort studies with findings based on 50 or more events observed standardized incidence ratios or odds ratio estimates of 1.15 (95% CI: 0.98, 1.35), 0.94 (95% CI: 0.83, 1.07), and 1.11 (95% CI: 0.93, 1.33) in Ji et al. (2005b), Pukkala et al. (2009) and Travier et al. (2002), respectively, for the association between kidney cancer risk and ever having a job title of dry-cleaner or laundry worker (refer to Table 4-7). The largest case-control study (n = 245 cases from Australia, Denmark, Germany, Sweden, and the United States) reported an odds ratio for the association between renal cell carcinoma and ever exposed to dry-cleaning solvents of 1.4 (95% CI: 1.1, 1.7) (Mandel et al., 1995). Dosemeci et al. (1999), whose subjects were included in the larger study of Mandel et al. (1995), reported an odds ratio estimate of 1.07 (95% CI: 0.7, 1.6) for the association between overall tetrachloroethylene exposure and renal cell carcinoma, based on 50 cases exposed to tetrachlorethylene. The other large case-control study by Pesch et al. (2000a) also included a high-quality exposure-assessment approach (JTEM) for tetrachloroethylene. This study observed odds ratio estimates of 1.2 (95% CI: 0.9, 1.7), 1.1 (95% CI: 0.7, 1.5), and 1.3 (95% CI: 0.7, 2.3) and, 2.2 (95% CI: 0.9, 5.2), 1.5 (95% CI: 0.6, 3.8), and 2.0 (0.5, 7.8) for medium, high, and substantial exposure in males and females, respectively. This study observed lower odds ratio estimates for the association between kidney cancer and tetrachloroethylene exposure assigned using a job-exposure-matrix, a less robust exposure-assessment approach compared to a JTEM.

Differences in risk estimates between males and females were reported in three studies; two studies observed higher point estimates in females (Ji et al., 2005b; Pesch et al., 2000a), with a higher risk estimate for males observed in Dosemeci et al. (1999). Pukkala et al. (2009), in contrast, did not observe differences in kidney cancer risk estimates between male and female subjects. It is unclear why apparent differences in sex-specific results were observed in some

[17] Andersen et al. (1999) is not included in this summary of the data from the individual studies because it was updated and expanded in the analysis by Pukkala et al. (2009).

studies, although different exposure potentials, different exposure intensities, chance, or residual confounding are possible alternative explanations (NRC, 2010; Dosemeci et al., 1999).

In addition to the large cohort and case-control studies, some evidence is found in studies whose effect estimates are based on fewer observed events and that carry less weight in the analysis. As expected, the magnitude of the point estimate of the association reported in these studies is more variable than in the larger studies. Because of the relatively small number of observed exposed cases in these cohort studies or exposed cases in case-control studies, ranging from 2 in Antilla et al. (1995) and Boice et al. (1999) to 29 in Seldén and Ahlborg (2011), the statistical power of these lesser-weighted studies is limited. The variation in the association observed in these studies is consistent with that from studies discussed above that carry greater weight in the analysis. For the association between kidney cancer and dry cleaning, six studies reported risk estimates from 0.69 to 0.94 [Andersen et al. (1999); Asal et al. (1988), males; Pukkala et al. (2009); Lynge et al. (2006); Boice et al. (1999); Lynge and Thygesen (1990)], three studies reported risk estimates from 1.0 to 1.08 (Seldén and Ahlborg, 2011; Blair et al., 2003; Aschengrau et al., 1993), four studies reported risk estimates from 1.35 to 1.92 (Parent et al., 2000; Anttila et al., 1995; Delahunt et al., 1995; Calvert, 1976), and four studies reported risk estimates from 2.3 to 2.8 [Asal et al. (1988), females; Schlehofer et al. (1995); Mellemgaard et al. (1994); McCredie and Stewart (1993)].

Several studies had been previously identified based on the relative strengths of their exposure-assessment methodology. The results from these studies are mixed. Some of these studies reported no evidence of an increased risk, with relative risks of 0.67 [(Lynge et al., 2006); dry cleaners], 0.69 [(Boice et al., 1999); routine exposure], 1.04 (Seldén and Ahlborg, 2011), and 1.07 [(Dosemeci et al., 1999); tetrachloroethylene exposure]. No cases were observed in the group above the 90th percentile of exposure based on modeling of residential exposure in Aschengrau et al. (1993), and the overall relative risk for any tetrachloroethylene exposure was 1.08. In contrast, data from other studies with relatively strong exposure-assessment methods provide more evidence of an effect, with relative risks of 1.35 [(Calvert, 1976); tetrachloroethylene-only], 1.5 [(Blair et al., 2003); medium-high exposure], 1.82 (Anttila et al., 1995); biological samples], and 2.52 [(Schlehofer et al., 1995); tetrachloroethylene or tetrachlorocarbonate exposure]. The data from Pesch et al. (2000a), as described earlier, do not indicate a pattern of increasing risk with increasing exposure among males (odds ratio [OR]: 1.2, 1.1, and 1.3 for medium, high, and substantial exposure, respectively), or among females, although the overall risk pattern is stronger among women (OR: 2.2, 1.5, and 2.0 for medium, high, and substantial exposure, respectively).

Two studies of the same population, an electronics factory in Taiwan, which did not use an exposure-assessment approach that allowed individual-level classification of exposure,

observed standardized mortality ratios (SMRs) for kidney and urinary organ cancer of 1.18 (95% CI: 0.24, 3.44) (Chang et al., 2003) and 1.10 (95% CI: 0.62, 1.82) (Sung et al., 2008), respectively. A geographic-based study reported a relatively constant prevalence rate ratio for the association between hospital discharge diagnoses for kidney cancer and density of dry cleaners by zip code of residence (Ma et al., 2009).

The two studies reporting findings for cancer of the renal pelvis and dry cleaner and laundry jobs were each based on 10 or fewer observations, with the standardized incidence ratio or odds ratio estimates in these studies of 1.23 (95% CI: 0.39, 2.86) and 6.09 (95% CI: 1.95, 8.9) in Wilson et al. (2008) and McCredie and Stewart (1993), respectively.

Establishment of an exposure or concentration-response relationship can add to the weight of evidence for identifying a cancer hazard, but only limited data pertaining to exposure-response relationships for kidney cancer and tetrachloroethylene exposure are available. Seven studies presented risk estimates for increasing exposure categories. Four studies used exposure duration as a proxy (Lynge et al., 2006; Ji et al., 2005b; Boice et al., 1999; Mandel et al., 1995); one of these included only five cases in three exposure categories (Boice et al., 1999), which limits the potential of this study to assess trends. Three studies used a semiquantitative exposure surrogate (Ma et al., 2009; Blair et al., 2003; Pesch et al., 2000a), but one of these was a relatively nonspecific and nonsensitive measure based on zip code area-based density of dry cleaners (Ma et al., 2009). A monotonic increasing trend in relative risk with increasing exposure surrogate was not observed in any of the larger occupational exposure studies with three or more exposure categories (Lynge et al., 2006; Pesch et al., 2000a; Mandel et al., 1995). In a smaller study, Blair et al. (2003) reported a higher risk in the higher of two exposure categories (SMR: 0.3 for little-to-no exposure and 1.5 for medium-to-high exposure). One other study provided data pertaining to the effect of duration of work. Ji et al. (2005b) reported a higher, but more imprecise, SIR for females employed as laundry workers and dry cleaners in the 1960 and 1970 Swedish Censuses (SIR: 1.67, 95% CI: 1.07, 2.37) compared to those who were classified in this type of work only in 1960 (SIR: 1.41, 95% CI: 1.13, 1.71). Neither of the two studies of renal pelvis cancer reported odds ratio estimates by exposure gradients.

Statistical analyses in all case-control studies except McCredie and Stewart (1993) and Lynge et al. (2006) controlled for cigarette smoking, a known risk factor for kidney cancer (Parent et al., 2000; Pesch et al., 2000a; Dosemeci et al., 1999; Delahunt et al., 1995; Mandel et al., 1995; Schlehofer et al., 1995; Auperin et al., 1994; Mellemgaard et al., 1994; Aschengrau et al., 1993; Asal et al., 1988). Fewer studies also controlled for body mass index, another risk factor for kidney cancer (Parent et al., 2000; Dosemeci et al., 1999; Mandel et al., 1995; Mellemgaard et al., 1994). Direct examination of possible confounders is less common in cohort studies relying on company-supplied or census work history data compared to case-control

studies where information is obtained from study subjects or their proxies. In cohort studies, however, use of internal controls rather than an external referent group (e.g., national mortality rates) can minimize effects of potential confounding due to smoking or socioeconomic status, because exposed and referent subjects are drawn from the same target population. However, only one of the available cohort studies included an analysis using internal controls, and that study is limited by the observation of only two kidney cancer cases with routine tetrachloroethylene exposure in the cohort (Boice et al., 1999). Effect of smoking as a possible confounder may be assessed indirectly through examination of risk ratios for other smoking-related sites such as lung cancer. Several studies observed roughly a 30% increase in lung cancer risk among dry cleaners (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji et al., 2005b; Blair et al., 2003). Any expected contribution of smoking to kidney cancer risk will be smaller than that for lung cancer.

In conclusion, the epidemiologic data provide limited evidence pertaining to tetrachloroethylene exposure and kidney cancer risk. The studies that support this finding include the largest international case-control study (245 exposed cases from Australia, Denmark, Germany, Sweden, and the United States), which reported a relative risk of 1.4 (95% CI: 1.1, 1.7) for any exposure to dry-cleaning solvents (Mandel et al., 1995). This study was able to adjust for smoking history, BMI, and other risk factors for kidney cancer. The large cohort studies, using a more general exposure classification based on national census occupation data, present more variable results, with relative risks of 0.94, 1.11, and 1.15 in Pukkula et al. (2009), Travier et al. (2002), and Ji et al. (2005b), respectively. One difference among these cohort studies is that Travier et al. (2002) and Ji et al. (2005b) were based on data from Sweden, while Pukkula et al. (2009) used data from Sweden, Denmark, Finland, Norway, and Iceland. Differences between these countries in tetrachloroethylene usage, as was noted by Lynge et al. (2006), may have introduced an additional source of exposure misclassification in this multicountry analysis. In addition to the large cohort studies, evidence also comes from cohort and case-control studies, whose effect estimates are based on fewer observed events. Smaller studies that do not also have a more sensitive or specific exposure metric carry lesser weight in the analysis. Eight studies were identified that used a relatively specific exposure-assessment approach to refine classification of potential tetrachloroethylene exposure in dry-cleaning settings (Calvert et al., 2011; Lynge et al., 2006; Blair et al., 2003), the aerospace industry (Boice et al., 1999), or within a variety of workplaces (Pesch et al., 2000a; Dosemeci et al., 1999; Anttila et al., 1995; Schlehofer et al., 1995) or a residential area setting (Aschengrau et al., 1993). The results from these studies are mixed, with some studies reporting little or no evidence of an association (Lynge et al., 2006; Pesch et al., 2000a; Boice et al., 1999; Dosemeci et al., 1999; Aschengrau et al., 1993), and other studies reporting elevated risks (Calvert et al.,

2011; Blair et al., 2003; Anttila et al., 1995; Schlehofer et al., 1995). An increasing trend in relative risk with increasing exposure surrogate was not observed in any of the larger occupational exposure studies with three or more exposure categories (Lynge et al., 2006; Mandel et al., 1995), but some indication of higher risk with higher exposure (or duration) was observed in other studies (Blair et al., 2003). As expected, the results from 16 other studies using a relatively nonspecific exposure measure (broad occupational title of launderers and dry cleaners, all workers at factory, density of dry-cleaning establishments by zip code) are more variable and less precise, reflecting a greater potential for misclassification bias.

Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Cohort Studies			
Biologically monitored workers			Anttila et al. (1995)
All subjects	1.82 (0.22, 6.56)	2	849 Finnish men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, external referents (SIR)
Aerospace workers (Lockheed)			Boice et al. (1999)
Routine exposure to PCE	0.69 (0.08, 2.47)	2	77,965 (n = 2,631 with routine PCE exposure and n = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, job exposure matrix without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm (mean) and 9.5 ppm (median), external reference for routine exposure (SMR) and internal references (workers with no chemical exposures) for routine-intermittent PCE exposure (RR)
Routine-intermittent exposure duration to PCE			
0	1.0[a]	22	
<1 yr	0.49 (0.07, 3.68)	1	
1−4 yr	0.56 (0.13, 2.41)	2	
≥5 yr	0.46 (0.10, 2.08)	2	
Electronic factory workers (Taiwan)			Chang et al. (2003); Sung et al. (2007)
All Subjects			86,868 (n = 70,735 female), follow-up 1979−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, cancer mortality, external referents (SMR) (Chang et al., 2003); 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, multiple solvents exposures and PCE not identified to individual subjects, cancer incidence, external referents, analyses lagged 5 yr (SIR) (Sung et al., 2007)
Males		0 1.31 exp	
Females	1.18 (0.24, 3.44)[b]	3	
Females	1.10 (0.62, 1.82)[c]	10	
Aircraft maintenance workers from Hill Air Force Base			Radican et al. (2008)
Any PCE exposure	Not reported		10,461 men and 3,605 women (total n = 14,066, n = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952−1956, follow-up 1973−2000, job exposure matrix (intensity), internal referent (workers with no chemical exposures) (RR)

Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Dry-cleaner and laundry workers				Andersen et al. (1999)
	All laundry worker and dry cleaners	0.92 (0.73, 1.15)	81	29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway), follow-up 1971−1987 or 1991, PCE not identified to individual subjects, external referents (SIR)
	Males	1.03 (0.66, 1.53)	24	
	Females	0.88 (0.67, 1.15)	57	
				Blair et al. (2003)
	All subjects	1.0 (0.4, 2.0)	8	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR)
	Semiquantitative exposure score			
	Little to no exposure	0.3 (<0.1, 1.6)	1	
	Medium to high exposure	1.5 (0.6, 3.1)	7	
				Ji et al. (2005b)
	Laundry workers and dry cleaners in 1960 Census	1.15 (0.98, 1.35)	153	9,255 Swedish men and 14,974 Swedish women employed in 1960 (men) or 1970 (women) as laundry workers or dry cleaners, follow-up 1961/1970−2000, PCE not identified to individual subjects, external referent (SIR) and adjusted for age, period, and socioeconomic status
	Males	0.90 (0.69, 1.14)	61	
	Females	1.41 (1.13, 1.71)	92	
	Laundry workers and dry cleaners in both 1960 and 1970 Censuses			
	Males	Not reported		
	Females	1.67 (1.07, 2.37)	26	
	Laundry workers and dry cleaners in 1960, 1970, and 1980 Censuses			
	Males	Not reported		
	Females	1.00 (0.90, 1.10)	3	

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Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Dry-cleaner and laundry workers (continued)				Lynge and Thygsen (1990)
	All laundry worker and dry cleaners	0.88 (0.44, 1.58)	11	10,600 Danish men and women, 20−64 yr old, employed in 1970 as laundry workers, dry cleaners, and textile dye workers, follow-up 1970−1980, external referents (SIR)
	Males	1.50 (0.55, 3.27)	6	
	Females	0.58 (0.19, 1.36)	5	
				Pukkala et al. (2009)
	Launderer and dry cleaner	0.94 (0.83, 1.07)	263	Men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30−64 yr, follow-up 2005, occupational title of launderer and dry cleaner in any census, external referents (SIR)
	Male	0.89 (0.68, 1.14)	62	
	Female	0.96 (0.84, 1.10)	201	
				Calvert et al. (2011)
	All subjects	1.14 (0.37, 2.67)	5	1,704 U.S. men and women dry-cleaning union members in CA, IL, MI, NY follow-up 1940−2004 (618 subjects worked for one or more yr prior to 1960 only at shops where PCE was the primary cleaning solvent, identified as PCE-only exposure), cancer mortality (SMR)
	Exposure duration/time since 1st employment			
	<5 yr/<20 yr	Not reported		
	<5 yr/≥20 yr	Not reported		
	≥5 yr/<20 yr	Not reported		
	≥5 yr/≥20 yr	Not reported		
	PCE-only subjects	1.35 (0.16, 4.89)	2	
				Seldén and Ahlborg (2011)
	Dry-cleaners and laundry workers	1.04 (0.69, 1.49)	100	9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−2000, exposure assigned using company self-reported information on PCE usage—PCE (dry cleaners and laundries with a proportion of PCE dry cleaning), laundry (no PCE use), and other (mixed exposures to PCE, CFCs, TCE, etc.), external referents (SIR)
	PCE	Not reported		
	Laundry	Not reported		

Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Travier et al. (2002)
All subjects, 1960 or 1970 Census in laundry and dry cleaner or related occupation and industry	1.11 (0.93, 1.33)	121	Swedish men and women identified as laundry worker, dry cleaner, or presser (occupational title), in the laundry, ironing, or dyeing industry or related industry in 1960 or 1970 (543,036 person-years); or, as laundry worker, dry cleaner, or presser (occupational and job title) (46,933 person-years) in both censuses, follow-up 1971−1989, external referents (SIR)
All subjects in 1960 and 1970 in laundry and dry cleaner occupation and industry	1.20 (0.71, 2.02)	14	
			Wilson et al. (2008)
All subjects, laundry and dry cleaning occupation			16,512 Swedish men (n = 3,375) and women (n = 13,137) identified in 1960 or 1970 as laundry worker or dry cleaner (occupation) or in laundry, ironing and dyeing industry, follow-up 1971−1989, external referents (SIR), cancer of the renal pelvis
Males	Not reported	<2 obs.	
Females	1.23 (0.39, 2.86)	5	
Case-Control Studies			
			Asal et al. (1988)
Dry-cleaning industry			315 histologically or radiologically confirmed renal cell carcinoma cases identified from 29 Oklahoma hospitals, 1981−1984, 336 population controls frequency matched on age and sex and 313 hospital controls matched by age, sex, race, hospital and time of interview to cases, in-person interview using questionnaire, longest job held was exposure surrogate, OR adjusted for age, smoking weight
Males	0.7 (0.2, 2.3)	3	
Females	2.8 (0.8, 9.8)	8	
Upper Cape Cod, MA (United States)			Aschengrau et al. (1993), Vieira et al. (2005)
Any PCE	1.08 (0.42, 2.79)	6	35 kidney cancer cases, 1983−1986, Massachusetts Cancer Registry, 777 population controls, residential history, ordinal estimate of PCE-contaminated water (RDD) from exposure model (Aschengrau et al., 1993) or geographical information system and proximity to groundwater plume (Vieira et al., 2005), OR adjusted for sex, age at diagnosis, vital status at interview, education, cigarette smoking, and urinary tract infection or stone (both studies)
RDD >90th percentile		0	

Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
10 hospitals (France)				Auperin et al. (1994)
	Dry cleaning occupation	Not reported		151 histologically confirmed renal cell carcinoma hospital cases, 1987−1991, 161 hospital cancer controls and 186 hospital controls with nonmalignant disease matched on age, sex, and interviewed to cases, in-person interview, lifetime occupational title as exposure surrogate, OR adjusted for age, smoking, weight
Population of New Zealand				Delahunt et al. (1995)
	Launderer and dry cleaner occupation	1.92 (0.37, 13.89)	Not reported	710 male histologically confirmed renal cell carcinoma cases, ≥20 yr of age, 1978−1986, 12,758 male controls randomly selected from same cancer registry as cases but with tumor outside urinary tract, interview method not reported, occupational title (ever employed or usual job title not reported) as exposure surrogate, Mantel-Haenszel OR stratified by smoking history and 10 yr age group
International Renal Cell Cancer Study (Australia, Denmark, Germany, Sweden, United States)				Mandel et al. (1995); Dosemeci et al. (1999); McCredie and Stewart (1993); Mellemgaard et al. (1994); Schlehofer et al. (1995)
	All Centers (Mandel et al., 1995)			1,732 histologically or cytologically confirmed renal cell carcinoma cases from 6 study centers (Mandel et al., 1995) [438 renal cell carcinoma cases from one United States center [Minnesota Cancer Surveillance System, a SEER reporting site] (Dosemeci et al., 1999), 368 cases from Denmark (Mellemgaard et al., 1994), 277 renal cell carcinoma cases from 10 local urology departments near Heidelberg, Germany (Schlehofer et al., 1995)], 20−79 yr (20−75 yr, Heidelberg), 1989−1991, identified from hospital surveillance (Germany) national cancer registries (all other countries), same birth country and cancer registry (except Australia and the United States), 2,309 population controls (all countries, with controls ≥65 yr in the United States identified from HCFA roles) (Mandel et al., 1995) [687 population controls (Dosemeci et al., 1999); 396 population referents (Mellemgaard et al., 1994), 286 population controls (Schlehofer et al., 1995)], matched on sex, and age, in-person interview with
	Ever exposed to dry-cleaning solvents	1.4 (1.1, 1.7)	245	
	Duration of exposure to dry-cleaning solvents (yr)			
	1−7	0.2 (0.9, 1.8)	70	
	8−25	1.7 (1.2, 2.4)	78	
	26−60	1.2 (0.9, 1.8)	75	
	Denmark (Mellemgaard et al., 1994)			
	≥1 yr exposure duration in dry-cleaning industry, 10 yr before interview			
	Males	2.3 (0.2, 27)	2	

Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer (continued)

	Exposure group	Relative risk (95% CI)	No. obs. events	Reference
	Females	2.9 (0.3, 33)	2	questionnaire inquiry on specific occupations (4 centers)
cont	**New South Wales, Australia (McCredie and Stewart, 1993)**[d]			or full occupational history (2 centers); occupation and chemical grouping as exposure surrogate, OR stratified by sex and adjusted for age, smoking, BMI, education, and study center, OR reported for males only (Mandel et al., 1995). In Mellemgaard et al. (1994), OR for occupational title/exposure ≥1 year duration and 10 years before interview and adjusted for age, BMI and smoking. In Dosemeci et al. (1999), OR reported for both sexes together and separately and adjusted for age, smoking hypertension, and/or diuretic use, and/or anti-hypertension drug use, and BMI. In Schlehofer et al. (1995), OR for exposure duration ≥5 years and adjusted for age and smoking
	Dry-cleaning industry occupation or job	2.70 (1.08, 6.72)	16	
	Germany (Heidelberg) (Schlehofer et al., 1995)			
	PCE and tetrachlorocarbonate	2.52 (1.23, 5.16)	27	
	United States (Minnesota) (Dosemeci et al., 1999)			
	PCE	1.07 (0.7, 1.6)	50	
	Male	1.12 (0.7, 1.7)	42	
	Female	0.82 (0.3, 2.1)	8	
Nordic Countries (Denmark, Finland, Norway, Sweden)				Lynge et al. (2006)
	Unexposed	1.00	129	Case-control study among 46,768 Danish, Finnish, Norwegian, and Swedish men and women employed in 1960 as laundry worker or dry cleaner, follow-up 1970−1971 to 1997−2001, 210 renal cell carcinoma cases, 3 controls per case randomly selected from cohort matched on country, sex, age, calendar period at diagnosis time, occupational task at 1970 Census proxy for exposure, kidney cancer incidence, RR adjusted for country, sex, age, calendar period at time of diagnosis
	Dry cleaner	0.67 (0.43, 1.05)	29	
	Other in dry-cleaning	1.15 (0.52, 2.53)	9	
	Unclassifiable	0.76 (0.50, 1.16)	3	
	Dry cleaner, employment duration, 1964−1979			
	≤1 yr	0.24 (0.03, 2.04)	1	
	2−4 yr	0.86 (0.28, 2.67)	4	
	5−9 yr	0.70 (0.32, 1.55)	8	
	≥10 yr	0.75 (0.39, 1.42)	14	
	Unknown	0.70 (0.15, 3.36)	2	

Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
New South Wales, Australia				McCredie and Stewart (1993)
	Dry-cleaning industry occupation or job	6.09 (1.95, 18.9)[e]	8	147 renal pelvic cancer cases, 20−79 yr, 1989−1990, identified from hospitals and physicians, 523 population controls, in-person or telephone interview, job title or industry as exposure surrogate, OR adjusted for age, sex, and method of interview (for renal cell carcinomas) and age, sex, interview methods and education (for renal pelvic cancers)
Finland				Partanen et al. (1991)
	Dry-cleaning operator	Not reported	1	338 renal cell carcinoma cases, 20−95 yr, 1977−1987, identified from Finnish Cancer Registry, 484 population controls matched on birth year, sex, and survival status at time of interview, mailed interview, job title or industry for all jobs held 1926−1968, OR adjusted for smoking, coffee consumption and obesity
Germany, 5 regions				Pesch et al. (2000a)
	PCE, JEM			935 histologically confirmed renal cell carcinomal cancer in men and women, hospital record study, 1991−1995, 4,298 age-sex-matched population controls, in-person interview, JEM and JTEM for PCE, OR adjusted for age, study center, smoking
	Medium exposure	1.1 (0.9, 1.4) M 1.2 (0.8, 1.8) F	135 28	
	High exposure	1.1 (0.9, 1.4) M 1.3 (0.8, 2.0) F	138 29	
	Substantial exposure	1.3 (0.9, 1.8) M 0.8 (0.3, 1.9) F	55 6	
	PCE, JTEM			
	Medium exposure	1.2 (0.9, 1.7) M 2.2 (0.9, 5.2) F	44 8	
	High exposure	1.1 (0.7, 1.5) M 1.5 (0.6, 3.8) F	39 6	
	Substantial exposure	1.3 (0.7, 2.3) M 2.0 (0.5, 7.8) F	15 3	

Table 4-7. Summary of human studies on tetrachloroethylene exposure and kidney cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Montreal, Canada			Parent, 2000
Launderers and dry cleaners			142 histologically confirmed renal cell carcinoma cancer, 1979−1985, 35−70 yr, 533 population control group and 1,900 cancer control group, in-person interviews, occupational title, OR adjusted age, smoking, and BMI
Any exposure	1.7 (0.6, 4.7)	4	
Substantial exposure		0	
Geographic Studies			
New York City, NY (United States)			Ma, 2010
Zip codes with number of dry cleaners/km^2			10,916 cases with hospital discharge diagnosis of renal or renal pelvis cancer, 1993−2004, zip code of residential address and dry-cleaner business number/zip code area as exposure surrogate, crude prevalence rate ratio (prevalence RR)
0−0.47	1.0a	1,458	
0.47−0.90	1.14 (1.03, 1.27)[f]	2,289	
0.90−1.50	1.09 (0.97, 1.21)[f]	1,838	
1.50−2.70	1.17 (1.05, 1.32)[f]	2,766	
2.70−16.43	1.15 (1.01, 1.30)[f]	2,565	

[a]Referent.
[b]For Chang et al. (2003), SMR for kidney and urinary organs.
[c]For Sung et al. (2007), SIR for kidney and urinary organs, 10 yr lag period.
[d]In McCredie and Stewart (1993), renal cell carcinoma cases from hospitals and physicians in New South Wales, Australia. Of the 489 renal cell carcinoma cases, 256 were from the Sydney Metropolitan area and were included in the National Cancer Institute's international study (Mandel et al., 1995).
[e]In McCredie and Stewart (1993), OR for renal pelvic cancer.
[f]In Ma et al. (2009), rate ratio from negative binomial regression model with main effect for zip code (crude rate ratio). Rate ratios from models with adjustment for age, race, sex, population density and median household and effect modifiers that vary by exposure category are 1.0 (referent), 1.15 (95% CI: 1.04, 1.27) [no effect modification], 1.10 (1.00, 1.24) [effect modification by population density], 1.27 (95% CI: 1.13, 1.42) [effect modification by race], and 1.16 (05% CI: 1.02, 1.33) [effect modification by mean household income and age], for numbers of dry cleaners of 0−0.47, 0.47−0.90, 0.90−1.50, 1.50−2.70, and 2.70−16.43/km^2, respectively.

JEM = job-exposure matrix, HCFA = Health Care Financing Administration, JTEM = job-task-exposure-matrix, PCE = tetrachloroethylene, RDD = relative delivered dose, TWA = time-weighted-average.

4.2.1.3. Bladder Cancer in Humans

Thirty-two epidemiologic studies reporting data on bladder cancer and tetrachloroethylene exposure were identified. This set of studies includes 13 cohort or nested case-control studies within a cohort (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Wilson et al., 2008; Sung et al., 2007; Lynge et al., 2006; Chang et al., 2005; Ji and Hemminki, 2005a; Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999; Boice et al., 1999; Lynge and Thygesen, 1990), 16 case-control studies of occupational exposures (Colt et al., 2011; Dryson et al., 2008; Reulen et al., 2007; Gaertner et al., 2004; Kogevinas et al., 2003; Zheng et al., 2002; Pesch et al., 2000b; Teschke et al., 1997; Swanson and Burns, 1995; Burns and Swanson, 1991; Siemiatycki, 1991; Steineck et al., 1990; Silverman et al., 1989a; Silverman et al., 1989b; Smith et al., 1985; Schoenberg et al., 1984), and 3 studies of residential exposure through contaminated drinking water (Vieira et al., 2005; Aschengrau et al., 1993; Mallin, 1990). These 32 studies represent the core studies evaluated by EPA, as described in more detail below. Two other cohort studies and one case-control study included information on tetrachloroethylene but did not report risk estimates for bladder cancer (Radican et al., 2008; Colt et al., 2004; Anttila et al., 1995), and so were not evaluated further. The peer-reviewed literature also contains a meta-analysis that examined dry cleaning and bladder cancer (Reulen et al., 2007).

There is some overlap in the study populations among these studies: Travier et al. (2002) used occupational data from the Swedish national census, and Lynge and Thygsen (1990) used a similar design in Denmark; Andersen et al. (1999) and Lynge et al. (2006) expanded these studies to include Denmark, Finland, and Norway in addition to Sweden, and Pukkala et al. (2009) added Iceland to this set. Pesch et al. (2000b) is a large case-control study examining urothelial cancers, a grouping of bladder, ureter, and renal pelvis neoplasms, with exposure information on tetrachloroethylene. Kogevinas et al. (2003), a pooled analysis of 11 studies conducted in European countries between 1976 and 1996, includes the dry cleaning but not the tetrachloroethylene exposure observations in males in Pesch et al. (2000b). Kogevinas does not provide information on women; 't Mannetje et al. (1999) pooled observations in women in these 11 studies but did not report findings on dry-cleaner and laundry workers.

Appendix B reviews the design, exposure-assessment approach, and statistical methodology for each study. Most studies were of the inhalation route, of occupational exposure, and unable to quantify tetrachloroethylene exposure.

4.2.1.3.1. Consideration of exposure-assessment methodology

Many studies examine occupational titles such as dry cleaner, launderer, and presser as surrogate for tetrachloroethylene, given its widespread use from 1960 onward in the United

States and Europe (Calvert et al., 2011; Colt et al., 2011; Pukkala et al., 2009; Dryson et al., 2008; Reulen et al., 2008; Wilson et al., 2008; Reulen et al., 2007; Lynge et al., 2006; Ji and Hemminki, 2005a; Gaertner et al., 2004; Blair et al., 2003; Kogevinas et al., 2003; Travier et al., 2002; Zheng et al., 2002; Andersen et al., 1999; Teschke et al., 1997; Swanson and Burns, 1995; Burns and Swanson, 1991; Lynge and Thygesen, 1990; Silverman et al., 1990; Steineck et al., 1990; Silverman et al., 1989a; Silverman et al., 1989b; Smith et al., 1985; Schoenberg et al., 1984). Six studies conducted in Nordic countries are either based on the entire Swedish population or combined populations of several Nordic countries; strengths of these studies are their use of job titles as recorded in census databases and ascertainment of cancer incidence using national cancer registries (Pukkala et al., 2009; Wilson et al., 2008; Lynge et al., 2006; Ji et al., 2005a; Travier et al., 2002; Andersen et al., 1999). Studies examining mortality among U.S. dry-cleaner and laundry workers (Calvert et al., 2011; Blair et al., 2003) are of smaller cohorts than the Nordic studies, with fewer observed bladder cancer events.

The exposure surrogate in studies of dry-cleaners and laundry workers is a broad category containing jobs of differing potential for tetrachloroethylene exposure. Thus, these studies have a greater potential for exposure misclassification bias compared to studies with exposure potential to tetrachloroethylene assigned by exposure matrix approaches. Two studies used additional information pertaining to work environment to refine the exposure (Calvert et al., 2011; Lynge et al., 2006). Lynge et al. (2006), using job titles reported in the 1970 Census, identified subjects based on an occupational code of "laundry and dry-cleaning worker" or an industry code of "laundry and dry cleaning." Additional information to refine this occupational classification was sought for incident cancer cases, including bladder cancer, within this defined cohort. Five controls, matched to the cases by country, sex, age, and calendar period, were also included in the study. The additional information included handwritten task information from the census forms from Denmark and Norway, pension databases in Denmark and Finland, and next-of-kin interviews in Norway and Sweden. Exposure classification categories were dry cleaner (defined as dry cleaners and supporting staff if employed in a business of <10 workers), other job titles in dry cleaning (launderers and pressers), unexposed (job title reported on 1970 census was other than dry cleaning), or unclassifiable (information was lacking to identify job title of subject). The unclassifiable category represented 57 of 351 bladder cancer cases (16%) and 234 out of 1,482 controls (16%). The study by Calvert et al. (2011) included an analysis of subjects who worked for one or more years before 1960 in one or more shops known to use tetrachloroethylene as the primary solvent (Calvert et al., 2011). The cohort was stratified into two groups based on the level of certainty that the worker was employed only in facilities using tetrachloroethylene as the primary solvent exposure; tetrachloroethylene-only and tetrachloroethylene plus. However, there were no bladder cancer deaths among this subset

(n = 618) of tetrachloroethylene-only subjects. Three additional studies used a semiquantitative or quantitative exposure metric. Blair et al. (2003) used an exposure metric for semiquantitative cumulative exposure between dry-cleaning and laundry workers. The case-control study by Siemiatycki (1991) used a JEM based on occupational titles for tetrachloroethylene, and another case-control study used a JEM and one JEM with information on specific tasks, a JTEM, with semiquantitative exposure assessment across a variety of jobs (Pesch et al., 2000b).

Two other cohorts with potential tetrachloroethylene exposure in manufacturing settings have been examined. These studies include aerospace workers in the United States (Boice et al., 1999) and electronic factory workers in Taiwan (Sung et al., 2007; Chang et al., 2005). Boice et al. (1999) used an exposure assessment based on a JEM to classify exposures. In contrast, the exposures in the Taiwan studies included multiple solvents, and tetrachloroethylene exposure was not linked to individual workers (Sung et al., 2007; Chang et al., 2005).

Three geographic studies focused on residential proximity to drinking water sources contaminated with tetrachloroethylene and other solvents. Mallin (1990) examined incidence and mortality by county in Illinois, with the exposure surrogate assigned uniformly to all subjects. Two other studies in Cape Cod, MA, used either an exposure model incorporating tetrachloroethylene leaching and characteristics of the community water distribution system (Aschengrau et al., 1993) or residential proximity to Superfund sites and a generalized additive model that incorporates smoothing approaches and adjusts for covariates (Vieira et al., 2005).

In summary, four studies with bladder cancer data assigned tetrachloroethylene exposure to individuals within the study using a job exposure matrix (Blair et al., 2003; Pesch et al., 2000b; Boice et al., 1999) or an exposure model (Aschengrau et al., 1993). One other study sought additional data using a questionnaire for use in refining potential exposure within dry-cleaning settings (Lynge et al., 2006). The relative specificity of these exposure-assessment approaches strengthens their ability to identify cancer hazards compared to studies with broader and less sensitive exposure-assessment approaches.

4.2.1.3.2. Summary of results

Seven studies evaluated by EPA reported estimated relative risks based on a large number of observed events; 50 or more deaths/incident cases in cohort studies (Pukkala et al., 2009; Wilson et al., 2008; 2005a; Travier et al., 2002; Andersen et al., 1999), or 50 or more exposed cases in case-control studies (Lynge et al., 2006; Pesch et al., 2000b), with sufficient power to detect a twofold elevation in estimated risk. Pukkala et al. (2009) updates the analysis of Andersen et al. (1999) adding data from a 5th country, Iceland, and extending follow-up to

2005, and is preferred over Andersen et al. (1999) for these reasons. The five[18] large cohort studies observed a standardized incidence ratio or odds ratio estimate of 1.01 (95% CI: 0.86, 1.19), 1.08 (95% CI: 0.98, 1.23), 1.14 (95% CI: 0.89, 1.45), 1.27 (95% CI: 1.08, 1.48), and 1.44 (95% CI: 1.07, 1.93) in Travier et al. (2002), Pukkala et al. (2009), Wilson et al. (2008), Ji et al. (2005a) and Lynge et al. (2006), respectively, for the association between bladder cancer risk and ever having a job title of dry cleaner or laundry worker (refer to Table 4-8). The Lynge et al. (2006) results were slightly higher among the subgroup from Denmark and Norway, in which the number of unclassifiable data was negligible (relative risk 1.69, 95% CI: 1.18, 2.43). The large case-control study by Pesch et al. (2000b) reported an odds ratio of 0.8 (95% CI: 0.6, 1.2), 1.3 (95% CI: 0.9, 1.7), and 1.8 (95% CI: 1.2, 2.7) for medium, high, and substantial exposure, respectively, compared to low exposure, based on the JTEM approach.

Additional evidence is found in studies whose effect estimates are based on fewer observed events and that carry lesser weight in the analysis. As expected, the magnitude of the point estimate of the association reported in these studies is more variable than in the larger studies: 4 studies report relative risks between 0.7 and 0.91 [Colt et al. (2011), males; Dryson et al. (2008); Boice et al. (1999); Lynge and Thygesen (1990)], 10 studies report relative risks between 1.2 and 1.9 [Colt et al. (2011), females; Gaertner et al. (2004); Blair et al. (2003); Kogevinas et al. (2003); Aschengrau et al. (1993); Burns and Swanson (1991); Siemiatycki (1991); Steineck et al. (1990); Smith et al. (1985); Schoenberg et al. (1984)], and 3 studies report relative risk estimates >2.0 (Reulen et al., 2007; Zheng et al., 2002; Teschke et al., 1997). Except for the estimate from Reulen et al. (2007) (RR: 2.7, 95% CI: 1.1, 6.6), all of the 95% CIs of these estimates overlap 1.0. Because of the relatively small number of observed cases in these cohort studies or exposed cases in case-control studies, ranging from 2 in Boice et al. (1999) to 19 in the pooled study of Kogevinas et al. (2003); the statistical power of these lesser-weighted studies is limited.

[18] Andersen et al. (1999) is not included in this summary of the data from the individual studies because it was updated and expanded in the analysis by Pukkala et al. (2009).

Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Cohort Studies			
Biologically monitored workers			Anttila et al. (1995)
All subjects	Not reported[a]		849 Finnish men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, external referents (SIR)
Aerospace workers (Lockheed)			Boice et al. (1999)
Routine exposure to PCE	0.70 (0.09, 2.53)	2	77,965 (n = 2,631 with routine PCE exposure and n = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, job exposure matrix without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm [mean] and 9.5 ppm [median], external reference for routine exposure (SMR) and internal references (workers with no chemical exposures) for routine-intermittent PCE exposure (RR)
Routine-Intermittent exposure to PCE	Not reported[b]		
Electronic factory workers (Taiwan)			Chang et al. (2005); Sung et al. (2007)
All Subjects			86,868 (n = 70,735 female), follow-up 1979−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, cancer incidence, external referents (SIR) (Chang et al., 2005); 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, multiple solvents exposures and PCE not identified to individual subjects, cancer incidence, external referents, analyses lagged 5 yr (SIR) (Sung et al., 2007)
Males	1.06 (0.45, 2.08)[c]	8	
Females	1.09 (0.56, 1.91)[c]	12	
Females	0.34 (0.07, 1.00)	12	
Aircraft maintenance workers from Hill Air Force Base			Radican et al. (2008)
Any PCE exposure	Not reported		10,461 men and 3,605 women (total n = 14,066, n = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952 to 1956, follow-up 1973−2000, job exposure matrix (intensity), internal referent (workers with no chemical exposures) (RR)

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Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Dry-cleaner and laundry workers			Andersen et al. (1999)
All laundry worker and dry cleaners	1.00 (0.83, 1.21)	119	29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway), follow-up 1971−1987 or 1991, PCE not identified to individual subjects, external referents (SIR)
Males	1.14 (0.87, 1.46)	62	
Females	0.89 (0.68, 1.16)	57	
			Blair et al. (2003)
All subjects	1.3 (0.7, 2.4)	12	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR)
Semiquantitative exposure score			
Little to no exposure	1.4 (0.4, 3.2)	5	
Medium to high exposure	1.5 (0.6, 3.1)	7	
			Ji et al. (2005a)
Male laundry workers and dry cleaners in 1960 Census	1.27 (1.08, 1.48)	157	9,255 Swedish men employed in 1960 as laundry worker or dry cleaner, follow-up 1961−2000, PCE not identified to individual subjects, external referent (SIR) and adjusted for age, period and socioeconomic status
Male laundry workers and dry cleaners in 1960 Census	1.13 (0.96, 1.31)[d]	157	
Male laundry workers and dry cleaners in both 1960 and 1970 Censuses	1.03 (0.80, 1.29)[d]	67	
Male laundry workers and dry cleaners in 1960, 1970 and 1980 Censuses	0.86 (0.51, 1.28)[d]	19	
Female laundry workers and dry cleaners	Not reported		
			Lynge and Thygsen (1990)
All laundry worker and dry cleaners	0.74 (0.41, 1.25)	14	10,600 Danish men and women, 20−64 yr old, employed in 1970 as laundry worker, dry cleaners and textile dye workers, follow-up 1970−1980, external referents (SIR)
Males	0.62 (0.23, 1.35)	6	
Females	0.88 (0.38, 1.73)	8	

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Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Pukkala et al. (2009)
Launderer and dry cleaner	1.08 (0.98, 1.23)	434	Men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30−64 yr, follow-up 2005, occupational title of launderer and dry cleaner in any census, external referents (SIR)
Male	1.10 (0.95, 1.27)	186	
Female	1.07 (0.95, 1.22)	248	
			Calvert et al. (2011)
All subjects	1.81 (0.87, 3.33)	10	1,704 U.S. men and women dry-cleaning union member in CA, IL, MI, NY follow-up 1940−2004 (618 subjects worked for one or more years prior to 1960 only at shops where PCE was the primary cleaning solvent, identified as PCE-only exposure), cancer mortality (SMR)
Exposure duration/time since 1st employment			
<5 yr/<20 yr		0	
<5 yr/≥20 yr	0.53 (0.03, 2.52)	1	
≥5 yr/<20 yr		0	
≥5 yr/≥20 yr	4.08 (2.13, 7.12)	9	
PCE-only subjects		0	
			Seldén and Ahlborg (2011)
Dry-cleaners and laundry workers (females)	0.92 (0.65, 1.26)	38	9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−2000
			Travier et al. (2002)
All subjects, 1960 or 1970 Census in laundry and dry cleaner occupation and industry	1.01 (0.86, 1.19)	145	Swedish men and women identified in 1960, 1970, or both Censuses as laundry worker, dry cleaner, or presser (occupational title) or in the laundry, ironing, or dyeing industry, follow-up 1971−1989, separates launders and dry cleaners form pressers, external referents (SIR)
All subjects in 1960 and 1970 in laundry and dry cleaner occupation and industry	1.00(0.61, 1.63)	16	

Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Wilson et al. (2008)
All subjects, laundry and dry cleaning occupation	1.14 (0.89, 1.45)	68	Swedish men and women identified in 1960 or 1970 as laundry worker or dry cleaner (occupation) or in laundry, ironing and dyeing industry, follow-up 1971−1989, external referents (SIR), transitional cell carcinoma
Males	1.23 (0.83, 1.74)	31	
Females	1.07 (0.75, 1.47)	37	
Case-Control Studies			
Upper Cape Cod, MA (United States)			Aschengrau et al. (1993), Vieira (2005)
Any PCE	1.39 (0.67, 2.91)	13	63 bladder cancer cases, 1968−1980, Massachusetts Cancer Registry, 852 population controls, residential history, ordinal estimate of PCE-contaminated water (RDD) from exposure model (Aschengrau et al., 1993) or geographical information system and proximity to groundwater plume (Vieira et al., 2005), OR adjusted for sex, age at diagnosis, vital status at interview, education, cigarette smoking, and urinary tract infection (both studies), and, past occupational exposure (Aschengrau et al., 1993)
RDD >90th percentile	4.03 (0.65, 25.10)	4	
"Hot spot" SW of MMR	~ 2.5 (CI not reported)		
Metropolitan Detroit, MI (United States)			Burns and Swanson (1991); Swanson and Burns (1995)
Usual occupation as dry-cleaning workers	1.9 (0.7, 4.9)	8	2,160 histologically confirmed bladder cancer cases in men and women, 40−84 yr old, Metropolitan Detroit Cancer Surveillance System, 3.979 rectal or colon cancer controls, telephone interview, longest period (usual) employed in occupation or industry, OR adjusted for cigarette smoking, race, sex, and age at diagnosis
Males	Not reported	2	
Females	2.0 (0.7, 6.2)	6	
Usual industry in dry cleaner and laundry	1.2 (0.6, 2.4)	15	
New Hampshire (United States)			Colt et al. (2004)
Launderers and dry cleaners			459 bladder cancer cases, 1994−1998, New Hampshire State Cancer Registry, 25−74 yr, 665 populations controls, 1993−1997, occupation as exposure surrogate, OR adjusted for 5-yr age group and smoking
Males	Not reported	5	
Females		0	

Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Maine, Vermont, and New Hampshire (United States)			Colt et al. (2011)
Occupation: Laundering and dry-cleaning machine operators and tenders			1,158 patients, aged 30−79, newly diagnosed with histologically confirmed bladder cancer, 2001−2004, ascertained from hospital pathology departments, hospital cancer registries and state cancer registries, 1,402 population controls frequency matched by age (within 5 yr), state and gender, occupational histories through interview coded by occupation (SOC 7658) and industry (SIC 721), OR for occupation or industry category compared to other never employed in that category, adjusted for age, race, Hispanic ethnicity, state, smoking status, and employment in a high risk occupation
Males	Not reported	5	
Females	0.45 (0.03, 7.46)	1	
Industry: Laundry, cleaning and garment services			
Males	0.91 (0.41, 2.03)	14	
Females	1.50 (0.50, 4.50)	10	
New Zealand			Dryson et al. (2008)
Textile bleaching, dyeing and cleaning machine operators	0.81 (0.19, 3.54)	3	213 bladder cancer cases, 25−70 yr, 2003−2004, New Zealand Cancer Registry, 471 population controls, occupational title, OR adjusted for sex, smoking, SES
Canada, 7 Provinces			Gaertner et al. (2004)
Drycleaner	1.24 (0.23, 6.64)	5	887 histologically confirmed bladder cancer, 20−74 yr, 2,847 population controls, Province Cancer Registry, mailed questionnaire, occupational title as exposure surrogate, OR adjusted for age, province, race, smoking status, consumption of fruit, fried food, and coffee, and past occupational exposure.
European Pooled Study (Denmark, France, Germany, Greece, Italy, Spain)			Kogevinas et al. (2003)[i]
Launderers, dry cleaners and pressers	1.24 (0.67, 2.31)	19	Pooled study of 3,346 male bladder cancer cases, 30−79 yr, study-specific groups of 6,840 controls, occupational title, OR adjusted for age, smoking, and study center
Nordic Countries (Denmark, Finland, Norway, Sweden)			Lynge et al. (2006)
Unexposed	1.00	188	Case-control study among 46,768 Danish, Finnish, Norwegian, and Swedish men and women employed in 1960 as laundry
Dry cleaner	1.44 (1.07, 1.93)[e]	93	

Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Other in dry-cleaning	1.08 (0.55, 2.11)[e]	12	worker or dry cleaner, follow-up 1970−1971 to 1997−2001, 351 bladder cancer cases, 3 controls per case randomly selected from cohort matched on country, sex, age, calendar period at diagnosis time, occupational task at 1970 Census proxy for exposure, bladder cancer incidence (excluding in-situ), RR adjusted for matching criteria
	Unclassifiable	1.24 (0.83, 1.83)[e]	57	
	Dry cleaner	1.69 (1.18, 2.43)[e,f]	15	
	Other in dry-cleaning	1.13 (0.51, 2.50)[e,f]	6	
	Unclassifiable	Not reported[e]	1	
	Dry cleaner, smoking adjusted	1.25 (0.79, 1.98)[g]		
	Dry cleaner, employment duration, 1964−1979			
	≤1 yr	1.50 (0.57, 3.96)[e]	6	
	2−4 yr	2.39 (1.09, 5.22)[e]	10	
	5−9 yr	0.92 (0.52, 1.59)[e]	17	
	≥10 yr	1.57 (1.07 2.29)[e]	53	
	Unknown	1.97 (0.64, 6.05)[e]	6	

Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Germany, 5 regions			Pesch et al. (2000b)
PCE, JEM			1,035 histologically confirmed urothelial cancer in men and women, hospital record study, 1991−1995, 4,298 population controls, in-person interview, JEM and JTEM for PCE, OR adjusted for age, study center, smoking
Medium exposure	1.1 (0.9, 1.3) M 1.8 (1.0, 3.0) F	162 21	
High exposure	1.2 (1.0, 1.5) M 1.0 (0.6, 1.9) F	172 16	
Substantial exposure	1.4 (1.0, 1.9) M 0.7 (0.2, 2.5)	71 3	
PCE, JTEM			
Medium exposure	0.8 (0.6, 1.2)	47	
High exposure	1.3 (0.9, 1.7)	74	
Substantial exposure	1.8 (1.2, 2.7)	36	
Belgium, Limburg Region			Reulen et al. (2007)
Domestic helpers, cleaners, and launderers	2.7 (1.1, 6.6)	14	202 histologically confirmed transitional cell carcinoma cases, 40−96 yr, Limburg Cancer Registry, 390 population controls, in-person interview, occupational title, OR adjusted for age, sex, smoking status, number cigarettes, years smoked, education
New Jersey (United States)			Schoenberg et al. (1984)
Dry-cleaning workers	1.33 (0.50, 3.58)	7	Histologically confirmed bladder cancer cases (658 Caucasian men), 1978−1979, 21−84 yr, age-stratified population controls (1,258 Caucasian men) identified through RDD or HCFA register, in-person interview with questionnaire, industry and job title surrogate exposure metric, OR adjusted for age and cigarette smoking

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Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Montreal, Canada			Siemiatycki (1991)
Launderers and dry cleaners			Histologically confirmed bladder cancer, 1979−1985, 35−70 yr, population control group and cancer control group, in-person interviews, occupational title and JEM for PCE, OR adjusted age, family income, and cigarette index, 90% CI
Any exposure	1.6 (0.9, 3.1)	10	
Substantial exposure	1.9 (0.9, 4.2)	7	
National Bladder Cancer study			Silverman et al. (1990; 1989a; 1989b); Smith et al. (1985)
Laundry and dry cleaners, males and females			Histologically confirmed bladder cancer cases (2,226 men, 733 women), 1977−1978, 21−64 yr, 5,757 population controls, in-person interview, occupational title as exposure surrogate, OR adjusted for smoking (Silverman et al., 1990) and employment in other high-risk occupation (Silverman et al., 1989b) and age, sex, and smoking (<20/d, ≥20 to <40/d, ≥40/d (Smith et al., 1985)
Nonsmoker	1.31 (0.85, 2.03)	Not reported	
Former smoker	2.99 (1.80, 4.97)	Not reported	
Current smoker	3.94 (2.39, 6.51)	Not reported	
Laundry and dry cleaners, non-Caucasian males	2.8 (1.1, 7.4)	11	
<5 yr employment duration	5.3 (CI not reported)	7	
≥5 yr employment duration	1.8 (CI not reported)	4	
p-value for linear trend	$p = 0.016$		
Laundry and dry cleaners, females	1.4 (0.8, 2.6)	23	
Stockholm, Sweden			Steineck et al. (1990)
Dry cleaner	1.2 (0.2, 9.2)	2	Bladder cancer cases in males, birth years, 1911−1945 and living in County of Stockholm 1985−1987, population controls, mailed questionnaire, occupational title as surrogate, OR adjusted for birth year and smoking
British Columbia, Canada			Teschke et al. (1997)
Laundry and dry-cleaner workers	2.3 (0.4, 13.9)	5	Histologically confirmed bladder cancer cases (excluding in situ) from British Columbia Cancer Agency in men and women,
Exposure surrogate lagged 20 yr	1.8 (0.3, 11.3)	4	

Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Dry cleaners	Not reported	3	1990−1991, ≥19 yr, population controls, in-person or telephone interviews, occupation and industry as surrogates, OR adjusted for sex, age, cigarette smoking
Iowa, United States				Zheng et al. (2002)
	Laundering and dry cleaning occupation			Histologically confirmed in situ and invasive bladder cancer from Iowa state health registry records in men and women, 1986−1989, 40−85 yr, population controls, in-person interview, occupation and industry as surrogate, OR adjusted for age, lifetime pack-years of cigarette smoking, and first-degree relative with bladder cancer
	Males	Not reported		
	Females	9.3 (0.9, 94.8)	3/1	
	Duration of employment			
	<10 yr		2/0	
	≥10 yr	2.1 (0.1, 36.9)	1/1	
Geographic Studies				
Illinois, 8 NW counties				Mallin (1990)
	Winnebago County			712 bladder cancer cases in Caucasian men and women, 1978−1985, residence as exposure surrogate, solvent-contaminated municipal drinking water wells in Winnebego County [multiple solvents including PCE, <1−5.1 ppb], incidence and mortality rates of U.S. population as referent (SIR, SMR)
	Males	0.96 (0.8, 1.1)[h]	250	
		1.39 (1.1, 1.7)[a]	76	
	Females	1.03 (0.8, 1.3)[h]	96	
		1.40 (1.0, 1.9)[a]	35	
Meta-analysis				
	Laundry and dry-cleaning workers	1.27 (0.95, 1.71)[j]		Reulen et al. (2008)
	Cohort studies	0.82 (0.54, 1.25)		
	Case-control studies	1.66 (1.23, 2.24)		

Table 4-8. Summary of human studies on tetrachloroethylene exposure and bladder cancer (continued)

[a]Incidence.
[b]For Boice et al. (1999), Relative risks for employment duration from Poisson regression with internal referents of factory workers not exposed to any solvent and with adjustment for date of birth, date first employed, date of finishing employment, race, and sex.
[c]For Chang et al. (2005), SIR for urinary organ neoplasms given bladder cancer SIR is not identified separately.
[d]Smoking-corrected SIR obtained by dividing SIR by 35% of the excess of lung cancer risk (assumed proportion of risk between lung and bladder cancer associated with smoking 20 cigarettes/d).
[e]In Lynge et al. (2006), odds ratio from logistic regression adjusted for country, sex, age, and calendar period at time of diagnosis.
[f] In Lynge et al. (2006), odds ratio—Norway and Denmark, countries with better exposure information.
[g]In Lynge et al. (2006), smoking adjusted odds ratio for subjects from Norway and Sweden.
[h]Mortality.
[i]In Kogevinas et al. (2003) includes the following case-control studies—Claude et al. (1988), Cordier et al. (1993), Gonzalez et al. (1989), Hours et al. (1994), Jensen et al. (1987), Pesch et al. (2000a), Pohlabeln et al. (1999), Porru et al. (1996), Rebelakos et al. (1985), Serra et al. (2000), and Vineis et al. (1985).
[j]Includes Andersen et al. (1999), Burns et al. (1991), Bouchardy et al. (2002), Colt et al. (2004), Gaertner et al. (2004), Schoenberg et al. (1984), Siemiatycki (1991), Silverman et al. (1989a), Silverman et al. (1990), Steineck et al. (1990), Swanson et al. (1995), Teschke et al. (1997) Travier et al. (2002), and Zheng et al. (2002).

HCFA = Health Care Financing Administration, JEM = job-exposure matrix, MMR = Massachusetts Military Reservation, NCI = National Cancer Institute, PCE = tetrachloroethylene, RDD = random digit dialing, SES = socioeconomic statuts.

Five studies had been previously identified based on the relative strengths of their exposure-assessment methodology. The results from four of these studies provide additional evidence of an association, with relative risks of 1.44 (Lynge et al., 2006), 1.5 (Blair et al., 2003) (medium-high exposure), 4.03 (Aschengrau et al., 1993) (>90th percentile exposure), and the exposure-response gradient observed in Pesch et al. (2000b). Although a SMR of 2.59 (95% CI: 1.24, 4.76) was reported among workers with exposure to tetrachloroethylene and possibly other dry-cleaning solvents (10 exposed cases), no bladder cancer deaths were observed among a subgroup with a higher certainty of exposure only to tetrachloroethylene (Calvert et al., 2011).

Statistical analyses in all case-control studies controlled for cigarette smoking, a known risk factor for bladder cancer. The potential effect modification by smoking history is also an important issue but has been examined in only one study (Smith et al., 1985). In the analysis stratified by smoking status, adjusted ORs for the association between laundry or dry-cleaning work (based on occupational title from interview data) and bladder cancer incidence of 1.31 (95% CI: 0.85, 2.03) among nonsmokers, 2.99 (95% CI: 1.80, 4.97) among former smokers, and 3.94 (95% CI: 2.39, 6.51) among current smokers were seen.

Three studies of weaker exposure-assessment approaches observed odds ratio or standardized incidence ratio estimates of 0.34 (95% CI: 0.07, 1.00), 1.39 (95% CI: 1.1, 1.7; males) and 1.40 (95% CI: 1.0, 1.9; females), and 2.5 (CI not reported) for the association between bladder cancer and employment in a manufacturing plant (Sung et al., 2007) or residential proximity to groundwater contamination (Aschengrau et al., 1993; Mallin, 1990). These studies carry lower weight in the analyses because of their low level of detail on tetrachloroethylene exposure.

The Reulen et al. (2008) meta-analysis of occupational titles and bladder cancer included 14 studies reporting relative risk estimates for dry-cleaners and laundry workers. The pooled relative risk estimate for employment in these industries was 1.27 (95% CI: 0.95, 1.71). While Reulen et al. (2008) included many of the studies identified above, they do not include the cohorts of Calvert et al. (2011), Blair et al. (2003), Ji et al. (2005b), and Pukkala et al. (2009), or the case-control studies of Kogevinas et al. (2003) and Lynge et al. (2006). Other differences between Reulen et al. (2008) and this analysis are the inclusion of Bouchardy et al. (2002), who reported a odds ratio estimate for the association between bladder cancer and cleaning, and personal services—a broad category that included dry cleaners, laundry workers, chimney sweeps, hairdressers, and other cleaning occupations not included in the EPA analysis due to the lack of data specific for dry-cleaners and laundry workers. Despite the differences in the specific studies included in this analysis, the results are similar to that of the EPA's evaluation, indicating a small (10−40%) increased risk.

Establishment of an exposure or concentration-response relationship can add to the weight of evidence for identifying a cancer hazard, but only limited data pertaining to exposure-response relationships for bladder cancer and tetrachloroethylene exposure are available. As described previously, effect estimates of 0.8 (95% CI: 0.6, 1.2), 1.3 (95% CI: 0.9, 1.7), and 1.8 (95% CI: 1.2, 2.7) for medium, high, and substantial exposure, respectively, based on JTEM exposure data were reported in the large case-control study by Pesch et al. (2000b). Some additional information on exposure-response relationships comes from lesser-weighted studies. Two of the smaller studies with semiquantitative exposure surrogates observed larger effect measures for the highest exposure category than for overall exposure. In Aschengrau et al. (1993), the adjusted OR was 4.03 (95% CI: 0.65, 25.10) for the >90th percentile of the relative delivered dose, compared with 1.39 (95% CI: 0.67, 2.91) for any tetrachloroethylene exposure. Siemiatycki (1991) reported an adjusted OR of 1.9 (95% CI: 0.9, 4.2) for substantial exposure and 1.6 (95% CI: 0.9, 3.1) for any exposure. In the third study with semiquantitative exposure measurement, the SMR in Blair et al. (2003) was 1.5 (95% CI: 0.6, 3.1) for the medium-to-high cumulative exposure, 1.4 (95% CI: 0.4, 3.2) for the little-to-no exposure category, and 1.3 (95% CI: 0.7, 2.4) among all cohort members (laundry and dry-cleaning union members). Other studies examined duration of laundry or dry-cleaning work. Two studies did not observe increasing patterns of risk with increasing employment durations as measured by census occupation codes from two or more periods (Ji et al., 2005a; Travier et al., 2002), and one study observed a lower risk with higher duration of laundry and dry-cleaning work based on employment duration data collected in interviews with cases and controls [trend p-value = 0.016 for the adjusted OR estimate of 5.3 for <5 years and 1.8 for ≥5 years duration in laundry and drying cleaning work, respectively (Silverman et al., 1989b)]. Another study using 1960 and 1970 Census data from Nordic countries reported a nonmonotonic pattern of increasing risk, with adjusted relative risks of 1.50, 2.39, 0.92, and 1.57 for duration of dry-cleaning work from 1964−1979 of ≤1, 2−4, 4−9, and ≥10 years, respectively, compared to subjects never employed as a dry cleaner or in a shop with <10 employees[19] (Lynge et al., 2006). For the job held in 1970, Lynge et al. (2006) relied upon a biography of dry-cleaning shop owners, the yellow pages of local telephone books for self-employed persons, and national pension system records to assess length of employment for Danish subjects; national pension records for Finnish subjects,[20] and self-reported information using questionnaires for subjects from Norway or Sweden. Several potential sources of exposure misclassification for these data should be noted, however, such as would be introduced by changing employers, starting dry-cleaning work at a later time

[19] Lynge et al. (2006), an analysis based only on the employment periods from 1965 through 1978, gave the following RRs: 0–1 year = 1.43 (95% CI, 0.52–3.97); 2–4 years = 2.38 (95% CI, 1.08–5.24); 5–9 years = 1.21 (95% CI, 0.58–2.50); ≥10 years = 2.84 (95% CI, 0.97–8.35); unknown = 2.12 (95% CI, 0.65–6.85).
[20] Finnish pension records started in 1962 for dry cleaning employees and in 1970 for self-employed persons.

period, employment during a time period outside the examined range or before recordkeeping began, or imperfect recall by proxy respondents on questionnaires. Moreover, exposure duration examined in all of these studies is a poorer surrogate than a semiquantitative or quantitative exposure metric because it does not account for potential temporal decreases in tetrachloroethylene intensity resulting from improved tetrachloroethylene recovery and technological changes (Gold et al., 2008) or for variation in tetrachloroethylene concentration across shops (Lynge et al., 2006). A fourth study that examined exposure duration and, also, time since first employment observed statistically significant associations with both increasing time since first employment and with increasing duration of exposure (Calvert et al., 2011).

Known risk factors for bladder cancer include smoking, aromatic amine dyes, chronic inflammation, infection with the parasite *Schistosoma heamatobium*, and pelvic irradiation (Kaufman et al., 2009). Of these identified risk factors, potential confounding related to smoking is most important to consider in the evaluation of bladder cancer and tetrachloroethylene in studies of occupational and residential exposures, as exposure to other known risk factors is much less common. Statistical control for smoking effects was used in all case-control studies, including those informing the hazard identification analysis and those contributing lesser weight (Colt et al., 2011; Dryson et al., 2008; Reulen et al., 2007; Vieira et al., 2005; Gaertner et al., 2004; Kogevinas et al., 2003; Zheng et al., 2002; 2000a; Teschke et al., 1997; Aschengrau et al., 1993; Burns and Swanson, 1991; Siemiatycki, 1991; Silverman et al., 1990; Steineck et al., 1990; Silverman et al., 1989a; Silverman et al., 1989b; Smith et al., 1985; Schoenberg et al., 1984). Lynge et al. (2006), a case-control study with subjects from four Nordic countries, presented smoking-adjusted and unadjusted effect measures for subjects from two countries for which smoking histories were obtained through interviews. Adjustment made little difference (<10%) in the magnitude of the effect measure, indicating that smoking history is not a strong confounder of the observed risk estimates [smoking unadjusted, 1.34, 95% CI: 0.86, 2.08; smoking adjusted, 1.25, 95% CI: 0.79, 1.98 (Lynge et al., 2006)].

Direct examination of possible confounders is less common in cohort studies relying on company-supplied or census work history data compared to case-control studies where information is obtained from study subjects or their proxies. In cohort studies, however, use of internal controls rather than an external referent group (e.g., national mortality rates) can minimize effects of potential confounding due to smoking or socioeconomic status, because exposed and referent subjects are drawn from the same target population. However, only one of the available cohort studies included an analysis using internal controls, and that study is limited by the observation of only two bladder cancer cases in the cohort (Boice et al., 1999). Effect of smoking as a possible confounder may be assessed indirectly through examination of risk ratios for other smoking-related sites such as lung cancer. Several studies observed roughly a 30%

increase in lung cancer risk among dry cleaners (Calvert et al., 2011; Pukkala et al., 2009; Ji and Hemminki, 2006a; Ji et al., 2005a; Blair et al., 2003) employed a method that assumed smoking accounted for 35% of their lung cancer observations and adjusted the bladder cancer standardized incidence ratio by this proportion. This method reduced slightly the effect measure for dry-cleaner and laundry workers (smoking unadjusted, 1.27, 95% CI: 1.08, 1.48; smoking adjusted, 1.13, 95% CI: 0.96, 1.31) (Ji et al., 2005a). Blair et al. (2003) addressed potential confounding by smoking and noted that if the magnitude of the difference in smoking for dry cleaners compared with the general population is in the range of 10% of less, confounding from smoking in their study of dry-cleaners and laundry workers was unlikely to result in increased excess of over >20%. In the case of bladder cancer in this study, smoking may explain the excess risk reported for overall exposure (SMR: 1.3). In contrast, the meta-analysis of Reulen et al. (2008) examined studies that did or did not adjust for smoking and found a stronger effect estimate with the smoking adjustment: the bladder cancer metarelative risk estimates for launderers and bladder cancer were 1.72 (95% CI: 1.25, 2.37) in studies that adjusted for smoking and 0.86 (95% CI: 0.59, 1.26) in studies that did not adjust for smoking. In conclusion, while smoking may potentially confound, to a small degree, observations in some cohort studies controlling for its effect in statistical analyses (Reulen et al., 2007; Lynge et al., 2006; Gaertner et al., 2004; Kogevinas et al., 2003; Zheng et al., 2002; Pesch et al., 2000b; Teschke et al., 1997; Aschengrau et al., 1993; Siemiatycki, 1991; Silverman et al., 1989a; Silverman et al., 1989b; Smith et al., 1985), these studies do provide evidence of an association with tetrachloroethylene or with holding a job as a dry cleaner or a laundry worker, a surrogate for tetrachloroethylene exposure potential.

In conclusion, the pattern of results from this collection of studies is consistent with an elevated risk for tetrachloroethylene of a relatively modest magnitude. The effect estimates from four of the five studies with the relatively high quality exposure-assessment methodologies provide evidence of an association, with relative risks of 1.44 to 4.03 (Calvert et al., 2011; Lynge et al., 2006; Blair et al., 2003; Pesch et al., 2000b; Aschengrau et al., 1993). The Lynge et al. (2006) results were slightly higher among the subgroup from Denmark and Norway, in which the number of unclassifiable data was negligible (relative risk 1.69, 95% CI: 1.18, 2.43). An exposure-response gradient was observed in a large case-control study by Pesch et al. (2000b) using a semiquantitative cumulative exposure assessment. A similar exposure-response pattern was not observed in the study by Lynge et al. (1995). This study examined exposure duration, however, rather than a measure that incorporated information on exposure concentration. In addition, relative risk estimates between bladder cancer risk and ever having a job title of dry-cleaner or laundry worker in four large cohort studies ranged from 1.01 to 1.44 (Pukkala et al., 2009; Wilson et al., 2008; Ji et al., 2005a; Travier et al., 2002). Confounding by smoking is an

unlikely explanation for the findings, given the adjustment for smoking by Pesch et al. (2000a) and other case-control studies.

4.2.2. Animal Studies

Kidney toxicity and cancer have been observed in laboratory animals exposed to tetrachloroethylene in multiple studies. The sections below describe studies of kidney toxicity (refer to Section 4.2.2.1) and cancer (refer to Section 4.2.2.2). These studies are summarized in Tables 4-9 and 4-10, respectively.

4.2.2.1. Kidney Toxicity in Animals

Tetrachloroethylene causes renal toxicity across multiple species, including several strains of rats and mice [for reviews, refer to Cal/EPA (2001), ATSDR (1997a), NYSDOH (1997)]. 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 increased kidney-to-body-weight ratios, hyaline droplet formation, cast formation, glomerular "nephrosis," karyomegaly (enlarged nuclei), and other lesions or indicators of renal toxicity. These nephrotoxic effects mainly occurred following relatively high subchronic (400−800 ppm) or chronic tetrachloroethylene exposures (100−200 ppm).

4.2.2.1.1. Inhalation

A long-term inhalation study examined the effects of tetrachloroethylene exposure in male and female rats by observation throughout the lifetime of the animals (0, 300, 600 ppm, 6 hours/day, 5 days/week, for 12 months) (Rampy et al., 1978). No increase in tumors compared to controls was observed in any animals in this study; however, an increase in mortality related to renal failure was observed in male rats starting at 5 months exposure in the high-dose group. No effects were observed in hematologic parameters measured (hemoglobin concentration, WBC counts) or various urinalysis endpoints (specific gravity, pH, presence of ketones, bilirubin, or blood, or sugar and albumin concentrations). The authors state that clinical chemistry measurements are not useful because most animals were deceased or moribund at the end of the study, and the study details show only measurements in a limited number of animals (1 male per group, 5 females per group). Although the authors conclude limited tetrachloroethylene toxicity, due to the large amount of morbidity in this study, it is difficult to make any conclusions as to the toxicity and/or carcinogenicity of tetrachloroethylene from this study.

Table 4-9. Summary of rodent kidney toxicity studies

Species/strain/ sex/number	Exposure level/duration	Effects	Reference
Mouse, B6C3F$_1$ (both sexes, 49 or 50 of each sex per dose group, total of ~300 mice)	0, 100, 200 ppm for 104 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed mice; nephrosis was observed in exposed females, casts increased in all exposed males and in high-dose females	NTP (1986)
Rat, F344 (both sexes, 50 of each sex per dose group, total of ~300 mice)	0, 200, 400 ppm for 104 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed rats	NTP (1986)
Mouse, Crj/BDF1 (both sexes, 50 of each sex per dose group, total of 400 mice)	0, 10, 50, 250 ppm for 110 wk, inhalation	Increased relative kidney weights and karyomegaly in the proximal tubules in 250 ppm exposed male and female mice; atypical tubular dilation in 250 ppm male and female mice but was not statistically significant	JISA (1993)
Rat, F344/DuCrj (both sexes, 50 of each sex per dose group, total of 400 rats)	0, 50, 200, 600 ppm for 110 wk, inhalation	Increased relative kidney weights and karyomegaly in the proximal tubules in 200 and 600 ppm exposed male and female rats; atypical tubular dilation in 600 ppm male and female rats; exacerbation of chronic renal disease in male rats only at 600 ppm	JISA (1993)
Rat, Osborne-Mendel (both sexes, 50 of each sex per dose group); Mouse, B6C3F$_1$ (both sexes, 50 of each sex per dose group)	0, 475, 950 mg/kg-day (rats); 0, 536, 1,072 mg/kg-day (male mice); 0, 386, 772 mg/kg-day (female mice) by oral gavage in corn oil for 78 wk, observed for 32 wk (rats) or 12 wk (mice) following exposure	Toxic nephropathy observed in all exposed animal groups, with an increased incidence in rats as compared to mice	NCI (1977)
Rat, Sprague-Dawley (both sexes, 96 per sex per exposure group; 192 per sex per control group)	0, 300, 600 ppm for 6 h/d, 5 d/wk for 12 mo; observed for the lifetime of the rat (up to 31 mo total)	Increased mortality related to renal failure in male rats exposed to 600 ppm starting at 5 mo of exposure	Rampy et al. (1978)
Rat, F344; and mouse, B6C3F$_1$ (both sexes, 5 of each sex per group)	0, 200 (28 d only), and 400 ppm (14, 21, 28 d) for 6 h/d, inhalation	Analysis in mice was limited to pooled tissue but showed slight increases in β-oxidation in mouse kidney; modest increases in PCO observed in male rat kidneys at 200 ppm for 28 d only, but elevated in female rat kidneys at all doses and times	Odum et al. (1988)
Mouse, Swiss-Webster (male, 4/group)	0, 150, 500, and 1,000 mg/kg-day, aqueous gavage for 30 d	No kidney injury or dysfunction was observed in this study	Philip et al. (2007)

Table 4-9. Summary of rodent kidney toxicity studies (continued)

Species/strain/ sex/number	Exposure level/duration	Effects	Reference
Rat, Wistar (female only, 10 rats in each control group; 5 rats in each treatment group)	0, 600, and 2,400 mg/kg-day for 32 d, corn oil gavage; alone or in combination with other compounds (trichloroethylene, hexachloro-1,2-butadiene, 1,1,2-trichloro-3,3,3-trifluoropropene)	Relative kidney weight was increased on exposure to PCE alone and in combination with other nephrotoxicants; nephrotoxic effects noted at high dose (urea, total protein, albumin, NAG); karyomegaly was also observed in high dose animals	Jonker et al. (1996)
Rat, F344 (male only, 5/group) and Mouse, $B6C3F_1$ (male only, 5/group)	0 or 1,000 mg/kg-day for 10 d, corn oil gavage	Increased kidney weight in exposed rats; increased PCO activity in all exposed mice	Goldsworthy and Popp (1987)
Rat, F344 (both sexes)	0 or 1,000 mg/kg-day for 10 d, corn oil gavage	Increases in α2μ-hyaline droplets in exposed male, but not female, rats, correlated with increased cell proliferation and protein droplet nephropathy	Goldsworthy et al. (1988)
Rat, F344 (both sexes, 12 per group)	0, 500 mg/kg-day daily for 4 wk, corn oil gavage	Increases in α2μ-hyaline accumulation in proximal tubule cells	Bergamaschi et al. (1992)
Mouse, Swiss (both sexes, 6 groups of 6 each (1996); male only; 8 groups of 6 each (2001))	0 or 3,000 mg/kg-day for 15 d, sesame oil gavage	Significant increase in kidney weight; decreased blood glucose (glucose effects mitigated by coexposures to 2-deoxy-D-glucose and vitamin E [1996]) Decreased membrane-bound Na^+K^+-ATPases and Mg_2^+-ATPases activity but increased Ca-ATPase activity; mitigated by coexposure to 2-deoxy-D-glucose and vitamin E, and taurine; hypercellular glomeruli in PCE-exposed only	Ebrahim et al. (2001; 1996)
Rat, F344 (both sexes) and Mouse, $B6C3F_1$ (both sexes) (10 per group for oral studies, 5 per group for inhalation studies)	0, 1,000, or 1,500 mg/kg-day daily by corn oil gavage for 42 d; 0 or 1,000 ppm for 10 d	Accumulation of α2u-globulin in proximal tubules of male rats; nephrotoxicity also observed in male rats (formation of granular tubular casts and evidence of tubular cell regeneration) Inhalation exposure demonstrated formation of hyaline droplets in kidneys of male rats	Green et al. (1990)
Rat, Sprague-Dawley (both sexes, 20 per group)	0, 14, 400, or 1,400 mg/kg-day for 90 d	Increased kidney weight observed in exposed animals; nephrotoxicity observed at 400 mg/kg-day	Hayes et al. (1986)
Rat, Sprague-Dawley (male only, 4 per group)	0, 115, 230 μmol/kg of TCVC or TCVCS in saline by one i.p. injection, sacrificed 24 h postexposure	High-dose exposed animals showed visible kidney necrosis; all other rats showed histological markers for mild acute tubular necrosis (TCVC) or severe acute tubular necrosis (TCVCS); prior exposure to AOAA increased toxicity	Elfarra et al. (2007)

Table 4-10. Kidney tumor incidence in laboratory animals exposed to tetrachloroethylene

Bioassay	Doses/exposures		Sex	Tumor incidence (%)
	Administered	Continuous equivalent		Kidney adenomas and carcinomas
NCI (1977)[a] $B6C3F_1$ mice Gavage: 5 d/wk, 78 wk	Vehicle control 450 mg/kg-day 900 mg/kg-day	0 332 mg/kg-day 663 mg/kg-day	Male	0/20 (0) 1/49 (2) 0/48 (0)
	Vehicle control 300 mg/kg-day 600 mg/kg-day	0 239 mg/kg-day 478 mg/kg-day	Female	0/20 (0) 0/48 (0) 0/45(0)
NCI (1977)[a] Osborn-Mendel rats Gavage: 5 d/wk, 78 wk	Vehicle control 500 mg/kg-day 1,000 mg/kg-day	0 471 mg/kg-day 941 mg/kg-day	Male	3/20 (5) 1/49 (2) 0/50 (0)
	Vehicle control 500 mg/kg-day 1,000 mg/kg-day	0 474 mg/kg-day 974 mg/kg-day	Female	0/20 (0) 0/50 (0) 1/50 (2)
NTP (1986) $B6C3F_1$ mice Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Male	0/49 (0) 1/49 (2) 0/50 (0)
	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Female	0/48 (0) 0/50 (0) 0/48 (0)
NTP (1986) F344/N rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 200 ppm 400 ppm	0 36 ppm 72 ppm	Male	1/49 (2) 3/47 (6) 4/50 (8)
	0 ppm 200 ppm 400 ppm	0 36 ppm 72 ppm	Female	0/50 (0) 0/50 (0) 0/50 (0)
JISA (1993) Crj:BDF1 mice Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Male	0/50 (0) 1/50 (2) 1/50 (2) 0/50 (0)
	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Female	0/50 (0) 0/47 (0) 0/49 (0) 0/50 (0)
JISA (1993) F344/DuCrj rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 50 ppm 200 ppm 600 ppm	0 9 ppm 36 ppm 108 ppm	Male	1/50 (2) 2/50 (4) 1/50 (2) 2/50 (4)
	0 ppm 50 ppm 200 ppm 600 ppm	0 9 ppm 36 ppm 108 ppm	Female	1/50 (2) 0/50 (0) 0/50 (0) 1/50 (2)

Acute, subchronic, and chronic exposures to tetrachloroethylene were examined in male and female F344 rats and $B6C3F_1$ mice (NTP, 1986). Single exposure studies and 14-day studies were performed, but no kidney effects were observed, with the first kidney effects observed in the subchronic (13 week) study. Groups of 10 rats and mice of each sex were exposed to air containing tetrachloroethylene for 6 hours/day, 5 days/week, for 13 weeks (0, 100, 200, 400, 800, or 1,600 ppm). Some rats in the high-dose group died before the end of the studies (4/10 male, 7/10 female), but no kidney effects were observed. In mice, 2/10 males and 4/10 females in the high-dose group died before the end of the studies, and karyomegaly (nuclear enlargement) of the renal tubule epithelial cells was observed in all but the lowest dose group.

Toxicity was observed in a 2-year cancer bioassay performed on groups of 50 F344 rats of each sex (0, 200, or 400 ppm tetrachloroethylene), or groups of 49 or 50 mice (0, 100, or 200 ppm tetrachloroethylene) exposed for 6 hours/day, 5 days/week, for 103 weeks (NTP, 1986). Karyomegaly and cytomegaly changes were observed in both sexes of rats at all doses but not in unexposed controls. These lesions were present primarily in the proximal convoluted tubules of the inner half of the cortex but not limited to this area. In mice, nephrosis (generally defined as noninflammatory degenerative disease of the kidney) was observed in exposed females, casts (cylindrical structures formed from cells and protein released from the kidney) were increased in exposed male and high-dose females, and karyomegaly of the tubular cells was observed in all dosed mice, with severity of lesions being dose related. Therefore, the LOAEL for renal toxicity reported in both mice and rats in this study is 100 ppm (678 mg/m^3) for inhalation exposure in mice and 200 ppm (1,356 mg/m^3) in rats (NTP, 1986).

Nephrotoxicity was observed in a second, 2-year inhalation cancer bioassay also performed in 50 male and female Fischer rats (0, 50, 200, or 600 ppm) and Crj:BDF1 mice (0, 10, 50, or 250 ppm) in each treatment group (6 hours/day, 5 days/week, for 104 weeks) (JISA, 1993). Survival compared to controls was decreased in all high-dose exposure groups, which was believed to be treatment related. Relative kidney weight was increased in male and female rats exposed to tetrachloroethylene (200 or 600 ppm) and in male and female mice (250 ppm). Karyomegaly in the proximal tubules of the kidneys was observed among males and females (200 and 600 ppm in male rats [23/50 and 48/50]; 600 ppm in female rats [18/50]; 50 and 250 ppm in male mice [6/50 and 38/50]; 250 ppm in female mice [49/50]), and an increase in atypical tubular dilation of the proximal tubules [male and female rats, 600 ppm (24/50 males, 6/50 females) and exacerbation of chronic renal disease in male rats only (600 ppm) was observed with tetrachloroethylene exposure (JISA, 1993). Atypical tubular dilation was also observed in mice but was not statistically significant (250 ppm in male mice [1/50] and female mice [6/50]).

The role of peroxisome proliferation in tetrachloroethylene-induced kidney toxicity and cancer was examined in male and female F344 rats and $B6C3F_1$ mice exposed to

tetrachloroethylene by inhalation (400 ppm, 6 hours/day, for 14, 21, or 28 days, or 200 ppm, 6 hours/day, for 28 days) in a study by Odum et al. (1988). Five animals per group were exposed. Insufficient mouse kidney tissue limited the analysis to pooled samples. Slight increases were observed in β-oxidation in mouse kidney (maximum 1.6-fold increase at 21 days, 400 ppm exposure). Modest palmitoyl-CoA oxidation (PCO) increases were observed in the kidney of male rats at 200 ppm at 28 days (1.3-fold) but not 400 ppm at 14, 21, or 28 days. In female rat kidney, PCO was elevated (approximately 1.6-fold) at all doses and times. However, peroxisome proliferation was not observed in rat or mouse kidney upon microscopy, suggesting that this does not play a role in kidney carcinogenesis. Short-term inhalation exposure to 1,000 ppm tetrachloroethylene for 10 days resulted in the formation of hyaline droplets in the kidneys of male rats. Although granular casts and tubule cell regeneration were not observed, the time period may have been too short to allow progression to this stage (Green et al., 1990).

4.2.2.1.2. Oral

Hayes et al. (1986) reported renal effects in rats exposed to 400 mg/kg-day tetrachloroethylene in drinking water for 90 days. Tetrachloroethylene was administered in the drinking water at 14, 400, and 1,400 mg/kg per day for 90 days, with no deaths reported before the end of the study. Increased kidney weight was observed.

A lifetime animal carcinogenicity study in which tetrachloroethylene was administered to 50 of each sex of Osborne-Mendel rats and $B6C3F_1$ 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. Animals were observed for 32 weeks (rats) or 12 weeks (mice) following the last dose. Toxic nephropathy was observed in almost all test animals, with a high incidence observed in treated rats, including those that died early in the study (as early as Week 20 in male rats, Week 28 in female rats). Similar results were observed in exposed mice, with no nephropathy observed in control mice. Therefore, the LOAEL for renal toxicity following oral exposure is 471 mg/kg-day in male rats and 474 mg/kg-day in female rats based on toxic nephropathy. The LOAEL for mice is 536 mg/kg-day for males and 386 mg/kg-day in females based on toxic nephropathy.

In a study by Jonker et al. (1996), tetrachloroethylene nephrotoxicity was observed in female Wistar rats administered tetrachloroethylene (600 or 2,400 mg/kg-day) in corn oil by daily oral gavage for 32 days. Relative kidney weight was increased upon exposure to tetrachloroethylene alone and in combination with other nephrotoxicants (trichloroethylene [TCE], hexachloro-1,2-butadiene, and 1,1,2-trichloro-3,3,3-trifluoropropene [TCTFP]). One high-dose animal died as a result of tetrachloroethylene treatment, and one animal exposed to the

high-dose combination of TCE, tetrachloroethylene, and TCTFP also died as a result of treatment. Nephrotoxic effects were noted at 2,400 mg/kg. Significant changes were observed following exposure to tetrachloroethylene at 2,400 mg/kg-day in all clinical chemistry markers related to kidney function (urea, total protein, albumin, NAG) as measured in the urine at the end of Week 1 or Week 4 except for urinary density, glucose, and creatinine. Karyomegaly was also observed at the high dose (2,400 mg/kg-day) in four of five animals exposed ($p < 0.01$) (Jonker et al., 1996).

Philip et al. (2007) exposed male 6−7-week-old Swiss Webster mice via aqueous gavage to three dose levels (150, 500, and 1,000 mg/kg-day) for 30 days. At the highest exposure, mortality was 10% due to apparent CNS toxicity (tremors and ataxia). Neither kidney injury nor dysfunction was observed following tetrachloroethylene exposure during the course of this study.

Goldsworthy and Popp (1987) administered tetrachloroethylene (1,000 mg/kg-day) by corn oil gavage to 5 male F344 rats and 5 male B6C3F$_1$ mice for 10 days. In tetrachloroethylene-exposed rats, PCO was modestly although not significantly elevated in the liver (1.4-fold increase) and kidney (1.7-fold increase). In mice, tetrachloroethylene exposure increased PCO activity 4.3-fold in liver and by 2.3-fold in kidney. Relative liver weight was increased in rats and mice with tetrachloroethylene exposure, but relative kidney weight was unaffected. A comparison of corn oil with methyl cellulose revealed no effect of the gavage vehicle on tetrachloroethylene-induced PCO. A less-than-additive effect of trichloroethylene (1,000 mg/kg) administered together with tetrachloroethylene on PCO induction was seen.

Oral administration of tetrachloroethylene in sesame oil (3,000 mg/kg-day for 15 days) to male and female albino Swiss mice caused a significant increase in kidney weight ($p < 0.001$) and a decrease in blood glucose levels ($p < 0.01$) as compared to control animals exposed to sesame oil alone as well as increases in glomerular nephrosis (Ebrahim et al., 1996). This study was designed to give support to the beneficial effect of 2-deoxy-D-glucose (2DG) and vitamin E on tetrachloroethylene-induced kidney damage. Based on previous experimental mouse tumor studies, administration of 2DG or vitamin E is hypothesized to have a beneficial effect on tetrachloroethylene-induced kidney damage, either by inhibition of tumor growth (2DG) or the auto-catalytic process of lipid peroxidation (vitamin E). In this study, concurrent administration of 2DG (500 mg/kg-day i.p.) or vitamin E (400 mg/kg-day oral gavage) prevented tetrachloroethylene-induced biochemical and pathological alterations. Tetrachloroethylene exposure alone led to a decrease in blood glucose levels, which was returned to near normal with concomitant exposure to 2DG and vitamin E. Elevated levels of glycolytic and gluconeogenic enzymes following exposure to tetrachloroethylene were also observed to return to near normal with exposure to 2DG and vitamin E. Histopathology of the kidney showed hypercellular glomeruli following exposure to tetrachloroethylene, but this was not observed in animals treated

with tetrachloroethylene and 2DG, or tetrachloroethylene and vitamin E (Ebrahim et al., 1996). A follow-up study by this group further examined the potential protective properties of 2DG and vitamin E as well as taurine against tetrachloroethylene-induced membrane damage (Ebrahim et al., 2001). This study exposed male albino Swiss mice to the same doses used in the previous study with the addition of a taurine-exposed group (tetrachloroethylene in sesame oil 3,000 mg/kg-day for 15 days orally by intubation; tetrachloroethylene plus 2DG 500 mg/kg-day by i.p. injection once a day for 15 days; tetrachloroethylene plus vitamin E 400 mg/kg-day by oral intubation once a day for 15 days; and tetrachloroethylene plus taurine 100 mg/kg-day by oral intubation once a day for 15 days). As compared to control cells in the kidney, membrane-bound Na^+K^+-ATPases and Mg_2^+-ATPases activity was significantly decreased ($p < 0.001$), while Ca-ATPases activity was increased ($p < 0.001$), following exposure to tetrachloroethylene alone. These levels remained near normal in the animals exposed to tetrachloroethylene along with 2DG, vitamin E, or taurine. This return to normal levels following exposure to vitamin E and taurine may be due to their antioxidant abilities, and reduced oxidative stress in exposed cells.

Goldsworthy et al. (1988) observed increases in α2μ-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. Cell replication was enhanced in the male rats specifically in damaged P_2 segments, suggesting a link between the α2u-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 α2μ-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 α2μ-accumulation in P_2 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 1,000 to 1,500 mg/kg of tetrachloroethylene daily for up to 42 days caused an accumulation of α2u-globulin in the proximal tubules of male rats. The animals were sacrificed within 24 hours 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.

4.2.2.1.3. Intraperitoneal injection

The role of the glutathione metabolites, particularly TCVC and TCVCS, in kidney toxicity was examined by Elfarra et al. (2007) in vivo. This study exposed two groups of four male Sprague-Dawley rats to a single i.p. injection of TCVC or TCVCS (115 or 230 μmol/kg in saline). Animals were sacrificed 24 hours following exposure. Serum was analyzed for BUN, and urine samples were analyzed for GGTP activity as markers of nephrotoxicity. Rats exposed to the high-dose of TCVCS showed visible signs of kidney necrosis, while all other exposed groups did not. Histologically, kidneys from rats exposed to low-dose TCVC or TCVCS showed slight-to-mild acute tubular necrosis. Analysis of kidneys at 24 hours postexposure showed mild-to-moderate acute tubular necrosis in animals exposed to high-dose (230 μmol/kg) TCVC, and severe tubular necrosis in animals exposed to high-dose (230 μmol/kg) TCVCS. Similar to the pattern of toxicity described above, significant increases in BUN (fourfold) were observed in rats exposed to 230 μmol/kg TCVCS as compared to control, but no significant increases were observed following exposure to TCVC. Variable increases were observed following exposure to TCVC or TCVCS in urine glucose levels and GGTP activity. A second part of this experiment involved a preexposure to a β-lyase inhibitor (AOAA) (500 μmol/kg bw) by i.p. injection 30 minutes prior to administration of 230 μmol/kg TCVC. Exposure to AOAA prior to exposure to TCVCS resulted in increased toxicity. In a third study, three groups of four rats were exposed to saline, TCVC, or TCVCS (230 μmol/kg) and sacrificed 2 hours after administration. The kidneys were removed at sacrifice and examined for NPT and NPT disulfide concentrations as a measure of thiol status in the kidney. Although no changes were observed in NPT status, histological examination of these kidneys showed scattered foci of mild acute tubular necrosis (TCVC) or widespread acute tubular necrosis, intratubular casts, and interstitial congestion and hemorrhage (TCVCS). These results suggest that while both TCVC and TCVCS are nephrotoxicants, TCVCS is more potent than TCVC.

In summary, exposure to tetrachloroethylene from all routes studied (oral, inhalation, i.p.) led to nephrotoxicity in multiple strains of rats and mice. These studies demonstrate karyomegaly, increased kidney weights, and atypical tubular dilation following subchronic high-dose exposures or lower dose chronic exposures. Limited studies have also examined the potential role for peroxisome proliferation or α2u-globulin in nephrotoxicity. Exposure to tetrachloroethylene glutathione conjugation metabolites led to similar effects in rats (mice not tested). Further, studies examining the impact of concomitant antioxidant exposures with tetrachloroethylene in mice suggest a role for oxidative stress in tetrachloroethylene-induced nephrotoxicity.

4.2.2.2. Kidney Cancer in Animals

4.2.2.2.1. Inhalation

In the studies conducted by NTP [(1986), described above], groups of 50 male and 50 female F344/N rats were exposed for 6 hours/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). In the same study (doses described above), one renal tubule adenocarcinoma was observed in a low-dose male mouse, but no other neoplastic lesions were observed.

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%. Thus, the appearance of tubule neoplasms in 8% of the treated animals in the NTP study (low-dose and high-dose groups combined) provided convincing evidence of a treatment-related effect Solleveld et al., 1984; Goodman et al., 1979). 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.[21] In addition, when compared with historical control incidences of renal tubule tumors at the NTP, a statistically 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.

No increase in renal cell cancers was observed in a second 2-year inhalation cancer bioassay that was also performed in 50 male and female Fischer rats (0, 50, 200, or 600 ppm) and Crj:BDF1 mice (0, 10, 50, or 250 ppm) in each treatment group (6 hours/day, 5 day/week, for 104 weeks) (JISA, 1993). Survival compared to controls was decreased in all high-dose exposure groups, which is believed to be treatment related. Renal cell adenoma was observed in male rats (1/50, control; 2/50, 50 ppm; 1/50, 200 ppm; 2/50, 600 ppm) and male mice (1/50, 50 ppm) but only in control female rats (1/50, control) and not in exposed female mice. Renal cell

[21] Assuming a binomial probability distribution, a background rate of 0.2%, and a sample size of 50 animals.

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carcinoma was not observed in male rats or female mice, but was observed in the high-dose female rats (1/50, 600 ppm) and male mice (1/50, 50 ppm). As described above for the NTP study (1986), these tumors are rare in Fischer rats, but the reported results are similar to those historical control rates for this study group (JISA, 1993).

The study authors reported a slight increase in renal tumors with tetrachloroethylene exposure in a study reporting increased mortality related to renal failure in male rats starting at 5 months exposure in the high-dose group (Rampy et al., 1978). This lifetime observation study exposed male and female rats to 0, 300, 600 ppm, 6 hours/day, 5 days/week, for 12 months (Rampy et al., 1978). The authors stated that most animals were deceased or moribund at the end of study, rendering difficult clear conclusions regarding the renal carcinogenicity of tetrachloroethylene.

4.2.2.2.2. Oral

No significant increased incidence of neoplastic lesions was observed in treated rats following oral exposure to tetrachloroethylene in a lifetime carcinogenicity bioassay (NCI, 1977; doses described above). However, a high rate of death occurred in the high-dose groups of both sexes, so the authors of the study determined carcinogenicity could not be evaluated. Only one kidney tumor was observed in mice in this study (high dose; doses described above), but this was a tumor that had metastasized from the liver.

In summary, an increase in rare kidney tumors was reported in one inhalation cancer bioassay of tetrachloroethylene (0, 200, or 400 ppm) in F344/N rats (NTP, 1986). The JISA (1993) rat inhalation bioassay of tetrachloroethylene (50, 200, and 600 ppm) reported no treatment-related increase in the incidence of kidney tubular cell adenoma or carcinoma in excess of that in the concurrent or historical control animals at administered concentrations. Another inhalation study, the interpretation of which is limited by high morbidity and mortality, reported a slight increase in renal tumors in male S-D rats (Rampy et al., 1978). Although the renal tumors were not significantly increased compared with controls, morbidity related to renal failure was increased in male rats beginning at 5 months of exposure. The NCI (1977) oral gavage bioassay of tetrachloroethylene (0, 475, 950 mg/kg-day) reported a high rate of death in the high-dose groups of both sexes, and, thus, carcinogenicity could not be evaluated in this study.

Other evidence supporting the conclusion of renal carcinogenicity of tetrachloroethylene includes low incidences of tubule neoplasms in male rats in NTP bioassays of other chlorinated ethanes and ethylenes (NTP, 1990a, 1989, 1988, 1987). In particular, the closely related compound trichloroethylene also induces low increases in the incidence of rare renal tumors in rats and in humans (U.S. EPA, 2011b).

4.2.2.2.3. In vitro

Lash et al. (1998) examined the role of glutathione conjugation of tetrachloroethylene in rats and mice in isolated renal cortical cells and hepatocytes from male and female F344 rats. All cells were exposed to tetrachloroethylene (0.5, 1, or 2 mM) and assayed for TCVG formation at 0, 15, 30, and 60 minutes. This study demonstrated that GSH metabolites from tetrachloroethylene are formed in kidney cells as well as hepatocytes in both species; however, the amount of TCVG produced varied depending on sex, species, and tissue assayed. TCVG formation was higher in male rats and mice as compared to their female counterparts and was also higher in hepatocytes as compared to kidney cells. Although rats are more susceptible to nephrocarcinogenicity as compared to mice (refer to Section 4.5.2.2), isolated mouse kidney and liver cells had a greater amount of TCVG formation (7- to 10-fold and 2- to 5-fold, respectively) as compared to rat cells (Lash et al., 1998). To further examine the species- and sex-dependent-differences in tetrachloroethylene cytotoxicity, Lash et al. (2002) measured acute cytotoxicity following exposure to tetrachloroethylene or TCVG (0.1 to 10 mM) in isolated rat kidney cells and renal mitochondria from rats and mice. Exposure to tetrachloroethylene or TCVG led to a marked increase in LDH release in isolated kidney cells from male but not female rats, but no significant effects were observed in rat hepatocytes from either gender (Lash et al., 1998). Isolated mitochondria from rats and mice showed a pattern of sensitivity similar to the kidney cell effects, with increased inhibition of respiration in isolated mitochondria from male rats as compared to their female counterparts. Inhibition of respiration was observed equally in male and female mice exposed to tetrachloroethylene or TCVG. The results of this in vitro study support those of the in vivo studies, which demonstrate increased nephrotoxicity in male rats following exposure to tetrachloroethylene or TCVG.

Lash et al. (2007) examined the effect of modulation of renal metabolism on toxicity of tetrachloroethylene in isolated cells and microsomes from male F344 rat kidney and liver. Oxidative-dependent metabolism of tetrachloroethylene was more than 30-fold increased in liver microsomes than in kidney. Pretreatment of rats with a P450-inhibitor had little to no effect on the tetrachloroethylene metabolism in either kidney or liver. Pretreatment of rats with a P450 inducer increased tetrachloroethylene metabolism by over twofold in the kidney microsomes, with no effect observed in liver. Following exposure to modulating chemicals, lactate dehydrogenase (LDH) was measured as a marker of cytotoxicity, and the presence of specific metabolites was documented (TCVG, TCOH, and CH). Tetrachloroethylene metabolism in kidney cells was slightly (but significantly) increased by the nonspecific inhibitors of P450s but not affected by the pretreatment with the CYP2E1-specific inhibitor. Increased cytotoxicity in kidney cells was observed following exposure to tetrachloroethylene (2 or 10 mM, 3 hours), and this was not affected by pretreatment with CYP inhibitors or inducers. However, increases in

GSH concentrations in the kidney cells led to increased cytotoxicity following exposure to tetrachloroethylene, but no effect was observed following pretreatment with GSH inhibitors. The results of this study highlight the role of different bioactivation pathways needed in both the kidney and the liver, with the kidney effects being more affected by the GSH conjugation pathways metabolic products.

Tetrachloroethylene effects in kidney cells have also been demonstrated in a variety of genotoxicity assays. Exposing kidney cells and/or microsomal fractions from kidneys to tetrachloroethylene or its some of its metabolites led to low levels of DNA binding (Mazzullo et al., 1987), micronuclei induction (Wang et al., 2001), single-stranded DNA breaks (Walles, 1986), unscheduled DNA synthesis (Vamvakas et al., 1989c), and gene mutations (Vamvakas et al., 1989d; Vamvakas et al., 1987; Dekant et al., 1986a). Negative studies were observed in kidney cells from exposed animals for DNA damage (Cederberg et al., 2010a; Potter et al., 1996), and DNA adduct formation (Toraason et al., 1999).

Limited DNA binding to calf thymus DNA was observed in the presence of microsomal fractions from mice and rats (Mazzullo et al., 1987). Binding to DNA in the in vitro study increased in the presence of microsomal fractions from both mouse and rat liver, but not kidney, lung, or stomach. Cytosolic fractions from rat and mouse liver, kidney, lung, and stomach, all induced binding of tetrachloroethylene to calf thymus DNA, with enzymes from both mouse and rat livers and mouse lung being the most efficient.

Wang et al. (2001) examined micronuclei induction following exposure to tetrachloroethylene (~63 ppm in culture medium at peak) in vitro in a closed system. Chinese hamster ovary (CHO-K1) cells were plated in a petri dish surrounding a glass dish of tetrachloroethylene and incubated for 24 hours. Tetrachloroethylene exposure led to a dose-dependent significant increase in micronuclei induction ($p < 0.001$) (Wang et al., 2001).

Vamvakas et al. (1989a) 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 β-lyase inhibitor. This effect was observed at exposure to 5×10^{-6}–10^{-5} M TCVC for 24 hours.

TCVG produced from tetrachloroethylene in isolated perfused rat liver and excreted into bile, in the presence of a rat kidney fraction, was mutagenic in *Salmonella*, as was purified TCVG (Vamvakas et al., 1989d). This study performed the Ames assay in *Salmonella typhimurium* TA100, TA98, and TA2638 with tetrachloroethylene, TCVG, and bile from liver perfusate following tetrachloroethylene exposure in rats and demonstrated that the GST-metabolites or tetrachloroethylene in the presence of bile containing GST led to gene mutations in *S. typhimurium* TA100. Dreessen (2003) also demonstrated for TCVG an unequivocal dose-dependent mutagenic response in the TA100 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 (1−10 nmol/plate) was also positive in *Salmonella* strains TA98 and TA100 but not strain TA2638, and inhibition of β-lyase activity was blocked by addition of amionoxyacetic acid (AOAA) (Dekant et al., 1986a). A subsequent study from this same group indicated that *Salmonella* also was capable of deacetylating the urinary metabolite NAcTCVC (50−100 nmol/plate) when TA100 showed a clear positive response in the Ames assay without exogenous activation (Vamvakas et al., 1987).

In summary, the limited in vitro studies performed in kidney cells exposed to tetrachloroethylene or its GSH conjugation metabolites demonstrate an increase in cytotoxicity. This cytotoxic effect was sex- and species-dependent, with increases observed in male rats and mice compared to their female counterparts, with rats showing the most cytotoxicity. Limited genotoxicity studies demonstrated the potential for tetrachloroethylene mutagenicity in *Salmonella* strains in the presence of the kidney S9 fraction, or in *Salmonella* exposed to GSH-conjugation metabolites (TCVC, TCVG, or NacTCVC) without activation.

4.2.3. Summary of Kidney Effects in Humans and Animals

Taken together, the epidemiologic studies support an association between inhalation tetrachloroethylene exposure and chronic kidney disease, as measured by urinary excretion of renal proteins and ESRD. The elevated urinary RBP levels observed in two studies (Verplanke et al., 1999; Mutti et al., 1992) and lysozyme or β-glucuronidase in Franchini et al. (1983) provide some evidence for effects to the proximal tubules from tetrachloroethylene exposure. Exposures in the studies that observed renal toxicity were 1.2 ppm, 10 ppm, and 15 ppm (means), representing an observational LOAEL for human kidney effects. An exposure-response relationship was reported in one study (Trevisan et al., 2000) but not in the other human studies that examined renal function, 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. Calvert et al. (2011) supports an association between inhalation tetrachloroethylene exposure and ESRD, particularly hypertensive ESRD. They observed a twofold elevated incidence (SIR: 2.66, 95% CI: 1.15, 5.23) among subjects who worked only in a shop where tetrachloroethylene was the primary cleaning solvent compared to that expected based on U.S. population rates. An exposure-response pattern was further suggested because hypertensive ESRD risk was highest among those employed for ≥5 years (SIR: 3.39, 95% CI: 1.10, 7.92). 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). The results from the other studies using a relatively specific exposure-assessment approach to refine classification of potential tetrachloroethylene exposure in dry-cleaning settings are mixed, with some studies reporting little or no evidence of an association (Lynge et al., 2006; Pesch et al., 2000a; Boice et al., 1999; Dosemeci et al., 1999; Aschengrau et al., 1993), and other studies reporting elevated risks (Calvert et al., 2011; Blair et al., 2003; Anttila et al., 1995; Schlehofer et al., 1995). An increasing trend in relative risk with increasing exposure surrogate was not observed in any of the larger occupational exposure studies with three or more exposure categories (Lynge et al., 2006; Mandel et al., 1995), but some indication of higher risk with higher exposure (or duration) was observed in other studies (Blair et al., 2003). As expected, the results from studies using a relatively nonspecific exposure measure (broad occupational title of launderers and dry cleaners, all workers at a factory, density of dry-cleaning establishments by zip code) are more variable and less precise, reflecting a greater potential for misclassification bias.

Adverse effects on the kidney have been observed in studies of animals exposed to high concentrations of tetrachloroethylene by inhalation (JISA, 1993; NTP, 1986), and oral gavage (Ebrahim et al., 2001; Ebrahim et al., 1996; Jonker et al., 1996; Green et al., 1990; Goldsworthy et al., 1988; NCI, 1977), as well as i.p. injection of tetrachloroethylene metabolites (Elfarra and Krause, 2007). The nephrotoxic effects include 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 one bioassay of male rats exposed to tetrachloroethylene by inhalation (NTP, 1986). The renal effects occurred following very high (or chronic, relatively high) doses of tetrachloroethylene exposures. Overall, multiple lines of evidence support the conclusion that tetrachloroethylene causes nephrotoxicity in the form of tubular toxicity, mediated potentially through the tetrachloroethylene GSH conjugation products: TCVC and TCVCS.

4.2.4. Hypothesized Mode(s) of Action for Kidney Carcinogenicity

There are multiple hypothesized MOAs for kidney carcinogenicity induced with tetrachloroethylene exposure, including α2u-globulin accumulation, peroxisome proliferation, genotoxicity, and cytotoxicity unrelated to α2u-globulin. These MOAs are addressed in the sections that follow.

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4.2.4.1. Role of Metabolism in Kidney Carcinogenicity

Except for α2u-globulin accumulation, which is more likely due to tetrachloroethylene itself (Lash and Parker, 2001), other mechanisms hypothesized to contributed to tetrachloroethylene-induced renal carcinogenicity are thought to be mediated by tetrachloroethylene metabolites rather than by the parent compound. Metabolites from the GSH conjugation pathway are posited to induce renal tumorigenicity, as opposed to (or to a greater extent than) the metabolites resulting from oxidative CYP processing. The glutathione conjugation of tetrachloroethylene in the kidney, discussed in Section 3, leads sequentially to TCVG and TCVC. TCVC can be further processed by β-lyase to yield an unstable thiol, 1,2,2-trichlorovinylthiol, which 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., 1999; 1997; Werner et al., 1996).

4.2.4.2. α2u-Globulin Accumulation

Generally, kidney tumors observed in cancer bioassays are assumed to be relevant for assessment of human carcinogenic potential. However, male rat-specific kidney tumors that are caused by the accumulation of α2u-globulin are not generally considered relevant to humans. Accumulation of α2u-globulin in hyaline droplets initiates a sequence of events that leads to renal nephropathy and, eventually, renal tubular tumor formation. The phenomenon is unique to the male rat because female rats and other laboratory mammals administered the same chemicals do not accumulate α2u-globulin in the kidney and do not subsequently develop renal tubule tumors (Doi et al., 2007; Swenberg and Lehman-McKeeman, 1999; U.S. EPA, 1991a).

4.2.4.2.1. Identification of key events

The histopathological sequence of events in mature male rats is hypothesized to consist of the following:

- Excessive accumulation of hyaline droplets containing α2u-globulin in renal proximal tubules
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium
- Sustained regenerative tubule cell proliferation
- Development of intralumenal granular casts from sloughed cellular debris associated with tubule dilatation and papillary mineralization

- Foci of tubule hyperplasia in the convoluted proximal tubules
- Renal tubule tumors

4.2.4.2.2. Data requirements for establishing the MOA

The EPA (1991a) Risk Assessment Forum Technical Panel report provides specific guidance for evaluating chemical exposure-related male rat renal tubule tumors for the purpose of risk assessment, based on an examination of the potential involvement of α2u-globulin accumulation. In particular, the following information from adequately conducted studies of male rats is used for demonstrating that the α2u-globulin process may be a factor in any observed renal effects. An affirmative response in each of the three categories is required. If data are lacking for any of the criteria in any one category, the available renal toxicity data should be analyzed in accordance with standard risk assessment principles. The three categories of information and criteria are as follows:

- *Increased number and size of hyaline droplets in the renal proximal tubule cells of treated male rats*. The abnormal accumulation of hyaline droplets in the P_2 segment helps differentiate α2u-globulin inducers from chemicals that produce renal tubule tumors by other modes of action.

- *Accumulating protein in the hyaline droplets is α2u-globulin*. Hyaline droplet accumulation is a nonspecific response to protein overload, and, thus, it is necessary to demonstrate that the protein in the droplet is, in fact, α2u-globulin.

- *Additional aspects of the pathological sequence of lesions associated with α2u-globulin nephropathy are present*. Typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia. If the response is mild, not all of these lesions may be observed. However, some elements consistent with the pathological sequence must be demonstrated to be present.

4.2.4.2.3. Induction of hypothesized key events by tetrachloroethylene

Three studies show that doses of tetrachloroethylene in excess of those observed to induce tumorigenesis are capable of precipitating hyaline droplet nephropathy in male rats (Bergamaschi et al., 1992; Green et al., 1990; Goldsworthy et al., 1988); refer to Table 4-11. Goldsworthy et al. (1988) observed increases in α2u-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 P_2 segments, suggesting a link between the α2u-globulin accumulation and kidney tumors. Bergamaschi et al. (1992) also demonstrated α2u-accumulation in P_2 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 1,000 to 1,500 mg/kg of tetrachloroethylene daily for up to 42 days caused an accumulation of α2u-globulin in the proximal tubules of male rats. These effects were not observed in female rats or in mice.

Table 4-11. Renal α2u-globulin formation in tetrachloroethylene-exposed rodents

Species/strain/ sex/number	Exposure level/duration	Effects	Reference
Mouse, B6C3F$_1$, both sexes (groups of 49 or 50 mice of each sex per dose group, total of ~300 mice)	0, 100, 200 ppm for 104 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed mice; nephrosis was observed in exposed females, casts increased in all exposed males and in high-dose females	NTP (1986)
Rat, F344, both sexes (groups of 50 mice of each sex per dose group, total of ~300 mice)	0, 200, 400 ppm for 104 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed rats	NTP (1986)
Rat, F344 (both sexes, 5 per group)	0 or 1,000 mg/kg-day for 10 d, corn oil gavage	Increases in α2u-hyaline droplets in exposed male but not female rats. Correlated to increased cell proliferation and protein droplet nephropathy	Goldsworthy et al. (1988)
Rat, F344 (both sexes, 12 per group)	0, 500 mg/kg-day daily for 4 wk, corn oil gavage	Increases in α2u-hyaline accumulation in proximal tubule cells	Bergamaschi et al. (1992)
Rat, F344 (both sexes) and mouse, B6C3F$_1$ (both sexes) (10 per group for oral studies, 5 per group for inhalation studies)	0, 1,000 or 1,500 mg/kg-day daily by corn oil gavage for 42 d; 0 or 1,000 ppm for 10 d	Accumulation of α2u-globulin in proximal tubules of male rats; nephrotoxicity also observed in male rats (formation of granular tubular casts and evidence of tubular cell regeneration) Inhalation exposure demonstrated formation of hyaline droplets in kidneys of male rats	Green et al. (1990)

Green et al. (1990) tested lower inhaled tetrachloroethylene doses in rats—up to 400 ppm for 6 hours/day, for 28 days, with the animals being sacrificed within 18 hours 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. 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. α2u-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 (NTP, 1990b; Charbonneau et al., 1987).

Lack of hyaline droplet formation, increase in α2u-globulin, or signs of the characteristic renal nephropathy at the high dose level of the NTP inhalation study (NTP, 1986) may, thus, diminish the likelihood that the renal tumors associated with exposure to tetrachloroethylene are induced through this mechanism (Green et al., 1990). 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 α2u-globulin accumulation in the kidney (NTP, 1990b). 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.

In contrast, the renal pathology reported in the NTP bioassay is not entirely consistent with the results generally found for chemicals where there is α2u-globulin accumulation (NTP, 1986) (letter from Scot Eustis, National Toxicology Program, to William Farland, Director, Office of Health and Environmental Assessment, U.S. 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 α2u-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.

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.

In summary, although a few studies show an increase in hyaline droplets in the proximal tubule cells of treated male rats, other studies demonstrate nephrotoxicity in both male and female rats and mice without hyaline droplet formation. Further, the studies that demonstrate hyaline droplet formation do not also have additional aspects of nephrotoxicity associated with α2u-globulin formation. The α2u-globulin response reported following exposure to tetrachloroethylene is relatively modest, and the fact that renal tumors have been observed at

doses lower than those shown to cause the α2u-globulin response is inconsistent with this phenomenon being responsible for tumorigenesis. Chronically induced tetrachloroethylene nonneoplastic kidney lesions exhibit neither species nor sex specificity. Unlike with other chemicals that induce α2u-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. Therefore, based on the criteria described above, there are insufficient data to demonstrate renal toxicity or cancers are caused by α2u-globulin formation.

4.2.4.3. Genotoxicity

A hypothesized mutagenic MOA entails the following key events leading to tetrachloroethylene-induced kidney tumor formation: following metabolism of tetrachloroethylene to one or more mutagenic intermediates, the genetic material is altered in a manner that permits changes to be transmitted during cell division through one or more mechanisms (gene mutations, deletions, translocations, or amplification); the resulting mutations advance acquisition of the multiple critical traits contributing to carcinogenesis. This MOA may apply to multiple cancer types.

The genotoxic potential of tetrachloroethylene is addressed in Section 4.8. To summarize, the results of a large number of in vitro genotoxicity tests in which tetrachloroethylene was the test agent support the conclusion that tetrachloroethylene does not exhibit direct mutagenic activity in the absence or presence of the standard S9 fraction (Watanabe et al., 1998; DeMarini et al., 1994; Roldán-Arjona et al., 1991; Milman et al., 1988; Warner et al., 1988; NTP, 1986; Connor et al., 1985; Shimada et al., 1985; Haworth et al., 1983; Hardin et al., 1981; Kringstad et al., 1981; Bartsch et al., 1979; Greim et al., 1975). However, the few in vitro mutagenicity studies of tetrachloroethylene under conditions that would generate the GSH conjugate were positive (Vamvakas et al., 1989c; Vamvakas et al., 1989d). While most of these intermediates have not been characterized for mutagenic potential, TCVG (Dreessen et al., 2003; Vamvakas et al., 1989d) and *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-*L*-cysteine (NAcTCVC) (Vamvakas et al., 1987) are mutagenic in the presence of activation while TCVC was mutagenic even in the absence of activation (Dreessen et al., 2003; Dekant et al., 1986a). 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. Studies of chromosomal aberrations following exposure to tetrachloroethylene are

mostly negative, but positive results have been reported from in vitro studies with enhanced metabolic activation (Doherty et al., 1996).

The limited in vivo studies of tetrachloroethylene are inconsistent, with only negative (NTP, 1986; Bronzetti et al., 1983) or equivocal (Cederberg et al., 2010a; Beliles et al., 1980) genotoxicity assay results demonstrated following inhalation or oral exposure. These include the finding that tetrachloroethylene at higher concentrations induces at most modest increases in DNA damage in liver tissue (Cederberg et al., 2010a). Following in vivo exposures, tetrachloroethylene induces SSB and DNA binding in kidney (Potter et al., 1996; Mazzullo et al., 1987; Walles, 1986). Intraperitoneal injection assays have demonstrated both negative (NTP, 1986) as well as positive results for different genotoxicity endpoints in other tissues (Murakami and Horikawa, 1995). Assays of clastogenic effects following inhalation exposure in humans have shown inconsistent results and are suggested to be related to coexposures (Seiji et al., 1990; Ikeda et al., 1980).

Thus, although tetrachloroethylene has largely yielded negative results in standard genotoxicity assays, uncertainties remain with respect to the possibility that genotoxicity contributes to renal carcinogenesis. Not all metabolites have been identified or characterized, but several known metabolites including those derived from P450 as well as GSH pathways are 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 modest increases in DNA damage and DNA binding in liver tissue (Cederberg et al., 2010a; Murakami and Horikawa, 1995). Given the demonstrated mutagenicity of several tetrachloroethylene metabolites, the hypothesis that mutagenicity contributes to the MOA for tetrachloroethylene carcinogenesis cannot be ruled out, although the specific metabolic species or mechanistic effects are not known.

4.2.4.4. Peroxisome Proliferation

The PPARα-agonism MOA is also hypothesized to induce rat kidney tumorigenesis. According to this hypothesis, the key events leading to tetrachloroethylene-induced kidney tumor formation constitute the following, after activation of tetrachloroethylene to one or more reactive metabolites: the PPARα receptor is activated, which then causes alterations in cell proliferation and apoptosis, followed by clonal expansion of initiated cells.

Limited data exist to support increased peroxisome proliferation in rodent kidney following exposure to tetrachloroethylene and are summarized in Table 4-12 (Odum et al., 1988; Goldsworthy and Popp, 1987). The role of peroxisome proliferation in tetrachloroethylene-induced kidney toxicity and cancer was examined in male and female F344 rats and B6C3F$_1$

mice exposed to tetrachloroethylene by inhalation (400 ppm, 6 hours/day, 14, 21, or 28 days or 200 ppm, 6 hours/day, 28 days) in Odum et al. (1988). Five animals per group were exposed. Insufficient mouse kidney tissue limited the analysis to pooled samples. Slight increases were observed in β-oxidation in mouse kidney (maximum 1.6-fold increase at 21 days, 400 ppm exposure). Modest palmitoyl-CoA oxidation (PCO) increases were observed in the kidney of male rats at 200 ppm at 28 days (1.3-fold) but not 400 ppm at 14, 21, or 28 days. In female rat kidney, PCO was elevated (approximately 1.6-fold) at all doses and times. However, peroxisome proliferation was not observed in rat or mouse kidney upon microscopy, suggesting that this does not play a role in kidney carcinogenesis.

Table 4-12. Renal peroxisome proliferation in tetrachloroethylene-exposed rodents

Species/strain/sex/number	Effect	Dose	Time
Rat, F344; and mouse, $B6C3F_1$ (both sexes, 5/group) Odum et al. (1988)	Mice of both sexes: Analysis in mice was limited to pooled tissue, but showed slight increases in β-oxidation in mouse kidney	200, and 400 ppm, inhalation	14, 21, 28 d
	Rats of both sexes: Modest increases in PCO observed in male rat kidneys at 200 ppm for 28 d only, but elevated in female rat kidneys at all doses and times	200, and 400 ppm, inhalation	14, 21, 28 d
F344 rats (male only, 5/group) and $B6C3F_1$ mice (male only, 5/group) Goldsworthy and Popp (1987)	Mice: Increased PCO activity in all exposed mice	1,000 mg/kg-day for 10 d, corn oil gavage	10 d
	Rats: Increased kidney weight in exposed rats	1,000 mg/kg-day for 10 d, corn oil gavage	10 d

Goldsworthy and Popp (1987) administered tetrachloroethylene (1,000 mg/kg-day) by corn oil gavage to 5 male F344 rats and 5 male $B6C3F_1$ mice for 10 days. In tetrachloroethylene-exposed rats, PCO was modestly—although not significantly—elevated in the liver (1.4-fold increase) and kidney (1.7-fold increase). In mice, tetrachloroethylene exposure increased PCO activity 4.3-fold in liver and by 2.3-fold in kidney. Relative liver weight was increased in rats and mice with tetrachloroethylene exposure, but relative kidney weight was unaffected. A comparison of corn oil with methyl cellulose revealed no effect of the gavage vehicle on tetrachloroethylene-induced PCO. A less-than-additive effect of trichloroethylene (1,000 mg/kg) administered together with tetrachloroethylene on PCO induction was seen.

4.2.4.5. Cytotoxicity/Sustained Chronic Nephrotoxicity Not Associated with α2u-Globulin Nephropathy

The hypothesis is that renal neoplasms induced by tetrachloroethylene arise secondary to renal cytotoxicity and subsequent cellular proliferation without regard to α2μ-accumulation. This MOA entails the following key events leading to tetrachloroethylene-induced kidney tumor formation: following metabolism of tetrachloroethylene to one or more reactive intermediates, toxicity to the kidney ensues and is sustained; via a variety of potential mechanisms (damage to and alteration of macromolecules, cell signaling alterations, etc.), the acquisition of the multiple critical traits contributing to carcinogenesis is advanced.

The kidney is a major target organ for tetrachloroethylene-induced toxicity through the reactive metabolites produced subsequent to GSH conjugation. Renal tubule neoplasia is observed to occur only in male rats. This species- and sex-specific response would not be expected based on the hypothesized MOA because tetrachloroethylene has been reported to produce nephrotoxicity across species, and in both sexes. 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 1/50 female rats exposed at the high dose developed tubule cell hyperplasia (NTP, 1986).

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 (NTP, 1986). 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 $B6C3F_1$ 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 (NCI, 1977).

Mechanistic studies of tetrachloroethylene nephrotoxicity are relatively sparse. 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 of the 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. (1989a; 1989b) have shown the tetrachloroethylene conjugate metabolites TCVG and TCVC to cause dose-related cytotoxicity in renal cell preparations and prevention of this toxicity by β-lyase enzyme inhibitor. Renal β-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., 1999; 1997; Werner et al., 1996). 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., 2002; Lash and Parker, 2001; 2000).

4.2.4.6. 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 cancer. Mutagenicity, peroxisome proliferation, α2u-globulin nephropathy, and cytotoxicity not associated with α2u-globulin accumulation are MOAs that have been investigated. Except for α2u-globulin accumulation, which is more likely due to tetrachloroethylene itself (Lash and Parker, 2001), other mechanisms hypothesized to contributed to tetrachloroethylene-induced renal carcinogenicity are thought to be mediated by tetrachloroethylene metabolites rather than with the parent compound.

Metabolites from the GSH conjugation pathway are posited to induce renal tumorigenicity, as opposed to or to a greater extent than the metabolites resulting from oxidative CYP processing. The glutathione conjugation of tetrachloroethylene in the kidney, discussed in Section 3, leads sequentially to TCVG and TCVC. TCVC can be further processed by β-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., 1999; Ripp et al., 1997; 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. 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 cannot be ruled out.

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 α2u-globulin accumulation, raising serious doubt that α2u-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, the chemical-specific data are limited and show only modest effects at exposures exceeding those required for renal carcinogenesis. There is no evidence causally linking PPARα-activation to kidney tumorigenesis for tetrachloroethylene or other compounds.

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 cannot be ruled out.

4.3. LIVER TOXICITY AND CANCER

4.3.1. Human Studies

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 (1997b)]. 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 (1997b)].

4.3.1.1. Liver Damage

Four cross-sectional studies were available that evaluated the prevalence of liver damage among dry-cleaner populations (Brodkin et al., 1995; Gennari et al., 1992; Cai et al., 1991; Lauwerys et al., 1983). 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 (Brodkin et al., 1995; Gennari et al., 1992).

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 lifestyles (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 phosphatase, 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 mean TWA exposure (8 hours) 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. The noninvasive imaged penetration of ultrasound into liver tissue can reveal the presence of fat accumulation and fibrous structures. Hepatic parenchymal changes 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 6 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 (OR: 4.2, 95% 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; but, 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 appears to be a more sensitive indicator of toxicity than 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 this study is poorly reported, and the authors 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-hour 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 4-13 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 4-13. Summary of studies of human liver toxicity

Subjects	Effects	Exposure	Author
27 PCE-exposed dry cleaners 26 nonexposed laundry workers	Sonographic scattering of fat in liver Severity greater with higher cumulative exposure No liver toxicity	Group mean TWA = 15.8 ppm Mean duration of exposure = 12 yr	Brodkin et al. (1995)
141 PCE-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 yr	Gennari et al. (1992)
24 PCE-exposed dry cleaners 33 controls nonoccupationally exposed to organic solvents	No effect on serum hepatic enzymes	Mean TWA = 21 ppm Mean duration of exposure = 6 yr	Lauwerys et al. (1983)
56 PCE-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 yr	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).

4.3.1.2. Liver Cancer

Eighteen epidemiologic studies reporting data on liver cancer and tetrachloroethylene exposure were identified. This set of studies includes 14 cohort or nested case-control studies on liver cancer (Calvert et al., 2011; Seldén and Ahlborg, 2011; Lindbohm et al., 2009; Pukkala et al., 2009; Sung et al., 2007; Lynge et al., 2006; Ji and Hemminki, 2005c; Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999; Boice et al., 1999; Lynge et al., 1995; Bond et al., 1990; Lynge and Thygesen, 1990), two liver cancer case-control studies of occupational exposures (Suarez et al., 1989; Stemhagen et al., 1983), and two liver cancer case-control studies of residential exposure (Lee et al., 2003; Vartiainen et al., 1993). Two other cohort studies included information on tetrachloroethylene but did not report risk estimates for liver cancer (Radican et al., 2008; Anttila et al., 1995), as well as an earlier report of mortality by Chang et al. (2003) for subjects in Sung et al. (2007), did not provide an estimate of the association for liver cancer. Additionally, three liver cancer case-control studies that examined occupational exposure did not report an odds ratio for holding an occupation or for work in a dry cleaner and laundry (Ferrand et al., 2008; Austin et al., 1987; Houten and Sonnesso, 1980) and so were not evaluated further. The 18 studies represent the core studies evaluated by EPA, as described in more detail below. Appendix B reviews the design, exposure-assessment approach, and statistical methodology for each study. Most studies were of the inhalation route of exposure, of occupational exposure, and lacked quantitative exposure information.

Thirteen studies reporting risk estimates for liver cancer examine occupational title as dry cleaner, launderer, and presser as surrogate for tetrachloroethylene, given its widespread use from 1960 onward in the United States and Europe (Calvert et al., 2011; Seldén and Ahlborg, 2011; Lindbohm et al., 2009; Pukkala et al., 2009; Lynge et al., 2006; Ji and Hemminki, 2005c; Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999; Lynge et al., 1995; Lynge and Thygesen, 1990; Suarez et al., 1989; Stemhagen et al., 1983). Seven studies conducted in Nordic countries are either based on the entire Swedish population or on combined populations of several Nordic countries; strengths of these studies are their use of job title as recorded in census databases and ascertainment of cancer incidence using national cancer registries (Seldén and

Ahlborg, 2011; Lindbohm et al., 2009; Pukkala et al., 2009; Lynge et al., 2006; Andersen et al., 1999; Lynge et al., 1995; Lynge and Thygesen, 1990). Lynge et al. (1995) is a nested case-control study of subjects in Lynge and Thygsen (1990). Subjects in the multi-Nordic country of Pukkala et al. (2009) overlapped those of Lynge and Thygesen (1990), Lynge et al. (1995), Andersen et al. (1999), Lynge et al. (2006), and Seldén and Ahlborg (2011). Studies examining mortality among U.S. dry-cleaner and laundry workers (Blair et al., 2003; Ruder et al., 2001) are of smaller cohorts than the Nordic studies, with fewer observed liver cancer events.

The exposure surrogate in studies of dry-cleaners and laundry workers is a broad category containing jobs of differing potential for tetrachloroethylene exposure. Thus, these studies have a greater potential for exposure misclassification bias compared to studies with exposure potential to tetrachloroethylene assigned by exposure matrix approaches applied to individual subjects. One dry-cleaning study included an analysis of subjects who worked for one or more years before 1960 in one or more shops known to use tetrachloroethylene as the primary solvent (Calvert et al., 2011). The cohort was stratified into two groups based on the level of certainty that the worker was employed only in facilities using tetrachloroethylene as the primary solvent exposure; tetrachloroethylene-only and tetrachloroethylene-plus. Lynge et al. (1995) separately classifies subjects in Lynge and Thygsen (1990) as either dry cleaners or laundry workers using occupation and workplace description from 1970 Census records. Lynge et al. (2006), using job title reported in the 1970 Census, identified subjects as dry cleaner (defined as dry cleaners and supporting staff if employed in business of <10 workers), other job titles in dry cleaning (launderers and pressers), unexposed (job title reported on 1970 Census was other than in dry cleaning), or unclassifiable (information was lacking to identify job title of subject). Selden and Alhborg (2011) identified subjects as either dry cleaners, assigned with potential for tetrachloroethylene exposure, or laundry workers, assigned as unexposed, and presented risk estimates separately by job title. Lindbohm et al. (2009), using a JEM approach based on job title and exposures, assigned a cumulative exposure index to chlorinated hydrocarbons to individual subjects. Tetrachloroethylene is one of several chlorinated solvents included in the broad category, but Lindbohm et al. (2009) do not present risk estimates for tetrachloroethylene-only subjects.

Three other cohorts with potential tetrachloroethylene exposure in industrial settings have been examined. These studies include aerospace or aircraft maintenance workers in the United States (Boice et al., 1999), workers, electronic factory workers in Taiwan (Sung et al., 2007), and workers at a Dow plant in Michigan (Bond et al., 1990). Boice et al. (1999) used an exposure assessment based on a JEM, and Bond et al. (1990), a nested case-control study, used company work history records to assign potential tetrachloroethylene exposure to individual subjects. In contrast and less sensitive, the exposures in the Taiwan studies included multiple solvents and

tetrachlorethylene exposure was not linked to individual workers, and cohorts included white-collar workers, who had an expected lower potential for exposure (Sung et al., 2007).

Two geographical studies focused on residential proximity to drinking water sources contaminated with tetrachloroethylene and other solvents. Vartiainen et al. (1993) examines liver cancer incidence in two southern Finnish municipalities, with the exposure surrogate assigned uniformly to all subjects. Lee et al. (2003), using a mortality odds ratio approach, examined residence in two communities surrounding the factory whose workers were studied by Chang et al. (2005; 2003) and Sung et al. (2007). One village upstream from the factory was considered as unexposed, and another village downstream from the factory was identified as exposed based on groundwater monitoring of drinking water wells during the period 1999−2000.

In summary, with respect to exposure-assessment methodologies, five studies with liver cancer data assigned tetrachloroethylene exposure to individuals within the study using a JEM (Boice et al., 1999; Bond et al., 1990), identified a subcohort whose subjects were employed only in facilities using tetrachloroethylene as the primary solvent exposure (Calvert et al., 2011), or restricting analyses to subjects identified as dry cleaners (Seldén and Ahlborg, 2011; Lynge et al., 1995). One other study sought additional data using a questionnaire for use in refining potential exposure within dry-cleaning settings (Lynge et al., 2006). The relative specificity of these exposure-assessment approaches strengthens their ability to identify cancer hazards compared to studies with broader and less sensitive exposure-assessment approaches. The least sensitive exposure assessments are those using very broad definitions such as working in a plant or factory (Sung et al., 2007; Chang et al., 2003).

Four[22] of the 16 liver cancer studies evaluated by EPA with exposure assessment to tetrachloroethylene or employment as dry-cleaner or laundry worker reported estimated relative risks based on 50 or more observed events (Pukkala et al., 2009; Lynge et al., 2006; Ji and Hemminki, 2005c; Travier et al., 2002). The observed number of liver cancer cases in these studies ranged from 58 (Lynge et al., 2006) to 113 (Pukkala et al., 2009). The four large studies observed a standardized incidence ratio of 0.76 (95% CI: 0.38, 1.52), 1.02 (95% CI: 0.84, 1.24), 1.22 (95% CI: 1.03, 1.45), and 1.23 (95% CI: 1.02, 1.49) in Lynge et al. (2006), Travier et al. (2002), Ji and Hemminki (2005c), and Pukkala et al. (2009), respectively, for the association between liver cancer risk and ever having a job title of dry-cleaner or laundry worker (refer to Table 4-14).

In addition to the evidence from the large cohort and case-control studies, 11 other studies reported effect estimates for liver cancer based on fewer observed events and carry lesser

[22] Lynge and Thygsen (1990) and Andersen et al. (1999) are not included in this summary of the data from the individual studies because they were updated and expanded in the analysis by Lynge et al. (1995) and Pukkala et al. (2009), respectively.

weight in the analysis. As expected, the magnitude of the point estimate of the association[23] reported in these studies is more variable than in the larger studies: 0.13 to 0.98 (Calvert et al., 2011; Sung et al., 2007; Blair et al., 2001; Vartiainen et al., 1993; Suarez et al., 1989), 1.2 to 1.8 (Seldén and Ahlborg, 2011; Lindbohm et al., 2009; Bond et al., 1990) and 2.05 to 2.57 (Lee et al., 2003; Boice et al., 1999; Stemhagen et al., 1983). Only the 95% CIs of the risk estimate of Lee et al. (2003) excluded 1.0.

Establishment of an exposure or concentration-response relationship can add to the weight of evidence for identifying a cancer hazard, but only limited data pertaining to exposure-response relationships for liver cancer and tetrachloroethylene exposure are available. Four studies of liver cancer presented risk estimates for increasing exposure categories using exposure duration, a proxy inferior to cumulative exposure due to inability to account for temporal changes in exposure intensity (Seldén and Ahlborg, 2011; Lynge et al., 2006; Travier et al., 2002; Boice et al., 1999). Boice et al. (1999) presents a statistical test for linear trend for subjects with intermittent-routine tetrachloroethylene exposure, a broader category than that used to examine overall tetrachloroethylene exposure (comprised of routine-exposed subjects only), and reported a *p*-value of >0.20. In Travier et al. (2002), the standardized incidence ratio estimate is 1.20 (95% CI: 073, 2.18) for dry-cleaners and laundry workers in both 1960 and 1970 Censuses, compared to 1.02 (95% CI: 0.84, 1.24) for only subjects in one of these census. Standardized incidence ratio estimates for both males and females with tetrachloroethylene exposure in Seldén and Ahlborg (2011) appeared to decrease monotonically with increasing employment duration.

[23]In Lynge et al. (1995), all 17 primary liver cancer deaths occurred among laundry workers, and a risk estimate and associated 95% CIs were not presented for dry cleaners.

Table 4-14. Summary of human studies on tetrachloroethylene exposure and liver cancer

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort Studies				
Biologically monitored workers				Anttila et al. (1995)
	All subjects	Not reported		849 Finnish men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, external referents (SIR)
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure to PCE	2.05 (0.83, 4.23)	7	77,965 (n = 2,631 with routine PCE exposure and n = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, job exposure matrix without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm [mean] and 9.5 ppm [median], external reference for routine exposure (SMR) and internal references (workers with no chemical exposures) for routine-intermittent PCE exposure (RR), liver and biliary tract (ICD-9, 155, 156)
	Routine-Intermittent exposure duration to PCE	Not reported		
	0	1.0[a]	22	
	<1 yr	1.38 (0.40, 4.69)	3	
	1−4 yr	1.17 (0.39, 3.47)	4	
	>5 yr	1.29 (0.46, 3.65)	5	
	p-value for trend	$p > 0.20$		
Chemical workers				Bond et al. (1990)
	PCE	1.8 (0.8, 4.3)	6	Nested case-control study with cohort (n = 21,437 males), follow-up 1940−1982, 44 liver and biliary tract deaths, unmatched controls randomly selected from cohort, PCE and 10 other potential exposures assigned to individual subjects based on company records, Mantel-Haenxzel χ^2 (OR)
Electronic factory workers (Taiwan)				Chang et al. (2003); Sung et al. (2007)
	All Subjects			86,868 (n = 70,735 female), follow-up 1979−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, cancer mortality, external referents (SMR) (Chang et al., 2003), primary liver cancer (A095) 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, multiple solvents exposures and PCE not identified to individual subjects, cancer incidence, external referents, analyses lagged 10 yr (SIR), liver and interhepatic bile ducts (Sung et al., 2007)
	Males	Not reported	0 0.69 exp	
	Females	Not reported	0 0.57 exp	
	Females	0.79 (0.55, 1.10)	36	

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Table 4-14. Summary of human studies on tetrachloroethylene exposure and liver cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aircraft maintenance workers from Hill Air Force Base				Radican et al. (2008)
	Any PCE exposure	Not reported		10,461 men and 3,605 women (total *n* = 14,066, *n* = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952 to 1956, follow-up 1973−2000, job exposure matrix (intensity), internal referent (workers with no chemical exposures (RR)
Dry-cleaner and laundry workers				Andersen et al. (1999)
	All laundry worker and dry cleaners	1.30 (0.93, 1.78)	39	29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway), follow-up 1971−1987 or 1991, PCE not identified to individual subjects, external referents (SIR), Primary liver cancer (ICD-7, 155.0)
	Males	1.26 (0.69, 2.21)	11	
	Females	1.32 (0.88, 1.91)	28	
				Blair et al. (2003)
	All subjects	0.8 (0.4, 1.5)	10	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR), liver and gallbladder (ICDA-8, 155)
	Semiquantitative exposure score	Not reported		
				Ji and Hemminki. (2005c)
	Laundry workers and dry cleaners in 1960 Census	1.22 (1.03, 1.45)[b]	138	9,255 Swedish men and 14,974 Swedish women employed in 1960 (men) or 1970 (women) as laundry worker or dry cleaner, follow-up 1961/1970−2000, PCE not identified to individual subjects, external referent (SIR) and adjusted for age, period and socioeconomic status
	Males	1.30 (0.97, 1.67)[b]	52	
		1.09 (0.70, 1.56)[c]	25	
		1.52 (0.83, 2.43)[d]	14	
		1.61 (0.88, 2.57)[e]	14	
	Females	1.18 (0.94, 1.44)[b]	86	
		1.26 (0.82, 1.81)[c]	25	
		1.05 (0.75, 1.40)[d]	39	
		1.39 (0.87, 2.04)[e]	22	

Table 4-14. Summary of human studies on tetrachloroethylene exposure and liver cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Lindbohm et al. (2009)
Launderers and dry cleaners	1.22 (0.56, 2.33)	9	Finnish population born 1906−1945 and participated in 1970 Census, follow-up 1971−1995, Finnish cancer registry, 1,691 males and 783 female primary liver cancers, longest held occupation reported on 1970 Census, laundry and dry-cleaner exposure surrogate, external referent for analyses examining job title (SIR) and all-other job titles for analyses for chlorinated hydrocarbon (RR) adjusted for age, period, social class, smoking and alcohol consumption
Males	2.91 (0.35, 4.26)	2	
Females	1.05 (0.42, 2.16)	7	
Cumulative exposure chlorinated HCs			
None	1.0[a]	1,618	
<5 ppm-yr	1.25 (0.80, 1.95)[b] 1.23 (0.68, 2.24)[c]	20 11	
5−49 ppm-yr	1.13 (0.84, 1.53)[b] 1.22 (0.83, 1.80)[c]	44 27	
≥50 ppm-yr	2.65 (1.38, 5.11)[b] 3.59 (1.71, 7.57)[c]	9 7	
			Lynge and Thygsen (1990); Lynge et al. (1995)
All laundry worker and dry cleaners	2.19 (0.88, 4.51)	7	10,600 Danish men and women, 20−64 yr old, employed in 1970 as laundry worker, dry cleaners and textile dye workers, follow-up 1970−1980, external referents (SIR), Primary liver cancer (ICD-7, 155) (Lynge and Thygesen, 1990) Nested case-control study within Lynge and Thygsen (1990), 17 primary liver cancer cases in men and women, follow-up 1970−1987, 85 controls randomly selected from within cohort, matched on sex, age, and occupation, dry cleaner assigned using occupation and workplace on 1970 Census form, logistic regression (OR) (Lynge et al., 1995)
Males		0 1.1 exp	
Females	3.33 (1.34, 6.87)	7	
Dry cleaner	Not reported	0 cases	
Laundry worker	Not reported	17 cases	
			Pukkala et al. (2009)
Launderer and dry cleaner	1.23 (1.02, 1.49)	113	Men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30−64 yr, follow-up 2005, occupational title of launderer and dry cleaner in any census, external referents (SIR), Primary liver cancer (ICD-7, 155)
Male	1.13 (0.76, 1.63)	29	
Female	1.27 (1.01, 1.57)	84	

Table 4-14. Summary of human studies on tetrachloroethylene exposure and liver cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Calvert et al. (2011)
All subjects	0.13 (0.00, 0.73)	1	1,708 U.S. men and women dry-cleaning union member in CA, IL, MI, NY follow-up 1940−2004, multiple solvent exposures (625 subjects entered union prior to 1960 and identified as PCE-only exposure), cancer mortality (SMR), liver and biliary tract (ICD-9, 155, 156)
Exposure duration/time since 1st employment	0.20 (0,00, 1.01)	1	
PCE-only subjects	Not reported	0	
			Seldén and Ahlborg (2011)
Dry-cleaners and laundry workers	1.12 (0.73, 1.64)	26	9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−2000, exposure assigned using company self-reported information on PCE usage—PCE (dry cleaners and laundries with a proportion of PCE dry cleaning), laundry (no PCE use), and other (mixed exposures to PCE, CFCs, TCE, etc.), external referents (SIR), liver and gallbladder (ICD-7, 155)
Males	1.93 (0.97, 3.46)	11	
Females	0.86 (0.48, 1.41)	15	
PCE	1.21 (0.72, 1.92)	18	
Males	2.14 (0.92, 4.21)	8	
Duration of employment			
<1 yr	(0.00, 9.71)	0	
1−4 yr	3.19 (0.66, 9.31)	3	
5−11 yr	2.06 (0.67, 4.80)	5	
Females	0.90 (0.43, 1.65)	10	
Duration of employment			
<1 yr	1.66 (0.20, 6.01)	2	
1−4 yr	1.50 (0.49, 3.50)	5	
5−11 yr	0.46 (0.09, 1.33)	3	
Laundry			
Males	1.74 (0.36, 5.09)	3	
Females	0.67 (0.18, 1.70)	4	

Table 4-14. Summary of human studies on tetrachloroethylene exposure and liver cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
				Travier et al. (2002)
	All subjects, 1960 or 1970 Census in laundry and dry cleaner or related occupation and industry	1.02 (0.84, 1.24)	105	Swedish men and women identified as laundry worker, dry cleaner, or presser (occupational title), in the laundry, ironing, or dyeing industry or related industry in 1960 or 1970 (543,036 person-years); or, as laundry worker, dry cleaner, or presser (occupational and job title) (46,933 person-years) in both censuses, follow-up 1971−1989, external referents (SIR), liver and biliary passages
	All subjects in 1960 and 1970 in laundry and dry cleaner occupation and industry	1.26 (0.73, 2.18)	13	
Case-Control Studies				
5 University Hospitals, United States (AL, FL, MA, NC, PA)				Austin et al. (1987)
	Laundry and dry cleaning occupation	Not reported	0	80 histologically confirmed hepatocellular carcinoma cases, 18−84 yr, years not identified, 161 hospital controls matched on sex, age, race, and study center, unknown interview methods, exposure surrogate jobs held ≥6 mo, OR from logistic regression
France				Ferrand et al. (2008)
	Laundry and dry cleaning occupation	Not reported		125 hepatocellular carcinoma in men, lacking HBV and HCV infection, identified from four hospitals, <75 yr, 2000−2003, 142 hospital controls in other departments, face-to-face interview, job title ≥6 mo as exposure surrogate, OR from logistic regression model and adjust for hospital, age, and alcohol consumption
				Houten and Sonnesso (1980)
	Laundry and dry-cleaning operatives	Not reported	2	102 primary liver cancer cases in men and women, identified from hospital records, 1956−1965, controls were all other hospitalized cancer cases, self-reported occupation at time of hospitalization, χ^2 comparing distribution of job titles

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Table 4-14. Summary of human studies on tetrachloroethylene exposure and liver cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Nordic Countries (Denmark, Finland, Norway, Sweden)				Lynge et al. (2006)
	Unexposed	1.0a	58	Case-control study among 46,768 Danish, Finnish, Norwegian, and Swedish men and women employed in 1960 as laundry worker or dry cleaner, follow-up 1970−1971 to 1997−2001, 72 incident esophageal cancer cases, 6 controls per case randomly selected from cohort matched on country, sex, age, calendar period at diagnosis time, occupational task at 1970 Census proxy for exposure, RR adjusted for matching criteria
	Dry cleaner	0.76 (0.38, 1.52)	95	
	Other in dry cleaning	0.42 (0.09, 1.89)	22	
	Unclassifiable	1.11 (0.59, 2.09)	121	
	Duration of employment in dry cleaning			
	≤1 yr	Not reported		
	2−4 yr	Not reported		
	3−9 yr	1.21 (0.43, 3.44)	5	
	≥10 yr	0.70 (0.26, 1.92)	5	
	Unknown	2.88 (0.21, 38.81)	1	
New Jersey (United States)				Stemhagen et al. (1983)
	Laundering, cleaning, and other garment services	2.50 (1.02, 6.14)[f]	10	265 histologically confirmed primary liver cancer cases and deaths, 1975−1980, New Jersey State Cancer Registry, 530 hospital controls matched on age, race, sex, county of residence, vital status, in-person interview, job title and industry coded to SIC/SOC, OR estimating using Mantel-Haenszel with matched case-control set and not adjusted for personal or lifestyle factors
	Laundering, cleaning, and other garment services	2.29 (0.85, 6.13)[g]	8	
				Suarez et al. (1989)
	Dry-cleaning services	0.98 (0.44, 2.20)	11	1,742 primary liver cancer deaths, 1969−1980, 1,742 dead controls, frequency matched on age, sex, race, and year death, Texas vital records, job tile on death certificate, OR from Mantel-Haenszel analyses for race and sex separately and adjusted for age
	Dry-cleaning operators	0.55 (0.17, 1.75)	4	

Table 4-14. Summary of human studies on tetrachloroethylene exposure and liver cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Geographic-Based Studies				
Taoyuan, Taiwan				Lee et al. (2003)
	Residence in upstream village	1.0[a]		Population of two villages surrounding electronic factory (Sung et al., 2007; Chang et al., 2005; Chang et al., 2003), 50 liver cancer deaths, primary, underlying, or underlying condition as cause of death, 1966−1997, residence as recorded on death certificate, MOR from logistic regression adjusted for age and period
	Residence in downstream village	2.57 (1.21, 5.46)	30	
Hausjarvi and Hattula, Finland				Vartiainen et al. (1993)
	Hausjarvi	0.7 (0.3, 1.4)	7	Lymphopoietic cancers, liver cancer and all cancers among residents with PCE and other solvents in drinking water, 1953−1991, no subject-level exposure information, cancer rates of Finnish population referent (SIR)
	Hattula	0.6 (0.2, 1.3)	6	

[a] Referent.
[b] SIR or RR for liver, biliary tract, and gallbladder cancers.
[c] SIR or RR for hepatocellular carcinoma.
[d] SIR for gallbladder cancer.
[e] SIR for all other liver cancers.
[f] In Stemhagen et al. (1983), odds ratio for primary liver cancer and work in laundering, cleaning, and other garment services industry.
[g] In Stemhagen et al. (1983), odds ratio for hepatocellular carcinoma and work in laundering, cleaning, and other garment services industry.

HBV = hepatitis B virus, HCV = hepatitis C virus, ICD = International Classification of Disease, ICDA = International Classification of Disease, Amended, ISCO = International Standard Classification of Occupation, ISIC = International Standard Industry Classification, JEM = job-exposure-matrix, MOR = mortality odds ratio, PCE = tetrachloroethylene, TWA = time-weighted-average.

Risk factors for liver cancer include alcohol and hepatitis B and C viruses, with diabetes mellitus suggested based on recent epidemiologic studies (El-Serag, 2007). None of the cohort or case-control studies on liver cancer and tetrachloroethylene controlled for these potential risk factors.

In conclusion, studies carrying greater weight in the analysis based on a large number of observed events or exposed cases or a strong exposure-assessment approach, show a mixed pattern of results. The one case-control study with a large number of exposed liver cancer cases and a relatively high quality exposure-assessment methodology reported an odds ratio estimate of 0.76 (95% CI: 0.38, 1.72) for liver cancer and dry cleaning (Lynge et al., 2006). A recent multiple Nordic country cohort study and two cohort studies of Swedish subjects with broad exposure-assessment approaches, and whose subjects overlapped with Lynge et al. (2006), reported SIRs of 1.02 (95% CI: 0.84, 1.24), 1.22 (95% CI: 1.03, 1.45), and 1.23 (95% CI: 1.02, 1.49) for liver and biliary tract cancer and work as a dry-cleaner or laundry worker (Pukkala et al., 2009; Ji and Hemminki, 2005c; Travier et al., 2002). The study of Lindbohm et al. (2009) of Finnish dry-cleaner and laundry workers whose subjects overlap the larger multiple-country study of Pukkala et al. (2009) and that carries less weight in the analysis due to fewer observed liver and biliary cancer cases supports observations in Swedish or the five Nordic country dry-cleaner and laundry worker studies (Pukkala et al., 2009; Ji and Hemminki, 2005c). Three other studies with strong exposure-assessment approaches specific to tetrachloroethylene but whose risk estimates are based on fewer observed liver cancer cases or deaths provide support for an association between liver cancer and tetrachloroethylene, risk estimates were 1.21 to 2.05 (Seldén and Ahlborg, 2011; Boice et al., 1999; Bond et al., 1990). However, dry cleaning workers did not have a higher liver cancer risk estimate than laundry workers or other categories of dry cleaning workers (Seldén and Ahlborg, 2011; Lynge et al., 2006). . An exposure-response relationship was not observed, and the SIR for tetrachloroethylene-exposed subjects with the longest employment duration in Seldén and Ahlborg (2011) was lower than that for shorter employment duration. Potential confounding may be an alternative explanation as no study adjusted for known and suspected risk factors for liver cancer (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Lynge et al., 2006; Ji and Hemminki, 2005c; Travier et al., 2002; Boice et al., 1999; Bond et al., 1990). Nine other cohort and case-control studies with fewer observed events and/or a broad exposure-assessment methodology carried less weight in the analysis; these studies also reported a mixed pattern of results (Calvert et al., 2011; Lindbohm et al., 2009; Sung et al., 2007; Blair et al., 2003; Lee et al., 2003; Lynge et al., 1995; Vartiainen et al., 1993; Suarez et al., 1989; Stemhagen et al., 1983). Lee et al. (2003) reported a risk estimate of 2.57 (95% CI: 1.21, 5.46) for the association between liver cancer and residence in a village with groundwater contamination, was in region with a high prevalence of HCV and did not control for

HCV status in the statistical analysis; potential confounding from HCV may be an alternative explanation for the observed association.

4.3.2. Animal Studies

Liver toxicity and cancer have been observed in laboratory animal studies following exposure to tetrachloroethylene through multiple routes of exposure. The sections below describe studies of liver toxicity (refer to Section 4.3.2.1) and cancer (refer to Section 4.3.2.2). These studies are summarized in Tables 4-15 and 4-16, respectively.

4.3.2.1. Liver Toxicity

Tetrachloroethylene causes hepatic toxicity in multiple species, including several strains of rats and mice. Adverse effects on the liver have been observed in studies of animals exposed to tetrachloroethylene by multiple routes of exposure, including inhalation and oral gavage. Hepatic effects observed after subchronic or chronic inhalation exposure to tetrachloroethylene include increased liver weight (Kyrklund et al., 1990; Kjellstrand et al., 1984); hypertrophy (Odum et al., 1988); fatty degeneration (Odum et al., 1988; Kylin et al., 1963); peroxisome proliferation (Odum et al., 1988); other histological changes (Odum et al., 1988; NTP, 1986; Kjellstrand et al., 1984); and degeneration and necrosis (JISA, 1993; NTP, 1986). When administered by oral gavage, tetrachloroethylene also causes hepatic toxicity, including increased liver enzymes, increased liver weights, histological changes, degeneration and necrosis, regenerative repair, and polyploidy (Philip et al., 2007; Ebrahim et al., 1996; Jonker et al., 1996; Berman et al., 1995; Goldsworthy and Popp, 1987; Buben and O'Flaherty, 1985; NCI, 1977). Table 4-15 presents a summary of inhalation and oral rodent liver toxicity studies, which are briefly described below. This review focuses on studies that identify critical effects commonly observed in tetrachloroethylene toxicity studies and could, accordingly, support oral and inhalation reference values. The database of liver toxicity studies is more extensively reviewed in prior assessments by ATSDR (1997a), NYSDOH (1997), and CalEPA (2001).

Table 4-15. Summary of inhalation and oral rodent liver toxicity studies

Species/strain/sex/number	Exposure level/duration	Effects	Reference
Mouse, B6C3F$_1$ (both sexes, 49 or 50 per sex per dose group, total of ~300 mice)	0, 100, 200 ppm for 104 wk, inhalation	Liver degeneration and necrosis at ≥100 ppm in males and at 200 ppm in females	NTP (1986)
Mouse, Crj/BDF1 (both sexes, 50 per sex per dose group, total of 400 mice)	0, 10, 50, 250 ppm for 110 wk, inhalation	Focal necrosis in males at ≥50 ppm; liver degeneration in males and females at 250 ppm	JISA (1993)
Rat, F344/DuCrj (both sexes, 50 per sex per dose group, total of 400 rats)	0, 50, 200, 600 ppm for 110 wk, inhalation	Spongiosis hepatitis in males at 200 ppm and higher; hyperplasia in males at 600 ppm	JISA (1993)
Mouse, NMRI (both sexes, 10 per dose group)	0, 9, 37, 75, 150 ppm, 30 d, inhalation, continuous (24 h); and 225 (16 h/d), 450 (8 h/d), 900 (4 h/d), 1,800 (2 h/d), or 3,600 (1 h/d), inhalation	Increase in liver weight (≥9 ppm); morphological changes (≥9 ppm); increased plasma butylcholinesterase (≥37 ppm)	Kjellstrand et al. (1984)
Mouse, B6C3F$_1$, and rat, Sprague-Dawley (males only, 3 per dose group)	Radiolabeled PCE by inhalation (10 or 600 ppm for 6 h), or as a single oral gavage dose (500 mg/kg)	Irreversible binding to hepatic macromolecules at all exposures in male mice and rats	Schumann et al. (1980)
Rat, F344; and mouse, B6C3F$_1$ (both sexes, 5 per dose group)	0, 200 ppm (28 d only) and 400 ppm (14, 21, 28 d) for 6 h/d, inhalation	Increased palmitoyl CoA in mice (3.7-fold) and rats (1.3-fold); increased peroxisome proliferation in mouse liver in all sex, dose and time groups; mitochondrial proliferation in male mice at 400 ppm for 28 d; increased relative liver weight, centrilobular lipid accumulation in exposed mice of both sexes	Odum et al. (1988)
Rat, Sprague-Dawley (males only, 8 per group)	0 or 320 ppm continuous for 90 d; 0 or 320 ppm continuous for 90 d followed by a 30 d recovery period, inhalation	Significantly increased relative liver weight after exposure; this was decreased following recovery; decreased cholesterol following the recovery period	Kyrklund et al. (1990)
Mice, albino (strain not specified) (females only, 20 per dose group, 240 total)	0 or 200 ppm 4 h/d, 6 d/w for 1, 2, 4 or 8 wk, inhalation	Fatty degeneration after 1 wk; incidence severity increased with longer exposure	Kylin et al. (1965)
Mouse, Swiss-Cox, male (males, 4−6 per 1,500 and 2,000 mg/kg-day doses; other doses, 12−15 mice/group)	0, 20, 100, 200, 500, 1,000, 1,500, 2,000 mg/kg-day for 6 wk, 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	Buben and O'Flaherty (1985)

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Table 4-15. Summary of inhalation and oral rodent liver toxicity studies (continued)

Species/strain/sex/number	Exposure level/duration	Effects	Reference
Mouse, Swiss-Webster, male (4 per dose group)	0, 150, 500, and 1,000 mg/kg-day, aqueous gavage for 30 d	Increased plasma ALT 24 hours to 14 d after initial exposure; mild to moderate fatty degeneration and necrosis, with focal inflammatory cell infiltration; increased mitotic figures and DNA synthesis (peaked on 7 d, sustained at 14−30 d at all doses); inhibition of PCE metabolism and TCA production; no change in CYP2E1; CYP4A increased at 7 but not 14 d, only at 1,000 mg/kg-day	Philip et al. (2007)
Rat, Wistar, female only (10 rats per each control group; 5 rats per dose group)	0, 600, and 2,400 mg/kg-day for 32 d, corn oil gavage; alone or in combination with other compounds (trichloroethylene, hexachloro-1,2-butadiene, 1,1,2-trichloro-3,3,3-trifluoropropene)	Relative liver weight increases in animals exposed to PCE alone or in combination; hepatotoxicity at 600 mg/kg	Jonker et al. (1996)
Rat, F344 (males only, 5 per dose group) and mouse, $B6C3F_1$ (males only, 5 per dose group)	0 or 1,000 mg/kg-day for 10 d, corn oil gavage	Increased relative liver weight in rats and mice; 4.3-fold PCO increase in mice; modest but not significant (1.4-fold) PCO increase in rats	Goldsworthy and Popp (1987)
Mouse, Swiss, both sexes; 6 groups of 6 mice each (Ebrahim et al., 1996); male only; 8 groups of 6 mice each (Ebrahim et al., 2001)	0 or 3,000 mg/kg-day for 15 d, sesame oil gavage	Significant increase in liver weight; degeneration and necrosis of hepatocytes; decreased blood glucose (glucose effects mitigated by coexposures to 2-deoxy-D glucose and vitamin E) (Ebrahim et al., 1996); Decreased membrane-bound Na^+K^+-ATPases and Mg_2^+-ATPases activity but increased Ca-ATPase activity; mitigated by coexposure to 2-deoxy-D-glucose and vitamin E, and taurine	Ebrahim et al. (2001; 1996)
Rat, F344 (females only, 8 per dose group)	0, 50, 150, 500, or 1,500 mg/kg-day, gavage, either once or for 14 consecutive days	Increased relative liver weight, elevated ALT and hepatocellular hypertrophy at 1,500 mg/kg-day	Berman et al. (1995)

4.3.2.1.1. Inhalation

Hepatic toxicity was observed in chronic lifetime inhalation bioassays of tetrachloroethylene in mice conducted by the National Toxicology Program (NTP, 1986), and the Japan Industrial Safety Association (JISA, 1993). The NTP study administered tetrachloroethylene to groups of 50 F344 rats of each sex (0, 200, or 400 ppm), or groups of 49 or 50 B6C3F$_1$ mice (0, 100, or 200 ppm), for 6 hours/day, 5 days/week, for 103 weeks (NTP, 1986). In addition to liver tumors in mice of both sexes, liver degeneration was reported in 2/49, 8/49, and 14/50 of males and in 1/49, 2/50, and 13/50 of females. Degeneration was characterized by a variety of histological features, including cytoplasmic vacuolation, hepatocellular necrosis, inflammatory cell infiltrates, pigment in cells, oval cell hyperplasia, and regenerative foci. Liver necrosis was observed at increased incidence in dosed males (1/49, 6/49, and 15/50) and in females at 400 ppm (3/48, 5/50, and 9/50). Nuclear inclusions increased in male mice (2/49, 5/49, and 9/50). No dose-related liver effects were reported in the rats.

In the 13-week NTP study, groups of 10 rats and mice of each sex were exposed to air containing tetrachloroethylene for 6 hours/day, 5 days/week, for 13 weeks (0, 100, 200, 400, 800, or 1,600 ppm). Some rats in the high-dose group died before the end of the studies (4/10 male, 7/10 female). In mice, 2/10 males and 4/10 females in the high-dose group died before the end of studies. Tetrachloroethylene (200 ppm and above) increased the incidence of hepatic congestion in male and female rats. In mice of both sexes, liver lesions (leukocytic infiltration, centrilobular necrosis, and bile stasis) were observed at 400, 800, or 1,600 ppm. Mitotic alterations were increased at 200 ppm in male mice. No hepatic effects were reported in the single exposure or 14-day studies.

In the Japan Industrial Safety Association (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. Hemangiomas or hemangiosarcomas, occurring primarily in the liver or spleen, were also reported in the male mice. This study also examined effects in F344/DuCrj rats exposed to 0, 50, 200, and 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 600 ppm.

The lowest reported level for liver effects by inhalation in laboratory animals is in female NMRI mice exposed for 30 days at 9 ppm [61 mg/m^3; Kjellstrand et al. (1984)]. Significant increases in liver weight as well as changes in liver morphology were observed in male and

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female mice exposed continuously to 9 ppm and higher concentrations of tetrachloroethylene for 30 days. Livers were enlarged, and vacuolization was evident. Reversible increases in levels of the blood plasma enzyme butyrylcholinesterase were reported at all tetrachloroethylene concentration levels at or above 37 ppm. The toxicological significance of the increased serum cholinesterase is uncertain, and this effect of tetrachloroethylene has not been reported by other investigators. After a recovery period, liver weight was still slightly elevated at 120 days after cessation of tetrachloroethylene exposure for 30 days at 150 ppm. Total dose administered in the continuous exposure experiment is not directly comparable to exposures during intermittent and pulsed exposure experiments, which also found increased liver weight and increased serum cholinesterase.

Schumann et al. (1980) administered radiolabeled tetrachloroethylene to male $B6C3F_1$ mice or Sprague-Dawley rats via inhalation (10 or 600 ppm for 6 hours). In mice, the percentage metabolized based on recovery of the radiolabeled material was determined to be 88% for a 6-hour inhalation exposure of 10 ppm (as compared to only 17% for a single oral gavage dose of 500 mg/kg). At all dose levels in both rats and mice, irreversible binding of radioactivity to hepatic macromolecules was observed. DNA binding was not seen. In mice, binding peaked at the termination of the 6-hour inhalation exposure and 6 hours after the single oral dose. In contrast, binding in the rat peaked 24 hours after either oral or inhalation exposure.

Odum et al. (1988) exposed groups of male and female F344 rats and $B6C3F_1$ mice by inhalation for 6 hours/day to 200 ppm (28 days only) or 400 ppm (for 14, 21, or 28 days) tetrachloroethylene. Five animals per group were exposed. In both sexes, hepatic palmitoyl coenzyme A (PCO) activity was increased in mice (up to 3.6-fold) and, to a lesser extent, in rats (up to 1.3-fold). Modest PCO increases were also observed in the kidney of male rats at 200 ppm at 28 days (1.3-fold) but not 400 ppm at 14, 21, or 28 days. In female rat kidney, PCO was elevated (approximately 1.6-fold) at all doses and times. However, peroxisome proliferation was not observed in rat kidney upon microscopy. In contrast, hepatic peroxisome proliferation was noted in mouse liver for all sexes, times, and dose groups on electron microscopy, and the percentage of cytoplasm occupied by peroxisomes also increased. Catalase, another peroxisomal enzyme, was unaffected by tetrachloroethylene; male mice exposed at 400 ppm showed the only moderate (1.4-fold) increase. Mitochondrial proliferation was observed at 28 days in 400 ppm male mice. In addition, a time-dependent proliferation of smooth endoplasmic reticulum in the liver of both sexes correlated well with centrilobular hypertrophy. Tetrachloroethylene caused centrilobular lipid accumulation in male and female mice. Relative liver weight was increased in mice of both sexes.

Kyrklund et al. (1990) exposed male Sprague-Dawley rats to 320 ppm tetrachloroethylene continuously for 90 days, followed by a 30 day recovery period. Relative

liver weight was significantly increased in rats examined at the end of the exposure period. A slight increase in relative liver weight was also observed in the recovered, solvent-treated group. Cholesterol was also decreased, but this effect was only statistically significant in the tetrachloroethylene-exposed group that also included a recovery period.

Kylin et al. (1965) exposed female albino mice (strain not specified) to 200 ppm tetrachloroethylene for 4 hours daily, 6 days a week, for 1, 2, 4, or 8 weeks. Hepatic effects were evaluated by histological examination and determination of extractable liver fat. The incidence and severity of fatty degeneration increased with longer exposure. Neither liver cell necrosis nor cirrhosis was observed.

4.3.2.1.2. Oral

In addition to studying the effects of inhalation and a single oral gavage dose (500 mg/kg), as described above, Schumann et al. (1980) also administered 100, 250, 500, or 1,000 mg/kg to male B6C3F$_1$ mice or Sprague-Dawley rats as a daily oral dose for 11 days. At all doses in mice, histopathological evidence of hepatocellular swelling in the centrilobular region, a decrease in liver DNA content, and an increase in DNA synthesis was observed. At $\geq$250 mg/kg, tetrachloroethylene increased the absolute or relative liver weights in mice. In rats, no statistically significant treatment-related effects were observed at 100, 250, or 500 mg/kg; however, increased liver DNA synthesis was observed in one rat in the 250 mg/kg-dose group, resulting in a large variation in liver DNA synthesis at that exposure level.

Buben and O'Flaherty (1985) exposed male Swiss-Cox mice to tetrachloroethylene doses of 0, 20, 100, 200, 500, 1,000, 1,500, or 2,000 mg/kg-day, 5 days/week, for 6 weeks. Liver/body-weight ratios and liver triglycerides were significantly increased at 100 mg/kg-day or more. Enlarged hepatocytes, karyorrhexis (disintegration of the nucleus), necrosis, polyploidy in the centrilobular region, and lipid accumulation were evident upon histopathological examination of mice exposed to 200 or 1,000 mg/kg. Other indices of tetrachloroethylene hepatotoxicity (decreased glucose-6-phosphatase activity, and increased serum glutamic pyruvic transaminase activity) were significantly increased at 500 or more mg/kg-day. The liver response (percentage increase in either liver weight/body-weight ratios or G6P inhibition) was highly correlated with the amount of tetrachloroethylene metabolized, and a plot of these measures against total urinary metabolites was linear (r^2 = 0.97 and 0.98 for increases in liver/body weight ratios and G6P inhibition, respectively). The LOAEL was 100 mg/kg-day.

Philip et al. (2007) exposed male 6−7-week old Swiss-Webster mice via aqueous gavage to three dose levels (150, 500, and 1,000 mg/kg-day) for 30 days. At the highest exposure, mortality was 10% due to apparent CNS toxicity (tremors and ataxia). Significant liver injury (as assessed by increased plasma ALT) was evident 24 hours after the first, single exposure at all

doses. ALT levels decreased transiently to control levels by 30 days thereafter. Histopathology was consistent with mild-to-moderate fatty degeneration and necrosis. Necrotic hepatocytes had either pyknotic, karyorrhectic, or karyolitic nuclei. Infiltration of neutrophils and macrophages was present near necrotic foci. Regenerative repair was evident in the two higher dose groups by 30 days of exposure, with observed increases in mitotic figures, tritiated thymidine incorporation with pulse-labeling, and PCNA immunostaining. At the two higher dose groups, a robust increase DNA synthesis peaked on 7 days, was sustained at 14 days, and had returned to control levels at 30 days of exposure. The amount of blood and liver TCA declined, while tetrachloroethylene levels increased, from 1 to 30 days. This is consistent with an inhibition of tetrachloroethylene metabolism. Because CYP2E1 levels and activity were unchanged, a different CYP isoform is suggested to be critical for tetrachloroethylene metabolism. The study found a transient increase in hepatic CYP4A expression, a marker of PPARα induction, which was evident at 7—but not 14—days at the highest dose. This finding suggests that peroxisome proliferation is not a sustained response in spite of continued tetrachloroethylene exposure.

In a study by Jonker et al. (1996), hepatotoxicity was observed in female Wistar rats administered tetrachloroethylene (600 or 2,400 mg/kg-day) daily via corn oil oral gavage for 32 days. Relative liver weight was increased on exposure to tetrachloroethylene alone and in combination with other hepatotoxicants (trichloroethylene, hexachloro-1,2-butadiene, and 1,1,2-trichloro-3,3,3-trifluoropropene). One high-dose animal died as a result of tetrachloroethylene treatment, and one animal exposed to the high-dose combination also died as a result of treatment. Hepatotoxic effects were noted at 600 mg/kg.

Goldsworthy and Popp (1987) administered tetrachloroethylene (1,000 mg/kg-day) by corn oil gavage to 5 male F344 rats and 5 male B6C3F_1 mice for 10 days. In tetrachloroethylene-exposed rats, cyanide-insensitive palmitoyl CoA oxidation (PCO) was modestly—although not significantly—elevated in the liver (1.4-fold increase) and kidney (1.7-fold increase). In mice, tetrachloroethylene exposure increased PCO activity by 4.3-fold in liver and by 2.3-fold in kidney. Relative liver weight was increased in rats and mice with tetrachloroethylene exposure, but relative kidney weight was unaffected. A comparison of corn oil with methyl cellulose revealed no effect of the gavage vehicle on tetrachloroethylene-induced PCO. A less-than-additive effect of trichloroethylene (1,000 mg/kg) administered together with tetrachloroethylene on PCO induction was seen.

Ebrahim et al. (1996) orally administered 3,000 mg/kg-day tetrachloroethylene in sesame oil to male and female Swiss 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. Blood glucose

levels were significantly decreased, and this effect was mitigated by concomitant exposure to 2-deoxy-D-glucose (2DG) and vitamin E. A follow-up study by this group further examined the potential protective properties of 2DG and vitamin E as well as taurine against tetrachloroethylene-induced membrane damage (Ebrahim et al., 2001). This study exposed male albino Swiss mice to the same doses used in the previous study with the addition of a taurine exposed group (tetrachloroethylene in sesame oil 3,000 mg/kg-day for 15 days orally by intubation; tetrachloroethylene plus 2DG 500 mg/kg-day by i.p. injection once a day for 15 days; tetrachloroethylene plus vitamin E 400 mg/kg-day by oral intubation once a day for 15 days; and tetrachloroethylene plus taurine 100 mg/kg-day by oral intubation once a day for 15 days). Compared to control cells in the liver, membrane-bound $Na^{+}K^{+}$-ATPases and Mg_{2}^{+}-ATPases activity was significantly decreased ($p < 0.001$), while Ca-ATPases activity was increased ($p < 0.001$), following exposure to tetrachloroethylene alone. These levels remained near normal in the animals exposed to tetrachloroethylene along with 2DG, vitamin E, or taurine. This return to normal levels following exposure to vitamin E and taurine may be due to their antioxidant abilities, and reduced oxidative stress in exposed cells.

Berman et al. (1995) reported liver and kidney toxicity in a study of female F344 rats exposed for 14 days by oral gavage to 0, 50, 150, 500, or 1,500 mg/kg-day tetrachloroethylene. The reported LOAEL was 1,500 mg/kg-day. Hepatic effects included increased relative liver weight, elevated ALT, and hepatocellular hypertrophy.

4.3.2.1.3. Intraperitoneal injection

Binding of radiolabelled tetrachloroethylene to hepatic DNA was observed in mice following i.p. injection (Mazzullo et al., 1987) but not inhalation and oral exposure [Schumann et al. (1980), described above]. Using a reportedly more sensitive assay, low levels of DNA binding were observed in vivo in BALB/C mouse liver 22 hours after i.p. injection (1.4 mg/kg), with 10 fold lower levels observed in Wistar rat liver than mouse liver (Mazzullo et al., 1987). Still lower levels of DNA binding were observed in the kidney and stomach of mice and rats in this study. Binding to RNA and protein was always higher than binding to DNA in both mice and rats. Binding to calf thymus DNA in an in vitro study increased in the presence of microsomal fractions from both mouse and rat liver, but not kidney, lung or stomach. Cytosolic fractions from rat and mouse liver, kidney, lung, and stomach all induced binding of tetrachloroethylene to calf thymus DNA, with enzymes from both mouse and rat livers and mouse lung being the most efficient. DNA binding in the presence of both cytosolic and microsomal fractions was similar to cytosolic fraction alone. Phenobarbital pretreatment of animals increased cytosol-mediated binding, but had only a slight effect on microsomal-mediated binding. Binding in the presence of rat liver microsomal fraction was also increased (17-fold)

with addition of GSH but decreased in the presence of superoxide dismutase or mannitol (Mazzullo et al., 1987).

4.3.2.2. Liver Cancer

In carcinogenicity bioassays, tetrachloroethylene caused a statistically significant increase in the incidence of hepatocellular carcinomas in both sexes of $B6C3F_1$ mice following either oral gavage administration or inhalation exposure (NTP, 1986; NCI, 1977). 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). Additionally, in male Crj:BDF1 mice, hemangiosarcomas (reported as malignant hemangioendotheliomas) in the liver and both hemangiosarcomas and combined hemangiosarcomas and hemangiomas (reported as benign hemangioendotheliomas) of the spleen were increased. The studies are presented in Table 4-16 and are briefly summarized here.

4.3.2.2.1. Inhalation

The NTP (1986) inhalation bioassay exposed groups of 50 $B6C3F_1$ mice of each sex to (epichlorohydrin free) tetrachloroethylene concentrations of 0, 100, or 200 ppm, 6 hours/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. Hepatocellular neoplasms (adenomas and carcinomas combined) were reported in 17/49, 31/49, and 41/50 males, and 4/45, 17/42, and 38/48 females. In male mice, hepatocellular carcinomas metastasized to the lungs in 2/49, 7/49, and 1/50 animals. Metastatic hepatocellular carcinomas were found in the lungs of 0/48, 2/50, and 7/50 female mice.

A Japan bioassay exposed groups of 50 Crj:BDF1 mice of each sex to 0, 10, 50, and 250 ppm tetrachloroethylene, 6 hours/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. The incidences of hepatocellular adenomas were 7/50, 13/50, 8/50, and 26/50 in males and 3/50, 3/47, 7/49, and 26/49 in females in control, 10, 50, and 250 ppm dose groups, respectively. Male hepatocellular carcinomas also increased, with reported incidences of 7/50, 8/50, 12/50, and 25/50 in males and 0/50, 0/47, 0/49, and 14/49 in females in control, 10, 50, and 250 ppm dose groups, respectively. Liver hemangiosarcomas (reported as malignant hemangioendotheliomas) were also increased in males. In the spleen, both hemangiosarcomas and combined hemangiosarcomas and hemangiomas (reported as benign hemangioendotheliomas) were increased in males.

4.3.2.2.2. Oral

In the NCI (1977) tetrachloroethylene mouse gavage study, 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). The test sample was estimated to contain epichlorohydrin concentrations of less than 500 ppm. 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.

Table 4-16. Incidence of hepatic tumors in rodents exposed to tetrachloroethylene

Bioassay	Administered dose/exposure	Continuous equivalent exposure	Sex	Hepatocellular adenomas and carcinomas	Hemangiomas or hemangiosarcomas[a]
NCI (1977) $B6C3F_1$ mice[b] Gavage: 5 d/wk, 78 wk	Vehicle 450 mg/kg-day 900 mg/kg-day	0 332 mg/kg-day 663 mg/kg-day	Male	2/20 (10) 32/48 (67) 27/45 (60)	None reported[c]
	Vehicle 300 mg/kg-day[a] 600 mg/kg-day	0 239 mg/kg-day 478 mg/kg-day	Female	0/20 (0) 19/48 (40) 19/48 (40)	None reported
NCI (1977)[d] Osborne-Mendel rats Gavage: 5 d/wk, 78 wk	Vehicle 500 mg/kg-day 1,000 mg/kg-day	0 471 mg/kg-day 941 mg/kg-day	Male	1/20 (0) 1/49 (0) 0/50 (0)	None reported
	Vehicle 500 mg/kg-day 1,000 mg/kg-day	0 474 mg/kg-day 974 mg/kg-day	Female	None reported	None reported
NTP (1986) $B6C3F_1$ mice Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Male	17/49 (35) 31/49 (70) 41/50 (82)	1/49 (2) 0/49 (0) 0/50 (0)
	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Female	4/45 (9) 17/42(40) 38/48 (79)	0/48 (0) 3/50 (6) 0/50 (0)
NTP (1986) F344/N rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 200 ppm 400 ppm	0 36 ppm 72 ppm	Male	0/50 (0) 1/50 (2) 1/50 (2)	0/50 0/50 0/50
	0 ppm 200 ppm 400 ppm	0 36 ppm 72 ppm	Female	0/50 0/50 0/50	0/50 0/50 0/50

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Table 4-16. Incidence of hepatic tumors in rodents exposed to tetrachloroethylene (continued)

Bioassay	Administered dose/exposure	Continuous equivalent exposure	Sex	Hepatocellular adenomas and carcinomas	Hemangiomas or hemangiosarcomasa
JISA (1993) Crj:BDF1 mice Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Male	13/50 (28) 21/50 (43) 19/50 (40) 40/50 (82)	4/50 (4) 2/50 (2) 7/50 (13) 11/50 (18)
	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Female	3/50 (6) 3/47 (6) 7/49 (15) 33/49 (67)	1/50 0/47 2/49 3/49
JISA (1993) F344/DuCrj rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 50 ppm 200 ppm 600 ppm	0 9 ppm 36 ppm 108 ppm	Male	4/50 0/50 1/50 2/50	0/50 0/50 0/50 0/50
	0 ppm 50 ppm 200 ppm 600 ppm	0 9 ppm 36 ppm 108 ppm	Female	1/50 (2) 0/50 (0) 1/50 (2) 0/50 (0)	1/50 0/50 0/50 0/50

[a] These tumors were reported as hemangioendotheliomas in the JISA (1993) report. The term has been updated to hemangioma (benign) or hemangiosarcoma (malignant). 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 hemangioendotheliomas from the individual animal data provided in the JISA report.

[b] Administered gavage doses listed were increased after 11 wk by 100 mg/kg-day in each low-dose group or by 200 mg/kg-day in each high-dose group. Mice received the listed TWA daily doses through Week 78, and surviving mice were observed up to study termination in Week 90.

[c] None reported: Individual animal data were not available, and summary data did not include a line item for this tumor type.

[d] Gavage doses listed were adjusted several times during the course of the study. Male rats received the listed TWA daily doses through Week 78, and surviving animals were observed up to study termination in Week 110.

4.3.3. 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 observed 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).

Liver toxicity has been reported in multiple animal species by inhalation and oral exposures to tetrachloroethylene. The effects are characterized by increased liver weight, fatty changes, necrosis, inflammatory cell infiltration, triglyceride increases, and proliferation. The mouse has been shown to be more sensitive to hepatic toxicity than the rat in multiple subchronic and chronic studies [e.g., JISA (1993); NTP (1986); Schumann et al. (1980); NCI (1977)]. After subchronic or chronic inhalation exposures in mice, liver toxicity is manifested by increased liver weight (Kjellstrand et al., 1984), liver enlargement (Odum et al., 1988; Kjellstrand et al., 1984), cytoplasmic vacuolation (fatty changes) (Odum et al., 1988; NTP, 1986; Kjellstrand et al., 1984), centrolobular hepatocellular necrosis (JISA, 1993; NTP, 1986), and inflammatory cell infiltrates, pigment in cells, oval cell hyperplasia, and regenerative foci (NTP, 1986). The LOAEL for the inhalation studies—9 ppm—is from a 30 day-exposure mouse study reporting increased liver weight and morphological changes and is supported by a finding of irreversible macromolecular binding in mouse liver following a single, 6-hour exposure at 10 ppm. The JISA (1993) chronic mouse inhalation bioassay reported 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 administration by oral gavage in mice, liver toxicity (increased liver weight, hepatocellular swelling, necrosis, lipid accumulation, and increased DNA synthesis) has been observed at 100 mg/kg-day (Buben and O'Flaherty, 1985; Schumann et al., 1980) and above (Ebrahim et al., 1996; Jonker et al., 1996; Berman et al., 1995; Goldsworthy and Popp, 1987). At 150 mg/kg-day administered for 30 days (Philip et al., 2007), tetrachloroethylene increased ALT levels transiently and stimulated fatty degeneration and necrosis, with ensuing regenerative repair. These findings support a LOAEL of 100 mg/kg-day and a NOAEL of 20 mg/kg-day.

For liver cancer, epidemiologic studies carrying greater weight in the analysis, based on a large number of observed events or exposed cases, or a strong exposure-assessment approach, show a mixed pattern of results. The one case-control study with a large number of exposed liver cancer cases and a relatively high quality exposure-assessment methodology reported an odds ratio estimate of 0.76 (95% CI: 0.38, 1.72) for liver cancer and dry cleaning (Lynge et al., 2006). A recent multiple Nordic country cohort study and two cohort study of Swedish subjects with broad exposure-assessment approaches and whose subjects overlapped with Lynge et al. (2006) reported SIRs of 1.02, 1.22, and 1.23 for liver and biliary tract cancer and work as a dry cleaner or laundry worker in Travier et al. (2002), Ji and Hemminki (2005c), and Pukkala et al. (2009), respectively. Three other studies with strong exposure-assessment approaches specific to tetrachloroethylene but whose risk estimates are based on fewer observed liver cancer cases or deaths reported risk estimates of 1.21 to 2.05 for the association between liver cancer and tetrachloroethylene (Seldén and Ahlborg, 2011; Boice et al., 1999; Bond et al., 1990). However,

dry cleaning workers did not have a higher liver cancer risk estimate than laundry workers or other categories of dry cleaning workers (Seldén and Ahlborg, 2011; Lynge et al., 2006). Exposure response was not observed, and the SIR for tetrachloroethylene-exposed subject with longest employment duration in Seldén and Ahlborg (2011) was lower than that for subjects with shorter employment duration. Potential confounding may be an alternative explanation as no study adjusted for known and suspected risk factors for liver cancer (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Lynge et al., 2006; Ji and Hemminki, 2005c; Travier et al., 2002; Boice et al., 1999; Bond et al., 1990). Nine other cohort and case-control studies with fewer observed events and broad exposure-assessment methodology carried less weight in the analysis and reported a pattern of mixed results (Calvert et al., 2011; Lindbohm et al., 2009; Sung et al., 2007; Blair et al., 2003; Lee et al., 2003; Lynge et al., 1995; Vartiainen et al., 1993; Suarez et al., 1989; Stemhagen et al., 1983). Lee et al. (2003) reported a risk estimate of 2.57 (95% CI: 1.21, 5.46) for the association between liver cancer and residence in a village with groundwater contamination, but subjects were from a region with a high prevalence of HCV infection, and HCV status may confound the observed association.

Tetrachloroethylene caused a statistically significant increase in the incidence of liver tumors in both sexes of mice in multiple carcinogenicity bioassays. A statistically significant increase in the incidence of hepatocellular carcinomas in both sexes of $B6C3F_1$ mice was observed following either oral gavage administration or inhalation exposure (NTP, 1986; NCI, 1977). Both sexes of Crj:BDF1 mice also showed an increased incidence of hepatocellular carcinomas and adenomas when exposed to tetrachloroethylene by inhalation (Nagano et al., 1998; JISA, 1993). Liver hemangiosarcomas were also increased in males. In the spleen, both hemangiosarcomas and combined hemangiosarcomas and hemangiomas were increased in males.

4.3.4. Mode of Action for Hemangiosarcomas or Hemangiomas in Mice

The incidence of hemangiomas or hemangiosarcomas occurring in the liver or spleen (and to a lesser extent in fat, subcutaneous skin, and the heart) was significantly increased in male Crj:BDF1 mice exposed to tetrachloroethylene by inhalation (JISA, 1993). This tumor type is distinct from the hepatocellular adenomas and carcinomas induced by tetrachloroethylene in male and female Crj:BDF1 mice by inhalation exposure (JISA, 1993), and in male and female $B6C3F_1$ mice by inhalation (NTP, 1986) or oral (NCI, 1977) exposure. No data are available concerning either the metabolites or the mechanisms that may contribute to the induction of hemangiosarcomas or hemangiomas occurring in the liver or spleen in male mice. It is concluded that the mechanisms or modes of action by which tetrachloroethylene induces this type of tumor are not known.

4.3.5. Mode of Action for Murine Hepatocellular Tumors

Multiple metabolites formed from tetrachloroethylene are toxic and carcinogenic in the liver. In particular, it is likely that TCA and DCA contribute to tetrachloroethylene-induced liver tumors in mice. However, the mode of action through which these (and potentially other) metabolites elicit the benign and malignant hepatocellular tumors induced with oral or inhalation exposure to tetrachloroethylene in multiple strains and both sexes of mice remains to be fully elucidated. As noted by NRC (2010), it is likely that key events from several pathways, comprising several simultaneous mechanisms, operate in tetrachloroethylene-induced liver cancer.

The discussion of mechanistic effects addresses the following topics: (1) contribution of tetrachloroethylene metabolism to hepatocarcinogenicity (refer to Section 4.3.5.1); (2) genotoxicity (refer to Section 4.3.5.2); (3) epigenetic effects, focusing on DNA hypomethylation (refer to Section 4.3.5.3); (4) oxidative stress (refer to Section 4.3.5.4); and (5) receptor activation, focusing on a hypothesized PPARα-activation mode of action (refer to Section 4.3.5.5). Because it has been suggested that hepatocarcinogenesis caused through a PPARα-activation MOA is not relevant to humans [e.g., Klaunig et al. (2003)], and such a conclusion would have significant implications for hazard conclusions and dose-response analyses, this hypothesized MOA is discussed in relatively more detail than other topics. In the NRC review of EPA's 2008 external review draft of tetrachloroethylene, a dissenting opinion put forth by one member was that "the weight of evidence strongly favors a key role of PPARα activation in tetrachloroethylene-induced hepatocarcinogenesis in mice; furthermore, this MOA lacks relevance for human hepatocarcinogenesis" [refer to Appendix B; NRC (2010)]. However, in their rebuttal [also presented in Appendix B; NRC (2010)], the committee as a whole did not support these conclusions. Overall, the committee judged that many gaps in knowledge remain with regard to the MOA of tetrachloroethylene. They stated that the relevance of the peroxisome proliferator MOA to tetrachloroethylene-induced mouse hepatic cancer and to tetrachloroethylene-induced human hepatic cancer remains hypothetical and requires further rigorous testing. Hence, they concluded that it is premature to draw conclusions on the relevance of the PPARα MOA to tetrachloroethylene-induced human hepatic carcinogenesis (NRC, 2010). They encouraged an in-depth presentation of the relevant issues and data, particularly with respect to tetrachloroethylene studies. The discussion below, especially that in Section 4.3.5.4, follows these recommendations.

4.3.5.1. Contribution of Tetrachloroethylene Metabolites to Mode of Action and Carcinogenicity

Several metabolites of tetrachloroethylene are 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. Oxidative metabolism is thought to predominate in the liver, and TCA is the major resultant urinary excretion product. As discussed in Section 3, TCA appears to be formed from spontaneous decomposition of trichloroacetyl chloride, which is known to bind to macromolecules. DCA may be formed from dechlorination of TCA, but DCA produced from this pathway is likely to be rapidly metabolized in the liver and not detected in blood or urine. DCA that has been detected in urine is thought to be the result of kidney-specific β-lyase metabolism of the results of GSH conjugation of tetrachloroethylene, and DCA produced from this pathway is presumed to not play a role in liver toxicity or cancer. The potential role of GST conjugates of tetrachloroethylene in liver carcinogenicity, although unknown, is presumed to be less important that the role of oxidative metabolites.

The focus of most hypotheses with respect to contributors to tetrachloroethylene hepatocarcinogenicity has been on TCA and, to a lesser extent, DCA. Data on the hepatocarcinogenicity of TCA and DCA in rodents, alone and in combination, are summarized in Tables 4-17, 4-18, and 4-19. In mice, TCA significantly increased the incidence of liver tumors in male and female B6C3F_1 mice exposed via drinking water for 52−104 weeks (DeAngelo et al., 2008; Bull et al., 2002; Pereira, 1996; Bull et al., 1990; Herren-Freund et al., 1987). Incidence of tumors increased with increasing TCA concentrations (DeAngelo et al., 2008; Bull et al., 2002; Bull et al., 1990). These results were obtained under conditions where the background incidence of tumors in control animals was generally low. The development of tumors in animals exposed to TCA progressed rapidly, as evidenced by significant numbers of tumors in less-than-lifetime studies of 82 weeks or less. Positive evidence for tumor promotion by TCA (following exposure to known tumor initiators) has been reported for liver tumors in B6C3F_1 mice (Pereira et al., 2001; Pereira et al., 1997) and for GGT-positive foci in livers of partially hepatectomized Sprague-Dawley rats (Parnell et al., 1988). DCA also causes liver cancer in mice (DeAngelo et al., 1999; Daniel et al., 1992; Bull et al., 1990; Herren-Freund et al., 1987). DCA and TCA are also hepatocarcinogenic in mice when coadministered in the drinking water for 52 weeks (Bull et al., 2002). Treatment-related liver tumors were observed in male F344/N rats exposed via drinking water to DCA (DeAngelo et al., 1996) but not TCA (DeAngelo et al., 1997) for 60 or 104 weeks. The carcinogenicity of TCA and DCA has not been evaluated in female rats or in other species of experimental animals.

Data on tumor phenotype support the view that TCA may not be the sole tumorigenic metabolite of tetrachloroethylene but also do not provide definitive evidence testing any

particular hypothesis. For instance, liver tumor genotypes (e.g., with regard to H-*ras* codon 61 mutation) and phenotypes (e.g., with regard to c-Jun staining) appear to differ among tumors induced by TCA, DCA, the combination of TCA and DCA, and the structurally related compound trichloroethylene (Bull et al., 2002). Bull et al. (2002) suggest that for trichloroethylene, the data are not consistent with the hypothesis that TCA is the sole active moiety, but a similar experiment has not been conducted for tetrachloroethylene. However, by analogy, it is possible that TCA and DCA, in combination with each other (and with other reactive intermediates produced during the oxidative metabolism of tetrachloroethylene) may contribute to the production of liver tumors. This appears to be the case for noncancer effects, as the spectrum of endpoints caused by tetrachloroethylene includes effects broader than that produced by TCA, and including fatty degeneration, focal necrosis and regenerative repair, some of which may play a role in liver carcinogenesis (discussed below).

The hepatocarcinogenic potencies of TCA and tetrachloroethylene have not been directly compared in a single rodent bioassay. Appendix D presents a comparative quantitative analysis of the carcinogenicity of TCA (including that predicted using PBPK modeling to be produced from tetrachloroethylene) with the carcinogenicity of tetrachloroethylene. This analysis suggests that TCA might explain the incidence of carcinomas observed in the available tetrachloroethylene bioassays, but that a wide range of possible contributions cannot be ruled out by the available data. Specifically, a contribution of TCA from as little as 12% up to 100% cannot be ruled out, under the assumptions that the tetrachloroethylene NTP and JISA bioassay data can be combined, and using the Chiu and Ginsberg (2011) PBPK model for tetrachloroethylene and the Chiu (2011) PBPK model for TCA and TCA bioavailability. If either of these assumptions is relaxed—i.e., given that residual uncertainties of about twofold exist in the PBPK model predictions for TCA internal dose and that there may be some underlying differences between the NTP and JISA bioassays—then the CIs will be greater. Furthermore, the high control tumor incidence reported in the TCA bioassay of DeAngelo et al. (2008) raises questions as to the representativeness of that bioassay for comparison with tetrachloroethylene bioassays. Overall, as discussed in Chiu (2011) with regards to the contribution of TCA to TCE-induced hepatomegaly, factors such as study-to-study experimental variability in kinetics (e.g., metabolism, bioavailability) or in dynamics (e.g., background tumor rates), different analytical methods used to quantify TCA in blood and tissues, and uncertainty in TCA dosing patterns in drinking water studies further limit the ability to discern the quantitative contribution of TCA. A more precise quantitative measure of the relative contribution of TCA to tetrachloroethylene-induced liver tumors requires an appropriately designed experiment to better control for these factors.

Table 4-17. Hepatocarcinogenicity of TCA in rodent drinking water studies

Species (sex)	Exposure	Results	Authors
B6C3F$_1$ mice (M)	0 and 5 g/L in drinking water for 61 wk	Carcinomas: 0/22, 7/22	Herren-Freund et al. (1987)
B6C3F$_1$ mice (M)	0, 1, and 2 g/L for 52 wk	Carcinomas: 0/35, 2/11, 4/24	Bull et al. (1990)
B6C3F$_1$ mice (M)	0, 0.05, 0.5, or 5 g/L TCA for 60 wk	Carcinomas: 7, 4, 21, 38%	DeAngelo et al. (2008)
B6C3F$_1$ mice (M)	0, 0.5, and 2 g/L for 52 wk	Carcinomas: 1/20, 11/20, 9/20	Bull et al. (2002)
B6C3F$_1$ mice (F)	0, 0.35, 1.2, 3.5 g/L for 51 wk 0, 0.35, 1.2, 3.5 g/L for 82 wk	Carcinomas (52 wk): 0/40, 0/40, 0/19, 5/20 Carcinomas (81 wk): 2/90, 0/53, 5/27, 5/18	Pereira (1996)
F344/N rats (M)	0, 0.05, 0.5, 5 g/L for 104 wk	Carcinomas: 0, 0, 0, 0%	DeAngelo et al. (1997)

Adapted from NRC (2006).

Table 4-18. Hepatocarcinogenicity of DCA in rodent drinking water studies

Species (sex)	Exposure	Results	Authors
B6C3F$_1$ mice (M)	0 and 5 g/L for 61 wk	Carcinomas: 0/22, 21/26	Herren-Freund et al. (1987)
B6C3F$_1$ mice (M)	0 and 2 g/L for 52 wk	Carcinomas: 0/35, 5/24	Bull et al. (1990)
B6C3F$_1$ mice (M)	0, 0.05, 0.5, 4.5, and 5 g/L for 60−95 wk	Carcinomas: 6.7−10, 22, 38, 98, 55%	DeAngelo et al. (1991)
B6C3F$_1$ mice (M)	0, 0.05 g/L for 60 wk 0, 0.5, 1, 2, 3.5 g/L for 100 wk	Carcinomas (60 wk): 8/12, 25/30 Carcinomas (100 wk): 5/50, 5/24, 16/32, 6/14, 4/8	DeAngelo et al. (1999)
B6C3F$_1$ mice (M)	0, 0.05 for 60 wk	Carcinomas: 2/20, 15/24	Daniel et al. (1992)
B6C3F$_1$ mice (F)	0, 0.28, 0.93, and 2.8 g/L for 52 wk 0, 0.28, 0.93, and 2.8 g/L for 81 wk	Carcinomas (52 wk): 0/40, 0/40, 0/20, 1/20 Carcinomas (81 wk): 2/90, 0/50, 1/28, 5/19	Pereira (1996)
F344 rats (M)	0, 0.05, 0.5, 2.4 g/L for 60 wk 0, 0.05, 0.5 g/L for 104 wk	Carcinomas (60 wk): 0/7, 0/7, 0/7, 1/27 Carcinomas (104 wk): 0/23, 0/26, 2/29	DeAngelo et al. (1996)

Adapted from NRC (2006).

Table 4-19. Incidence of mouse liver tumors with drinking water administration of TCA and DCA, alone and in combination

Species (sex)	Exposure (52 wk)	Liver tumor incidence	Authors
B6C3F$_1$ mice (M)	0 (drinking water vehicle) 0.5 g/L TCA 2 g/L TCA 0.1 g/L DCA 0.5 g/L DCA 2 g/L DCA 0.1 g/L DCA + 0.5 g/L TCA 0.5 g/L DCA + 0.5 g/L TCA 0.1 g/L DCA + 2 g/L TCA 0.5 g/L DCA + 2 g/L TCA	1/20 11/20 9/20 2/20 5/20 12/19 9/20 13/19 15/20 13/20	Bull et al. (2002)

4.3.5.2. Genotoxicity

A hypothesized mutagenic MOA entails the following key events leading to tetrachloroethylene-induced liver tumor formation: following metabolism of tetrachloroethylene to one or more mutagenic intermediates, the genetic material is altered in a manner that permits changes to be transmitted during cell division through one or more mechanisms (gene mutations, deletions, translocations, or amplification); the resulting mutations advance acquisition of the multiple critical traits contributing to carcinogenesis. This MOA may apply to multiple cancer types.

The genotoxic potential of tetrachloroethylene is addressed in Section 4.8. To summarize, the results of a large number of in vitro genotoxicity tests in which tetrachloroethylene was the test agent support the conclusion that tetrachloroethylene does not exhibit direct mutagenic activity in the absence or presence of the standard S9 fraction (Watanabe et al., 1998; DeMarini et al., 1994; Roldán-Arjona et al., 1991; Milman et al., 1988; Warner et al., 1988; NTP, 1986; Connor et al., 1985; Shimada et al., 1985; Haworth et al., 1983; Hardin et al., 1981; Kringstad et al., 1981; Bartsch et al., 1979; Greim et al., 1975). However, the few in vitro mutagenicity studies of tetrachloroethylene under conditions that would generate the GSH conjugate were positive (Vamvakas et al., 1989c; Vamvakas et al., 1989d). Several other known (DCA) and putative (tetrachloroethylene oxide) P450 metabolites also exhibit in vitro mutagenicity. Studies of chromosomal aberrations following exposure to tetrachloroethylene are mostly negative, but positive results have been reported from in vitro studies with enhanced metabolic activation (Doherty et al., 1996).

TCA, the primary oxidative metabolite of tetrachloroethylene, exhibits little, if any, genotoxic activity in vitro. TCA did not induce mutations in *S. typhimurium* strains in the absence of metabolic activation or in an alternative protocol using a closed system (Kargalioglu et al., 2002; Nelson et al., 2001b; Giller et al., 1997; DeMarini et al., 1994; Rapson et al., 1980; Waskell, 1978), but a mutagenic response was induced in TA100 in the Ames fluctuation test (Giller et al., 1997). However, in vitro experiments with TCA should be interpreted with caution if steps have not been taken to neutralize pH changes caused by the compound (Mackay et al., 1995). Measures of DNA-repair responses in bacterial systems have shown induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations (Harrington-Brock et al., 1998). TCA was positive in some genotoxicity studies in vivo, in mouse, newt, and chick test systems (Giller et al., 1997; Bhunya and Jena, 1996; Birner et al., 1994; Bhunya and Behera, 1987). DNA unwinding assays have either shown TCA to be much less potent than DCA (Nelson and Bull, 1988) or negative (Styles et al., 1991; Nelson et al., 1989). Due to limitations in the genotoxicity database, the possible contribution of TCA to tetrachloroethylene genotoxicity is unclear.

The limited in vivo studies of tetrachloroethylene are inconsistent, with only negative (NTP, 1986; Bronzetti et al., 1983) or equivocal (Cederberg et al., 2010a; Beliles et al., 1980) genotoxicity assay results demonstrated following inhalation or oral exposure. These include findings that tetrachloroethylene at higher concentrations induces, at most, modest increases in DNA damage and DNA binding in liver tissue (Cederberg et al., 2010a; Murakami and Horikawa, 1995). Intraperitoneal injection assays have demonstrated both negative (NTP, 1986) as well as positive results for different genotoxicity endpoints (Walles, 1986). Assays of clastogenic effects following inhalation exposure in humans have shown inconsistent results and are suggested to be related to coexposures (Seiji et al., 1990; Ikeda et al., 1980).

Thus, although tetrachloroethylene has largely yielded negative results in standard genotoxicity assays, uncertainties remain with respect to the possibility that genotoxicity contributes to hepatocarcinogenesis. 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 modest increases in DNA damage and DNA binding in liver tissue (Cederberg et al., 2010a; Murakami and Horikawa, 1995). 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, the hypothesis that mutagenicity contributes to the MOA for tetrachloroethylene carcinogenesis cannot be ruled out, although the specific metabolic species or mechanistic effects are not known.

4.3.5.3. Altered DNA Methylation

Another hypothesis is that tetrachloroethylene induces hepatocarcinogenesis via the induction of epigenetic changes, particularly DNA methylation. This MOA entails the following key events leading to tetrachloroethylene-induced liver tumor formation: following metabolism of tetrachloroethylene to one or more reactive intermediates, particularly TCA, DCA, and other reactive species, epigenetic changes ensue; the resulting alterations advance acquisition of the multiple critical traits contributing to carcinogenesis. This MOA may apply to multiple cancer types.

No tetrachloroethylene-specific data are available regarding a role of alteration in DNA methylation in tumorigenesis. However, experimental evidence supports the hypothesis that hypomethylation of DNA may be related to the carcinogenicity of TCA and DCA in mice. In female B6C3F_1 mice that received an i.p. injection of *N*-methyl-*N*-nitrosourea (MNU) and were then administered TCA or DCA in drinking water, DNA methylation in the resulting hepatocellular adenomas and carcinomas was about half that observed in noninvolved tissue from the same animal or from animals given only MNU (Tao et al., 1998). Drinking water exposure of female B6C3F_1 mice to TCA or DCA for 11 days also decreased total liver DNA methylation by 60% (Tao et al., 1998). The same investigators (Tao et al., 2004) also demonstrated hypomethylation of a region of the IGF-II gene in liver and tumors from mice initiated with MNU and subsequently exposed to TCA or DCA. An association between hypomethylation and cell proliferation in liver of TCA- or DCA-exposed mice was demonstrated by Ge et al. (2001). An increase in DNA replication (evidenced by increased proliferating cell nuclear antigen labeling index and mitotic labeling index) was observed 72 hours and 96 hours after the first daily gavage dose of either TCA or DCA. Hypomethylation of the internal cytosine of CCGG sites in the promoter region of the *c-myc* gene began between 48 and 72 hours from the initiation of TCA or DCA exposure and continued to 96 hours. These observed effects of TCA and DCA, together with the fact that methylation changes represent common early molecular events in most tumors (Baylin et al., 1998; Zingg and Jones, 1997), support the plausibility of a hypothesis that dysregulation of gene methylation plays a role in tetrachloroethylene-induced tumorigenesis. However, no data are available specifically testing this hypothesis for tetrachloroethylene.

4.3.5.4. Cytotoxicity and Secondary Oxidative Stress

Another hypothesis is that oxidative stress produced secondary to tetrachloroethylene-induced cytotoxicity plays a critical role in hepatocarcinogenesis. This MOA entails the following key events leading to tetrachloroethylene-induced liver tumor formation: following metabolism of tetrachloroethylene to one or more reactive intermediates, toxicity to the liver ensues; oxidative stress is produced during hepatocyte injury, from infiltrating inflammatory cells, and/or as part of the intracellular/extracellular repair processes; the resultant oxidative stress, via a variety of potential mechanisms (damage to and alteration of macromolecules, cell signaling alterations, etc.), advances acquisition of the multiple critical traits contributing to carcinogenesis. This MOA may apply to multiple cancer types.

Numerous studies, including chronic bioassays, have demonstrated that tetrachloroethylene is hepatotoxic. Reported characteristics of the hepatic injury induced by tetrachloroethylene and the ensuing tissue repair include increased liver weight, fatty changes, necrosis, inflammatory cell infiltration, triglyceride increases, and proliferation. The NTP chronic bioassay reported a variety of histological changes, including cytoplasmic vacuolation, hepatocellular necrosis, inflammatory cell infiltrates, pigment in cells, oval cell hyperplasia, and regenerative foci. Liver tissue repair is a complex process involving cell division, angiogenesis, ductulogenesis, cell mobility, and extracellular matrix repair, all in a coordinated manner (Mehendale, 2005). Reactive oxygen species can play a role in mediating many of these processes and are produced during hepatocyte injury, from infiltrating inflammatory cells, and/or as part of the intracellular/extracellular repair processes.

A limited database of studies is available on tetrachloroethylene-induced hepatic oxidative stress. Two studies by Ebrahim et al. (2001; 1996) have examined the ability of 2-deoxy-glucose (2DG), vitamin E, or taurine to modulate hepatic effects following short-term exposure. Ebrahim (1996) orally administered 3,000 mg/kg-day tetrachloroethylene in sesame oil to male and female Swiss 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. Blood glucose levels were significantly decreased, and this effect was mitigated by concomitant exposure to 2-deoxy-D-glucose and vitamin E.

In a follow-up study, Ebrahim et al. (2001) further examined the potential protective properties of 2DG and vitamin E as well as taurine against membrane damage induced with a similar exposure paradigm. This study exposed male albino Swiss mice to the same doses used in the previous study with the addition of a taurine-exposed group (tetrachloroethylene in sesame oil 3,000 mg/kg-day for 15 days by oral gavage; tetrachloroethylene plus 2DG 500 mg/kg-day by

i.p. injection once a day for 15 days; tetrachloroethylene plus vitamin E 400 mg/kg-day by oral gavage once a day for 15 days; and tetrachloroethylene plus taurine 100 mg/kg-day by oral gavage once a day for 15 days). Compared to control cells in the liver, membrane-bound Na^+K^+-ATPases and Mg_2^+-ATPases activity was significantly decreased ($p < 0.001$), while Ca-ATPases activity was increased ($p < 0.001$), following exposure to tetrachloroethylene alone. These levels remained near normal in the animals exposed to tetrachloroethylene along with 2DG, vitamin E, or taurine. This return to normal levels following exposure to vitamin E and taurine may be due to their antioxidant abilities, and reduced oxidative stress in exposed cells.

A recent in vitro investigation examined tetrachloroethylene-induced gene expression changes in the HepG2 cultured human hepatoma cell line using an Affymetrix platform (Kawata et al., 2009). HepG2 cells retain Phase 1 and Phase 2 metabolic enzymes. Tetrachloroethylene (2 mM) altered the expression of 445 genes, of which, 367 were annotated in Gene Ontology terms to represent 261 biologic processes. The major processes included cell death, regulation of metabolic processes, phosphorylation, lipid biosynthesis, steroid metabolism, intracellular transport, DNA repair, and regulation of cell cycle. Based on KEGG pathway mapping, "cell cycle" and "MAPK signaling" pathways were prominent; a similar finding was reported for other chemicals (dimethyl nitrosamine and the phorbol ester 12-O-tetradecanoylphorbol-13-acetate) and metals (nickel, cadmium, and arsenic). The authors noted that this pathway has been shown to be activated by reactive oxygen species and metals in earlier studies (Guyton et al., 1996; Liu et al., 1996) and demonstrated that metal-induced gene changes associated with this pathway could be inhibited by vitamin C. Upregulation of the oncogene PTT1G was noted in all exposures. This hypothesis-generating in vitro experiment may aid in elucidating molecular pathway-based biomarkers of tetrachloroethylene.

4.3.5.5. Peroxisome Proliferator-Activated Receptor (PPAR) Activation Mode of Action

4.3.5.5.1. Description of hypothesized MOA

Another hypothesis is that tetrachloroethylene acts by a PPARα-agonism MOA in inducing mouse hepatocarcinogenesis. According to this hypothesis, the key events leading to tetrachloroethylene-induced liver tumor formation constitute the following: tetrachloroethylene metabolites (primarily the oxidative metabolite, TCA), after being produced in the liver, activate the PPARα receptor, which then causes alterations in cell proliferation and apoptosis, followed by clonal expansion of initiated cells. This MOA is assumed to apply only to the liver. This corresponds to the widely cited version of the hypothesized MOA for hepatocarcinogenesis induced by PPARα agonists posited by Klaunig et al. (2003), in which three key causal events were proposed: (1) activation of the receptor, (2) perturbation of hepatocellular apoptosis and proliferation, and (3) selective clonal expansion. A number of intermediary events were

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considered associative (e.g., expression of peroxisomal and nonperoxisomal genes, peroxisome proliferation, inhibition of gap junction intracellular communication, hepatocyte oxidative stress and Kupffer cell-mediated events). The data requirements suggested by Klaunig et al. (2003) for demonstrating that the PPARα-activation MOA is operative did not comprise all purportedly causal events; instead, these requirements included PPARα-agonism combined with microscopic evidence for peroxisome proliferation (or, in lieu of evidence of peroxisome proliferation, increased liver weight together with in vivo markers such as increases in peroxisomal β-oxidation, CYP4A, or acyl CoA oxidase). Alterations in proliferation and apoptosis were considered corroborative evidence.

4.3.5.5.2. Induction of hypothesized key events by tetrachloroethylene and metabolites

4.3.5.5.2.1. Activation of PPARα and associated markers

As summarized in Table 4-20, several in vivo studies have examined the effect of tetrachloroethylene on peroxisome proliferation or its markers (Philip et al., 2007; Odum et al., 1988; Goldsworthy and Popp, 1987). Odum et al. (1988) exposed groups of male and female F344 rats and B6C3F$_1$ mice by inhalation for 6 hours/day to 200 ppm (28 days only) or 400 ppm (for 14, 21, or 28 days) tetrachloroethylene. Five animals per group were exposed. In both sexes, hepatic PCO activity was increased in mice (up to 3.6-fold) and, to a lesser extent, in rats (up to 1.3-fold). Modest PCO increases were also observed in the kidney of male rats at 200 ppm at 28 days (1.3-fold) but not 400 ppm at 14, 21, or 28 days. In female rat kidney, PCO was elevated (approximately 1.6-fold) at all doses and times. However, peroxisome proliferation was not observed in rat kidney upon microscopy. In contrast, hepatic peroxisome proliferation was noted in all exposed mice on electron microscopy, and the percentage of cytoplasm occupied by peroxisomes also increased in mice. In rats, variable increases in peroxisome volume were noted at 200 ppm, but results lacked statistical significance. Catalase, another peroxisomal enzyme, was unaffected by tetrachloroethylene; male mice exposed at 400 ppm showed the only moderate (1.4-fold) increase. Mitochondrial proliferation was observed at 28 days in 400 ppm male mice. In addition, a time-dependent proliferation of smooth endoplasmic reticulum in the liver of both sexes correlated well with centrilobular hypertrophy. Tetrachloroethylene caused centrilobular lipid accumulation in male and female mice. Relative liver weight was increased in mice of both sexes.

Goldsworthy and Popp (1987) administered tetrachloroethylene (1,000 mg/kg-day) by corn oil gavage to 5 male F344 rats and 5 male B6C3F$_1$ mice for 10 days. In tetrachloroethylene-exposed rats, PCO was modestly—although not significantly—elevated in the liver (1.4-fold increase) and kidney (1.7-fold increase). In mice, tetrachloroethylene exposure increased PCO activity 4.3-fold in liver and by 2.3-fold in kidney. Relative liver

weight was increased in rats and mice with tetrachloroethylene exposure, but relative kidney weight was unaffected. A comparison of corn oil with methyl cellulose revealed no effect of the gavage vehicle on tetrachloroethylene-induced PCO. Administration of trichloroethylene (1,000 mg/kg) together with tetrachloroethylene had a less-than-additive effect on PCO induction.

Table 4-20. Rodent studies of induction of peroxisome proliferation or its markers by tetrachloroethylene

Species/strain/sex/number	Effect	Dose	Time
Rat, F344; and mouse, $B6C3F_1$; both sexes (5/group) Odum et al. (1988)	Mice of both sexes: increased relative liver weight, centrilobular lipid accumulation and peroxisome proliferation; increased PCO (up to 3.7-fold)	200 and 400 ppm, inhalation	14, 21, 28 d
	Male mice: mitochondrial proliferation	400 ppm, inhalation	28 d
	Rats of both sexes: increased PCO (up to 1.3-fold)	200 and 400 ppm, inhalation	14, 21, 28 d
Rat, F344 (male only, 5/group) and mouse, $B6C3F_1$ (male only, 5/group) Goldsworthy and Popp (1987)	Mice: Increased relative liver weight; 4.3-fold PCO increase	1,000 mg/kg-day for 10 d, corn oil gavage	10 d
	Rats: Increased relative liver weight; modest but not significant (1.4-fold) PCO increase	1,000 mg/kg-day for 10 d, corn oil gavage	10 d
Mouse, Swiss-Webster, male (4/group) Philip et al. (2007)	Increased plasma ALT	150, 500, and 1,000 mg/kg-day, aqueous gavage	24 hours to 14 d after initial exposure
	Mild-to-moderate fatty degeneration and necrosis, with focal inflammatory cell infiltration	150, 500, and 1,000 mg/kg-day, aqueous gavage	24 hours to 30 d after initial exposure
	Increased mitotic figures and DNA synthesis	150, 500, and 1,000 mg/kg-day, aqueous gavage	Peaked on 7 d, sustained at 14−30 d
	CYP4A increased at 7 but not 14 d, only at 1,000 mg/kg-day	1,000 mg/kg-day, aqueous gavage	7 but not 14 d

The peroxisome-related effects of tetrachloroethylene are most likely mediated primarily through TCA based on tetrachloroethylene metabolism producing more TCA than DCA, and the lower doses of TCA required to elicit a response relative to DCA. Bull (2004) and Bull et al. (2004) have recently suggested that peroxisome proliferation occurs at higher exposure levels than those that induce liver tumors for TCA and DCA. They report that a direct comparison of the no-effect level or low-effect level for induction of liver tumors in the mouse and several other endpoints shows that, for TCA, liver tumors occur at lower concentrations than peroxisome

proliferation in vivo but that PPARα-activation occurs at a lower dose than either tumor formation or peroxisome proliferation. A similar comparison for DCA shows that liver tumor formation occurs at a much lower exposure level than peroxisome proliferation or PPARα-activation. In vitro transactivation studies have shown that human and murine versions of PPARα are activated by TCA and DCA, while tetrachloroethylene itself is relatively inactive in the in vitro system, at least with mouse PPARα (Maloney and Waxman, 1999; Zhou and Waxman, 1998). In addition, Laughter et al. (2004) reported that the responses of ACO, PCO, and CYP4A induction by TCA and DCA were substantially diminished in PPARα null mice. Therefore, evidence suggests that tetrachloroethylene activates PPARα in vivo, and that the role of TCA in activating PPARα is likely to predominate at doses relevant to tetrachloroethylene-induced hepatocarcinogenesis.

4.3.5.5.2.2. Alterations of cell proliferation and apoptosis and clonal expansion of initiated cells

As discussed above, increased cell proliferation in mice has been reported following exposure to tetrachloroethylene. However, few data are available to inform the hypothesis that activation of PPARα after tetrachloroethylene exposure causes alterations in cell proliferation and apoptosis, followed by clonal expansion of initiated cells. Moreover, available data suggest that PPARα-activation may not be the predominant cause of the observed cell proliferative response. For example, transient increases in DNA synthesis and PCNA staining in the liver were reported by Philip et al. (2007), similar to that observed with other PPARα agonists (with the exception of WY-14,643, which induces sustained proliferation) (refer to Section 4.3.5.2.4.2). However, Philip et al. (2007) suggest that PPARα-activation is not required for the observed cell proliferative response, and rather that this is a regenerative response following cytotoxicity. This is based on evidence of significantly increased CYP4A expression at only the highest dose (1,000 mg/kg-day) and at the earliest time point (7 days), in contrast to the robust dose-dependent proliferative response of a more prolonged nature (lasting for 14−30 days post exposure) observed at the same and lower (150, 500 and 1,000 mg/kg-day) levels of tetrachloroethylene. The authors concluded that their findings suggest peroxisome proliferation is not a sustained response in spite of continued tetrachloroethylene exposure and, therefore, are not supportive of a close mechanistic relationship of carcinogenicity and PPARα induction for tetrachloroethylene-derived TCA. This interpretation is limited by the possible lack of sensitivity of CYP4A protein expression as a marker of peroxisome proliferation, and the lack of other supporting data for the observed absence of sustained peroxisome proliferation in the context of a robust regenerative proliferative response. Additionally, the sensitivity of the SW mouse to tetrachloroethylene hepatocarcinogenicity is unknown, somewhat limiting the significance of these findings for the interpretation of hepatocellular tumor findings in other

mouse strains. However, other studies of the toxicity of tetrachloroethylene in the $B6C3F_1$ strain discussed above [e.g., Schumann et al. (1980)] have reported liver toxicity and repair at 100 mg/kg-day, whereas Odum et al. (1988) reported only modest increases in peroxisomal markers in $B6C3F_1$ mice with repeated exposures to 1,000 mg/kg-day. Another noteworthy finding in Odum et al. (1988) was the modest increase in peroxisome proliferation observed in rats.

Data on TCA are also informative of the extent to which tetrachloroethylene alters cell proliferation and apoptosis through PPARα-activation, as it was concluded above that the PPARα-agonism following tetrachloroethylene is mostly likely caused by its metabolism to TCA. Data that inform the hypothesis that activation of PPARα after TCA exposure causes alterations in cell proliferation and apoptosis, followed by clonal expansion of initiated cells, are discussed in the EPA *Toxicological Review of Trichloroacetic Acid* (2011c). To summarize, several studies have observed hepatocyte proliferation in response to TCA in mice (DeAngelo et al., 2008; Stauber and Bull, 1997; Pereira, 1996; Dees and Travis, 1994; Sanchez and Bull, 1990). For instance, Dees and Travis (1994) observed relatively small (two- to threefold)—but statistically significant—increases in [^{3}H]thymidine incorporation in hepatic DNA in mice exposed for 11 days at TCA doses (100−1,000 mg/kg) that increased relative liver weight. Increased hepatic DNA labeling was observed at doses lower than those associated with evidence of necrosis, suggesting that TCA-induced cell proliferation is not due to regenerative hyperplasia. PPARα-null mice exposed to 2 g/L TCA in drinking water for 7 days do not show the characteristic responses of ACO, PCO, and CYP4A induction associated with PPARα-activation and peroxisome proliferation in wild-type mice (Laughter et al., 2004). In addition, the livers from wild-type—but not PPARα-null—mice exposed to TCA developed centrilobular hepatocyte hypertrophy, although no significant increase in relative liver weight was observed. Therefore, while there are data associating TCA exposure, PPARα-activation, and cell proliferation, it is not clear the extent to which PPARα-activation is the cause of the observed cell proliferation.

Data informing the hypothesis that PPARα-activation following tetrachloroethylene exposure causes clonal expansion of initiated cells, are limited to studies of its metabolite TCA. Mechanistic studies reveal that the mode of action for TCA hepatocarcinogenesis is complex and that TCA may induce tumors by multiple modes of action that may not be mutually exclusive (U.S. EPA, 2011c). In particular, tumor induction by TCA appears to involve perturbation of cell growth, reduced intercellular communication (Benane et al., 1996), release of cytokines and oxidants by activated Kupffer cells, and hypomethylation of DNA.

4.3.5.5.2.3. Conclusions regarding induction of hypothesized key events by tetrachloroethylene and metabolites

The available evidence from tetrachloroethylene and its metabolites supports the conclusion that tetrachloroethylene exposure leads to PPARα-activation predominantly through its metabolite TCA. There is more limited evidence supporting the hypothesis that PPARα-activation is the cause of the cell proliferative responses observed, and some evidence suggesting that PPARα-activation is not the cause of these responses. Data informing the hypothesis that PPARα-activation following tetrachloroethylene exposure causes clonal expansion of initiated cells are even more limited.

4.3.5.5.3. Are activation of PPAR α and its sequelae key events in tetrachloroethylene-induced hepatocarcinogenesis?

No tetrachloroethylene-specific data have directly tested the hypothesis that tetrachloroethylene-induced PPARα-activation, along with its sequelae, are key or causative events in tetrachloroethylene-induced hepatocarcinogenesis (e.g., bioassays with knockout mice or involving the blocking of hypothesized key events). With respect to more associative data, Philip et al. (2007) found increases in CYP4A, a marker for PPARα-activation, to be transient (only increased at 7 days) rather than sustained, and only occurring at the highest dose (1,000 mg/kg-day). These data are not supportive of PPARα-activation as a key event in tetrachloroethylene-induced hepatocarcinogenesis for two reasons: (1) chronic activation would be needed to sustain changes in cell proliferation, apoptosis, and clonal expansion, and (2) statistically significant increases in liver tumors have been reported at doses around 500 mg/kg-day (NCI, 1977), at which no increased CYP4A activity was reported. However, the SW strain of mouse used by Philip et al. (2007) may differ in tumor responsiveness from those used in the cancer bioassays discussed above.

Support for this MOA is based primarily on the hypothesis that TCA induces tumors through PPARα-activation, and the fact that TCA is formed after in vivo exposure to tetrachloroethylene. The experimental evidence related to the hypothesis that TCA induces tumors through PPARα-activation is discussed extensively in the EPA *Toxicological Review of TCA* (U.S. EPA, 2011c). TCA activates PPARα, and induces peroxisome proliferation and hepatocyte proliferation. However, a number of inconsistencies and data gaps reduce the confidence in the conclusion that TCA induces hepatocarcinogenesis solely through a PPARα-activation MOA. First, while TCA induces peroxisome proliferation (a marker for PPARα-agonism) in both rats and mice, to date, TCA has been shown to be tumorigenic in $B6C3F_1$ mice but not F344 rats (DeAngelo et al., 1997) (the only strains tested for carcinogenicity). In addition, the tumor phenotype of TCA-induced mouse liver tumors has been reported to have a different pattern of H-ras mutation frequency from DCA and other peroxisome proliferators

(Bull et al., 2002; Stanley et al., 1994; Hegi et al., 1993; Fox et al., 1990). Other effects of TCA, including increased *c-myc* expression and hypomethylation of DNA, are not specific to the PPARα-activation MOA, and other data (discussed below in Section 4.3.4.2.4) also contribute uncertainty as to whether PPARα independent mechanisms may be involved in TCA-induced tumors in mice.

To summarize, based on data from tetrachloroethylene and its metabolites alone, there is only limited evidence that activation of PPARα and its sequelae are key events in tetrachloroethylene-induced hepatocarcinogenesis. In all, the modest peroxisome proliferation observed in response to tetrachloroethylene may lack specificity and consistency with respect to tissue, species, and dose, and studies of the temporal sequence of events are limited. Given the limitations in the database of tetrachloroethylene-specific studies, it can be concluded that the few studies demonstrating activation of PPARα and related markers by tetrachloroethylene are insufficient to demonstrate a causative role of this effect in the induction of other key events posited for the PPARα mode-of-action hypothesis, and for hepatocarcinogenesis by tetrachloroethylene.

4.3.5.5.4. Other experimental evidence for the hypothesized MOA

4.3.5.5.4.1. Evidence from PPARα-null mouse bioassays

An apparent reduction was observed in tumor response to an 11-month exposure to the prototypical agonist 4-chloro-6-(2,3-xylidino)-2-pyrimidyl-thio]acetic acid (Wy-14,643) in PPARα-null mice in comparison to wild-type mice (Peters et al., 1997). Peters et al. reported the absence of tumors in nine PPARα-null mice exposed to Wy-14,643 at 11 months, whereas each of the six similarly exposed wild-type mice had multiple hepatocellular neoplasms.

As has also has been shown for Wy-14,643, the monoester metabolite (mono-2-ethylhexylphthalate, MEHP) of DEHP activates PPARα in vitro (Maloney and Waxman, 1999; Issemann and Green, 1990). Other evidence for DEHP includes induction of peroxisome proliferation (or an increase in peroxisomal enzyme activity), an associative event in the MOA, by tumorigenic doses of DEHP in the liver of mice and rats and of MEHP in rat hepatocytes (David et al., 1999; Hasmall et al., 1999; Reddy et al., 1986; Mitchell et al., 1985; Mitchell et al., 1984; Gray et al., 1983; Gray et al., 1982). Additionally, an absence of peroxisomal enzyme induction and peroxisome proliferation in PPARα-null mice exposed to DEHP for 24 weeks was demonstrated (Ward et al., 1998).

However, as reviewed recently by Guyton et al. (2009), a 2-year bioassay found that DEHP (100 or 500 ppm) induces liver tumors in PPARα-null mice (Ito et al., 2007a). Ito et al. (2007a) reported a significant trend for the observed increase in total liver tumors with DEHP in PPARα-null male mice with Sv/129 genetic background generated as described in Lee et al.

(1995). Guyton et al. (2009) performed additional statistical analyses to compare the Ito et al. (2007a) results with those of a prior DEHP bioassay in B6C3F$_1$ wild-type mice (David et al., 1999). A pair-wise analysis found that DEHP (500 ppm) significantly increased adenomas in PPARα-null—but not in companion wild-type—mice compared to their respective controls (refer to Figure 4-3, single asterisks). In the David et al. study of B6C3F$_1$ mice, DEHP (500 ppm) also significantly increased adenomas and adenomas plus carcinomas (refer to Figure 4-3B, single asterisks). Moreover, a significant dose-response trend for adenomas and for adenomas plus carcinomas was observed in both the Ito et al. (2007a) PPARα-null mice and the David et al. B6C3F$_1$ mice after exposure to DEHP (refer to Figure 4-3B, double asterisks). Additionally, Guyton et al. (2009) found no statistically significant differences between groups at the same dose, including controls, consistent with mouse strain and PPARα genotype having no influence on carcinogenicity under the study conditions.

The observed lack of difference in reported control incidences across groups lends support to the approach of basing comparative analyses on concurrent controls. Historical data on spontaneous liver tumor incidences in PPARα-null mice are limited; Ito et al. (2007a) is the largest published 2-year bioassay in PPARα-null mice, reporting findings for 24/25 surviving unexposed animals at 23 months of age. A different laboratory that had established a distinct breeding colony reported mouse liver tumor incidences in 12 PPARα-null Sv129/C57BL/6 mice ~2 years of age (Howroyd et al., 2004). Adenomas and carcinomas were reported in 6/12 and 2/12 PPARα-null mice, respectively, compared with adenomas in 5/22 wild-type animals. As Howroyd et al. note, "The relatively small number of animals available made it difficult to draw robust conclusions concerning enhancement of spontaneous findings in PPARα-null mice." In addition, cross-laboratory differences [particularly the low survival of PPARα-null mice in the Howroyd et al. relative to the Ito et al. (2007a) study] limit statistical comparisons based on this data set.

In summary, the Ito et al. (2007a) study indicates that DEHP carcinogenesis can occur independently of PPARα-activation. As noted in a recent National Research Council report on risk assessment (NRC, 2008), this finding "calls into question" the 2000 IARC conclusions regarding the carcinogenic risks of DEHP (IARC, 2000). The 2011 IARC Working Group evaluating DEHP also concluded that "the human relevance of the molecular events leading to DEHP-induced cancer in several target tissues (e.g., liver and testis) in rats or mice could not be ruled out, resulting in the evaluation of DEHP as a Group 2B agent, rather than Group 3" (Grosse et al., 2011). Although new hypotheses are being generated based on more detailed comparisons between wild-type and PPARα-null mice (Eveillard et al., 2009; Takashima et al., 2008; Ito et al., 2007a), the available data indicate that the mechanisms of cancer induction by DEHP are complex.

4.3.5.5.4.2. Quantitative analyses of hypothesized key events and carcinogenic potency

If potency for PPARα-activation or its attendant sequelae is quantitatively associated with carcinogenic activity or potency, then it might be possible to predict differences in sensitivity for carcinogenesis (such as may occur across species) for environmental contaminants that activate PPARα (e.g., certain phthalates and chloroacetic acids) using quantitative information about the key events alone. It is, thus, of interest to assess whether potency for inducing these events is quantitatively related to hepatocarcinogenic potential by these and other compounds that also activate PPARα. However, there are limitations in the dose-response data available for such analyses, specifically for precursor events in the proposed PPARα-activation MOA as well as for liver tumor induction. Most tumor data, including for the best characterized PPARα agonists, are for exposure concentrations inducing well above 50% tumor incidence, with less-than-lifetime administration. Precursor events have typically been studied at a single dose, often eliciting a near maximal response, thus, precluding benchmark-based comparisons across studies. This is especially true for Wy-14,643, which has been administered most often at only one exposure concentration (1,000 ppm) that elicits a 100% tumor incidence after 1 year or less (Peters et al., 1997) and that also appears to be necrogenic (Woods et al., 2007). On the other hand, hypothesized precursor events such as hepatomegaly, peroxisome proliferation, and increased DNA synthesis appear to have reached their maximal responses at 50 ppm Wy-14,643, with some statistically significant responses as low as 5 ppm (Marsman et al., 1992; Wada et al., 1992). Potencies across compounds have rarely been compared in a single study using the same experimental paradigm. These deficits in the database notwithstanding, provided below is an assessment of the quantitative predictive power of the potency for four proposed data elements for establishing the hypothesized MOA for hepatocarcinogenesis: (1) PPARα-activation in mice; and (2) hepatomegaly, (3) DNA synthesis, and (4) increased peroxisome proliferation in rats.

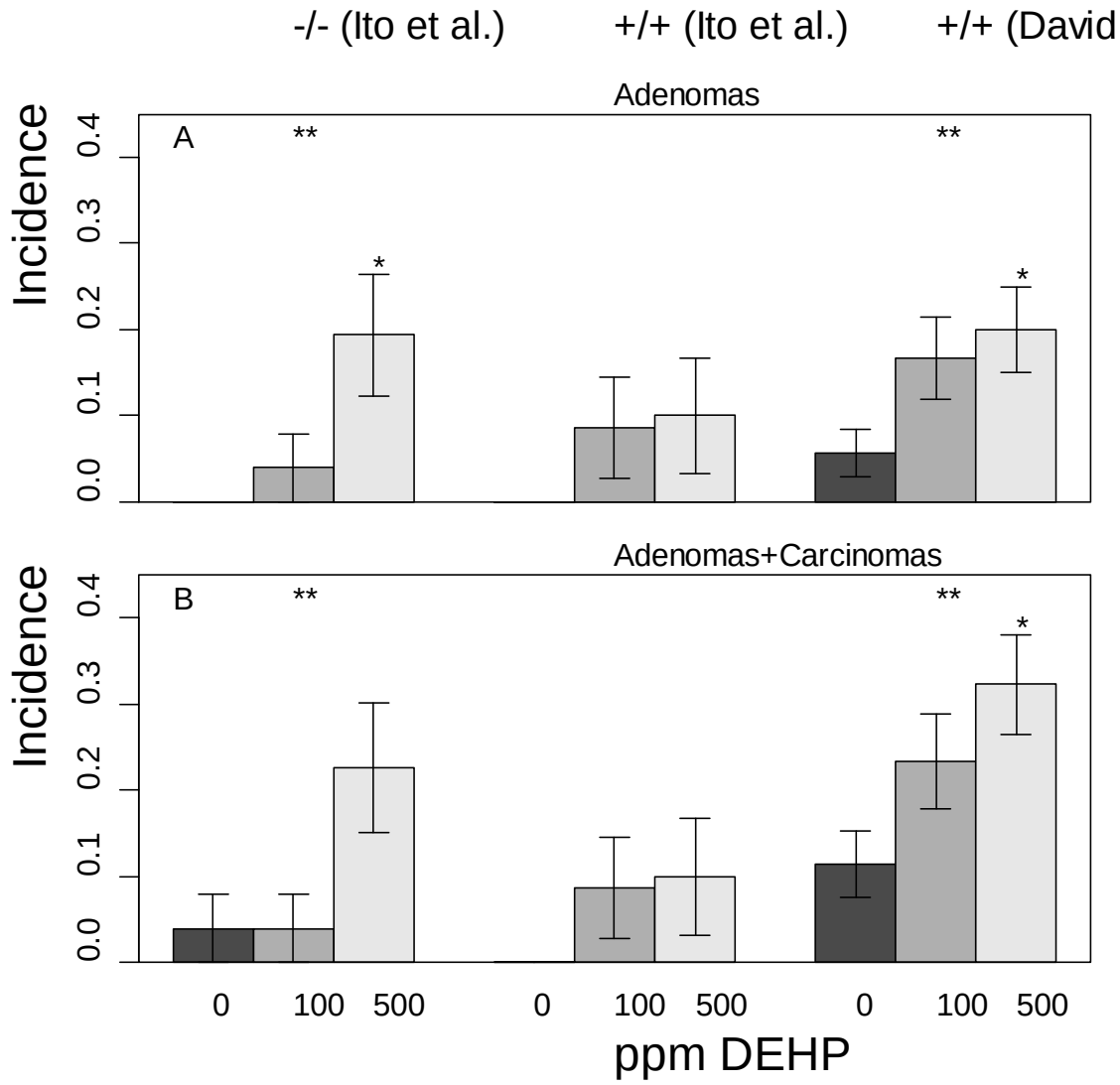

Figure 4-3. Incidences of hepatocellular adenomas (A) and hepatocellular adenomas and carcinomas (B) in mice exposed to DEHP.
Ito et al. (2007a) exposed PPARα null [-/-] and wild-type [+/+] Sv/129 mice for 22 months; David et al. (1999) exposed B6C3F1 wild-type [+/+] mice for up to 104 weeks. Data are presented as incidence +/- SD assuming a binomial distribution for each group. Single asterisks (*) indicate a significant difference from controls of the same genotype in the same study (Fisher's exact test, $p < 0.05$). Double asterisks (**) indicate a significant trend with dose in the study (Cochran Armitage test, $p < 0.05$). All pair-wise cross-study comparisons between like dose groups (e.g., Ito et al. [-/-] 500 ppm compared with David et al. [+/+] 500 ppm) were not significant (Fisher exact test $p > 0.05$). Because David et al. (1999) reported only adenomas and carcinomas, the cholangiocellular carcinoma reported by Ito et al. (2007a) in DEHP-exposed PPARα null mice was excluded from analyses. Adapted from Guyton et al. (2009).

4.3.5.5.4.2.1 *PPARα-activation in mice*

Table 4-21 presents data for four peroxisome proliferators in order of decreasing potency for inducing mouse liver tumors. These compounds were selected because of their importance to environmental human health risk assessments and because data to derive receptor activation potency indicators were available from a single study (Maloney and Waxman, 1999). The transactivation potencies of MEHP, Wy-14,643, dichloroacetic acid (DCA), and TCA for the mouse PPARα were monitored using a luciferase reporter gene containing multiple PPAR response elements derived from the rat hydratase/dehyrogenase promoter in transiently transfected COS-1 monkey kidney cells. The derived potency indicators were compared to the TD_{50} (i.e., the daily dose inducing tumors in half of the mice that would otherwise have remained tumor-free) from the Carcinogenic Potency Database (CPDB) of Gold et al. (2005). Note that for Wy-14,643, the dose listed yielded a maximal response and, thus, represents an upper limit to the TD_{50} (indicated by "<"). Two estimates of PPARα transactivation potency are given, the first based on 50% of the maximal response (i.e., EC_{50}) and the second based on the effective concentration required for a twofold increase in activity (i.e., $EC_{2\text{-fold}}$) (Maloney and Waxman, 1999). Because unmetabolized DEHP does not exhibit PPARα activity, the transactivation activity of its metabolite MEHP is given but compared to the hepatocarcinogenic potency indicator for DEHP. In addition, unmetabolized tetrachloroethylene does not exhibit PPARα activity, so is not included in the table. No data on the potency for transactivation of rat PPARα by chemicals in the CPDB were located to enable a similar comparison in rats.

These data clearly show a lack of correlation between the potencies for in vitro PPARα transactivation and in vivo tumorigenesis across different PPARα agonists. Especially notable is that MEHP exhibited orders of magnitude more potency for transactivating mouse PPARα than DCA, but DEHP was sixfold less potent as a mouse hepatocarcinogen. TCA was more similar in potency to DCA for both outcomes, i.e., was also dramatically less active at transactivating PPARα than DEHP despite exhibiting comparable hepatocarcinogenic potency. Wy-14,643 and MEHP activate PPARα at comparable concentrations when directly compared in the transactivation assay, but the carcinogenic potency of Wy-14,643 was estimated to be at least 70-fold higher than DEHP. This difference cannot be explained by pharmacokinetics (Kessler et al., 2004; Pollack et al., 1985). Possible explanations for these results include one or more of the following: (1) the transactivation assay is not an accurate quantitative indicator of in vivo receptor activation, (2) the rate and nature of effects downstream of PPARα-activation depends on the ligand or, (3) there are rate-limiting events independent of PPARα-agonism that contribute to mouse hepatocarcinogenesis by the agonists examined.

Table 4-21. Potency indicators for mouse hepatocarcinogenicity and in vitro transactivation of mouse PPARα for four PPARα agonists[a]

Chemical	Carcinogenic potency indicators (mg/kg-day)	Transactivation potency indicators (μM)	
	TD_{50}	EC_{50}	$EC_{2\text{-fold}}$
Hepatocarcinogens			
Wy-14,643	<10.8	0.63	~0.4
DCA	119	~300	~300
TCA	584	~300	~300
DEHP/MEHP	700	~0.7	~0.7

[a] TD_{50}, the daily dose inducing tumors in half of the mice that would otherwise have remained tumor-free, estimated from the Carcinogenic Potency Database (Gold et al., 2005). EC_{50}, the effective concentration yielding 50% of the maximal response; $EC_{2\text{-fold}}$, the effective concentration required for a twofold increase in activity. Transactivation potencies were estimated from Maloney and Waxman (1999). The "<" symbol denotes an upper limit due to maximal response. A "~" symbol indicates that the transactivation potency was approximated from figures in Maloney and Waxman (1999).

Adapted from Guyton et al. (2009).

4.3.5.5.4.2.1 *Hepatomegaly, DNA synthesis, and peroxisome proliferation in rats*

Table 4-22 compares potency indicators for various precursor effects at the TD_{50} for four PPARα agonists and rat hepatocarcinogens. The analysis of whether there are consistent levels of in vivo precursor effect induction across peroxisome proliferators at the TD_{50} does not include all of the data from a similar, prior analysis by Ashby et al. (1994) for several reasons. First, unlike the CPDB, Ashby et al. did not adjust carcinogenicity data for less-than-lifetime dosing, which is relevant for most compounds. Second, for those mouse carcinogens reported in the CPDB, only acute data are available regarding DNA synthesis effects from Ashby et al. Therefore, this analysis was restricted to rat precursor and potency data for the four compounds Wy-14,643, nafenopin, clofibrate, and DEHP and included both 1-week and 13-week data to separately address transient and sustained changes in DNA synthesis. Even for this small set of compounds, several limitations in the rat database were apparent. Because no single study provided comparative data for the precursor endpoints of interest, four separate reports were used. In the Wada et al. (1992) and Tanaka et al. (1992) studies of Wy-14,643 and clofibrate, respectively, administered doses were within 10% of the TD_{50}. However, nafenopin data were only available at a single dose of 500 ppm (Lake et al., 1993), which was linearly interpolated to the TD_{50}. The highest administered dose of DEHP was 12,500 ppm (David et al., 1999), a dose

notably below the TD_{50}, and, thus, a lower limit based on the assumption of monotonicity with dose is shown. A further data limitation is that in the CPDB, only the TD_{50} for one of the four compounds—DEHP—incorporates data from studies administering more than one dose for 2 years.

The results shown in Table 4-22 indicate that potency for the occurrence of short-term in vivo markers of PPARα-agonism varies widely in magnitude and lacks any apparent correlation with carcinogenic potency. Such differences have been noted previously. Similar to the results presented in Table 4-22, Marsman et al. (1988) noted that although DEHP (12,000 ppm) and Wy-14,643 (1,000 ppm) induced a similar extent of hepatomegaly and peroxisome proliferation (measured either morphologically or biochemically) after 1 year, the frequency of hepatocellular lesions was over 100-fold higher in Wy-14,643 relative to DEHP-exposed rats. In addition, a higher labeling index was reported for 12,500 ppm DEHP than the maximal level attained after 50 to 1,000 ppm Wy-14,643 (David et al., 1999; Tanaka et al., 1992; Wada et al., 1992). Such differences in response with dose and time observed among PPARα agonists are prominent enough to prevent displaying dose-response data on a common scale. For instance, labeling differences in maximal responses were not examined in this analysis.

Table 4-22. Potency indicators for rat hepatocarcinogenicity and common short-term markers of PPARα-agonism for four PPARα agonists[a]

Chemical	Tumor TD_{50} (ppm in diet)	Fold-increase over control at tumor TD_{50}					
		1 wk			13 wk		
		RLW	LI	PCO	RLW	LI	PCO
Wy-14,643	109	1.8	12	13	2.6	6.8	39
Nafenopin	275	1.4	3.6	7.6	1.5	1.12	6.7
Clofibrate	4,225	1.4	4.4	4.2	1.4	0.95	3.7
DEHP	17,900	≥1.4	≥19	≥3.6	≥1.9	≥1.25	≥4.9

[a]For ease of comparison with precursor effect studies, administered doses for the tumor TD_{50}s in the Carcinogenic Potency Database were back-converted to equivalent ppm in diet using the formula of Gold et al. (2005), i.e., TD_{50} (mg/kg-day) = TD_{50} (ppm in diet) * 0.04 (for male rats). Administered doses for precursor data on Wy-14,643 (Wada et al., 1992) and clofibrate (Tanaka et al., 1992) were within 10% of the TD_{50}. Because nafenopin precursor data were only available at 0 and 500 ppm (Lake et al., 1993), these doses were linearly interpolated to the TD_{50}. Because the highest administered dose of DEHP in precursor effect studies was 12,500 ppm (David et al., 1999), a lower limit is shown, based on the assumption of monotonicity with dose. RLW = relative liver weight, LI = labeling index, PCO = cyanide insensitive palmitoyl CoA oxidation.

Adapted from Guyton et al. (2009).

Together, these findings underscore the significant chemical-specific quantitative differences in these markers that limit their utility for predicting carcinogenic dose-response relationships.

4.3.5.5.4.3. Evidence from transgenic animals

Data from transgenic animals suggest the key events in the hypothesized MOA—PPARα-activation, hepatocellular proliferation, and clonal expansion—are not sufficient to cause tumors. This suggests that other events not mediated by PPARα-activation, either independently or in combination with PPARα-activation, are necessary to induce tumors. The discussion below is based on the review by Guyton et al. (2009).

Yang et al. (2007) raises questions regarding whether PPARα-activation in hepatocytes is causally linked to hepatocarcinogenesis as a sole operant MOA. The experimental approach entailed fusing the mouse PPARα to the potent viral transcriptional activator VP16 under control of the liver enriched activator protein (LAP) promoter, resulting in targeted constitutive expression of activated PPARα in hepatocytes. In LAP-VP16PPARα transgenic mice, ligand-independent hepatocyte PPARα-activation evoked many of the same hepatic responses (in type and magnitude) as observed with PPARα ligand treatment of companion wild-type 129/Sv mice. For instance, DNA synthesis was increased in LAP-VP16PPARα transgenic mice; the effect was persistent and still evident at 11 months of age. In addition, increases were reported in markers of peroxisome proliferation (including increases in expression of peroxisomal membrane protein 70, acyl CoA oxidase and CYP4A family genes, and enhanced cyanide insensitive palmitoyl CoA oxidation). Other effects included an increase in cell-cycle genes (cyclin D1 and cyclin-dependent kinases 1 and 4) and a decrease in serum triglycerides and free fatty acids. Together, these results are consistent with the view that PPARα-activation and its sequelae are alone sufficient to induce increased hepatocyte DNA synthesis and peroxisome proliferation.

However, constitutive PPARα-activation in hepatocytes in the LAP-VP16PPARα transgenic mouse model was not sufficient to induce several important hepatic responses stimulated by PPARα ligand treatment of wild-type mice. Notably, no preneoplastic hepatic lesions or hepatocellular neoplasia were found in ">20 LAP-VP16PPARα mice at the age of over 1 year" (Yang et al., 2007). In sharp contrast, wild-type mice exposed to the PPARα agonist Wy-14,643 for 11 months developed grossly visible lesions consistent with previous reports of its hepatocarcinogenicity [e.g., Peters et al. (1997)]. Interestingly, nonparenchymal cell proliferation was observed with Wy-14,643 exposure of wild-type mice but was absent in the LAP-VP16PPARα transgenic mice. In addition, although liver weight was increased in LAP-VP16PPARα transgenic mice, the extent of hepatomegaly was reduced in comparison to Wy-14,643-exposed wild-type mice, and hepatocellular hypertrophy was absent.

Thus, the Yang et al. (2007) study provides evidence that, by itself, PPARα-activation (and its sequelae) is not sufficient to induce hepatocarcinogenesis. These data are, therefore, inconsistent with the hypothesis that effects mediated through PPARα-activation constitute a complete MOA for carcinogenesis. Notably, key events in the proposed MOA such as the robust and sustained elevation in hepatocyte proliferation (evidenced by enhanced DNA synthesis), accompanied by enzyme changes commonly associated with peroxisome proliferation, did not evoke hepatocarcinogenesis. In fact, a comparable extent of sustained increases in hepatocyte DNA synthesis was observed with constitutive PPARα-activation in the LAP-VP16PPARα transgenic mouse model and Wy-14,643 exposure in wild-type mice, but only the latter developed liver tumors under comparable experimental paradigms.

4.3.5.5.5. Rationale for species differences

Toxicodynamic differences across species, including in the absolute or allometrically scaled amount or activity of the receptor, may contribute to differences in sensitivity of response to PPARα agonists. Absolute levels of PPARα are generally thought to be lower in human compared with rodent liver. However, PPARα amount varies by an order of magnitude among individuals (Palmer et al., 1998; Tugwood et al., 1996), e.g., 1 of the 6 human samples examined expressed levels comparable to the mouse in one study (Walgren et al., 2000). The pattern of PPARα expression across tissues also differs across species (Melnick, 2001; Tugwood et al., 1996), e.g., human levels are higher in kidney and skeletal muscle than in liver, while the highest rodent levels are in liver and kidney. In addition, considerable interindividual variation in PPARα structure and function among humans has been reported (Tugwood et al., 1996), and polymorphisms have been shown to increase or decrease receptor levels and to modulate baseline lipid and apolipoprotein levels, atherosclerotic progression, and the presence of diabetes mellitus and insulin resistance (Tanaka et al., 2007; Tai et al., 2006; Flavell et al., 2005; Foucher et al., 2004; Flavell et al., 2002; Jamshidi et al., 2002). An impact of PPARα polymorphisms on preexisting disease status and response to PPARα agonists is also suggested from bezafibrate [2-(4-(2-[(4-chlorophenyl)formamido]ethyl)phenoxy)-2-methylpropanoic acid] and gemfibrozil [5-(2,5-dimethylphenoxy)-2,2-dimethyl-pentanoic acid] trials (Tai et al., 2006; Jamshidi et al., 2002).

The human PPARα is functional in in vitro transactivation assays and is responsive to a number of PPARα agonists (e.g., nafenopin, clofibrate, and WY-14,643) (Maloney and Waxman, 1999; Mukherjee et al., 1994; Sher et al., 1993). Compared with the mouse PPARα, human PPARα is suggested to be 10- to 20-fold less responsive to Wy-14,643 (Maloney and Waxman, 1999; Palmer et al., 1998; Mukherjee et al., 1994). However, this magnitude of interspecies difference has not been demonstrated for other compounds. Hurst and Waxman

(2003) reported a fivefold lower sensitivity to the DEHP metabolite MEHP of human—compared with mouse—PPARα (EC_{50} = 3.2 μM vs. 0.6 μM) in transfected COS-1 monkey kidney cells, but acknowledged that they could not quantify the relative amount of each receptor. Using a similar experimental paradigm, Wolf et al. (2008) found an approximately twofold lower slope of the dose-response curve for activation of human—compared with mouse—PPARα for perfluorooctanoic acid and other perfluoroalkyl acids. For other PPARα agonists, including TCA and DCA, little (<twofold) or no species difference in receptor transactivation sensitivity was evident (Maloney and Waxman, 1999). Some compounds appear to more efficiently activate human compared with rodent PPARα, as was demonstrated for the synthetic polyunsaturated fatty acid 5,8,11,14-eicosatetraynoic acid in transfected human liver cancer HepG2 cells (Mukherjee et al., 1994) and for perflourobutane sulfonate in transfected COS-1 monkey kidney cells (Wolf et al., 2008).

Using adenovirus expression in PPARα null mice, Yu et al. (2001) also found little (<twofold) or no difference between the mouse and human receptor in terms of induction of in vivo markers of peroxisome proliferation. Wy-14,643, ciprofibrate, DEHP and nafenopin enhanced mRNA and protein levels of peroxisomal genes regardless of whether the human or mouse PPARα was expressed (Yu et al., 2001). Transgenic mice stably expressing human PPARα in the liver only (Morimura et al., 2006; Cheung et al., 2004) or in all tissues (Yang et al., 2008) exhibit increases in both DNA synthesis (with Wy-14,643) and hepatomegaly (with Wy-14,643 and fenofibrate [propan-2-yl 2-(4-[(4-chlorophenyl)carbonyl]-phenoxy)2-methylpropanoate]). However, these increases were much diminished from the response in wild-type mice and lacked statistical significance due largely to the small number of animals studied (n = 5 to 9). With regard to mouse liver tumor induction, Wy-14,643 (1,000 ppm) exposure for up to 44 weeks induced one liver adenoma in 20 PPARα-humanized mice while none were observed in 10 untreated animals (Morimura et al., 2006); in comparison, Wy-14,643 (1,000 ppm) caused lethality in 5/10 wild-type mice at 38 weeks and tumors in the 5 surviving animals. These findings are suggestive of differential sensitivity of humanized mice to Wy-14,643. However, the accuracy of estimates of the extent of this difference is limited by the short exposure duration, the substantial mortality and morbidity in wild-type mice, the small number of animals studied, and potential differences in the interaction of the human receptor with mouse-specific coactivators and response elements.

Several key or associative events in the hypothesized MOA have been observed directly in some but not all primate studies (Ito et al., 2007b; Hoivik et al., 2004; Kurata et al., 1998). Studies of cultured primary human hepatocytes have generally reported little or no proliferative response to peroxisome proliferators [for reviews, refer to Ashby et al. (1994), Peters et al. (2005), and Rusyn et al. (2006)]. The culture conditions, including lack of cocultured

nonparenchymal cells (e.g., Kupffer cells), may limit the in vitro hepatocyte proliferative response, as observed for other species [e.g., Parzefall et al. (2001)]. The extent of peroxisome proliferation in human liver following exposure to fibrate drugs (e.g., with clofibrate, gemfibrozil, or fenofibrate) or dialysis treatment (possibly due to DEHP exposure) is reported to be generally less than the rodent response (Ganning et al., 1987; Gariot et al., 1987; Ganning et al., 1984; Blümcke et al., 1983; Hanefeld et al., 1983; De La Iglesia et al., 1982; Hanefeld et al., 1980). However, the ability to quantitatively characterize human sensitivity to this effect is limited (e.g., by the small number of subjects studied).

In sum, despite notable qualitative similarities, quantitative differences in receptor activation and the subsequent events in the hypothesized MOA are evident across species. The magnitude of these differences has been best characterized for Wy-14,643, to which rodents appear to have 10-fold or more greater sensitivity for response (Morimura et al., 2006; Cheung et al., 2004; Yu et al., 2001; Maloney and Waxman, 1999; Palmer et al., 1998; Mukherjee et al., 1994). Although more limited, studies of other agonists suggest a smaller magnitude of difference in sensitivity for response across species than is observed for Wy-14,643 (Hurst and Waxman, 2003; Yu et al., 2001; Maloney and Waxman, 1999). Considerable interindividual variation in PPARα amount, structure, and function has been reported among humans (Tugwood et al., 1996), and some studies have suggested variability in human response to PPARα agonists (Tai et al., 2006; Jamshidi et al., 2002). However, few studies have examined directly how these factors may affect sensitivity—as well as the potential for heterogeneity of response—to hepatocarcinogenesis induced by PPARα agonists in humans.

Another consideration is whether human epidemiologic data on fibrates offer an indirect test of the PPARα-activation MOA hypothesis. Human exposures to exogenous and endogenous PPARα agonists encompass a broad group of chemicals, including environmental contaminants known to activate the receptor, as well as a number of therapeutic agents whose molecular target is one or more receptors in the PPAR family. Indeed, fibrate drugs were developed using rodent models to treat hyperlipidemia in humans before the receptor was identified. These agents have varying degrees of affinity for PPARα (Shearer and Hoekstra, 2003), and some have multiple mechanisms of action. Drugs that have PPARα agonist activity include fibrates or fibric acid derivatives (which are primarily PPARα agonists), bezafibrate (which also shows PPARγ activity), dual PPARα/γ agonists currently under development, the glitazones, and nonsteroid anti-inflammatory drugs (e.g., ibuprofen) (Sertznig et al., 2007).

Some human data on PPARα agonist effects are available from fibrate clinical trials and population case-control studies of site-specific cancer (Freeman et al., 2006; Tenkanen et al., 2006; Keech et al., 2005; Diabetes Atherosclerosis Intervention Study Investigators, 2001; Meade and clinics, 2001; BIP Study Group, 2000; Rubins et al., 1999; Frick et al., 1997; de Faire

et al., 1995; Huttunen et al., 1994; Rubins et al., 1993; Frick et al., 1987; Canner et al., 1986; WHO, 1984, 1980, 1978; Coronary Drug Project Research Group, 1977, 1975). These studies examined a range of human responses to PPARα agonists, which included atherosclerosis, cardiovascular disease, serum biomarkers of fatty acid metabolism, acute toxicity, and, more limitedly, organ-specific chronic toxicity, including cancer. However, examination of hepatotoxicity in the fibrate clinical trials has been limited to alterations in hepatic metabolic pathways and changes in liver enzymes as assessments of drug tolerance, because the primary focus of these trials was cardiovascular events.

Reviews of the PPARα-activation MOA hypothesis have generally focused on liver cancer response in two fibrate clinical trials, the Helsinki Heart Study (Tenkanen et al., 2006; Huttunen et al., 1994; Frick et al., 1987) and the World Health Organization's Cooperative Trial on Primary Prevention of Ischemic Heart Disease (WHO, 1984, 1980, 1978), and have concluded that, while limited, those data did not provide evidence of an increased liver cancer risk from fibrate exposure (Klaunig et al., 2003; Ashby et al., 1994). However, the available studies have low power to detect statistical differences in the risk of liver cancer; an estimated five or fewer liver cancer deaths would have been expected in these studies using data from the National Cancer Institute's Surveillance, Epidemiology, and End Results database (Ries et al., 2008). This low statistical power, in addition to the studies' exclusion or removal of subjects showing signs of liver (or other) toxicity from treatment, precludes a strong conclusion about the presence or lack of liver cancer risk. These studies and the other fibrate trials did not examine site-specific causes of mortality or morbidity and did not follow subjects for a sufficient period to adequately consider cancer latency; in addition, placebo subjects were offered fibrate therapy at the end of the clinical trials, making analyses after further follow-up difficult to interpret. For example, the three trials that did assess mortality after a follow-up period longer than 10 years included liver cancers in a larger category of contiguous sites or in the category of all cancers, introducing disease misclassification and a downward bias for any site-specific treatment-related cancers (Tenkanen et al., 2006; Huttunen et al., 1994; Canner et al., 1986; WHO, 1984, 1980, 1978). In voluntary postmarketing safety reports to the U.S. Food and Drug Administration (FDA), rates of liver adverse event reports for gemfibrozil and fenofibrate (2.6 and 6.9 per 1,000,000 prescriptions, respectively) were similar to that of statins (Holoshitz et al., 2008). However, an examination of liver cancer is precluded by the general under-reporting of chronic toxicities to FDA, and the lack of specific FDA reporting requirements for cancer, even premarketing. Because of these inadequacies, the available epidemiologic data for fibrate drugs cannot inform conclusions about the relevance of PPARα-activation to human cancer.

4.3.5.6. Mode of Action Conclusions for Hepatocellular Tumors

There is only limited experimental support for the position that tetrachloroethylene-induced hepatocarcinogenesis is mediated solely by the hypothesized PPARα-activation MOA. Chemical-specific data for PPARα-activation support the view that this is not the primary MOA for hepatocarcinogenesis. Philip et al. (2007) suggest that PPARα-activation is not required for the observed cell proliferative response. This is based on evidence of significantly increased CYP4A expression at only the highest dose (1,000 mg/kg-day) and at the earliest time point (7-days), in contrast to the robust dose-dependent proliferative response of a more prolonged nature (lasting for 14−30 days post exposure) observed at the same and lower (150, 500, and 1,000 mg/kg-day) levels of tetrachloroethylene. The authors concluded that their findings suggest peroxisome proliferation is not a sustained response in spite of continued tetrachloroethylene exposure and, therefore, are not supportive of a close mechanistic relationship of carcinogenicity and PPARα induction for tetrachloroethylene-derived TCA. Limitations of this interpretation include the possible lack of sensitivity of CYP4A protein expression as a marker of peroxisome proliferation, and the unknown sensitivity of the SW mouse to tetrachloroethylene hepatocarcinogenicity. However, other investigators [e.g., Schumann et al. (1980)] have reported liver toxicity and repair at 100 mg/kg-day in the $B6C3F_1$ strain, whereas repeated exposures to 1,000 mg/kg-day were reported by Philip et al. (2007) and Odum et al. (1988) to only modestly increase peroxisomal markers in SW and $B6C3F_1$ mice, respectively. Odum et al. (1988) also observed moderate increases in peroxisome proliferation in rats, a species insensitive to tetrachloroethylene hepatocarcinogenicity. In all, these findings indicate that the modest peroxisome proliferation observed in response to tetrachloroethylene may lack specificity with respect to species, tissue, and dose. Studies of the temporal sequence of events are limited. Given the limitations in the database of tetrachloroethylene-specific studies, it can be concluded that the few studies demonstrating peroxisome proliferation by tetrachloroethylene are insufficient to demonstrate a causative role of this effect in the induction of other key events posited for the PPARα mode of action hypothesis, and for hepatocarcinogenesis by tetrachloroethylene.

Other data and analyses more generally support the view that the hypothesized PPARα-activation MOA is not a sole causative factor in rodent hepatocarcinogenesis. PPARα-agonism may play a significant role in mouse liver tumor induction by some compounds, such as Wy-14,643. However, recent studies suggest that DEHP can induce tumors in a PPARα independent manner without any loss of potency (Ito et al., 2007a), and that PPARα-agonism in hepatocytes is itself insufficient to cause tumorigenesis (Yang et al., 2007). Additional analyses presented above demonstrate that peroxisome proliferation and associated markers are poor quantitative predictors of hepatocarcinogenesis in rats or mice. These data and analyses raise

serious concerns about basing human health risk assessment conclusions exclusively on evidence of key events in the hypothesized PPARα-activation MOA, given that other modes, mechanisms, toxicity pathways, and molecular targets may contribute to or be required for the observed adverse effects. Indeed, for most PPARα agonists, chemical-specific data to define the range of effects that may contribute to human carcinogenesis are insufficient. Similarly, the epidemiologic data are inadequate to inform conclusions of human relevance.

A recent review (Rusyn et al., 2006) addressed other mechanistic effects of the PPARα agonist DEHP and proposed that tumors arise from a combination of molecular signals and pathways, rather than from a single event such as PPARα-activation. Indeed, the PPARα agonists are pleiotropic and have been reported to exhibit a diversity of responses in addition to the hallmark effect of peroxisome proliferation, including genotoxicity [reviewed by Melnick (2001)], epigenetic alterations (e.g., hypomethylation) (Pogribny et al., 2007), oxidative stress [reviewed in O'Brien et al. (2005)] and effects on other receptors [e.g., Guo et al. (2007)] and other organelles (e.g., mitochondria) within parenchymal cells (Scatena et al., 2003; Zhou and Wallace, 1999; Youssef and Badr, 1998; Lundgren et al., 1987). As reviewed above, the metabolites of tetrachloroethylene have been shown to induce a number of effects that may contribute to carcinogenicity, including mutagenicity, alterations in DNA methylation, and oxidative stress. Given the demonstrated mutagenicity of several tetrachloroethylene metabolites, the hypothesis that mutagenicity contributes to the MOA for tetrachloroethylene carcinogenesis cannot be ruled out, although the specific metabolic species or mechanistic effects are not known. Epigenetic effects and oxidative stress, including that produced secondary to cytotoxicity, may also contribute. Currently, the available database of tetrachloroethylene-specific studies addressing these mechanisms is very limited and merits further exploration.

Cancer is a complex, multicausal process that is characterized by the acquisition and/or activation of multiple critical traits. As described by Hanahan and Weinberg (2000), these traits or hallmarks comprise six essential features: (1) self-sufficiency in growth signals, (2) insensitivity to growth-inhibitory (antigrowth) signals, (3) evasion of programmed cell death (apoptosis), (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. Epigenetic changes (e.g., in the expression of microRNAs that negatively regulate gene expression by targeting mRNA for translational repression or cleavage) appear to contribute to many of the observed phenotypic alterations. The acquisition of these six capabilities can also be facilitated by genomic instability, another feature of the cancer phenotype. A number of factors, such as inflammation (Grivennikov et al., 2010), diet, and physiological factors [e.g., obesity (Park et al., 2010)], can affect the tumor microenvironment in ways that advance these features of tumor development. Studies of human hepatocarcinogenesis

reveal significant heterogeneity, with evidence of aberrant signaling in multiple, overlapping pathways involved in cellular proliferation (e.g., EGF, HGF, RAS/mitogen-activated protein kinase), survival, differentiation (e.g., Wnt, Hedgehog), and angiogenesis (e.g., VEGF, PDFG, FGF) [refer to recent review by Hoshida et al. (2010)]. Other studies have provided support for a hypothesized role of stem cells in hepatocarcinogenesis (Marquardt and Thorgeirsson, 2010). In contrast to the stochastic cancer model, the cancer stem cell hypothesis posits a hierarchical model in which a minor cell population possessing stemness undergoes epigenetic changes to generate heterogeneous tumors [refer to review by Reya et al. (2001). The potential cell types of origin of liver cancer stem cells include mature hepatocytes possessing stem-like characteristics, as well as circulating cells (Kim et al., 2009) including bone-marrow derived stem cells (Marquardt and Thorgeirsson, 2010). Such stem cells have been posited to play a role in liver development and regeneration in addition to carcinogenesis [refer to review by Kung et al. (2010)]. Thus, although significant knowledge gaps remain, particularly with respect to the particular pathways and processes necessary and sufficient for the disease to originate and develop, the etiology of hepatocarcinogenesis appears complex.

Given the multiple metabolites and mechanisms that may contribute, and the known complexity and heterogeneity in liver cancer development, in general, it is unlikely that a single causative metabolite, mechanism, pathway, or mode of action will be identified for tetrachloroethylene-induced hepatocarcinogenesis. A single, linear sequence of key events does not seem likely to explain the observed hepatocarcinogenicity, given the multiple cell types and processes involved. Instead, a plausible hypothesis may be posited of multiple, contributing mechanistic effects that may, in turn, be affected by multiple modifying factors. Accordingly, the mechanisms described in this review are not intended to be interpreted as being mutually exclusive. Altogether, the described mechanistic effects may aid in identifying sources of human vulnerability, as well as informing the likelihood of other outcomes influenced by the same mechanisms, pathways, and biological processes. They may be informative of future analysis integrating data on human "upstream" biomarkers of hepatocarcinogenesis with chemically induced perturbations. In this manner, the mechanistic data may be informative for addressing the issues of cumulative assessment across exposures as well as overall population risk.

In summary, as noted by NRC (2010), there are significant gaps in the scientific knowledge of mechanisms contributing to tetrachloroethylene-induced mouse liver cancer. Multiple metabolites formed from tetrachloroethylene are toxic and carcinogenic in the liver. Given this knowledge, and the known complexity and heterogeneity in liver cancer development, in general, the available evidence supports a hypothesis of multiple, contributing mechanistic effects that may, in turn, be affected by multiple modifying factors.

4.4. ESOPHAGEAL CANCER

Thirteen epidemiologic studies reporting data on esophageal cancer and tetrachloroethylene exposure were identified. This set of publications includes 11 cohort or nested case-control studies (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Sung et al., 2007; Lynge et al., 2006; Blair et al., 2003; Chang et al., 2003; Travier et al., 2002; Andersen et al., 1999; Boice et al., 1999; Lynge and Thygesen, 1990) and two case-control studies of occupational exposures (Vaughan et al., 1997; Siemiatycki, 1991). No studies of residential exposure through contaminated drinking water were identified in the literature review. These 13 studies represent the core studies evaluated by EPA, as described in more detail below. Two other cohort studies included information on tetrachloroethylene but did not report risk estimates for esophageal cancer (Radican et al., 2008; Anttila et al., 1995), and one case-control study did not observe any cases exposed as a dry cleaner (Siemiatycki, 1991), and so were not evaluated further. There is some overlap in the study populations among these studies: Travier et al. (2002) used occupational data from the Swedish national census, and Lynge and Thygsen (1990) used a similar design in Denmark; Andersen et al. (1999) and Lynge et al. (2006) expanded these studies to include Denmark, Finland, and Norway in addition to Sweden, and Pukkala et al. (2009) added Iceland to this set. Appendix B reviews the design, exposure-assessment approach, and statistical methodology for each study. All studies were of the inhalation route, of occupational exposure, and, except for the case-control study of Vaughan et al. (1997), unable to quantify tetrachloroethylene exposure.

4.4.1. Consideration of Exposure-Assessment Methodology

Many studies examine occupational title as dry cleaner, launderer, and presser as surrogate for tetrachloroethylene, given its widespread use from 1960 onward in the United States and Europe (Calvert et al., 2011; Pukkala et al., 2009; Lynge et al., 2006; Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999; Lynge and Thygesen, 1990). Six studies conducted in Nordic countries are based on either the entire Swedish population or on combined populations of several Nordic countries; strengths of these studies are their use of job title as recorded in census databases and ascertainment of cancer incidence using national cancer registries (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Lynge et al., 2006; Travier et al., 2002; Andersen et al., 1999; Lynge and Thygesen, 1990). Studies examining mortality among U.S. dry-cleaner and laundry workers (Calvert et al., 2011; Blair et al., 2003) are of smaller cohorts than most Nordic studies, with fewer observed esophageal cancer events.

The exposure surrogate in studies of dry-cleaners and laundry workers is a broad category containing jobs of differing potential for tetrachloroethylene exposure. Thus, these studies have a greater potential for exposure misclassification bias compared to studies with

exposure potential to tetrachloroethylene assigned by exposure matrix approaches. Three studies used additional information pertaining to work environment to refine the exposure classification (Calvert et al., 2011; Seldén and Ahlborg, 2011; Lynge et al., 2006). Seldén and Ahlborg (2011) obtained information about the dry-cleaning establishment (e.g., washing techniques, chemicals used, number of employees, and work history of individual employees) in a questionnaire sent to businesses in Sweden in the 1980s. Lynge et al. (2006), using job title reported in the 1970 Census, identified subjects based on occupational code of "laundry and dry-cleaning worker" or industry code of "laundry and dry cleaning." Additional information to refine this occupational classification was sought for incident cancer cases, including esophageal cancer, within this defined cohort. Five controls, matched to the cases by country, sex, age, and calendar period, were also included in the study. The additional information included handwritten task information from the census forms from Denmark and Norway, pension databases in Denmark and Finland, and next-of-kin interviews in Norway and Sweden. Exposure classification categories were dry cleaner (defined as dry cleaners and supporting staff if employed in business of <10 workers), other job titles in dry cleaning (launderers and pressers), unexposed (job title reported on 1970 Census was other than in dry cleaning), or unclassifiable (information was lacking to identify job title of subject). The unclassifiable category represented 18 out of 72 esophageal cancer cases (25%) and 108 out of 567 controls (19%). The study by Calvert et al. (2011) of unionized dry cleaners in the United States included an analysis of subjects who worked for one or more years before 1960 in a shop known to use tetrachloroethylene as the primary solvent (Calvert et al., 2011; Ruder et al., 2001, 1994). The cohort was stratified into two groups based on the level of certainty that the worker was employed only in facilities using tetrachloroethylene as the primary solvent; tetrachloroethylene-only and tetrachloroethylene plus. There were 6 esophageal cancer deaths among this subset (n = 618) of the study subjects. Calvert et al. (2011) also presented risk estimates by exposure duration and by latent periods for the full set of study subjects. Two additional studies used an exposure metric for semiquantitative or quantitative exposure within a dry-cleaning setting. Blair et al. (2003) used an exposure metric for semiquantitative cumulative exposure, and the case-control study of Vaughan et al. (1997) used a JEM with quantitative exposure assessment for dry-cleaning and laundry jobs.

Two other cohorts with potential tetrachloroethylene exposure in manufacturing settings have been examined. These studies include aerospace workers in the United States (Boice et al., 1999) and electronic factory workers in Taiwan (Sung et al., 2007; Chang et al., 2003). Boice et al. (1999) used an exposure assessment based on a job-exposure matrix to classify exposures. In contrast, the exposures in the Taiwan studies included multiple solvents, tetrachloroethylene

exposure was not linked to individual workers, and cohorts included both white- and blue-collar workers (Sung et al., 2007; Chang et al., 2003).

In summary, with respect to exposure-assessment methodologies, five studies with esophageal cancer data assigned tetrachloroethylene exposure to individuals using a semiquantitative surrogate or a job exposure matrix (Blair et al., 2003; Boice et al., 1999; Vaughan et al., 1997), information about working conditions obtained through a questionnaire (Seldén and Ahlborg, 2011), or a classification of the cohort by certainty of tetrachloroethylene exposure (Calvert et al., 2011). One other study based on occupational census data sought additional data for use in refining potential exposure within dry-cleaning settings (Lynge et al., 2006). The relative specificity of these exposure-assessment approaches strengthens their ability to identify cancer hazards compared to studies with broader and less sensitive exposure-assessment approaches.

4.4.2. Summary of Results

All studies evaluated by EPA reported estimated relative risks based on a small number of observed events; 35 or fewer deaths/incident cases in cohort studies (Calvert et al., 2011; Sung et al., 2007; Lynge et al., 2006; Blair et al., 2003; Chang et al., 2003; Travier et al., 2002; Andersen et al., 1999; Boice et al., 1999; Lynge and Thygesen, 1990), except Pukkala et al. (2009), whose esophageal cancer findings are based on 95 exposed subjects. The few esophageal cancers in cohort studies and exposed cases in case-control studies contribute to reduced statistical power and limited ability to inform an evaluation of tetrachloroethylene exposure, particularly for esophageal cancer, whose estimated incidence is lower than for other cancer sites discussed in Section 4 (Edwards et al., 2010).

The largest cohort study observed an SIR estimate of 1.18 (95% CI: 0.96, 1.46) (Pukkala et al., 2009). Some evidence for an association between esophageal cancer risk and ever having a job title of dry cleaner or laundry worker or routine exposure to tetrachloroethylene is also found in cohort studies[24] whose effect estimates are based on fewer observed events and that carry lesser weight in the analysis. As expected, the magnitude of the point estimate of the association reported in these studies is more variable than in the larger study. The smaller cohort studies reported risks of 0.74 (95% CI: 0.41, 1.25), 1.16 (95% CI: 0.14, 4.20), 1.32 (95% CI: 0.94, 1.85), 1.47 (95% CI: 0.54, 3.21), 2.2 (95% CI: 1.15, 3.3), and 2.44 (95% CI: 1.4, 3.97) in Lynge and Thygsen (1990), Sung et al. (2007), Travier et al. (2002), Boice et al. (1999), Blair et al. (2003), and Calvert et al. (2011), respectively (refer to Table 4-23). The 10-year follow-up period in Lynge and Thygsen (1990) may represent an insufficient latent period with respect to

[24] Andersen et al. (1999) is not included in this summary of the data from the individual studies because it was updated and expanded in the analysis by Pukkala et al. (2009).

the development of cancer, reducing the study's sensitivity compared to Pukkala et al. (2009), whose follow-up was ≥15 years.

The nested case-control study of Lynge et al. (2006) reported an odds ratio of 0.76 (95% CI: 0.34, 1.69) for dry cleaners, with 8 exposed cases, compared to no exposure. In this study, job title could not be classified for 25% of the cases and 19% of the controls. The odds ratio for risk cancer in this "unclassifiable" group was 2.04 (95% CI: 0.91, 4.62). Lynge et al. (2006) carried out sensitivity analyses using different assumptions regarding the true classification for these subjects. In these analyses, the odds ratio for the association between dry cleaner and esophageal cancer was 0.66 (95% CI: 0.30, 1.45) assuming all unclassified subjects were unexposed and 1.19 (95% CI: 0.67, 2.21) assuming all unclassified subjects were dry cleaners. One other case-control study that adopted a JEM approach to assign exposure reported odds ratios of 6.5 (95% CI: 0.6, 68.9) and 0.9 (0.1, 10.0) for overall exposure to tetrachloroethylene, based on two and one exposed case, respectively, for squamous cell carcinoma and adenocarcinoma of the esophagus (Vaughan et al., 1997).

Several studies had been previously identified based on the relative strengths of their exposure-assessment methodology. The results from these studies are mixed. Lynge et al. (2006) reported no evidence of an increased risk among individuals classified as dry cleaners, with relative risks of 0.76, but a higher risk was observed in the "unclassifiable" group (RR: 2.04). Seldén and Ahlborg (2011) reported similar but slightly higher relative risks for laundry workers (SIR: 1.56) compared with dry cleaners (SIR: 1.25). In contrast, data from other studies with relatively strong exposure-assessment methods provide more evidence of an effect, with relative risks of 1.47 [Boice et al. (1999); routine exposure], 2.2 (Blair et al., 2003), and 2.68 [Calvert et al. (2011); tetrachloroethylene-only workers], and 6.4 (Vaughan et al., 1997).

Table 4-23. Summary of human studies on tetrachloroethylene exposure and esophageal cancer

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Cohort Studies			
Biologically monitored workers			Anttila et al. (1995)
All subjects	Not reported		849 Finnish men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, external referents (SIR)
Aerospace workers (Lockheed)			Boice et al. (1999)
Routine exposure to PCE	1.47 (0.54, 3.21)	6	77,965 (*n* = 2,631 with routine PCE exposure and *n* = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, job exposure matrix without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm [mean] and 9.5 ppm [median], external reference for routine exposure (SMR) and internal references (workers with no chemical exposures) for routine-intermittent PCE exposure (RR)
Routine-Intermittent exposure to PCE[a]			
Duration of exposure			
Never exposed	1.0[b]	28	
<1 yr	1.0 (0.30, 3.34)	3	
1−4 yr	0.79 (0.27, 2.50)	4	
≥5 yr	0.91 (0.13, 1.60)	3	
p for trend	*p* = 0.07		
Electronic factory workers (Taiwan)			Chang et al. (2003); Sung et al. (2007)
All Subjects			86,868 (*n* = 70,735 female), follow-up 1985−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, cancer mortality, external referents (SMR) (Chang et al., 2003); 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, multiple solvents exposures and PCE not identified to individual subjects, cancer incidence, external referents, analyses lagged 10 yr (SIR) (Sung et al., 2007)
Males		0	
Females		0	
Females	1.16 (0.14, 4.20)	2	
Aircraft maintenance workers from Hill Air Force Base			Radican et al. (2008)
Any PCE exposure	Not reported		10,461 men and 3,605 women (total *n* = 14,066, *n* = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952 to 1956, follow-up 1973−2000, job exposure matrix (intensity), internal referent (workers with no chemical exposures) (RR)

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Table 4-23. Summary of human studies on tetrachloroethylene exposure and esophageal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Dry cleaner and laundry workers				Andersen et al. (1999)
	All laundry worker and dry cleaners	0.91 (0.57, 1.40)	21	29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway), follow-up 1971−1987 or 1991, PCE not identified to individual subjects, external referents (SIR)
	Males	0.82 (0.33, 1.70)	7	
	Females	0.97 (0.53, 1.62)	14	
				Blair et al. (2003)
	All subjects	2.2 (1.5, 3.3)	26	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR)
	Semiquantitative exposure score			
	Little to no exposure	2.1 (0.9, 4.4)	7	
	Medium to high exposure	2.2 (1.2, 3.5)	16	
				Lynge and Thygsen (1990)
	All laundry worker and dry cleaners	0.74 (0.41, 1.25)	14	10,600 Danish men and women, 20−64 yr old, employed in 1970 as laundry worker, dry cleaners and textile dye workers, follow-up 1970−1980, external referents (SIR)
	Males	0.62 (0.23, 1.35)	6	
	Females	0.88 (0.38, 1.73)	8	
				Pukkala et al. (2009)
	Launderer and dry cleaner	1.18 (0.96, 1.46)	95	Men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30−64 yr, follow-up 2005, occupational title of launderer and dry cleaner in any census, external referents (SIR)
	Male	0.99 (0.66, 1.44)	28	
	Female	1.29 (1.00, 1.64)	67	

Table 4-23. Summary of human studies on tetrachloroethylene exposure and esophageal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
				Calvert et al. (2011)
	All subjects	2.44 (1.4, 3.97)	16	1,704 U.S. men and women dry-cleaning union member in CA, IL, MI, NY follow-up 1940−2004 (618 subjects worked for one or more years prior to 1960 only at shops where PCE was the primary cleaning solvent, identified as PCE-only exposure), cancer mortality (SMR)
	Exposure duration/time since 1st employment			
	<5 yr/<20 yr		0	
	<5 yr/≥20 yr	2.16 (0.85, 4.54)	5	
	≥5 yr/<20 yr		0	
	≥5 yr/≥20 yr	4.78 (2.68, 7.91)	11	
	PCE-only subjects	2.68 (0.98, 5.83)	6	
				Seldén and Ahlborg (2011)
	Dry-cleaners and laundry workers (females)	1.33 (0.43, 3.10)	5	9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−2000, exposure assigned using company self-reported information on PCE usage—PCE (dry cleaners and laundries with a proportion of PCE dry cleaning), laundry (no PCE use), and other (mixed exposures to PCE, CFCs, TCE, etc.), external referents (SIR). No observed cases in males
	PCE (females)	1.25 (0.26, 3.25)	3	
	Laundry (females)	1.56 (0.19, 5.65)	2	
				Travier et al. (2002)
	All subjects, 1960 or 1970 Census in laundry and dry cleaner occupation and industry	1.32 (0.94, 1.85)	34	Swedish men and women identified in 1960, 1970, or both Censuses as laundry worker, dry cleaner, or presser (occupational title) or in the laundry, ironing, or dyeing industry, follow-up 1971−1989, separates launders and dry cleaners form pressers, external referents (SIR)
	All subjects in 1960 and 1970 in laundry and dry cleaner occupation and industry	0.34 (0.05, 2.39)	1	

Table 4-23. Summary of human studies on tetrachloroethylene exposure and esophageal cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Case-Control Studies			
Nordic Countries (Denmark, Finland, Norway, Sweden)			Lynge et al. (2006)
Unexposed	1.00	41	Case-control study among 46,768 Danish, Finnish, Norwegian, and Swedish men and women employed in 1960 as laundry worker or dry cleaner, follow-up 1970−1971 to 1997−2001, 72 incident esophageal cancer cases, 6 controls per case randomly selected from cohort matched on country, sex, age, calendar period at diagnosis time, occupational task at 1970 Census proxy for exposure, RR adjusted for matching criteria
Dry cleaner	0.76 (0.34, 1.69)[b]	8	
Assume unclassifiable exposed as dry cleaner	1.19 (0.67, 2.21)[b]	26	
Assume unclassifiable unexposed	0.66 (0.30, 1.45)[b]	59	
Other in dry-cleaning	1.22 (0.41, 3.63)[b]	5	
Unclassifiable	2.04 (0.91, 4.62)[b]	18	
Dry cleaner, employment duration, 1964−1979			
Unexposed	1.0	41	
≤1 yr		0	
2−4 yr	1.20 (0.14, 10.41)[b]	1	
5−9 yr	0.66 (0.19, 2.29)[b]	3	
≥10 yr	0.70 (0.20, 2.49)[b]	3	
Unknown	1.65 (0.18, 14.98)[b]	1	
Montreal, Canada			Siemiatycki (1991)
Launderers and dry cleaners			Histologically confirmed esophageal cancers (n = 99), 1979−1985, 35−70 yr, population control group and cancer control group, in-person interviews, occupational title, OR adjusted age, family income, and cigarette index, 90% CI
Any exposure	(0.0, 2.4)	0	
Substantial exposure	(0.0, 4.3)	0	

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Table 4-23. Summary of human studies on tetrachloroethylene exposure and esophageal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Washington State (United States)				Vaughan et al. (1997)
	Squamous cell carcinoma			Esophageal cancer cases (404 cases), 1983−1987, 20−74 yr, 724 population controls, in-person interview, occupational title and JEM for PCE, blinded exposure assessment, OR adjusted for age, sex, education, study period, alcohol consumption and cigarette smoking
	Ever exposed to PCE (probable exposure)	6.4 (0.6, 68.9)	2	
	Cumulative PCE exposure (possible exposure)			
	1−29 ppm-yr	11.9 (1.1, 124.0)	2	
	30+ ppm-yr		0	
	Adenocarcinoma			
	Ever exposed to PCE (Probable exposure)	0.9 (0.1, 10.0)	1	

[a]For Boice et al. (1999), relative risks for employment duration from Poisson regression with internal referents of factory workers not exposed to any solvent and with adjustment for date of birth, date first employed, date of finishing employment, race and sex.
[b]In Lynge et al. (2006), odds ratio from logistic regression adjusted for country, sex, age, calendar period at time of diagnosis.

JEM = job-exposure matrix, PCE = tetrachloroethylene.

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Establishment of an exposure or concentration-response relationship can add to the weight of evidence for identifying cancer hazard, but only limited data pertaining to exposure-response relationships for esophageal cancer and tetrachloroethylene exposure are available. Five studies reported risk by exposure categories using exposure duration (Calvert et al., 2011; Lynge et al., 2006; Boice et al., 1999) or a semiquantitative or quantitative surrogate (Blair et al., 2003; Vaughan et al., 1997). However, Boice et al. (1999) and Vaughan et al. (1997) were based on relatively few observed cases, with <5 cases in individual exposure categories, greatly limiting the usefulness of these exposure-response examinations. Boice et al. (1999) presented a formal statistical test of linear trend ($p = 0.07$) for exposure duration and esophageal cancer deaths among workers with routine or intermittent exposure; three of the 10 esophageal cancer deaths in this group had exposure durations 5 years or longer (RR: 0.91, 95% CI: 0.13, 1.60, with an internal comparison group of factory workers not exposed to any solvents as the referent). This analysis included subjects whose exposure was infrequent and likely of lesser certainty than subjects identified as having routine exposure. The overall SMR for any tetrachloroethylene exposure in this study was 1.47 (95% CI:0.54, 3.21). Both exposed cases in Vaughan et al. (1997) were identified with lower cumulative exposure, 1−29 ppm-years (OR: 11.9, 95% 1.1, 124.0) compared to no cases with 30+ ppm-years. Effect estimates in one of the two larger studies that examined exposure duration was not suggestive of a trend (Lynge et al., 2006) (refer to Table 4-23). However, all 16 exposed esophageal deaths in Calvert et al. (2011) had ≥20 years since first employment, with effect estimates of 2.16 (95% CI: 0.85, 4.54) and 4.78 (95% CI: 2.68, 7.91) for <5 years and ≥5 years exposure duration, respectively. Sixteen of the 26 esophageal cancer deaths in Blair et al. (2003) had medium-to-high cumulative exposure to dry-cleaning solvents with an effect estimate of 2.2 (95% CI: 1.2, 3.5).

Only Vaughan et al. (1997) directly evaluated possible effects due to smoking or alcohol, which are risk factors for the squamous cell histologic type of esophageal cancer; all other studies lacked control for these potential confounders. Both Calvert et al. (2011) and Blair et al. (2003) noted that the magnitude of the risks for esophageal cancer was greater than could be explained by smoking alone; any smoking effect was estimated to contribute to no more than a 20% increase in risk. This suggests a further contribution from another risk factor, such as occupational exposure. The incidence of esophageal cancer is generally higher for non-Caucasian males than for Caucasian males (Brown et al., 2001; Blot and McLaughlin, 1999). In contrast, Calvert et al. (2011) observed similar SMRs for esophageal cancer across all race-sex groupings (supplementary table at http://www.cdc.gov/niosh/dc-mort.html), suggesting the contribution of another factor such as occupational exposure. However, the inability to adjust for potential effects of alcohol use in cohort studies is an uncertainty.

In conclusion, the SIR in the only large cohort study (n = 95 cases), a study using broad exposure categories, was 1.18 (95% CI: 0.96, 1.46) (Pukkala et al., 2009). The point estimates of the association in seven of eight smaller studies, four studies with specific exposure assessments and four other studies with less precise assessments, were between 1.16 and 2.44 (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Sung et al., 2007; Blair et al., 2003; Travier et al., 2002; Boice et al., 1999; Lynge and Thygesen, 1990). Two small case-control studies with relatively high quality exposure-assessment approaches, Lynge et al. (2006) and Vaughan et al. (1997) reported an odds ratio of 0.76 (95% CI: 0.34, 1.69) and of 6.4 (95% CI: 0.6, 68.9), respectively. Some uncertainties in these estimates arise from the lack of job title information for 25% of the cases and 19% of the controls, and the variability in the results from the sensitivity analysis using different assumptions regarding the correct classification of individuals in this group in Lynge et al. (2006) and the small numbers of exposed cases in Vaughan et al. (1997). One of the two larger studies examining exposure-response suggested a positive relationship, with SMRs of 2.16 (95% CI: 0.85, 4.54) and 4.78 (95% CI: 2.68, 7.91) for durations of <5 years and ≥5 years, respectively (Calvert et al., 2011). None of the cohort studies can exclude possible confounding from alcohol and smoking—risk factors for squamous cell carcinoma of the esophagus. Based on smoking rates in blue-collar workers, the twofold risk estimate reported in Calvert et al. (2011) and Blair et al. (2003) was higher than that attributable to smoking.

4.5. LUNG AND RESPIRATORY CANCER

Nineteen epidemiologic studies reporting data on lung cancer and tetrachloroethylene exposure were identified. This set of studies includes 12 cohort or nested case-control studies within a cohort (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Sung et al., 2007; Ji et al., 2005b; Blair et al., 2003; Chang et al., 2003; Travier et al., 2002; Andersen et al., 1999; Boice et al., 1999; Anttila et al., 1995; Lynge and Thygesen, 1990), 6 case-control studies of occupational exposures (Consonni et al., 2010; MacArthur et al., 2009; Richiardi et al., 2004; Pohlabeln et al., 2000; Brownson et al., 1993; Siemiatycki, 1991), and one case-control study of residential exposure through contaminated drinking water (Paulu et al., 1999). Some of these studies represent overlapping populations. For example, Travier et al. (2002) and Lynge and Thygsen (1990) used occupational data from Sweden and Denmark, respectively; Andersen et al. (1999) included Denmark, Finland, and Norway in addition to Sweden, and Pukkala et al. (2009) added Iceland to the study population. Additionally, nonsmoking cases in Richiardi et al. (2004), whose lung cancer cases included both smokers and nonsmokers, were included in the International Agency for Research on Cancer (IARC) multicenter study of lung cancer among nonsmokers (Pohlabeln et al., 2000). These studies represent the core studies evaluated by EPA,

as described in more detail below. One other cohort study included information on tetrachloroethylene but did not report risk estimates for lung cancer (Radican et al., 2008). Also, one other lung cancer case-control study did not identify any cases as a dry cleaner or launderer (Zeka et al., 2006) and was not evaluated further. Appendix B reviews the design, exposure-assessment approach, and statistical methodology for each study. Most studies were of the inhalation route, of occupational exposure, and unable to quantify tetrachloroethylene exposure.

4.5.1. Consideration of Exposure-Assessment Methodology

Most of these studies examine occupational titles such as dry cleaner, launderer, and presser as surrogates for tetrachloroethylene, given its widespread use from 1960 onward in the United States and Europe (Calvert et al., 2011; Seldén and Ahlborg, 2011; Consonni et al., 2010; MacArthur et al., 2009; Pukkala et al., 2009; Zeka et al., 2006; Ji et al., 2005a; Ji and Hemminki, 2005a; Ji et al., 2005b; Ji and Hemminki, 2005b, c; Richiardi et al., 2004; Blair et al., 2003; Travier et al., 2002; Pohlabeln et al., 2000; Andersen et al., 1999; Brownson et al., 1993; Siemiatycki, 1991; Lynge and Thygesen, 1990). Seven studies conducted in Nordic countries are based on either the entire Swedish population or on combined populations of several Nordic countries; the strengths of these studies are their use of job titles as recorded in census databases and ascertainment of cancer incidence using national cancer registries (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Lynge et al., 2006; Ji et al., 2005a; Ji and Hemminki, 2005a; Ji et al., 2005b; Ji and Hemminki, 2005b, c; Travier et al., 2002; Andersen et al., 1999; Lynge and Thygesen, 1990). Studies examining mortality among U.S. dry-cleaner and laundry workers (Calvert et al., 2011; Blair et al., 2003) are of smaller cohorts than the Nordic studies, with fewer observed lung cancer events.

The exposure surrogate in studies of dry-cleaners and laundry workers is a broad category containing jobs of differing potential for tetrachloroethylene exposure. Thus, these studies have a greater potential for exposure misclassification bias compared to studies with exposure potential to tetrachloroethylene assigned by exposure matrix approaches applied to individual subjects. Three studies used additional information pertaining to work environment to refine the exposure classification. Seldén and Ahlborg (2011) obtained information about the dry-cleaning establishment (e.g., washing techniques, chemicals used, number of employees, and work history of individual employees) in a questionnaire sent to businesses in Sweden in the 1980s. Blair et al. (2003) used an exposure metric for semiquantitative cumulative exposure within the dry-cleaning setting. The study by Calvert et al. (2011) of unionized dry cleaners in the United States included an analysis of subjects who worked for one or more years before 1960 in a shop known to use tetrachloroethylene as the primary solvent (Calvert et al., 2011; Ruder et al., 2001, 1994). The cohort was stratified into two groups based on the level of certainty that

the worker was employed only in facilities using tetrachloroethylene as the primary solvent; tetrachloroethylene-only and tetrachloroethylene plus. Twenty-six of the 77 observed lung cancer deaths were among this subset (n = 618) of the study subjects.

Four other cohorts with potential tetrachloroethylene exposure in manufacturing settings have been examined. These studies include aerospace workers in the United States (Boice et al., 1999), workers, primarily in the metal industry, in Finland (Anttila et al., 1995) and electronic factory workers in Taiwan (Sung et al., 2007; Chang et al., 2005). Boice et al. (1999) used an exposure assessment based on a job-exposure matrix, and Anttila et al. (1995) used biological monitoring of tetrachloroethylene in blood to assign potential tetrachloroethylene exposure to individual subjects. In contrast, the exposures in the Taiwan studies included multiple solvents, and tetrachloroethylene exposure was not linked to individual workers. These cohorts also included white-collar workers, who had an expected lower potential for exposure (Sung et al., 2007; Chang et al., 2003).

Paulu et al. (1999) is a case-control study that examined residential proximity to drinking water sources contaminated with tetrachloroethylene in Cape Cod, MA. This study used an exposure model incorporating leaching and characteristics of the community water distribution system to assign a household relative dose of tetrachloroethylene.

In summary, with respect to exposure-assessment methodologies, six studies with lung cancer data assigned tetrachloroethylene exposure to individuals within the study using biological monitoring data (Anttila et al., 1995), a job exposure matrix (Boice et al., 1999), a semiquantitative metric (Blair et al., 2003), an exposure model (Paulu et al., 1999), additional details pertaining to work environment (Seldén and Ahlborg, 2011), or a classification of the cohort by certainty of tetrachloroethylene exposure (Calvert et al., 2011). The relative specificity of these exposure-assessment approaches strengthens their ability to identify cancer hazards compared to studies with broader and less sensitive exposure-assessment approaches. The least sensitive exposure assessments are those using very broad definitions such as working in a plant or factory (Sung et al., 2007; Chang et al., 2003).

4.5.2. Summary of Results

Lung cancer is a relatively common cancer, and six of the cohort studies of dry-cleaners and laundry workers evaluated by EPA reported estimated relative risks based on 100 or more deaths/incident cases (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji et al., 2005b; Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999); Pukkala et al. (2009) was the largest study, with 965 incident lung cancers. Two other cohort studies, Lynge and Thygsen (1990) and Calvert et al. (2011), observed 60 and 77 lung cancers, respectively. In contrast, the number of exposed cases in the case-control studies ranged from 3 cases each of small cell and

adenocarcinoma histological subtypes in MacArthur et al. (2009) to 30 (all histological types) in Brownson et al. (1993). The three cohort studies with exposure assessment specific to tetrachloroethylene observed 5 incident cancer cases, 46 lung cancer deaths, and 125 lung cancer deaths in Anttila et al. (1995), Boice et al. (1999), and Blair et al. (2003), respectively. The geographic-based case-control study of Paulu et al. (1999) observed 33 of the 326 lung cancer cases living in a residence receiving tetrachloroethylene contaminated water, and only 5 of these cases were identified as highly exposed.

The seven[25] cohort studies with findings based on 50 or more events observed a standardized incidence ratio estimate between 1.15 and 1.4 for the association between lung cancer risk and ever having a job title of dry-cleaner or laundry worker, each with relatively tight 95% CIs (refer to Table 4-24). These estimates by study were 1.15 (95% CI: 1.02, 1.31) in Travier et al. (2002), 1.2 (0.9, 1.5) in Lynge and Thygsen (1990), 1.26 (95% CI: 1.18, 1.34) in Pukkala et al. (2009), 1.32 (1.07, 1.60) in Ji et al. (2005b), 1.32 (95% CI: 1.20, 1.45) in Seldén and Ahlborg (2011), 1.31 (1.04, 1.64) in Calvert et al. (2011), and 1.4 (1.1, 1.6) in Blair et al. (2003), respectively. Seldén and Alhborg (2011) examined separately subjects working in a dry cleaner using tetrachloroethylene (potential tetrachloroethylene exposure) and laundry workers, subjects without potential tetrachloroethylene exposure. The standardized incidence ratios were 1.16 (95% CI: 0.89, 1.51) and 1.62 (95% CI: 1.15, 2.19) for dry cleaners and for laundry workers, respectively.

In addition to the large cohort studies, evidence also comes from cohort and case-control studies whose effect estimates are based on fewer observed events. Smaller studies that do not also have a more sensitive or specific exposure metric carry lesser weight in the analysis. As expected, the magnitude of the point estimate of the association reported in these studies is more variable than in the larger studies: one study reported an odds ratio estimate below 1.0 (Siemiatycki, 1991), four studies reported a relative risk estimate between 1.0 and 1.3 (Consonni et al., 2010; MacArthur et al., 2009; Boice et al., 1999; Paulu et al., 1999), three studies reported relative risks between 1.8 and 2.0 (Pohlabeln et al., 2000; Anttila et al., 1995; Brownson et al., 1993), and two studies reported odds ratios estimates over 2.0 (MacArthur et al., 2009; Richiardi et al., 2004). Except for the estimate from Brownson et al. (1993) (OR: 1.8, 95% CI: 1.1, 3.0) and McArthur et al. (2009) (small cell carcinoma, OR: 3.55, 95% CI: 1.13, 11.17), all of the 95% CIs of these estimates overlap 1.0.

[25] Andersen et al. (1999) is not included in this summary of the data from the individual studies because it was updated and expanded in the analysis by Pukkala et al. (2009).

Table 4-24. Summary of human studies on tetrachloroethylene exposure and lung cancer

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Cohort studies			
Biologically monitored workers			Anttila et al. (1995)
All subjects	1.92 (0.62, 4.48)	5	849 Finnish men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, external referents (SIR)
Aerospace workers (Lockheed)			Boice et al. (1999)
Routine exposure to PCE	1.08 (0.79, 1.44)	46	77,965 (n = 2,631 with routine PCE exposure and n = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, job exposure matrix without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm [mean] and 9.5 ppm [median], external reference for routine exposure (SMR) and internal references (workers with no chemical exposures) for routine-intermittent PCE exposure (RR)
Routine-Intermittent exposure duration to PCE			
0	1.0[a]	288	
<1 yr	1.15 (0.80, 1.66)	33	
1−4 yr	1.09 (0.80, 1.48)	51	
≥5 yr	0.71 (0.49, 1.02)	36	
p-value for linear trend	p = 0.02		
Electronic factory workers (Taiwan)			Chang et al. (2003); Sung et al. (2007)
All Subjects	0.97 (0.69, 1.33)	38	86,868 (n = 70,735 female), follow-up 1979−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, cancer mortality, external referents (SMR) (Chang et al., 2003); 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, multiple solvents exposures and PCE not identified to individual subjects, cancer incidence, external referents, analyses lagged 10 yr (SIR) (Sung et al., 2007)
Males	0.90 (0.48, 1.53)	13	
Females	1.01 (0.65, 1.49)	25	
Females	0.92 (0.67, 1.23)	46	
Aircraft maintenance workers from Hill Air Force Base			Radican et al. (2008)
Any PCE exposure	Not reported		10,461 men and 3,605 women (total n = 14,066, n = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952 to 1956, follow-up 1973−2000, job exposure matrix (intensity), internal referent (workers with no chemical exposures) (RR)

Table 4-24. Summary of human studies on tetrachloroethylene exposure and lung cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Dry-cleaner and laundry workers				Andersen et al. (1999)
	All laundry worker and dry cleaners	1.19 (1.07, 1.34)	313	29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway), follow-up 1971−1987 or 1991, PCE not identified to individual subjects, external referents (SIR)
	Males	1.24 (1.05, 1.46)	141	
	Females	1.16 (1.00, 1.35)	172	
				Blair et al. (2003)
	All subjects	1.4 (1.1, 1.6)	125	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR)
	Semiquantitative exposure score			
	Little to no exposure	1.0 (0.7, 1.4)	34	
	Medium to high exposure	1.5 (1.2, 1.9)	78	
				Ji et al. (2005b);
	Laundry workers and dry cleaners in 1960 Census	1.32 (1.20, 1.46)	403	9,255 Swedish men and 14,974 Swedish women employed in 1960 (men) or 1970 (women) as laundry worker or dry cleaner, follow-up 1961/1970−2000, PCE not identified to individual subjects, external referent (SIR) and adjusted for age, period and socioeconomic status
	Males	1.36 (1.20, 1.54)	247	
	Females	1.26 (1.07, 1.47)	156	
	Laundry workers and dry cleaners in both 1960 and 1970 Censuses			
	Males	Not reported		
	Females	Not reported		
	Laundry workers and dry cleaners in 1960, 1970, and 1980 Censuses			
	Males	Not reported		
	Females	Not reported		

Table 4-24. Summary of human studies on tetrachloroethylene exposure and lung cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Lynge and Thygsen (1990)
All laundry worker and dry cleaners	1.2 (0.9, 1.5)	60	10,600 Danish men and women, 20−64 yr old, employed in 1970 as laundry worker, dry cleaners and textile dye workers, follow-up 1970−1980, external referents (SIR)
Males	1.1 (0.8, 1.7)	28	
Females	0.3 (0.9, 1.8)	32	
			Pukkala et al. (2009)
Launderer and dry cleaner	1.26 (1.18, 1.34)	965	Men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30-64 yr, follow-up 2005, occupational title of launderer and dry cleaner in any census, external referents (SIR)
Male	1.28 (1.15, 1.42)	353	
Female	1.25 (1.15, 1.35)	612	
			Calvert et al. (2011)
All subjects	1.31 (1.04, 1.64)	77	1,704 U.S. men and women dry-cleaning union member in CA, IL, MI, NY follow-up 1940−2004 (618 subjects worked for one or more years prior to 1960 only at shops where PCE was the primary cleaning solvent, identified as PCE-only exposure), cancer mortality (SMR)
Exposure duration/time since 1st employment			
<5 yr/<20 yr	0.63 (0.21, 1.44)	4	
<5 yr/≥20 yr	1.75 (1.33, 2.26)	32	
≥5 yr/<20 yr	1.27 (0.55, 2.50)	6	
≥5 yr/≥20 yr	1.08 (0.75, 1.51)	26	
PCE-only subjects	1.25 (0.82, 1.83)	26	
			Seldén and Ahlborg (2011)
Dry-cleaners and laundry workers	1.32 (1.07, 1.60)	100	9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−2000, exposure assigned using company self-reported information on PCE usage—PCE (dry cleaners and laundries with a proportion of PCE dry cleaning), laundry (no PCE use), and other (mixed exposures to PCE, CFCs, TCE, etc.), external referents (SIR)
PCE	1.16 (0.89 1.51)	58	
Males	1.30 (0.82, 1.94)	23	
Females	1.09 (0.76, 1.51)	35	
Laundry	1.62 (1.15, 2.21)		
Males	1.60 (0.85, 2.74)	13	
Females	1.63 (1.06, 2.39)	26	

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Table 4-24. Summary of human studies on tetrachloroethylene exposure and lung cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Travier et al. (2002)
All subjects, 1960 or 1970 Census in laundry and dry cleaner or related occupation and industry	1.15 (1.02, 1.31)	248	Swedish men and women identified as laundry worker, dry cleaner, or presser (occupational title), in the laundry, ironing, or dyeing industry or related industry in 1960 or 1970 (543,036 person-years); or, as laundry worker, dry cleaner, or presser (occupational and job title) (46,933 person-years) in both censuses, follow-up 1971−1989, external referents (SIR)
All subjects in 1960 and 1970 in laundry and dry cleaner occupation and industry	1.20 (0.84, 1.72)	30	
Case-Control Studies			
Missouri, United States			Brownson et al. (1993)
Dry-cleaning industry			429 female primary lung cancer cases, 30−84 yr, 1986−1991, never smokers or ex-smokers (>15 yr prior to diagnosis), identified from Missouri Cancer Registry, 1,021 female population controls matched on age, identified from state driver's licenses (<65 yr) or HFCA roles (65−84 yr), telephone and in-person interview using questionnaire, dry cleaner occupation or job title exposure surrogate, OR adjusted for age, smoking, and history of previous lung disease
All subjects	1.8 (1.1, 3.0)	30	
Lifetime nonsmokers	2.1 (1.2, 3.7)	23	
Former smokers	1.1 (not reported)	7	
Exposure duration			
<1.125 yr	0.8 0.2, 1.7)	Not reported	
≥1.125 yr	2.9 (1.5, 5.4)	Not reported	
Lombardy, Italy (EAGLE study)			Consonni et al. (2010)
Dry-cleaning industry			1,943 histologically or cytologically confirmed hospital lung cancer cases in men and women, 35−79 yr, 2002−2005, and 2,116 population controls matched on residence, sex, and age, in-person and self-administered questionnaire, job title and industry coded to ISCO and ISIC surrogate for exposure, dry-cleaning industry identified *a priori* suspected lung hazard, OR adjusted for residential area, age, smoking and number of jobs held
Males	Not reported	3	
Females	1.26 (0.46, 3.41)	12	

Table 4-24. Summary of human studies on tetrachloroethylene exposure and lung cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
British Columbia, Canada			MacArthur et al. (2009)
Dry cleaner and launderer occupation			2,998 male histologically confirmed lung cancer cases, ≥20 yr, 1983−1990, 10,233 all-other sites-cancer controls matched on age and diagnosis year, identified from British Columbia Cancer Registry, self-administered questionnaire, job title and industry coded to Canadian SOC and Canadian SIC as exposure surrogate, OR adjusted for smoking duration, respondent status, and education
Squamous cell carcinoma	1.25 (0.47, 3.35)	4	
Adenocarcinoma	1.28 (0.44, 3.70)	3	
Small cell	3.55 (1.13, 11.17)	3	
Large cell		0	
International Lung Cancer Study (IARC Study) (France, Germany, Italy, Portugal, Spain, Sweden, United Kingdom)			Pohlabeln et al. (2000)
All Centers			660 nonsmoking lung cancer cases, ≤75 yr, 1988−1994, 1,542 nonsmoking controls, 12 study centers in 7 countries, various sources of nonsmoking controls (community based in 6 centers, hospital-based in 1 center, both sources in 5 centers), hospital controls with diseases not related to smoking, in-person interview, job title and industry coded to ISCO and ISIC exposure surrogate, dry-cleaning industry identified *a priori* suspected lung hazard, OR adjusted for age and center
Dry-cleaning industry			
Males	Not reported	1	
Females	1.83 (0.98, 3.40)	19	
Turin and Veneto Regions, Italy			Richiardi et al. (2004)
Dry Cleaners and Launderers			1,132 histologically or cytologically confirmed lung cancer cases, <75 yr, 1990−1991 or 1991−1992, population controls identified from population registries and matched on sex and age, in-person interview, job title and industry ≥6 mo duration coded to ISCO and ISIC exposure surrogate, dry-cleaning industry identified *a priori* suspected lung hazard, OR adjusted for age, study area, cigarette smoking, other tobacco product use, and number of jobs. Cases and controls included in international multicenter study of Pohlaban et al. (2000)
Males	1.6 (0.2, 12)	3	
Females	2.1 (0.8, 5.6)	9	

Table 4-24. Summary of human studies on tetrachloroethylene exposure and lung cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Montreal, Canada			Siemiatycki (1991)
Launderers and dry cleaners			857 histologically confirmed lung cell carcinoma cancer, 1979−1985, 35−70 yr, 533 population control group and 1,900 cancer control group, in-person interviews, occupational title, OR adjusted age, family income, ethnic origin, respondent status, cigarette smoking, and alcohol consumption, 90% CI
Any exposure	0.8 (0.5, 1.5)[b]	12	
Substantial exposure	0.6 (0.2, 1.4)[b]	5	
International Lung Cancer Study (IARC Study) (Czech Republic, Hungary, Poland, Romania, Russia, Slovakia, United Kingdom)			Zeka et al. (2006)
Launderers and dry cleaners			223 hospital lung cancer cases, 20−74 yr, 1998−2002, lifetime nonsmokers, identified from 16 hospitals or clinics in 7 countries, hospital (14 centers) or population controls (2 centers) frequency-matched on sex and age, in-person interview, industry and job title exposure surrogate, dry-cleaning industry identified *a priori* suspected lung hazard, OR adjusted for age, sex, and study center, with ETS exposure included as additional covariate in some analyses
Males	Not reported	0	
Females	Not reported	0	
Geographic-Based Studies			
Cape Cod, MA			Paulu et al. (1999)
Overall PCE exposure	1.1 (0.7, 1.7)	33	326 histologically confirmed lung cancer cases in males and females, 1983−1986, MA Cancer Registry, 2,236 population controls identified by random digit dialing, vital records for deceased controls, and HCFA records if >65 yr, telephone interview, algorithm of Webler and Brown (1993) to estimate mass of PCE in drinking water entering residence was surrogate exposure metric, OR adjusted for age of diagnosis or index year, vital status at interview, sex, occupation exposure to PCE, other solvents, and exposures associated with lung cancer, usual number of cigarettes smoked, history of cigar/pipe use, living with a smoker
PCE RDD >90th percentile	2.7 (1.0, 11.7)	5	

[a]Referent.
[b]In Siemiatycki (1991), 90% CI.
CFC = chloroflourocarbon, HCFA = Health Care Financing Administration, ISCO = International Standard Classification of Occupation, ISIC = International Standard Industry Classification, JEM = job-exposure-matrix, PCE = tetrachloroethylene, RDD = relative delivered dose, TCE = trichloroethylene, TWA = time-weighted-average.

Five occupational studies were identified as having a relatively strong exposure-assessment methodology. The results from four of these studies provide support for an increased risk in the dry-cleaning cohorts with a relative risk of 1.4 (95% CI: 1.1, 1.6) in Blair et al. (2003), 1.31 (95% CI: 1.04, 1.64) in Calvert et al. (2011), and in other settings, a relative risk of 1.08 (95% CI: 0.79, 1.44) in Boice et al. (1999) and 1.92 (95% CI: 0.62, 4.48) in Anttila et al. (1994). In contrast, Seldén and Ahlborg (2011) reported similar, but slightly higher, relative risks for laundry workers compared with dry-cleaning workers in their study. Two studies of an electronics factory using relatively weak exposure-assessment approaches (i.e., no classification of individuals within the study) observed relative risks or SMRs of 0.97 (95% CI: 0.69, 1.33) (Chang et al., 2003) and 0.92 (95% CI: 0.67, 1.23) (Sung et al., 2008).

Establishment of an exposure or concentration-response relationship can add to the weight of evidence for identifying a cancer hazard, but only limited data pertaining to exposure-response relationships for lung cancer and tetrachloroethylene exposure are available. Seven studies presented risk estimates for increasing exposure categories: three studies using exposure duration as a proxy (Calvert et al., 2011; Travier et al., 2002; Boice et al., 1999) and four studies with a semiquantitative exposure surrogate (Blair et al., 2003; Paulu et al., 1999; Brownson et al., 1993; Siemiatycki, 1991). Boice et al. (1999) was the only study to present a formal statistical test for trend and reported a statistically significant decreasing trend between lung cancer risk estimates and duration among subjects with routine and intermittent tetrachloroethylene exposure ($p = 0.02$). A monotonic increasing trend in risk estimates and exposure surrogate was apparent in four studies (Blair et al., 2003; Travier et al., 2002; Paulu et al., 1999; Brownson et al., 1993).

A known risk factor for lung cancer is cigarette smoking (NTP, 2005). Subjects in both Brownson et al. (1993) and Pohlabeln et al. (2000) were either lifetime nonsmokers or ex-smokers who had terminated smoking 15 years before cancer diagnosis, reducing any potential role of confounding from smoking. Furthermore, in the case of Pohlabeln et al. (2000), the inclusion of occasional smoking (ever smoked occasionally but fewer than 400 cigarettes total) and exposure to tobacco smoking as possible confounders did not significantly affect the odds ratio estimate and were not included in the final model. Statistical analyses in all other case-control studies controlled for cigarette smoking (Consonni et al., 2010; MacArthur et al., 2009; Richiardi et al., 2004; Paulu et al., 1999; Siemiatycki, 1991). However, both Brownson et al. (1993) and MacArthur et al. (2009) had a high percentage of surrogate or proxy respondents, 58 and 27%, respectively. Proxy respondents may have motivations to report or not report specific exposures leading to differential information bias that could result in the relative risk estimate towards or away from the null depending on whether controls were more or less likely to recall or report such exposure than cases (Pearce et al., 2007).

Direct examination of possible confounders is less common or feasible in cohort studies relying on company-supplied or census work history data compared to case-control studies where information is obtained from study subjects or their proxies. In cohort studies, however, use of internal controls rather than an external referent group (e.g., national mortality rates) can minimize effects of potential confounding due to smoking or socioeconomic status, because exposed and referent subjects are drawn from the same target population. Only one of the available cohort studies included an analysis using internal controls and reported a decreasing trend between lung cancer and tetrachloroethylene exposure duration, $p = 0.02$ (Boice et al., 1999). Blair et al. (2003) considered the potential effect of differences in the prevalence of smoking in their study of laundry and dry-cleaning workers. Surveys from 1970 to 1990 indicated that smoking rates among dry cleaners were 5−10% higher than the general population. With this level of difference, confounding from smoking is unlikely to result in a relative risk greater than 1.2 but may explain most of the observed 40% excess in lung cancer. The magnitude of relative risk estimates in cohort studies of dry-cleaners and laundry workers (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji et al., 2005b; Travier et al., 2002; Lynge and Thygesen, 1990) is similar to or less than that of Blair et al. (2003) and suggests smoking may contribute to the observed association.

In conclusion, the results from seven large cohort studies of dry cleaners are consistent with an elevated lung cancer risk of 10−40%. Similar results were observed in four of the five occupational studies that were identified as having a relatively strong exposure-assessment methodology (Calvert et al., 2011; Blair et al., 2003; Boice et al., 1999; Anttila et al., 1994). However, Seldén and Ahlborg (2011) observed similar, but slightly higher, relative risks for laundry workers compared with dry-cleaning workers in their study. These studies were unable to control for potential confounding from cigarette smoking; however, and the magnitude of the association in these studies is consistent with that expected, assuming the prevalence of smoking among dry-cleaners and laundry workers was slightly higher (e.g., 10% higher) than among the general population. Features of the selection of study participants and study analysis in the available case-control studies reduce the potential for confounding by smoking, however. Two case-control studies were limited to either nonsmokers or ex-smokers who had ceased smoking 15 years before diagnosis (Pohlabeln et al., 2000; Brownson et al., 1993). Both of these studies indicate an approximate twofold increased risk with a history of work in the dry-cleaning industry [OR: 1.8, 95% CI: 1.1, 3.0, in Brownson et al. (1993); and OR: 1.83, 95% CI: 0.98, 3.40, among women in Pohlabeln et al. (2000)]. The other case-control studies adjusted for smoking history, and the results for these (somewhat smaller studies) are similar to the previously cited estimates. Among the studies that evaluated exposure-response gradients, the

evidence for a trend in risk estimates was mixed (Calvert et al., 2011; Blair et al., 2003; Travier et al., 2002; Boice et al., 1999; Paulu et al., 1999; Brownson et al., 1993).

4.6. IMMUNOTOXICITY, HEMATOLOGIC TOXICITY, AND CANCERS OF THE IMMUNE SYSTEM

Chemical exposures may result in a variety of adverse immune-related effects, including immunosuppression (decreased host resistance), autoimmunity, and allergy-hypersensitivity, and may result in specific diseases such as infections, systemic or organ-specific autoimmune diseases, or asthma. Measures of immune function (e.g., T-cell counts, immunoglobulin [Ig] E levels, specific autoantibodies, cytokine levels) may provide evidence of an altered immune response that precedes the development of clinically expressed diseases. This section discusses effects relating to immunotoxicity and hematotoxicity. It also discusses evidence pertaining to tetrachloroethylene in relation to lymphoid tissue cancers, including childhood leukemia.

4.6.1. Human Studies

4.6.1.1. Noncancer Immune and Hematologic Effects

Adverse effects on the immune system resultingincluded the following: 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 an 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 (Tanios 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 chemical sensitivity to tetrachloroethylene in a municipal water supply (Rea et al., 1991).

4.6.1.1.1. Immunologic and hematologic 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.

Other studies have examined immunological parameters in dry-cleaning workers in the Czech Republic (Andrys et al., 1997) and in Egypt (Emara et al., 2010) (refer to Table 4-25). Andrýs et al. (1997) included 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 ages of the exposed workers and office controls were 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 immunoglobulin (Ig) A, IgG, IgM, and IgE 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 the 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.

Table 4-25. Immune and hematological parameters in studies of dry-cleaning workers or tetrachloroethylene exposure in children

Study details	Measure(s)	Results	Authors
Adults			
Czech Republic, period not reported. 21 dry-cleaning workers (20 women; mean age 45.7 yr); 16 office workers in the dry-cleaning plant (14 women; mean age 31.9 yr); reference values based on samples from blood donors and "healthy persons in the same region" (n = 14−311, depending on the test)	Ig (IgA, IgG, IgM) levels, complement (C3 and C4) levels, phagocyte activity, C-reactive protein, α-macroglobulin, T-lymphocytes	Higher levels serum complement C3 and C4, salivary IgA in exposed workers compared with office workers. Reduced T-lymphocytes, higher phagocytic activity, higher C3 levels in exposed workers compared with reference controls. Reduced T-lymphocytes in office workers compared with reference controls	Andrýs et al. (1997)
Egypt, period not reported. 40 adult men (ages 20−38 yr), dry-cleaning workers; 40 healthy male controls (matched by age and smoking history): n = 20 in 4 groups (controls, never smoked; controls, smoked; PCE-exposed, never smoked; PCE exposed, smoked). Amount and duration of smoking similar among exposed and nonexposed. Mean years of PCE exposure 7 yr. Blood PCE levels in exposed: 1,685 µg/L	RBC counts	RBC counts and hemoglobin levels decreased with exposure. No difference in MCV, MCH, or MCHC	Emara et al. (2010)
	WBC counts	Total white cell and lymphocyte counts increased with exposure. No difference in eosinophils, monocytes, or platelets	
	lymphocyte subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, $CD3^+CD16CD56^+$, $CD19^+$ cells)	$CD4^+$ and $CD8^+$ T-lymphocytes and $CD3^+CD16CD56$+ NK cells increased with exposure	
	Ig levels (IgA, IgE, IgG, IgM)	IgE increased with exposure. No difference in IgA, IgG, or IgM levels across groups	
	serum and lymphocytic interferon-γ and interleukin-4	Interleukin-4 levels increased with exposure. No differences with interferon-γ	
Germany, 1995−1996. 121 children (ages 36 mo), selected based on high risk profile for allergic diseases, blood sample and indoor air sampling (child's bedroom) of 26 volatile organic chemicals (4 wk around age 36 mo)	IgE levels	no association between PCE measures and total IgE or IgE-specific allergen antibodies	Lehmann et al. (2001)
Germany 1997−1999. 85 newborns, cord blood and indoor air sampling (child's bedroom) of 28 volatile organic chemicals (4 wk immediately after birth)	CD3 T-cell subpopulations from cord blood	Decreased interferon-γ cells No association with interleukin-4, interleukin-2, or tumor necrosis factor-α	Lehmann et al. (2002)

Ig = immunoglobulin; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RBC = red blood cells; WBC = white blood cells.

Emara et al. (2010) examined immunological and hematologic parameters in 80 men, ages 20−38 years, in Tanta City, Egypt. Forty men were dry-cleaning workers, with a mean duration of work of 7 years. They were matched by age and smoking history to 40 healthy controls from the same area. The study, thus, included four groups, each with 20 men: controls who had never smoked; controls who were smokers; tetrachloroethylene-exposed workers who had never smoked; and tetrachloroethylene-exposed workers who were smokers. The amount smoked and duration of smoking were similar in the exposed and nonexposed groups (mean: 17.9 and 17.5 cigarettes per day, respectively; mean: 4.5 and 5.0 years smoking, respectively). Tetrachloroethylene levels were measured at five sites within each worksite, and blood levels of tetrachloroethylene were also measured in all study participants. The mean air level was <140 ppm tetrachloroethylene, and mean blood levels were 1,681 and 1,696 µg/L among nonsmoking and smoking workers (compared with 0.11 µg/L in each of the control groups), respectively. Blood samples were obtained from each study participant for measurement of a differential blood cell count, serum Ig levels (IgA, IgE, IgG, and IgM), and interferon-γ and interleukin-4 levels in serum and lymphocytes.

Red blood cell counts and hemoglobin levels were decreased with exposure ($p < 0.05$ for smoking-stratified comparisons), but there was no difference in mean corpuscular volume, mean corpuscular hemoglobin, or mean corpuscular hemoglobin concentration across groups (Emara et al., 2010). In contrast, total white cell counts and total lymphocytes increased significantly with exposure ($p < 0.05$ for smoking-stratified comparisons). There was no difference in eosinophils, monocytes, or platelets counts across groups. Neutrophil counts were increased in smokers compared with nonsmokers but did not differ by tetrachloroethylene-exposure group. $CD4^+$ and $CD8^+$ T-lymphocytes and natural killer ($CD3^+CD16CD56^+$) cells were increased in smoking and nonsmoking exposed workers ($p < 0.05$), but $CD3^+$ T-lymphocytes were only increased in the exposed smoking group. This study demonstrated statistically significant effects of tetrachloroethylene exposure on hematological parameters including decreased red blood cell counts, increased white blood cells counts, total lymphocytes, and specific T- and NK cell subpopulations.

Th2 cytokines (e.g., interleukin-4) stimulate production of IgE, and Th1 cytokines (e.g., interferon-γ) act to inhibit IgE production. The results from Emara et al. (2010) indicate that tetrachloroethylene exposure results in an increase in serum and lymphocytic interleukin-4 levels, as well as increased IgE levels ($p < 0.05$ for smoking-stratified comparisons). As determined from Figure 5 of Emara et al. (2010), the mean levels were approximately 90, 160, 170, and 195 IU/mL in nonexposed nonsmokers, nonexposed smokers, exposed nonsmokers, and

exposed smokers, respectively ($p < 0.05$ for smoking-stratified comparisons). No difference was observed in IgA, IgG, or IgM levels across groups.

Two studies examined variation in cytokines and in IgE levels in children (Lehmann et al., 2002; Lehmann et al., 2001) (refer to Table 4-25). 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 a double positive family history of atopy. These eligibility criteria were met by 429 children; 200 of these children participated in the allergy study described below, but complete data (IgE and volatile organic compound measurements) were available for only 121 of the study participants.

Lehmann et al. (2001) 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 observed 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-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 (refer to Table 4-25). 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 birth (a period that is likely to reflect the exposures during the prenatal period close to the time of

delivery). The levels were generally similar or slightly higher than the levels observed in the previous study using samples from the bedrooms of the 36-month-old children. The highest levels of exposure were observed for limonene (median 24.3 μg/m^3), α-pinene (median 19.3 μg/m^3), and toluene (median 18.3 μg/m^3), and the median exposure of tetrachloroethylene was 3.4 μg/m^3 (1.8 μg/m^3 and 7.3 μg/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 percentages of interferon-γ cells were 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.

4.6.1.1.2. Immune-related conditions and diseases

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.

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. Delfino et al. (2003a;

2003b) 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 suggests 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 participated 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 levels of tetrachloroethylene were 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.

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 and Meira, 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 two- to threefold increased risk of systemic sclerosis (scleroderma) in epidemiologic studies summarized in a recent meta-analysis (Aryal et al., 2001) and in subsequent studies (Maitre et al., 2004; Garabrant et al., 2003). Similar results were observed in studies of other systemic autoimmune diseases including undifferentiated connective tissue disease (Lacey et al., 1999), rheumatoid arthritis (Sverdrup et al., 2005; Lundberg et al., 1994), and antineutrophil-cytoplasmic antibody (ANCA)-related vasculitis (Beaudreuil et al., 2005; Lane et al., 2003). In contrast, there was little evidence of an association between solvent exposure and systemic lupus erythematosus in two recent case-control studies (Finckh et al., 2006; Cooper et al., 2004).

As described in the preceding paragraph, the epidemiologic data in relation to the role of solvents, as a broad category, in systemic autoimmune diseases, vary among these conditions. Much more limited data are available pertaining to specific solvents, including tetrachloroethylene, and risk of autoimmune diseases. One case report describes 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). Another case report describes 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 (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 observed 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.

Two registry-linkage studies from Sweden of rheumatoid arthritis (Li et al., 2008; 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 (refer to Table 4-26). 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 for the broad classification of laundry and dry-cleaning work, with odds ratios for dry cleaning of 1.0 in the largest study of rheumatoid arthritis (Li et al., 2008) and 1.4 in two studies of scleroderma (Garabrant et al., 2003) and undifferentiated connective tissue disease (Lacey et al., 1999). None of the individual studies are statistically significant. The studies from Sweden linking occupational census data to risk of rheumatoid arthritis (Li et al., 2008; Lundberg et al., 1994) are also limited by the difficulty in defining time of diagnosis for this disease based on hospitalization data. The results observed for the exposure to tetrachloroethylene in the three studies that attempted this kind of assessment were more varied (Beaudreuil et al., 2005; Garabrant et al., 2003; Lacey et al., 1999). 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.

Table 4-26. Immune-related conditions in studies of dry cleaning or tetrachloroethylene exposure in humans[a]

Condition and study details	Results	Authors
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)
Sweden, hospitalized 1964−2004 (men) or 1970 to 2004 (women). 13,280 male cases and 14,509 female cases; population comparison (full population), ages ≥30 yr, Registry linkage to 1960 or 1970 Census occupation data for men and women, respectively	launderers and dry cleaning men: 57 exposed cases; OR: 0.8 (95% CI: 0.6−1.0) women: 204 exposed cases; OR: 1.0 (95% CI: 0.8−1.1)	Li et al. (2008)
Other autoimmune diseases		
Undifferentiated connective tissue disease, Michigan and Ohio, diagnosed 1980−1991 (Michigan) 1980−1992 (Ohio). 205 cases, 2,095 population controls. Women, ages 18 and older. Structured interview (specific jobs and materials; jobs held 3 or more mo)	dry cleaning cases: 4.3%, controls 3.8% OR: 1.4 (95% CI: 0.68, 2.8) PCE cases: 0%, controls 1% OR: 0.00	Lacey et al. (1999)
Scleroderma, Michigan and Ohio. Diagnosed 1980−1991 (Michigan), 1980−1992 (Ohio). 660 cases, 2,227 population controls. Women, ages 18 and older. Structured interview (specific jobs and materials; jobs held 3 or more mo)	dry cleaning cases: 4.7%, controls 3.7% OR: 1.4 (95% CI: 0.9, 2.2) PCE 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 yr	PCE cases: 8.3%, controls 4.1% OR: 2.0 (0.6−6.9)	Beaudreuil et al. (2005)
Allergy and hypersensitivity		
Exacerbation of asthma symptoms, Los Angeles, 1999−2000. 21 children (ages 10−16 yr), 3 mo diaries, ambient levels and exhaled breath measures of 8 volatile organic compounds and 8 criteria pollutants	Little evidence of an association between ambient PCE exposure or exhaled PCE measures and asthma symptoms	Delfino et al. (2003a; 2003b)

[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).

4.6.1.1.3. Summary of human noncancer immune and hematologic effects

The strongest study examining immunologic and hematologic effects of tetrachloroethylene exposure in terms of sample size and use of an appropriately matched control group is of 40 male dry-cleaning workers (mean exposure levels <140 ppm; mean duration: 7 years) by Emara et al. (2010). Statistically significant decreases in red blood cell count and hemoglobin levels and increases in total white cell counts and lymphocyte counts were observed in the exposed workers compared to age- and smoking-matched controls. In addition, increases in several other immunological parameters, including T-lymphocyte and natural killer cell subpopulations, IgE, and interleukin-4 levels were observed. These immunologic effects suggest an augmentation of Th2 responsiveness. However, the limited available data from studies in children (Delfino et al., 2003a; Delfino et al., 2003b; Lehmann et al., 2002; Lehmann et al., 2001) do not provide substantial evidence of an effect of tetrachloroethylene exposure during childhood on allergic sensitization or exacerbation of asthma symptomology. 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. The available data pertaining to risk of autoimmune disease in relation to tetrachloroethylene exposure are limited by issues regarding ascertainment of disease incidence and exposure-assessment difficulties in population-based studies.

4.6.1.2. Cancers of the Immune System, Including Childhood Leukemia

Forty-one epidemiologic studies report on adult lymphopoietic cancer and tetrachloroethylene exposure. These publications include numerous cohort or nested case-control studies (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Radican et al., 2008; Sung et al., 2007; Ji and Hemminki, 2006b; Lynge et al., 2006; Chang et al., 2005; Ji and Hemminki, 2005b; Blair et al., 2003; Travier et al., 2002; Cano and Pollán, 2001; Andersen et al., 1999; Boice et al., 1999; Blair et al., 1998; Anttila et al., 1995; Spirtas et al., 1991; Lynge and Thygesen, 1990), case-control studies (Gold et al., 2010b; McLean et al., 2009; Schenk et al., 2009; 't Mannetje et al., 2008; Costantini et al., 2008; Seidler et al., 2007; Mester et al., 2006; Miligi et al., 2006; Kato et al., 2005; Costantini et al., 2001; Fabbro-Peray et al., 2001; Miligi et al., 1999; Clavel et al., 1998; Aschengrau et al., 1993; Blair et al., 1993; Scherr et al., 1992; Siemiatycki, 1991; Malone et al., 1989; Hardell et al., 1981), and three geographical-based studies (Cohn et al., 1994; Vartiainen et al., 1993; Morton and Marjanovic, 1984). Some of these papers represent studies of related populations. For example, three papers examined cancer incidence or mortality in a cohort of aircraft maintenance workers at an air force base in the United States, with follow-up through 1982 (Spirtas et al., 1991), 1990 (Blair et al., 1998), and

2000 (Radican et al., 2008). Six papers examined cancer risk among occupational groups defined by census or employer-provided data in Sweden (Seldén and Ahlborg, 2011; Ji and Hemminki, 2006b, 2005b; Travier et al., 2002; Cano and Pollán, 2001; Lynge and Thygesen, 1990), two papers were based on census data from Sweden, Denmark, Finland, and Norway (Lynge et al., 2006; Andersen et al., 1999), and a third paper added data from Iceland (Pukkala et al., 2009). Four papers examined different subsets of lymphopoietic cancers from a large population-based case-control study in Italy (Costantini et al., 2008; Miligi et al., 2006; Costantini et al., 2001; Miligi et al., 1999). Additionally, five epidemiologic studies—one cohort and four case-control—report on childhood lymphopoietic cancer and tetrachloroethylene exposure (Sung et al., 2008; Infante-Rivard et al., 2005; Costas et al., 2002; Shu et al., 1999; Lowengart et al., 1987). Appendix B reviews the design, exposure-assessment approach, and statistical methodology for each study; the adult lymphopoietic cancer studies are also summarized in Table 4-27, and the childhood lymphopoietic cancer studies are summarized in Table 4-28. Most studies were primarily of the inhalation route, of occupational exposure, and, generally, unable to quantify tetrachloroethylene exposure. Two studies of contaminated drinking water containing multiple solvents including tetrachloroethylene were available (Cohn et al., 1994; Vartiainen et al., 1993). Collectively, these studies have varying sensitivities for identifying cancer hazards.

4.6.1.2.1. Adult lymphopoietic cancer: consideration of exposure assessment

Since the 1960s in Western Europe and the United States, the dry-cleaning industry has accounted for about 90% of tetrachloroethylene consumption (Gold et al., 2008; Johansen et al., 2005; IARC, 1995), with more infrequent and lower volume use of trichloroethylene and CFC-113 for specialized cleaning (IARC, 1995). As described previously, eight publications used occupational data derived from national census data or by the employer for one or more northern European countries, focusing on dry cleaners and other laundry workers (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji and Hemminki, 2006b; Lynge et al., 2006; Ji and Hemminki, 2005b; Travier et al., 2002; Cano and Pollán, 2001; Andersen et al., 1999; Lynge and Thygesen, 1990). Lynge et al. (2006) used national databases and pension schemes to identify subjects as dry cleaners versus other job titles held in 1970; however, these databases were not available for subjects from two of the four countries (i.e., Norway and Finland), nor was information on a subject's workplace and length of employment available for Swedish subjects. In the absence of national databases, Lynge et al. (2006) collected this information through interviews, many with a subject's next of kin. A higher likelihood for recall bias is possible with next of kin or proxy information, particularly for knowledge of solvent exposures as shown by Boyle et al. (1992). Additionally, workers who may have switched to jobs as dry cleaners after

1970 would be misclassified using a classification system based on job held in 1970. Two smaller cohort studies examining mortality using cause of death data from death certificates were conducted among laundry and dry-cleaning union members in the United States (Calvert et al., 2011; Blair et al., 2003; Ruder et al., 2001, 1994).

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Cohort Studies					
Biologically monitored Finnish workers					Anttila et al. (1995)
	All subjects	Lymphopoietic	1.38 (0.28, 4.02)	3	849 men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, cancer incidence, external referents (SIR).
		Non-Hodgkin	3.76 (0.77, 11.0)	3	
		Multiple myeloma		0 0.38 exp	
		Leukemia	Not reported		
Aerospace workers (Lockheed)					Boice et al. (1999)
	Routine exposure to PCE	Lymphopoietic	1.13 (0.62, 1.89)[a]	14	77,965 (n = 2,631 with routine PCE exposure and n = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, JEM without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm [mean] and 9.5 ppm [median], mortality, external referents for routine exposure (SMR) and internal referents (workers with no chemical exposures) for routine-intermittent PCE exposure (RR).
		Non-Hodgkin	1.70 (0.73, 3.34)	8	
		Hodgkin		0 0.98 exp	
		Multiple myeloma	0.40 (0.01, 2.25)	1	
		Leukemia	0.55 (0.18, 1.29)	5	
	Routine-intermittent PCE-exposure duration				
	0 yr	Non-Hodgkin	1.0[b]	32	
	<1 yr		1.25 (0.43, 3.57)	4	
	1−4 yr		1.11 (0.46, 2.70)	6	
	≥5 yr		1.41 (0.67, 3.00)	10	
	Test for trend		$p > 0.20$		

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Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
	0 yr	Multiple myeloma	1.0[b]	24	Boice et al. (1999) (continued)
	<1 yr		0.46 (0.06, 3.48)	1	
	1−4 yr		1.13 (0.38, 3.35)	4	
	≥5 yr		0.24 (0.03, 1.84)	1	
	Test for Trend		$p < 0.01$		
Electronic factory workers (Taiwan)					Chang et al. (2005); Sung et al. (2007)
	All subjects	Lympho- and hemato-poietic	0.67 (0.42, 1.01)	22	86,868 (n = 70,735 female), follow-up 1979−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, lympho- and hematopoietic cancer incidence, external referents (SIR) (Chang et al., 2005); 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, PCE not identified to individual subjects, leukemia cancer incidence, external referents, analyses lagged 5 yr (SIR) (Sung et al., 2007).
	Males		0.73 (0.27, 1.60)	6	
	Females		0.65 (0.37, 1.05)	16	
	Females	Leukemia	0.78 (0.49, 1.17)	5	
Aircraft maintenance workers from Hill Air Force Base					Spirtas et al. (1991); Blair et al. (1998); Radican et al. (2008)
	Ever-exposed to PCE				14,066 (10,461 men and 3,605 women) (n = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952 to 1956, follow-up to 2000, PCE used for parachute cleaning, JEM without quantitative estimate of PCE intensity, mortality, internal referent (workers with no chemical exposures) (RR).
	Males	Non-Hodgkin	2.32 (0.75, 7.15)[b]	5	
	Females		2.35 (0.52, 10.71)[b]	2	
	Males	Multiple myeloma	1.71 (0.42, 6.91)[b]	3	
	Females		7.84 (1.43, 43.06)[b]	2	

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Dry-cleaner and laundry worker				
				Andersen et al. (1999)
All subjects	Lymphopoietic	1.0 (0.87, 1.15)[c]	204	29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway) with occupation as launderers or dry cleaners, follow-up 1971−1987 or 1991, PCE not identified to individual subjects, incidence, country-specific cancer rates referent (SIR).
Males		1.05 (0.79, 1.38)[c]	53	
Females		0.98 (0.84, 1.16)[c]	151	
All subjects	Non-Hodgkin	1.07 (0.86, 1.34)	82	
Males		1.46 (0.96, 2.13)	27	
Females		0.95 (0.71, 1.23)	55	
All subjects	Hodgkin	1.34 (0.81, 2.10)	19	
Males			0 0.4 exp	
Females		1.88 (1.13, 2.93)	19	
All subjects	Multiple myeloma	1.0 (0.73, 1.34)	45	
Males		1.38 (0.75, 2.31)	14	
Females		0.89 (0.60, 1.26)	31	
All subjects	Leukemia	0.85 (0.65, 1.10)	58	
Males		0.67 (0.35, 1.17)	12	
Females		0.90 (0.66, 1.21)	46	

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Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
					Blair et al. (2003)
	All subjects	Non-Hodgkin	0.9 (0.5, 1.6)	12	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, PCE exposure potential higher for subcohort entering union after 1960, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR).
		Hodgkin lymphoma	2.0 (0.6, 4.6)	5	
		Multiple myeloma	0.8 (0.3, 1.6)	7	
		Leukemia	0.8 (0.4, 1.4)	12	
	Semiquantitative exposure score				
	Any exposure	Lympho- and hemato-poietic	1.0 (0.7, 1.3)	39	
	Little to no exposure		1.0 (0.6, 1.5)	18	
	Medium to high exposure		0.9 (0.5, 1.4)	17	
					Cano and Pollán (2001)
	Males	Non-Hodgkin	1.76 (0.97, 3.17)[d]	11	Swedish men and women aged 25−64 yr reporting occupation as "launderers and dry cleaners" in 1970 Census, employed and counted in 1960 Census, follow-up 1971−1989, NHL incidence from Swedish Cancer Registry, PCE not identified to individual subjects, all other occupations referent (RR).
			1.85 (0.83, 4.12)[e]	6	
	Females		Not reported		
					Ji and Hemminki (2006b, 2005b)
	Males	Non-Hodgkin[f,g]	0.99 (0.75, 1.26)	59	9,255 men and 14,974 women reporting laundry and dry-cleaning work 1970 Swedish Census, follow-up 1960−2002, cases identified from Swedish Cancer Registry, PCE not assigned to individual subjects, cancer incidence from Swedish Cancer Registry, Swedish cancer rates referent (SIR).
	Females		1.05 (0.82, 1.32)	67	
	Males	Multiple myeloma[f]	0.99 (0.66, 1.38)	52	
	Females		1.07 (0.75, 1.45)	36	
	Males	Leukemia[g]	0.84 (0.62, 1.90)	47	
	Females		1.30 (1.03, 1.60)	80	

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Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
				Lynge and Thygesen (1990)
All subjects	Non-Hodgkin	1.03 (0.44, 2.02)	8	10,600 men and women reporting work in dry cleaner and laundries in Swedish 1970 Census, follow-up 1970−1980, job title surrogate for exposure, cancer incidence from Swedish Cancer Registry, Swedish cancer rates referents (RR).
	Multiple myeloma	1.75 (0.70, 3.61)	7	
	Leukemia	0.74 (0.30, 1.52)	7	
				Pukkala et al. (2009)
Launderer and dry cleaner	Lymphopoietic	0.98 (0.83, 1.11)	653	15 million men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30−64 yr, follow-up to 2005, occupational title of launderer and dry cleaner in any census [*n* = 8,744 men, *n* = 34,752 women], PCE not identified to individual subjects, cancer incidence from national cancer registries, national population cancer incidence rates referent (SIR).
Male		0.94 (0.79, 1.08)	140	
Female		0.99 (0.83, 1.06)	513	
Launderer and dry cleaner	Non-Hodgkin	0.98 (0.86, 1.10)	264	
Male		0.96 (0.72, 1.25)	54	
Female		0.98 (0.86, 1.13)	210	
Launderer and dry cleaner	Hodgkin	0.97 (0.67, 1.36)	33	
Male		0.77 (0.31, 1.58)	7	
Female		1.04 (0.68, 1.53)	26	
Launderer and dry cleaner	Multiple myeloma	1.02 (0.86, 1.20)	152	
Male		1.31 (0.95, 1.78)	42	
Female		0.94 (0.78, 1.33)	110	
Launderer and dry cleaner	Leukemia[h]	0.95 (0.83, 1.09)	204	
Male		0.71 (0.50, 0.99)	37	
Female		1.03 (0.88, 1.19)	167	

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
				Calvert et al. (2011)
All subjects	Lympho- and hemato-poietic	0.88 (0.53, 1.38)	19	1,704 U.S. men and women dry-cleaning union member in CA, IL, MI, NY follow-up 1940−2004 (618 subjects worked for one or more years prior to 1960 only at shops where PCE was the primary cleaning solvent, identified as PCE-only exposure), cancer mortality (SMR).
Exposure duration/time since 1st employment		Not reported		
PCE-only subjects	Lympho- and hemato-poietic	1.51 (0.75, 2.70)	11	
All subjects	Non-Hodgkin lymphoma	1.57 (0.78, 2.81)	11	
Exposure duration/time since 1st employment		Not reported		
PCE-only subjects	Non-Hodgkin lymphoma	2.46 (0.90, 5.36)	6	
				Seldén and Ahlborg (2011)
Dry-cleaners and laundry workers	Non-Hodgkin	1.38 (1.02, 1.82)	49	9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−2000, exposure assigned using company self-reported information on PCE usage—PCE (dry cleaners and laundries with a proportion of PCE dry cleaning), laundry (no PCE use), and other (mixed exposures to PCE, CFCs, TCE, etc.), external referents (SIR).
PCE				
Males	Non-Hodgkin	2.02 (1.13, 3.34)	15	
Duration of exposure				
<1 yr		6.02 (2.21, 13.09)	6	
1−4 yr		1.00 (0.12, 3.61)	2	
5−11 yr		1.19 (0.64, 3.27)	7	
Females		1.14 (0.68, 1.81)	18	
Duration of exposure				
<1 yr		1.95 (0.53, 5.00)	4	
1−4 yr		1.04 (0.34, 2.44)	5	
5−11 yr		1.10 (0.46, 1.92)	9	

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Laundry				Seldén and Ahlborg (2011) (continued)
Males	Non-Hodgkin	2.33 (1.01, 4.59)	8	
Females		0.99 (0.43, 1.95)	8	
				Travier et al. (2002)
All subjects	Non-Hodgkin[i]	0.86 (0.43, 1.72)	8	Men and women with occupation as dry cleaners, launderers, and pressers in Swedish 1960 or 1970 Census and employed in laundry, ironing, or dyeing industries, followed 1971−1989, cancer incidence from Swedish Cancer Registry, PCE not identified to individual subjects, all other occupations/industries referent (RR).
Males		1.32 (0.75, 2.32)	5	
Females		0.52 (0.17, 1.61)	3	
All subjects	Hodgkin[i]	2.69 (1.01, 7.19)	4	
Males		1.58 (0.22, 11.26)	1	
Females		3.57 (1.15, 11.13)	3	
All subjects	Leukemia	1.84 (1.11, 3.06)	15	
Males		0.93 (0.30, 2.88)	3	
Females		2.53 (1.44, 4.46)	12	
Case-Control Studies				
Upper Cape Cod, MA (United States)				Aschengrau et al. (1993)
Any PCE, no lag	Leukemia	2.13 (0.88, 5.19)	7	34 men and women incident leukemia cases, 737 population controls, stratified by age, vital status, year of death, sex, telephone or in-person interviews, water distribution model of Webler and Brown (1993), adjusted for sex, age, vital status, education, job exposures (OR).
RDD >90th percentile, no lag	Leukemia	8.33 (1.53, 25.29)	2	
Any PCE, ≥5 yr lag	Leukemia	1.96 (0.71, 5.37)	Not reported	
RDD >90th percentile, ≥5 yr lag	Leukemia	5.84 (1.37, 24.91)	Not reported	

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Iowa and Minnesota (United States)				Blair et al. (1993)
Dry-cleaning industry	Non-Hodgkin	2.0 (0.97, 4.3)	16	622 histologically confirmed incident NHL cases in men, 1,245 population controls matched on state, age, and year deaths [for dead cases], in-person interview, JEM for solvent group but not PCE individually; adjusted for age, state, smoking, family history lymphopoietic disease, agricultural pesticide use, hair dye use, and proxy respondent (OR).
Solvents other than benzene				
Any exposure	Non-Hodgkin	1.1 (0.9, 1.4)	359	
Low intensity		1.1 (0.8, 1.4)	334	
High intensity		1.4 (0.8, 2.5)	25	
France, 18 provinces				Clavel et al. (1998)
Launderer and dry cleaner	Hairy cell leukemia (a type of NHL)	3.0 (0.2, 49.2)	1	226 males histologically confirmed hospital HCL cases, 1980−1990,425 hospital controls from orthopedic and rheumatological departments matched on sex, birth date, admission date, residence, self-administered questionnaire, JEM for solvent exposures, adjusted for smoking and farming (OR).
Solvents, more confident exposure assessment	Hairy cell leukemia (a type of NHL)	0.7 (0.4, 1.2)	32	
Italy, 12 regions				Costantini et al. (2001); Miligi et al. (2006); Costantini et al. (2008)
PCE				2,737 incident lymphomas in men and women (1,450 NHL, 365 HD, 652 leukemia, 270 multiple myeloma) 20−74 yr, 1991−1993, 1,779 population controls stratified by sex and age, in-person interview, exposure proxy of job title and JEM for PCE, adjusted for sex, age, education, and area (OR).
Very low/low intensity	Non-Hodgkin + CLL	0.6 (0.3, 1.2)	18	
Medium/high intensity		1.2 (0.6, 2.5)	14	
Very low/low intensity	Leukemia	0.6 (0.2, 1.6)	6	
Medium/high intensity	Leukemia	1.0 (0.4, 2.7)	7	
	Hodgkin	Not reported		

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
	Launderer, dry cleaner, presser				Costantini et al. (2001); Miligi et al. (2006); Costantini et al. (2008) (continued)
	Males	Non-Hodgkin + CLL	1.6 (0.3, 9.1)	3	
	Females		0.7 (0.3, 1.5)	10	
	Males	Hodgkin	2.5 (0.3, 24.6)	1	
	Females		3.5 (1.5, 8.2)	7	
	Males	Multiple myeloma	Not reported		
	Females		1.0 (0.3, 3.8)	3	
	Males	Leukemia	3.3 (0.1, 32.4)	2	
	Females		1.1 (0.4, 3.2)	5	
Languedoc-Roussillon region (France)					Fabbro-Peray et al. (2001)
	Dry-cleaning solvents	Non-Hodgkin	1.0 (0.6, 1.6)	35	445 histologically confirmed Hodgkin and NHL hospital cases in men and women recruited, 1992−1996, 1,025 population controls stratified on municipalities size and population distribution, in-person or telephone interview, self-reported exposure, exposed defined as duration >1 yr, 5 yr prior to diagnosis, information, adjusted for age, sex, urban setting, education level (OR).

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Puget Sound-Seattle (Washington State), Detroit (Michigan) (United States)				Gold et al. (2010b)
Ever exposed to PCE	Multiple myeloma	1.5 (0.8, 2.9)[j]	16	180 histologically confirmed multiple myeloma cases in men and women reported to cancer registries, 2000−2002, 481 population controls, RDD or Medicare/Medicaid services files, in-person interview, self-reported or proxy-assisted reply to all jobs held ≥12 mo since 1945, adjusted for age, gender, race, education, study site (OR).
Cumulative exposure (ppm-wk)				
Referent	Multiple myeloma	1.0[a]	164	
1−353		0.3 (0.04, 3.0)[j]	1	
354−1,430		0.5 (0.1, 4.4)[j]	1	
1,431−4,875		1.5 (0.4, 5.4)[j]	4	
4,876−13,500		3.3 (1.2, 9.5)[j]	10	
p-value for trend		$p = 0.02$		
Textile, apparel, furnishing machine operators and tenders (includes dry-cleaning machine operators)	Multiple myeloma	6.0 (1.7, 21)	9	
Exposure duration				
1−5 yr	Multiple myeloma	3.6 (0.7, 1.7)	4	
>5 yr		12 (1.3, 110)	5	
Trend test		$p = 0.001$		
Dry-cleaning machine operators	Multiple myeloma	Not reported	5 cases, 3 controls	
Umea (Sweden)				Hardell et al. (1981)
Any styrene, TCE, PCE, benzene exposure	Non-Hodgkin	4.6 (1.9, 11.4)	10	169 men histologically confirmed incident NHL and Hodgkin cases, 1974−1978, population controls, 25−85 yr, matched for sex, age, and residence, and death [for dead cases], self-administered questionnaire, OR from univariate χ^2 test.

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
New York (United States)				Kato et al. (2005)
Dry-cleaning fluids	Non-Hodgkin	1.59 (0.49, 5.13)	7	376 cases histologically confirmed NHL in women, 20−79 yr, 1995−1998, NY State Cancer registry, 463 population controls stratified on age, telephone interview, occupation exposure to solvents, dry-cleaning fluids, adjusted for age, family history hematologic cancer, education, interview year, proxy respondent, BMI, prescription/over-counter drugs, pesticide exposures (OR).
Population of Denmark, Finland, Norway, Sweden				Lynge et al. (2006)
Dry cleaner	Non-Hodgkin	1.0 (0.7, 1.4)[k]	42	46,768 subjects with occupation "laundry and dry-cleaning worker" or industry "laundry and dry cleaning" in 1970 Censuses in Denmark, Finland, Norway, Sweden followed 1970−1971 through 1997−2001; 247 incident cases NHL, controls randomly selected from cohort, matched on country, sex, age, and calendar period at time of diagnosis. Dry cleaner assigned by job title or employed in shop ≤10 employees using pension data in Denmark and Finland or by questionnaire for subjects from Sweden and Norway; mean PCE during study period, 24 ppb (165 mg/m^3), nested case-control study (OR [RR]).
Other job in DC	Non-Hodgkin	0.7 (0.3, 1.6)[k]	8[j]	
Unclassifiable	Non-Hodgkin	0.9 (0.6, 1.4)[k]	52[j]	
Dry cleaner, employment duration, 1964−1979	Non-Hodgkin	1.0 (referent)	145	
≤1 yr		1.35 (0.44, 4.14)	5	
2−4 yr		0.61 (0.17, 2.21)	3	
5−9 yr		0.92 (0.49, 1.72)	14	
≥10 yr		0.66 (0.36, 1.22)	15	
Unknown		1.47 (0.49, 4.47)	5	

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
United States (SEER)				Malone et al. (1989)
Dry cleaner occupation	Chronic lymphocytic leukemia (a type of NHL)	1.1 (0.6, 2.0) (all respondents) 0.9 (0.4, 1.8) (self-respondents, no NOK information)	14	427 men and women incident CLL cases and 1,683 population controls, <80 yr of age, SEER sites, matched on sex, race, age, education, study site, questionnaire, chlorinated HC surrogate exposure metric, adjusted for race, age, education, sex, study site (OR).
New Zealand				‘t Mannetje et al. (2008); McLean et al. (2009)
Textile bleaching, dyeing and cleaning machine operators	Non-Hodgkin	0.75 (0.24, 2.32)	5	291 NHL cases ('t Mannetje et al., 2008) and 225 leukemia cases (McLean et al., 2009), in men and women, 20 or 25−75 yr, 2003−2004, New Zealand Cancer Registry, 471 population controls frequency matched on age, in-person interview, occupational title as surrogate exposure metric, adjusted for age, sex, and smoking (OR).
	Leukemia	2.07 (0.70, 6.09)	6	
Germany, 6 regions				Mester et al. (2006); Seidler et al. (2007)
Launderer, dry cleaner, presser				710 histologically confirmed Hodgkin and NHL in men and women, 18−80 yr, 1998−2003, 710 population controls matched on sex, region, and age, in-person interviews, exposure assessed by job title and JEM for semiquantitative intensity metric, adjusted for smoking and alcohol consumption (OR).
Any exposure	Non-Hodgkin and Hodgkin	1.3 (0.5, 3.2)	11	
1−10 yr duration		0.8 (0.3, 2.5)	6	
>10 yr duration		3.4 (0.6, 18.5)	5	
PCE				
0 ppm-yr	Non-Hodgkin and Hodgkin	1.0 (reference)	667	
>0− ≤9.1 ppm-yr		1.1 (0.5, 2.3)	16	
>9.1− ≤78.8 ppm-yr		1.0 (0.5, 2.2)	14	
>78.8 ppm-yr		3.4 (0.7, 17.3)	6	
Test for trend		$p = 0.12$		

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
	0 ppm-yr	Multiple myeloma	1.0 (reference)	33	Mester et al. (2006); Seidler et al. (2007) (continued)
	>0− ≤9.1 ppm-yr		1.8 (0.5, 6.7)	3	
	>9.1− ≤78.8 ppm-yr			0	
	>78.8 ppm-yr			0	
	Test for trend		p = 0.34 (negative)		
4-SEER reporting sites (CA, IO, MI, WA, United States)					Schenk et al. (2009)
	Launderers and ironers	Non-Hodgkin	3.89 (1.06, 14.20)	12	2,046 histologically confirmed NHL in men and women, 20−74 yr, 1998−2000, 1,057 population controls frequency matched on age, sex, race and study center, mailed questionnaire, occupational title exposure surrogate, adjusted for age, group, sex, ethnicity, and study center (OR).
Montreal, Canada					Siemiatycki (1991)
	Launderer and dry cleaner				215 men and women histologically confirmed incident NHL cases, 1979−1985, 35−70 yr, 533 population control group and cancer control group, in-person interviews, occupational title and JEM for PCE, adjusted age, family income, and cigarette index, 90% CI (OR).
	Any exposure	Non-Hodgkin	0.9 (0.3, 2.4)	3	
	Substantial exposure		(0.00, 1.7)	0	
Geographic-based and Other Studies					
Northern New Jersey, 75 Municipalities (United States)					Cohn et al. (1994)
	PCE in town water >5 ppb				1,190 leukemia cases identified from NJ State Cancer Registry, 1979−1987, residence in 1 of 17 NJ municipalities, PCE and other chlorinated solvents in municipal water supplies, log-linear regression adjusted for age, stratified by sex (RR).
	Males	Non-Hodgkin[1]	1.20 (0.94, 1.52)	78	
	Females		1.38 (1.08, 1.70)	87	
	Males	Leukemia	0.84 (0.66, 1.06)	63	
	Females		1.20 (0.94, 1.52)	56	

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Portland-Vancouver Metropolitan Area, Oregon (United States)					Morton and Marjanovic (1984)
	Dry cleaners and launderers				1,622 leukemia cases identified from 24 hospitals and death certificates, 1963−1977, 16−74 yr, occupational title as exposure surrogate, 1,611 dry cleaners and launderers in 1970 population census, age-standardized rates using 1970 population.
	Males	All leukemia	55.7 per 100,000[m]	2	
	Females		23.7 per 100,000[m]	5	
	Males	Lymphatic leukemia	27.8 per 100,000[m]	1	
	Females		20.9 per 100,000[m]	4	
	Males	Nonlymphatic leukemia	27.8 per 100,000[m]	1	
	Females		9.0 per 100,000[m]	2	
Hausjarvi and Hattula, Finland					Vartiainen et al. (1993)
	Hausjarvi	Non-Hodgkin	0.6 (0.3, 1.1)	14	Lymphopoeitic cancers, liver cancer and all cancers among residents with PCE and other solvents in drinking water, 1953−1991, no subject-level exposure information, cancer rates of Finnish population referent (SIR).
	Hattula		1.4 (1.0, 2.0)	31	
	Hausjarvi	Hodgkin	0.8 (0.3, 1.7)	6	
	Hattula		1.4 (0.7, 2.5)	11	
	Hausjarvi	Multiple myeloma	0.7 (0.3, 1.3)	7	
	Hattula		0.7 (0.2, 1.3)	6	
	Hausjarvi	Leukemia	1.2 (0.8, 1.7)	33	
	Hattula		0.7 (0.4, 1.1)	19	

[a] For Boice et al. (1999), all lymphopoetic cancers is the sum of ICD 9th Edition, 200−208.
[b] Internal referent population as comparison.
[c] For Andersen et al. (1999), all lymphopoeitic cancer is the sum of ICD 7th Edition, 200−204.
[d] For Cano and Pollán (2001), relative risk for male dry cleaner and launderers in 1970 Census.
[e] For Cano and Pollán (2001), relative risk for male dry cleaner and launderers in 1960 and 1970 Censuses.

Table 4-27. Summary of epidemiologic studies on tetrachloroethylene exposure and hematopoietic cancers, including leukemia (continued)

[f] For Ji and Hemminki (2006b), female subjects reporting occupation as launderers and dry cleaner in two consecutive censuses, 1960−1970, SIRs for NHL were 0.76 (95% CI: 0.39, 1.25) [*n* = 12] and 0.87 (95% CI: 0.76, 1.10) [*n* = 64], respectively, and, for multiple myeloma, 1.01 (0.46, 1.78) [*n* = 9] and 0.88 (0.60, 1.21) [*n* = 31], respectively.

[g] For Ji and Hemminki (2006b, 2005b), SIR for launderers and dry cleaners in 1960 Census. For lymphopoietic subtypes in launderers and dry cleaners in 1960 Census, for males, SIR: 0.85 (0.51, 1.28) [*n* = 19] for chronic lymphocytic leukemia, a form of NHL; 0.63 (0.25, 1.18) [*n* = 7] for acute myelogenous leukemia; 0.91 (0.29, 1.87) [*n* = 5] for chronic myelogenous leukemia; and, 1.04 (0.41, 1.96) [*n* = 7] for polycythemia vera; and, for females, SIR: 1.54 (1.05, 2.12) [*n* = 32] for chronic lymphocytic leukemia; 0.1.36 (0.83, 2.02) [*n* = 20] for acute myelogenous leukemia; 0.33 (0.03, 0.94) [*n* = 2] for chronic myelogenous leukemia; and, 1.71 (0.93, 2.73) [*n* = 14] for polycythemia vera.

[h] For Pukkala et al. (2009), SIR for chronic lymphatic leukemia, a form of NHL, were 0.90 (95% CI: 0.50-1.49) [males, *n* = 15 cases] and 1.02 (95% CI: 0.74, 1.36) [females, *n* = 46 cases].

[l] For Travier et al. (2002), RRs for subjects reporting occupation as dry cleaners, launderers, or pressers and employed in dry-cleaning industry in 1960 and 1970 Censuses (Group 2). RRs for these subjects for chronic lymphocytic leukemia, a form of NHL, were 0.67 (0.09, 4.76) [males, *n* = 1] and 2.89 (1.20, 6.96) [females, *n* = 5].

[j] For Gold et al. (2010b), odds ratio for PCE exposure with jobs assessed with low confidence considered unexposed.

[k] Lynge et al. (2006) is a nested case-control study. RR adjusted for matching criteria (country, sex, 5-yr age group and 5-yr calendar period at the time of diagnosis of the case).

[l] For Cohn et al. (1994), RRs for chronic lymphocytic leukemia, a form of NHL, were 0.98 (0.65, 1.47) [males, *n* = 28] and 0.93 (0.56, 1.52) [females, *n* = 19].

[m] For Morton and Marjanovic (1984), age-standardized incidence rate is statistically significantly different from rate for all men or all women.

CLL = chronic lymphocytic leukemia; Exp = expected number of cancers; JEM = job-exposure matrix; NOK = next of kin; RDD = relative delivered dose.

Table 4-28. Summary of epidemiologic studies on tetrachloroethylene exposure and childhood hematopoietic cancers, including leukemia

Exposure group	Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Cohort Studies				
Offspring of Electronic factory workers (Taiwan)				Sung et al. (2009)
Nonexposed	All leukemia (ICD 9, 204−208)	1.0	9	40,647 first singleton births among 47,356 women employed at factory, 1978−2001, 8,506 births among women employed 3 mo prepregnancy and 3 mo postconception, incident childhood cancers from national cancer registry, 1979−2001, does not identify PCE exposure to individual mothers, Poisson regression adjusted for maternal age, maternal education, sex and birth year, internal referents [offspring of subjects not employed during period] (RR).
Exposed pregnancy to organic solvents		3.83 (1.17, 12.55)	6	
Case-Control Studies				
Residents of ages <19 in Woburn, MA (United States)				Costas et al. (2002)
Maternal exposure 2 yr before conception to diagnosis				19 leukemia, 1969−1989, identified through physician or hospital records pre-1982 and MA Cancer Register 1982 onward, 37 local public school controls matched on race, sex, birth date, residential status, in-person interview, questionnaire to parents included information on use of public drinking water in the home, hydraulic mixing model used to estimate fraction of month that TCE, PCE and other solvents in drinking water were delivered to residence 1964−1979 (Murphy, 1990), logistic regression with composite covariate for socioeconomic status, maternal smoking during pregnancy, maternal age at birth of child, and breastfeeding (OR).
Never	Acute lymphocytic leukemia	1.00	3	
Least		5.00 (0.75, 33.5)	9	
Most		3.56 (0.51, 24.8)	7	
(*p* for linear trend)		≥0.05		
Maternal exposure 2 yr before conception				
Never	Acute lymphocytic leukemia	1.00	11	
Least		2.48 (0.42, 15.2)	4	
Most		2.82 (0.30, 26.4)	5	
(*p* for linear trend)		≥0.05		

Table 4-28. Summary of epidemiologic studies on tetrachloroethylene exposure and childhood hematopoietic cancers, including leukemia (continued)

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
	Birth to pregnancy				Costas et al. (2002) (continued)
	Never	Acute lymphocytic leukemia	1.00	7	
	Least		1.82 (0.31, 10.8)	7	
	Most		0.90 (0.18, 4.56)	5	
	(*p* for linear trend)		≥0.05		
	Maternal exposure during pregnancy				
	Never	Acute lymphocytic leukemia	1.00	9	
	Least		3.53 (0.22, 58.1)	3	
	Most		14.3 (0.92, 224)	7	
	(*p* for linear trend)		<0.05		
Residents of ages ≤14 yr Quebec (Canada)					Infante-Rivard et al. (2005)
	Probable/definite exposure to PCE	Acute lymphocytic leukemia ICD 9 204.0	0.87 (0.35−2.18)	18	790 acute lymphoblastic leukemia, 1980−2000, 790 population controls from family stipend records, 1980−1993, or health insurance records, 1994−2000, matched on sex and age, telephone interview with questions on maternal occupation, blinded JEM for PCE, logistic regression stratified by time period and adjusted for maternal age and education (OR).
	Maternal exposure 2 yr before conception to birth		0.96 (0.41−2.25)	11	
	During pregnancy		0.84 (0.30−2.34)	7	
	Cumulative exposure score				
	<4	Acute lymphocytic leukemia ICD 9 204.0	0.95 (0.35−2.55)		
	≥4		0.55 (0.05−6.34)		

Table 4-28. Summary of epidemiologic studies on tetrachloroethylene exposure and childhood hematopoietic cancers, including leukemia (continued)

Exposure group		Cancer site	Relative risk (95% CI)	No. obs.	Reference(s) and study description
Residents of ages <10 yr Los Angeles (CA) Cancer Surveillance Program					Lowengart et al. (1987)
	Maternal occupational exposure to PCE	Acute lymphatic and nonlymphatic leukemia	Not reported		123 case-control pairs—acute lymphocytic and nonlymphocytic leukemia cases, 1980−1984, and maternal friend controls or population controls matched on age, sex, race, nonblinded telephone interview, self-reported occupational exposure, logistic regression (OR).
	Paternal occupational exposure to PCE				
	1 yr before pregnancy	Acute lymphatic and nonlymphatic leukemia	∞ (p = 0.39)	1:0[a]	
	During pregnancy		∞ (p = 0.39)	1:0[a]	
	After pregnancy		∞ (0.19−∞)	2:0[a]	
Children's Cancer Group Study (children ≤15 yr of age) (Australia, Canada, United States)					Shu et al. (1999)
	Maternal occupational exposure to PCE				1,842 acute lymphocytic leukemia cases identified in 37 participating institutions, 1989−1933, 1,986 population controls, RDD, matched on age, race and telephone area code/exchange, telephone interview with structured questionnaire to assess parental exposure to PCE using job-industry title and self-reported exposure history, logistic regression adjusted for maternal education, race and family income (maternal exposures) or paternal education, race, family income, age and sex of case (OR).
	Anytime	Acute lymphocytic leukemia	0.4 (0.1−1.4)	4	
	Preconception		1.4 (0.2−8.6)	3	
	During pregnancy		1.3 (0.2−8.4)	3	
	Postnatal		0.4 (0.1−1.5)	4	
	Paternal occupational exposure to PCE				
	Anytime	Acute lymphocytic leukemia	0.9 (0.5−1.6)	25	
	Preconception		0.8 (0.5−1.5)	21	
	During pregnancy		0.5 (0.2−1.1)	8	
	Postnatal		0.5 (0.2−1.2)	10	

[a] For Lowengart et al. (1987), the number of case:control pairs.

Exp = expected number of cancers; JEM = job-exposure-matrix; RDD = relative delivered dose; Obs = observed number of cancers.

The exposure surrogate in studies of dry cleaners and launderers is a broad category and will have some associated measurement error as this broad category does not account for individual characteristics that modify one's exposure potential. For example, some variation can be expected within an occupational group between countries, as Lynge et al. (2006) reported, average tetrachloroethylene usage in 1960−1970 in Sweden was higher than in Finland or Norway. The more general the exposure surrogate, such as job title, the greater the likelihood for misclassification errors, as differences in tasks and exposure conditions within a job title may be considerable. For some occupations, these differences may be gender related, making it difficult to interpret differences in relative risk that may be observed between men and women within a specific occupational group (Messing et al., 1994). Blair et al. (2003) recruited members of a laundry and dry-cleaning workers union and attempted to increase the specificity of the classification of tetrachloroethylene exposure by examining a subgroup who entered the cohort after 1960, a time of widespread tetrachloroethylene use in dry cleaning. However, this restriction resulted in a considerable decrease in the number of observed cases of lymphopoietic cancers, from 39 in the full cohort to 2 in the group that joined after 1960. Blair et al. (2003) also developed a semiquantitative exposure intensity score using published monitoring data. The available data indicated a high degree of consistency in exposure levels to tetrachloroethylene between establishments and provided information that could be used to categorize differences in potential exposures based on types of jobs. Exposure was characterized with respect to distance from the washers (cleaners were assigned a high-exposure score, pressers, sewers, and counter clerks were assigned a medium-exposure score, and those who worked at locations that did not include washing facilities were assigned a no-exposure score) (Blair et al., 2003; Blair et al., 1990). Another study by Calvert et al. (2011) of unionized dry cleaners in the United States included an analysis of subjects who worked for one or more years before 1960 in a shop known to use tetrachloroethylene as the primary solvent. The cohort was stratified into two groups based on the level of certainty that the worker was employed only in facilities using tetrachloroethylene as the primary solvent: tetrachloroethylene-only and tetrachloroethylene plus. Another approach to improving the exposure measure was used by Lynge et al. (2006). In this study, effect measures were presented for dry cleaners separately from other laundry workers. Seldén and Ahlborg (2011) obtained information about the dry-cleaning establishment (e.g., washing techniques, chemicals used, number of employees, and work history of individual employees) in a questionnaire sent to businesses in Sweden in the 1980s to identify subjects as either dry cleaners or laundry workers. Travier et al. (2002) presented estimates for launderers, dry cleaners, and pressers, using job classifications based on the 1960 or 1970 Census data, and for subjects holding a dry-cleaning job in both census years.

A variety of exposure-assessment approaches have been used in studies in other work settings and in population-based case-control studies. One occupational study assessed tetrachloroethylene potential for individual subjects using biological monitoring data (Anttila et al., 1994). The cohort studies of aerospace workers (Boice et al., 1999) and aircraft maintenance workers (Radican et al., 2008; Blair et al., 1998; Spirtas et al., 1991) developed a job exposure matrix referencing historical industrial monitoring data. In case-control studies, attributes that strengthen the quality of the exposure assessment include ascertainment of a complete job history (i.e., all jobs held for ≥6 or 12 months rather than limiting to most recent job or longest-held job), inclusion of information on job tasks or duties as well as job title, inclusion of additional modules for specific jobs that collect more detailed information pertaining to exposure conditions, and blinded exposure assessment and development of job-exposure matrices focusing on tetrachloroethylene based on this complete set of information. These attributes were used in the case-control studies in Italy (Costantini et al., 2008; Miligi et al., 2006) and a case-control study of multiple myeloma in Washington (Gold et al., 2010a). One case-control study of potential residential tetrachloroethylene exposure used a statistical model of water distribution system to estimate delivered dose to a subject's home (Aschengrau et al., 1993). Because a nondifferential misclassification of exposure most often leads to an attenuation of the observed effect estimates (Dosemeci et al., 1990), the relative specificity of these exposure-assessment approaches, particularly those that allow assignment of values to individuals within the study, strengthens their ability to identify cancer hazards compared to studies with broader exposure-assessment approaches.

4.6.1.2.2. Adult lymphopoietic cancer: consideration of disease subtypes

The broad category of lymphopoietic cancers can be divided into specific types of cancers, including non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, and various types of leukemia (e.g., acute and chronic forms of lymphoblastic and myeloid leukemia). The classification criteria for these cancers have changed over the past 30 years, reflecting improved understanding of the underlying stem cell origins of these specific subtypes. For example, hairy cell leukemia, chronic lymphocytic leukemia, non-Hodgkin lymphoma, and multiple myeloma may arise from mature B cells. This understanding may help elucidate common etiologic pathways and exposures. The studies of tetrachloroethylene exposure examine various outcomes, including the broad category of lymphopoietic cancers, as well as non-Hodgkin lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma plus chronic lymphocytic leukemia, hairy cell leukemia, multiple myeloma, and leukemia.

All of the studies of dry cleaning and other occupations from the Nordic countries ascertained cancer incidence using national cancer registries. Four other cohort studies from the United States (Calvert et al., 2011; Radican et al., 2008; Blair et al., 2003; Boice et al., 1999)

relied on cause-of-death data from death certificates or the National Death Index. For diseases with a relatively high survival rate such as non-Hodgkin lymphoma (5-year survival: 67.4% based on 1999−2006 data), use of cause-of-death data may underestimate cancer risk. Most of the case-control studies relied on histologically confirmed cases of incident cancers in a defined geographic area, as ascertained from cancer registries.

4.6.1.2.3. Adult lymphopoietic cancer: consideration of potential confounding and other factors

Common behaviors, such as smoking and use of alcohol, have not been strongly associated with non-Hodgkin lymphoma and multiple myeloma, so there is little reason to be concerned about potential confounding of observed results pertaining to specific jobs or tetrachloroethylene measures by these factors. Smoking is a risk factor for some kinds of leukemia, however, and so its role as a potential confounder for this outcome should be considered. Tetrachloroethylene was the primary, or in Nordic countries, the exclusive solvent used in dry cleaning (Lynge et al., 2006; Johansen et al., 2005). In studies of some types of occupations, participants may also have been exposed to other solvents.

4.6.1.2.4. Adult lymphopoietic cancer: summary of results

All of the studies examining the broad category of lymphopoietic cancers were cohort studies, with the number of exposed cases ranging from 3, in a study of biologically monitored workers in Finland (Anttila et al., 1995), to 653, in a study using occupational census codes in five Nordic countries (Pukkala et al., 2009) (refer to Table 4-29). The relative risk estimates among these seven studies ranged from 0.67 (95% CI: 0.42, 1.01) to 1.51 (95% CI: 0.75, 2.70), with values from the largest studies around 1.0 (Pukkala et al., 2009; Andersen et al., 1999). The three studies with relative risk estimates greater than 1.0 were studies that used a relatively high quality exposure-assessment methodology: an standardized incidence ratio (SIR) of 1.39 (95% CI: 0.28, 4.02) in a small study in Finland examining risk among workers who had been monitored using blood tetrachloroethylene levels (Anttila et al., 1995), an SMR of 1.51 (95% CI: 0.75, 2.70) among laundry and dry-cleaning union workers employed prior to 1960 only in facilities using tetrachloroethylene as the primary solvent (tetrachloroethylene-only) (Calvert et al., 2011), and an SMR of 1.13 (95% CI: 0.62, 1.89) for routine exposure to tetrachloroethylene, based on a job exposure matrix, in a cohort study of workers in the aerospace industry (Boice et al., 1999). In the other study with a relatively detailed exposure-assessment methodology (a semiquantitative exposure score based on job titles and proximity to washers), no increased risk was observed (SMR: 0.9, 95% CI: 0.5, 1.4, for the medium/high intensity score group) (Blair et al., 2003).

Table 4-29. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult lymphopoietic cancer and leukemia, by cancer type and study design

Cancer type, *n* exposed cases	Relative risk (95% CI)	Design, location, exposure assessment[a]	Reference
Lymphopoietic (all)		Cohort	
3	1.38 (0.28, 4.02)	biological monitored workers (SIR), Finland, blood PCE[a]	Antilla et al. (1995)
11	1.51 (0.75, 2.70)	laundry and dry-cleaning workers (SMR), United States, union employment records (PCE-only exposure based on history of solvent use by shops)	Calvert et al. (2011)
14	1.13 (0.62, 1.89)	aerospace workers (SMR), United States, job exposure matrix (PCE routine exposure)[a]	Boice et al. (1999)
22	0.67 (0.42, 1.01)	electronic factory workers (SIR), Taiwan	Chang et al. (2005)
39	1.0 (0.7, 1.3)	laundry and dry-cleaning workers (SMR), United States, union records (all workers)	Blair et al. (2003)
	0.9 (0.5, 1.4)	(medium/high intensity score)[a]	Blair et al. (2003)
204	1.0 (0.87, 1.15)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, census occupation codes	Andersen et al. (1999)
653	0.98 (0.30, 1.52)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, Iceland, census occupation codes	Pukkala et al. (2009)
Leukemia (all)		Cohort	
5	0.55 (0.18, 1.29)	aerospace workers (SMR), United States, job exposure matrix (PCE routine exposure)[a]	Boice et al. (1999)
5	0.78 (0.49, 1.17)	electronic factory workers (SIR), Taiwan (females)	Sung et al. (2007)
7	0.74 (0.30, 1.52)	laundry and dry-cleaning workers (SIR), Sweden, census occupation codes	Lynge and Thygesen (1990)
12	0.8 (0.4, 1.4)	laundry and R workers (SMR), United States, union records (all workers)	Blair et al. (2003)
3	0.93 (0.30, 2.88)	laundry and dry-cleaning workers and pressers, Sweden, census occupation codes, 1960 and 1970 (males)	Travier et al. (2002)
12	2.53 (1.44, 4.46)	laundry and dry-cleaning workers and pressers, Sweden, census occupation codes, 1960 and 1970 (females)	Travier et al. (2002)
15	1.84 (1.11, 2.88)	laundry and dry-cleaning workers and pressers, Sweden, census occupation codes, 1960 and 1970 (males and females)	Travier et al. (2002)
58	0.85 (0.65, 1.0)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, census occupation codes	Andersen et al. (1999)
47	0.84 (0.62, 1.90)	laundry and dry-cleaning workers (SIR), Sweden (males)	Ji and Hemminki (2005b)

Table 4-29. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult lymphopoietic cancer and leukemia, by cancer type and study design (continued)

Cancer type, *n* exposed cases	Relative risk (95% CI)	Design, location, exposure assessment[a]	Reference
80	1.30 (1.03, 1.60)	laundry and dry-cleaning workers (SIR), Sweden (females)	Ji and Hemminki (2005b)
204	0.95 (0.83, 1.09)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, Iceland, census occupation codes	Pukkala et al. (2009)
Leukemia (all)		Case-control	
2	3.3 (0.3, 32.4)	Italy, job titles (launderer, dry cleaner, presser) (males)	Costantini et al. (2001)
5	1.1 (0.4, 3.2)	Italy, job titles (launderer, dry cleaner, presser) (females)	Miligi et al. (1999)
6	2.07 (0.70, 6.09)	New Zealand, occupational title (textile bleaching, dyeing and cleaning machine operators)	McLean et al. (2009)
7	1.0 (0.4, 2.7)	Italy, job exposure matrix (PCE, medium/high intensity)[a]	Costantini et al. (2008)
Leukemia (all)		Geographic based	
7	2.13 (0.88, 5.19)	Massachusetts, water distribution model (any PCE)[a]	Aschengrau et al. (1993)
19	0.7 (0.4, 1.1)	Finland (Hattula), PCE in drinking water	Vartiainen et al. (1993)
33	1.2 (0.8, 1.7)	Finland (Hausjarvi), PCE in drinking water	Vartiainen et al. (1993)
56	1.20 (0.94, 1.52)	New Jersey, PCE in town water >5 ppb (females)	Cohn et al. (1994)
64	0.84 (0.66, 1.06)	New Jersey, PCE in town water >5 ppb (males)	Cohn et al. (1994)

[a] Studies with relatively high quality exposure assessment methodologies, based on biological monitoring data, cohort studies with job exposure matrix based on historical industrial monitoring data, or case-control studies with job exposure matrix focusing on PCE based on information on job title and tasks or duties, and additional modules for specific jobs, or studies of residential PCE exposure using a statistical model of water distribution system to estimate delivered dose to a subject's home.

Studies of leukemia risk include occupational cohorts and case-control studies and geographic-based studies of residential exposure (refer to Table 4-30). The cohort studies range from 5 to 204 cases. Two studies using Swedish census data on occupation reported elevated relative risks among women, but not men, who reported jobs as launderers or dry cleaners.

Table 4-30. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult non-Hodgkin lymphoma, by study design

Cancer type, *n* exposed cases	Relative Risk (95% CI)	Design, location, exposure assessment[a]	Reference
Adult non-Hodgkin lymphoma		Cohort	
3	3.76 (0.77, 11.0)	biological monitored workers (SIR), Finland, blood PCE[a]	Antilla et al. (1995)
2	2.35 (0.52, 10.7)	aircraft maintenance workers (RR-internal referent), United States, job exposure matrix (PCE) (females)[a]	Radican et al. (2008)
5	2.32 (0.75, 7.15)	aircraft maintenance workers (RR-internal referent), United States, job exposure matrix (PCE) (males)[a]	Radican et al. (2008)
6	2.46 (0.90, 5.36)	laundry and dry-cleaning workers (SMR), United States, union employment records (PCE-only exposure based on history of solvent use by shops)	Calvert et al. (2011)
8	1.70 (0.73, 3.34)	aerospace workers (SMR), United States, job exposure matrix (routine exposure to PCE)[a]	Boice et al. (1999)
8	1.03 (0.44, 2.02)	laundry and dry-cleaning workers (SIR), Sweden, census occupation codes	Lynge and Thygesen (1990)
8	0.86 (0.43, 1.72)	laundry and dry-cleaning workers and pressers, Sweden, census occupation codes	Travier et al. (2002)
11	1.76 (0.97, 3.17)	laundry and dry-cleaning workers (SIR), Sweden, census occupation codes	Cano and Pollán (2001)
12	0.9 (0.5, 1.6)	laundry and dry-cleaning workers (SMR), United States, union records (all workers)	Blair et al. (2003)
15	2.02 (1.13, 3.34)	dry-cleaning workers (SIR), Sweden, census occupation codes and questionnaire (dry cleaner) (males)[a]	Seldén and Ahlborg (2011)
18	1.14 (0.68, 1.81)	dry-cleaning workers (SIR), Sweden, census occupation codes and questionnaire (dry cleaner) (females)[a]	Seldén and Ahlborg (2011)
27	1.46 (0.96, 2.13)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, census occupation codes (males)	Andersen et al. (1999)
55	0.95 (0.71, 1.23)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, census occupation codes (females)	Andersen et al. (1999)
59	0.99 (0.75, 1.26)	laundry and dry-cleaning workers (SIR), Sweden (males)	Ji and Hemminki (2006b)
67	1.05 (0.82, 1.32)	laundry and dry-cleaning workers (SIR), Sweden (females)	Ji and Hemminki (2006b)
264	0.98 (0.86, 1.10)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, Iceland, census occupation codes	Pukkala et al. (2009)

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Table 4-30. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult non-Hodgkin lymphoma, by study design (continued)

Cancer type, *n* exposed cases	Relative Risk (95% CI)	Design, location, exposure assessment[a]	Reference
42	1.0 (0.7, 1.4)	nested case-control, Sweden, Denmark, Finland, Norway, census occupation codes and pension data/questionnaires (dry cleaners)	Lynge et al. (2006)
Adult non-Hodgkin lymphoma		Case-control	
1	3.0 (0.2, 49.2)	France, jobs held 6 or more mo, launderer and dry cleaner[b]	Clavel et al. (1998)
3	0.9 (0.3, 2.4)	Canada, job exposure matrix for PCE (any exposure)	Siemiatycki (1991)
3	1.6 (0.3, 9.1)	Italy, job titles (launderer, dry cleaner, presser) (males)	Costantini et al. (2001)
5	0.75 (0.24, 2.32)	New Zealand, occupational title (textile bleaching, dyeing and cleaning machine operators)	't Mannetje et al. (2008)
7	1.59 (0.49, 5.13)	United States, self-reported exposure to dry-cleaning fluids	Kato et al. (2005)
9	1.6 (0.6, 4.0)	United States, laundering, dry cleaning, leather products fabrication[c]	Scherr et al. (1992)
10	0.7 (0.3, 1.5)	Italy, job titles (launderer, dry cleaner, presser) (females)	Miligi et al. (1999)
10	4.6 (1.9, 11.4)	Sweden, JEM using self-reported information (any styrene, TCE, PCE, or benzene exposure)	Hardell et al. (1981)
12	3.89 (1.06, 14.2)	United States, occupation title (launders and ironers)	Schenk et al. (2009)
14	1.2 (0.6, 2.5)	Italy, job exposure matrix (PCE, medium/high intensity)[a, d]	Miligi et al. (2006)
14	1.1 (0.6, 2.0)	United States, ever employed in dry-cleaning industry[e]	Malone et al. (1989)
16	2.0 (0.97, 4.3)	United States, all jobs held ≥1 yr (dry-cleaning industry)	Blair et al. (1993)
35	1.0 (0.6, 1.6)	France, self-reported exposure to dry-cleaning solvents	Fabbro-Peray et al. (2001)
Adult non-Hodgkin lymphoma		Geographic-based (residential exposure)	Vartiainen et al. (1993)
14	0.6 (0.3, 1.1)	Finland (Hausjarvi), PCE and other solvents in drinking water	Vartiainen et al. (1993)
31	1.4 (1.0, 2.0)	Finland (Hattula), PCE and other solvents in drinking water	Vartiainen et al. (1993)
78	1.20 (0.94, 1.52)	New Jersey, PCE in town water >5 ppb (males)	Cohn et al. (1994)
87	1.38 (1.08, 1.70)	New Jersey, PCE in town water >5 ppb (females)	Cohn et al. (1994)

Table 4-30. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult non-Hodgkin lymphoma, by study design (continued)

[a] Studies with relatively high quality exposure-assessment methodologies, based on biological monitoring data, cohort studies with job exposure matrix based on historical industrial monitoring data, or case-control studies with job exposure matrix focusing on PCE based on information on job title and tasks or duties, and additional modules for specific jobs, or studies of residential PCE exposure using a statistical model of water distribution system to estimate delivered dose to a subject's home.
[b] Includes patients with hairy cell leukemia.
[c] Number of exposed cases estimated based on report of a prevalence of 3% in the population (*n* cases = 303); job history limited to most recent job, job held 15 yr ago, major occupation, and second most major occupation.
[d] Includes patients with non-Hodgkin lymphoma and chronic lymphocytic leukemia.
[e] Includes patients with chronic lymphocytic leukemia.

Travier et al. (2002) examined cancer incidence from 1971 through 1989. The relative risk among women who reported work as a launderer, dry cleaner, or presser in the laundry, ironing, or dyeing industry in 1960 and 1970 was 2.53 (95% CI: 1.44, 4.46), and among men, the relative risk was 0.93 (95% CI: 0.30, 2.28). Ji and Hemminki (2005b) used a similar approach, with cancer incidence ascertained through 2002. The start of follow-up began at the time of the relevant census data (i.e., 1961 for analyses based on jobs held in 1960). The SIR among women who worked as a launderer or dry cleaner in 1970 was 1.30 (95% CI: 1.03, 1.60), and the SIR among men who worked as a launderer or dry cleaner in 1960 was 0.84 (95% CI: 0.62, 1.09). The latter time period was used for women because of the increase of women in the workforce during the 1960s. A limitation of these studies is the lack of detailed information pertaining to job tasks for individuals, information that could be particularly useful with respect to the interpretation of the observed gender-related differences. No increased risk was observed in the cohort study of aerospace workers using a job exposure matrix to estimate tetrachloroethylene exposure [SMR: 0.55, 95% CI: 0.18, 1.29 in Boice et al. (1999)]. The number of exposed cases in the case-control studies range from 2 to 7 leukemia cases. The odds ratio in the study with a relatively strong exposure-assessment methodology was 1.0 (95% CI: 0.4, 2.7) (Costantini et al., 2008). The three geographic-based studies of residential exposure involved 7 to 64 exposed cases. The case-control study in Cape Cod, MA, that estimated exposure using a statistical model of the water distribution reported an adjusted odds ratio of 2.13 (95% CI: 0.88, 5.19) for any tetrachloroethylene exposure and 8.33 (95% CI: 1.53, 25.29) for exposures above the 90th percentile (Aschengrau et al., 1993). Relative risk estimates were lower, ranging from 0.7 to 1.2, in two other residential studies with poorer quality exposure-assessment approaches (Cohn et al., 1994; Vartiainen et al., 1993).

The data pertaining to non-Hodgkin lymphoma are more extensive, with 14 cohort studies ranging in size from 3 (Anttila et al., 1995) to 264 (Pukkala et al., 2009) cases, 13 publications based on case-control studies from six countries ranging in size from 3

(Siemiatycki, 1991) to 35 exposed cases (Fabbro-Peray et al., 2001), and two geographic-based studies of residential exposures through drinking water (Cohn et al., 1994; Vartiainen et al., 1993) (refer to Table 4-30). Six of the relative risk estimates from the cohort studies, including the four with the largest number of non-Hodgkin lymphoma cases, were between 0.95 and 1.05 (Pukkala et al., 2009; Ji and Hemminki, 2006b, 2005b; Andersen et al., 1999). Among the nine smaller cohorts (*n* cases <30) (Calvert et al., 2011; Radican et al., 2008; Blair et al., 2003; Travier et al., 2002; Cano and Pollán, 2001; Andersen et al., 1999; Boice et al., 1999; Anttila et al., 1995; Lynge and Thygesen, 1990), three effect estimates were between 0.86 and 1.03, and six ranged from 1.46 to 3.76. Five cohort studies using relatively high quality exposure-assessment methods reported the highest relative risks, but these studies were also based on only 2 to 18 exposed cases, so the estimates are imprecise: RR: 2.35 (95% CI: 0.52, 10.7) for females and 2.32 (95% CI 0.75, 7.15) for males in Radican et al. (2008); RR: 3.76 (95% CI: 0.77, 11.0) in Antilla et al. (1995); RR: 1.70 (95% CI: 0.73, 3.34) in Boice et al. (1999); SIR: 2.02 (95% CI: 1.13, 3.34) for males and 1.14 (95% CI: 0.68, 1.68) for females in Seldén and Ahlborg (2011); and SMR: 2.46 (95% CI: 0.90, 5.36) in Calvert et al. (Calvert et al., 2011). Results from the case-control studies are also quite variable, with ORs ranging from 0.7 to 4.6 (Schenk et al., 2009; Lynge et al., 2006; Miligi et al., 2006; Fabbro-Peray et al., 2001; Miligi et al., 1999; Blair et al., 1993; Siemiatycki, 1991; Malone et al., 1989; Hardell et al., 1981). The studies with the higher quality exposure estimate reported ORs of 1.2 (95% CI: 0.6, 2.5) and 1.0 (95% CI: 0.7, 1.4) (Lynge et al., 2006; Miligi et al., 2006). Both of the geographic studies provide some evidence of an association between residential exposures via drinking water. Cohn et al. (1994) reported RR: 1.38 (95% CI: 1.08, 1.70) in females and RR: 1.20 (95% CI: 0.94. 1.52) for residence in a town with municipal water supplies containing >5 ppb tetrachloroethylene. In the second, a study of two towns with tetrachloroethylene and other solvents in the drinking water in Finland, an association was observed in one town (SIR: 1.4, 95% CI: 1.0, 2.0) but not in the other (SIR: 0.6, 95% CI: 0.3, 1.1) (Vartiainen et al., 1993). The ability of these studies to provide clear and specific evidence pertaining to cancer hazard and tetrachloroethylene is limited by their ecological designs and examination of several solvents in addition to tetrachloroethylene.

Six studies provide data pertaining to tetrachloroethylene and Hodgkin lymphoma (refer to Table 4-31). Four cohort studies (Pukkala et al., 2009; Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999) and one case-control study reported in two published papers (Costantini et al., 2001; Miligi et al., 1999) examine risk among laundry and dry-cleaning workers, and one is a geographic-based study of drinking water exposure in two towns in Finland (Vartiainen et al., 1993). No association is observed in the largest cohort, with 33 cases in the cohort of laundry and dry-cleaning workers from 5 Nordic countries (SIR: 0.97, 95% CI: 0.67, 1.36) (Pukkala et

al., 2009). A two- to threefold increased risk is observed in each of the smaller occupational studies, with number of cases ranging from 4 to 19 (Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999). The exposure-assessment methodology in these studies is relatively limited, and none were considered to be of high quality.

The studies of multiple myeloma are summarized in Table 4-31. As was observed in the compilation of studies of other types of lymphopoietic cancers, the larger cohort studies that use a relatively nonspecific exposure measure (broad occupational title of launderers and dry cleaners, based on census data) do not report an increased risk, with effect estimates ranging from 0.99 to 1.07 (Pukkala et al., 2009; Ji and Hemminki, 2006b; Andersen et al., 1999). Results from the cohort and case-control studies with a higher quality exposure-assessment methodology, with an exposure measure developed specifically for tetrachloroethylene, do provide evidence of an association, however, with relative risks of 7.84 (95% CI: 1.43, 43.1) in women and 1.71 (95% CI: 0.42, 6.91) in men in the cohort of aircraft maintenance workers (Radican et al., 2008) and 1.5 (95% CI: 0.8, 2.9) in the case-control study (Gold et al., 2010b). Boice et al. (1999) also used a relatively high quality exposure measure, but because the results

Table 4-31. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult Hodgkin lymphoma and multiple myeloma, by study design

Cancer type, *n* exposed cases	Relative Risk (95% CI)	Design, location, exposure assessment[a]	Reference
Hodgkin		Cohort	
4	2.69 (1.01, 7.19)	laundry and dry-cleaning workers and pressers, Sweden, census occupation codes	Travier et al. (2002)
5	2.0 (0.6, 4.6)	laundry and dry-cleaning workers (SMR), United States, union employment records	Blair et al. (2003)
19	1.88 (1.13, 2.93)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, census occupation codes (females)	Andersen et al. (1999)
33	0.97 (0.67, 1.36)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, Iceland, census occupation codes	Pukkala et al. (2009)
Hodgkin		Case-control	
1	2.5 (0.3, 24.6)	Italy, job titles (launderer, dry cleaner, presser) (males)	Costantini et al. (2001)
7	3.5 (1.5, 8.2)	Italy, job titles (launderer, dry cleaner, presser) (females)	Miligi et al. (1999)
Hodgkin		Geographic-based	
6	0.8 (0.3, 1.7)	Finland (Hausjarvi), PCE in drinking water	Vartiainen (1993)

11	1.4 (0.7, 2.5)	Finland (Hattula), PCE in drinking water	Vartiainen (1993)

Table 4-31. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult Hodgkin lymphoma and multiple myeloma, by study design (continued)

Cancer type, *n* exposed cases	Relative Risk (95% CI)	Design, location, exposure assessment[a]	Reference
Multiple myeloma		Cohort	
1	0.40 (0.01, 2.25)	aerospace workers (SMR), United States, job exposure matrix (PCE routine exposure)[a]	Boice et al. (1999)
2	7.84 (1.43, 43.1)	aircraft maintenance workers (RR-internal referent), United States job exposure matrix (females)[a]	Radican et al. (2008)
3	1.71 (0.42, 6.91)	aircraft maintenance workers (RR-internal referent), United States, job exposure matrix (males)[a]	Radican et al. (2008)
7	0.8 (0.3, 1.6)	laundry and dry-cleaning workers (SMR), United States, union records (all workers)	Blair et al. (2003)
7	1.75 (0.70, 3.61)	laundry and dry-cleaning workers (SIR), Sweden, census occupation codes	Lynge and Thygesen (1990)
36	1.07 (0.75, 1.45)	laundry and dry-cleaning workers (SIR), Sweden (females)	Ji and Hemminki (2006b)
45	1.0 (0.73, 1.34)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, census occupation codes	Andersen et al. (1999)
52	0.99 (0.66, 1.38)	laundry and dry-cleaning workers (SIR), Sweden (males)	Ji and Hemminki (2006b)
152	1.02 (0.86, 1.20)	laundry and dry-cleaning workers (SIR), Sweden, Denmark, Finland, Norway, Iceland, census occupation codes	Pukkala et al. (2009)
Multiple myeloma		Case-control	
3	1.0 (0.3, 3.8)	Italy, job titles (launderer, dry cleaner, presser) (females)	Miligi et al. (1999)
9	6.0 (1.7, 21)	United States, all jobs held ≥12 mo (textile, apparel, furnishing machine operators and tenders)	Gold et al. (2010a)
16	1.5 (0.8, 2.9)	United States, all jobs held ≥12 mo, job exposure matrix (PCE)[a,b]	Gold et al. (2010b)
Multiple myeloma		Geographic-based	
6	0.7 (0.2, 1.3)	Finland (Hattula), PCE in drinking water	Vartiainen (1993)
7	0.7 (0.3, 1.3)	Finland (Hausjarvi), PCE in drinking water	Vartiainen (1993)

[a] Studies with relatively high quality exposure-assessment methodologies, based on biological monitoring data, cohort studies with job exposure matrix based on historical industrial monitoring data, or case-control studies with job exposure matrix focusing on PCE based on information on job title and tasks or duties, and additional modules for specific jobs, or studies of residential PCE exposure using a statistical model of water distribution system to estimate delivered dose to a subject's home.

[b] Results for analysis in which low confidence jobs were considered unexposed. Similar results observed in the primary analysis in which low confidence jobs were included in the exposure group.

are based on only one observed case, the imprecision of the estimate (RR: 0.40, 95% CI: 0.01, 2.25) limits this study for insights on multiple myeloma and tetrachloroethylene.

Variation in risk in relation to variation in exposure levels is examined in one study of lymphopoietic cancer (Blair et al., 2003), five studies of non-Hodgkin lymphoma (Lynge et al., 2006; Miligi et al., 2006; Boice et al., 1999; Blair et al., 1993) or of non-Hodgkin combined with Hodgkin lymphoma (Seidler et al., 2007), four studies of multiple myeloma (Gold et al., 2010a; Gold et al., 2010b; Seidler et al., 2007; Boice et al., 1999) and two studies of leukemia (Miligi et al., 2006; Aschengrau and Seage, 2003) (refer to Table 4-32). Gold et al. (2010b) and Seidler et al. (2007) examined exposure gradients using a cumulative tetrachloroethylene measure. The aerospace worker cohort study by Boice et al. (1999), the dry cleaners cohort study by Blair et al. (2003), and the Italian case-control studies (Costantini et al., 2008; Miligi et al., 2006) used a semiquantitative measure of exposure intensity or frequency, and two studies used a less-specific measure of job duration (Gold et al., 2010a; Lynge et al., 2006). Inability to account for temporal changes in exposure intensity makes duration an inferior exposure surrogate compared to semiquantitative or quantitative measures. The tetrachloroethylene-based measures in the non-Hodgkin lymphoma studies (Seidler et al., 2007; Miligi et al., 2006; Boice et al., 1999) provide evidence of a higher risk at the higher exposure levels, particularly in the highest category of cumulative exposure (>78.8 ppm-years) in the case-control study by Seidler et al. (2007). Similar results are observed in one of the multiple myeloma studies (Gold et al., 2010b), but the smaller study by Seidler et al. (2007) observed no cases among the highest exposure groups (refer to Table 4-32).

There is considerable variation in the databases (e.g., number of studies, study design, and quality of the exposure assessment) for the different types of lymphopoietic cancers. In general, studies with relatively strong exposure assessments are based on a small number of observed deaths or incident cases, with a relatively low statistical power resulting from few observed events, or, for population case-control studies, low exposure prevalence. For non-Hodgkin lymphoma and multiple myeloma, the presence of higher relative risk estimates in studies with better exposure-assessment methodologies and evidence of an exposure-response trend in one or more studies provide the basis for considering the collection of studies as supportive of a role of tetrachloroethylene as a likely carcinogen. The collection of studies for leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, and multiple myeloma is summarized below.

There is little evidence for an association with leukemia. The two studies with a relatively high quality exposure-assessment methodology had few exposed cases (≤7) and did

not provide evidence of an association (RRs of 0.55 and 1.0 in Boice et al. (1999) and Costantini et al. (2008), respectively), although a case-control study reported a twofold increased risk of leukemia with the highest exposure level of tetrachloroethylene-contaminated drinking water (Aschengrau et al., 1993). The results from studies using more general (i.e., nonspecific) exposure methods (e.g., occupational codes for laundry or dry-cleaning workers) generally showed no association with leukemia (i.e., relative risk estimates <1.0 in 6 of the 9 cohorts) (Pukkala et al., 2009; Sung et al., 2007; Blair et al., 2003; Andersen et al., 1999; Boice et al., 1999; Lynge and Thygesen, 1990). Two of the increased leukemia relative risks (RR of 2.53 and 1.30) were observed in studies limited to female workers, which may represent a more homogenous group in terms of potential exposures (Ji and Hemminki, 2005b; Travier et al., 2002).

Table 4-32. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult lymphopoeitic cancers, with data pertaining to exposure-response gradients, by cancer type

Cancer type	Exposure measure	Results		Design, location, exposure assessment	Reference
		n	RR (95% CI)		
Lymphopoeitic	Exposure score Little to no Medium to high	 18 17	 1.0 (0.6, 1.5) 0.9 (0.5, 1.4)	Cohort, laundry and dry-cleaning workers, union records (exposure score based on proximity to washers)	Blair et al. (2003)
Non-Hodgkin	Job duration (yr) 0 >0− ≤1 2−4 5−9 ≥10	 145 5 3 14 15	 1.0 (referent) 1.35 (0.44, 4.14) 0.61 (0.17, 2.21) 0.92 (0.49, 1.72) 0.66 (0.36, 1.22)	Nested case-control within cohort of laundry and dry-cleaning workers, Sweden, Denmark, Finland, Norway, census occupation codes[a]	Lynge et al. (2006)
	PCE (duration, yr) 0 <1 1−4 ≥5	 32 4 6 10	 1.0 (referent) 1.25 (0.43, 3.57) 1.11 (0.46, 2.70) 1.41 (0.67, 3.00) (trend $p > 0.20$)	Cohort, aerospace workers, job exposure matrix (routine or intermittent exposure to PCE)	Boice et al. (1999)
	PCE (intensity) Very low/low Medium/high	 18 14	 0.6 (0.3, 1.2) 1.2 (0.6, 2.5) (trend $p = 0.72$)	Case-control, Italy, job exposure matrix	Miligi et al. (2006)
	PCE (duration, yr) ≤15 >15	 10 3	 1.3 (0.5, 3.3) not reported[a]	Case-control, Italy, job exposure matrix	Miligi et al. (2006)

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	PCE (cumulative, ppm-yr) 0 >0− ≤9.1 >9.1− ≤78.8 >78.8	 67 16 14 6	 1.0 (referent) 1.1 (0.5, 2.3) 1.0 (0.5, 2.2) 3.4 (0.7, 17.3) (trend $p = 0.12$)	Case-control, Germany (PCE, job exposure matrix)[b] (Includes non-Hodgkin and Hodgkin lymphoma; similar results observed with B-non-Hodgkin)	Seidler et al. (2007)

Table 4-32. Results of epidemiologic studies of potential tetrachloroethylene exposure and adult lymphopoeitic cancers, with data pertaining to exposure-response gradients, by cancer type (continued)

Cancer type	Exposure measure	Results		Design, location, exposure assessment	Reference
		n	RR (95% CI)		
Multiple myeloma	Job duration (yr) 1−5 >5	 4 5	 3.6 (0.7, 1.7) 12 (1.3, 110) (trend $p < 0.01$)	Case-control, United States, all jobs held ≥12 mo (textile, apparel, furnishing machine operators and tenders)	Gold et al. (2010a)
	PCE duration (yr) 0 <1 1−4 ≥5	 24 1 4 1	 1.0 (referent) 0.46 (0.06, 3.48) 1.13 (0.38, 3.35) 0.24 (0.03, 1.84) (trend $p < 0.01$)	Cohort, aerospace workers, job exposure matrix (routine or intermittent exposure to PCE)	Boice et al. (1999)
	PCE duration (yr) 1−4 5−11 12−29 3−51	 3 3 4 6	 0.9 (0.2, 3.5) 2.0 (0.4, 9.2) 1.3 (0.3, 4.6) 2.1 (0.7, 6.8) (trend $p = 0.18$)	Case-control, United States, all jobs held ≥12 mo (PCE, job exposure matrix)[c]	Gold et al. (2010b)
	PCE (cumulative) 1−318 319−2,218 2,219−7,713 7,794−57,000	 1 1 4 10	 0.3 (0.04, 3.0) 0.3 (0.1, 4.4) 1.5 (0.4, 5.4) 3.3 (1.2, 9.5) (trend $p = 0.02$)	Case-control, United States, all jobs held ≥12 mo (PCE, job exposure matrix)[c]	Gold et al. (2010b)
	PCE (cumulative) 0 >0− ≤9.1 ppm-yr >9.1− ≤78.8 ppm-yr >78.8 ppm-yr	 5 6 0 0	 1.0 (referent) 1.8 (0.5, 6.7) (inverse trend $p = 0.34$)	Case-control, Germany (PCE, job exposure matrix)[b]	Seidler et al. (2007)
Leukemia	PCE intensity Very low/low Medium/high	 6 7	 0.6 (0.2, 1.6) 1.0 (0.4, 2.7)	Case-control, Italy, job exposure matrix (PCE)	Costantini et al. (2008)
	Any PCE RDD >90th percentile	7 2	2.13 (0.88, 5.19) 8.33 (1.53, 25.3)	Geographic based, United States, water distribution model (any PCE)	Aschengrau et al. (1993)

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[a] Relative risk estimates only reported for strata with at least five exposed cases.
[b] Cumulative score based on summation of the product of intensity score (low, 5 ppm; medium, 50 ppm; high, 200 ppm), frequency score (low, 3%; medium, 7.5%; high, 65%) of workweek, and duration for each job.
[c] Results for analysis in which low confidence jobs were considered unexposed. Similar results observed in the primary analysis in which low confidence jobs were included in the exposure group. Cumulative measure based on summation of the product of intensity (ppm), frequency (h/wk), and duration (yr) for each job.

The results from the collection of studies pertaining to non-Hodgkin lymphoma indicate an elevated risk associated with tetrachloroethylene exposure. The results from five cohort studies that used a relatively high quality exposure-assessment methodology generally reported relative risks between 1.7 and 3.8 (Calvert et al., 2011; Seldén and Ahlborg, 2011; Radican et al., 2008; Boice et al., 1999; Anttila et al., 1995) and support an association with tetrachloroethylene. The studies with tetrachloroethylene-specific exposure measures and exposure-response analysis (based on intensity, duration, or cumulative exposure) (Seidler et al., 2007; Miligi et al., 2006; Boice et al., 1999) provide further support for an association, reporting higher non-Hodgkin lymphoma risks in the highest exposure category, with the strongest evidence from the large case-control study in Germany, in which a relative risk of 3.4 (95% CI: 0.7, 17.3) was observed in the highest cumulative exposure category (trend p-value = 0.12) (Seidler et al., 2007). Lynge et al. (2006) distinguished dry cleaners from other workers but used an approach with greater potential for misclassification because exposure was assigned only for jobs held in 1970. This study did not report an association between dry cleaners and non-Hodgkin lymphoma, nor did risk estimates increase with exposure duration. Effect estimates in studies with broader exposure assessments showed a more variable pattern (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji and Hemminki, 2006b; Blair et al., 2003; Travier et al., 2002; Cano and Pollán, 2001; Lynge and Thygesen, 1990). Confounding by lifestyle factors are unlikely explanations for the observed non-Hodgkin lymphoma results because common behaviors, such as smoking and alcohol use, are not strong risk factors for non-Hodgkin lymphoma (Besson et al., 2006; Morton et al., 2005).

With respect to Hodgkin lymphoma, the data are more limited, with only four cohort studies (Pukkala et al., 2009; Blair et al., 2003; Travier et al., 2002; Andersen et al., 1999), one case-control study from Italy reported in two publications (Costantini et al., 2001; Miligi et al., 1999), and one geographic-based study from Finland (Vartiainen et al., 1993). None of the exposure-assessment methods used in these studies were considered to be relatively high quality. A two- to threefold increased risk is observed in all of the occupational studies except Pukkala et al. (2009) [SIR: 0.97 (95% CI: 0.67, 1.36)].

The larger cohort studies that use a relatively nonspecific exposure measure (broad occupational title of launderers and dry cleaners, based on census data) do not report an increased risk of multiple myeloma, with effect estimates ranging from 0.99 to 1.07 (Pukkala et al., 2009; Ji and Hemminki, 2006b; Andersen et al., 1999). Some uncertainty in these estimates

arises from these studies' broader exposure-assessment methodology. Results from the cohort and case-control studies with a higher quality exposure-assessment methodology, with an exposure measure developed specifically for tetrachloroethylene, do provide evidence of an association, however, with relative risks of 7.84 (95% CI: 1.43, 43.1) in women and 1.71 (95% CI: 0.42, 6.91) in men in the cohort of aircraft maintenance workers (Radican et al., 2008) and 1.5 (95% CI: 0.8, 2.9) in the case-control study in Washington [Gold et al. (2010b); tetrachloroethylene exposure]. Gold et al. (2010a; 2010b) also reported increasing risks with increasing exposure duration [based on job titles (Gold et al., 2010a) and based on a cumulative tetrachloroethylene exposure metric (Gold et al., 2010b)]. A smaller case-control study (n = 76 cases) with tetrachloroethylene-specific exposure measures based on intensity, duration, or cumulative exposure, Seidler et al. (2007), observed no cases among the highest exposure groups. A small study by Boice et al. (1999) of aerospace workers observed one death among routinely exposed subjects and six deaths among subjects with a broader definition of routine or intermittent exposure.

4.6.1.2.5. Childhood leukemia

One cohort and 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 (Sung et al., 2008; Infante-Rivard et al., 2005; Costas et al., 2002; Shu et al., 1999; Lowengart et al., 1987) (Table 4-28; Appendix B). Some studies suggest a vulnerability for ALL with maternal exposure either preconception or during pregnancy (Sung et al., 2009; Costas et al., 2002; Shu et al., 1999; Lowengart et al., 1987). These studies, however, are insensitive for assessing association, or lack thereof, between ALL and tetrachloroethylene exposure because observations are based on a few exposed cases (all studies) or a weak exposure assessment (Sung et al., 2008). Only Lowengart et al. (1987) and Shu et al. (1999) examined paternal exposure and tetrachloroethylene exposure with inconsistent observations. Other studies are needed to clarify the role of tetrachloroethylene in ALL.

4.6.2. Animal Studies

4.6.2.1. Noncancer Effects

4.6.2.1.1. Immunotoxicity

The animal evidence for immunotoxicity following exposure to tetrachloroethylene is very limited. These studies consist of mixed solvent exposures and some inhalation and oral studies in which experimental animals were dosed with tetrachloroethylene alone.

Immune system parameters were altered in a mouse study (female $B6C3F_1$) administered tetrachloroethylene by inhalation (maximum concentration: 6.8 ppm) along with a mixture of 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 observed, with a dose-related decrease in antibody response to sheep red blood cells and decreased host resistance following challenge to *Plasmodium yoelli*. There were no changes in lymphocyte number, T-cell subpopulations, NK cell activity, or in response to challenge to *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).

Aranyi et al. (1986) studied the effects of acute inhalation exposures to 25 or 50 ppm tetrachloroethylene on two measures of immune response (susceptibility to respiratory infection and mortality due to *Streptoccocus zooepidemicus* exposure and ability of pulmonary macrophages to clear infection with *Klebsiella pneumoniae*). Female CD1 mice that were 5−7 weeks of age at the start of the exposure portion of the experiment were used for both assays. Up to five replicate groups of about 30 mice were challenged with viable *S. zooepidemicus* during simultaneous exposure to tetrachloroethylene or to filtered air. Deaths were recorded over a 14-day observation period. Clearance of ^{35}S-labeled *K. pneumoniae* by pulmonary macrophages was determined by measuring the ratio of the viable bacterial counts to the radioactive counts in each animal's lungs 3 hours after infection; 18 animals were used per dose group. A single 3-hour exposure to 50 ppm tetrachloroethylene significantly increased the susceptibility to respiratory infection and greater mortality following exposure to *S. zooepidemicus* ($p \leq 0.01$). Forty-four deaths occurred in 140 (31.4%) mice challenged during a 3-hour exposure to 50 ppm tetrachloroethylene; in contrast, 21 deaths occurred in 140 mice (15.0%) exposed to filtered air. The 3-hour exposure to 50 ppm tetrachloroethylene was associated with a statistically significant ($p \leq 0.05$) 6.6% decrease in pulmonary bactericidal activity (80.5 and 73.9% of bacteria killed in controls and 50 ppm group, respectively). No difference was observed in either mortality rate or bactericidal activity in experiments using a single 3-hour exposure to 25 ppm, or 3-hour exposures to 25 ppm tetrachloroethylene repeated daily for 5 days compared with control animals exposed to filtered air.

In a study by Hanioka et al. (1995a), atrophy of the spleen and thymus was observed in rats receiving 2,000 mg/kg-day tetrachloroethylene via corn oil gavage for 5 days. No effect was observed in the 1,000 mg/kg-day group. In a 14-day corn oil gavage (1,000 mg/kg-day) study of tetrachloroethylene, no effects were observed on thymus and spleen weights of adult rats at a dose that produced liver toxicity (Berman et al., 1995). Another study employed 3 daily i.p.

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.

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. The data from Aranyi et al. (1986) suggest that short-term exposures may result in decreased immunological competence (immunosuppression) in CD-1 mice. The relative lack of data, taken together with the concern that other structurally related solvents (Cooper et al., 2009) have been associated with immunotoxicity, contributes to uncertainty in the database for tetrachloroethylene.

4.6.2.1.2. Hematologic toxicity

Several studies by Marth (1987) or Marth et al. (1989; 1985a; 1985b) and a study by Seidel et al. (1992) have demonstrated hematopoietic toxicity of tetrachloroethylene in female mice. In the Marth (1987) and Marth et al. (1989; 1985a; 1985b) studies, 135 female NMRI mice were exposed in drinking water to tetrachloroethylene at 0.05 or 0.1 mg/kg per day beginning at 2 weeks of age for 7 weeks and examined 8 or 16 weeks after exposure cessation. The mice exhibited a reversible hemolytic anemia and had microscopic evidence of splenic involvement (Marth et al., 1985a; Marth et al., 1985b). Tetrachloroethylene was found to accumulate in the spleen to a significantly greater extent than in the liver, brain, or kidney; levels of tetrachloroethylene were 20-fold higher in spleen than in liver at the end of the exposure period (Marth, 1987). Tetrachloroethylene was found in the spleen and fatty tissue of test animals up to 2 months (56 days) after initial exposure (Marth et al., 1989). Reversible body-weight decreases and increases in the relative weight of the spleen compared with the kidneys were reported. Serum triglycerides increased, and cholesterol levels decreased. These effects persisted as long as 16 weeks after cessation of exposure. Liver function (as assessed by serum protein levels) and hepatic protein synthesis were within normal limits, and there was no evidence of hepatic fatty accumulation or necrosis. Compared with brain, kidney, or liver, the erythropoietic system was found to be most susceptible to tetrachloroethylene in these studies.

Seidel et al. (1992) exposed female hybrid mice (C57/BL/6 × DBA/2) to tetrachloroethylene at 270 ppm (11.5 weeks) and 135 ppm (7.5 weeks), 6 hours/day, 5 days/week. Reductions in the numbers of lymphocytes/monocytes and neutrophils were observed, with a return to control values over the next 3 weeks. There were no effects on spleen colony-forming units (CFU-Ss), but evidence of a reduction in red cells was supported by decreases in erythroid colony-forming units and erythroid burst-forming units and evidence of reticulocytosis. A partial regeneration was observed in the exposure-free follow-up period of 3 weeks. It was noted that the slight CFU-C depression, which persisted in the exposure-free

period, could indicate the beginning of a disturbance at all progenitor cell levels. These data suggest a reversible bone marrow depression.

Hematological parameters were examined following oral administration of tetrachloroethylene in sesame oil (3,000 mg/kg-day for 15 days) to male albino Swiss mice with and without concurrent administration of 2-deoxy-D-glucose (2DG; 500 mg/kg-day i.p.), vitamin E (400 mg/kg-day oral gavage) or taurine (100 mg/kg-day by oral intubation) (Ebrahim et al., 2001). This study was designed to examine the potential protective properties of 2DG and vitamin E as well as taurine against tetrachloroethylene-induced cytotoxicity in various organ systems. Animals exposed to tetrachloroethylene alone demonstrated significantly decreased hemoglobin and RBC counts ($p < 0.01$), and significantly decreased HCT (packed cell volume) and platelet counts ($p < 0.001$). The WBC count was found to be significantly increased ($p < 0.001$). These changes were reverted back to near normal in the animals coexposed to 2DG, vitamin E, or taurine.

In summary, the limited laboratory animal studies of hematological toxicity demonstrated an effect of tetrachloroethylene exposure on RBC [decreased RBC (Ebrahim et al., 2001), or decreased erythrocyte colony-forming units (Seidel et al., 1992)], with reversible hemolytic anemia observed in female mice exposed to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age in one series of studies (Marth et al., 1989; Marth, 1987; Marth et al., 1985a; Marth et al., 1985b). Ebrahim et al. (2001) also observed decreased hemoglobin, platelet counts and packed cell volume, and increased WBC counts. Although limited studies are available in the peer-reviewed published literature, the results of these studies support the results observed in the study of dry-cleaning workers by Emara et al. (2010) described in Section 4.6.1.1.1.

4.6.2.2. Cancer Effects

4.6.2.2.1. Mononuclear cell leukemia in rats

The incidence of mononuclear cell leukemia in rats chronically exposed to tetrachloroethylene is summarized in Table 4-33. The NCI oral gavage study in Osborne-Mendel rats was considered to be inconclusive because of the high incidence of respiratory disease, and high mortality with tetrachloroethylene exposure. Lesions indicative of pneumonia were observed in almost all rats at necropsy. A high incidence of toxic nephropathy was evident in tetrachloroethylene-exposed male and female rats. Early mortality was also observed in tetrachloroethylene-exposed animals; 50% of the high dose males and females had died by Weeks 44 and 66, respectively. Therefore, this bioassay is not considered further in the below evaluation of the mononuclear cell leukemia induction by tetrachloroethylene in rats.

NTP (1986) reported that the chronic inhalation administration of tetrachloroethylene at concentration levels of 0, 200, and 400 ppm caused statistically significant positive trends in the incidence of MCL in male ($p = 0.004$) and female ($p = 0.018$) F344/N rats. The incidences of MCL in male and female rats exposed to tetrachloroethylene at 0, 200, and 400 ppm (6 hours/day, 5 days/week, for 104 weeks) were 56, 77, and 74% and 36, 60, and 58%, respectively. Interpretation of these data is somewhat complicated 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%]).

Table 4-33. Mononuclear cell leukemia incidence in rats exposed to tetrachloroethylene

Bioassay	Exposure	Sex	Mononuclear cell leukemia incidence (%)[a]
NCI (1977)[b] Osborne-Mendel rats Gavage: 5 d/wk, 78 wk	Vehicle control 500 mg/kg-day 1,000 mg/kg-day	Male	None reported
	Vehicle control 500 mg/kg-day 1,000 mg/kg-day	Female	None reported
NTP (1986) F344/N rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 200 ppm 400 ppm	Male	28/50 (56) 37/50 (77) 37/50 (74)
	0 ppm 200 ppm 400 ppm	Female	18/50 (36) 30/50 (60) 29/50 (58)
JISA (1993) F344/DuCrj rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 50 ppm 200 ppm 600 ppm	Male	11/50 (22) 14/50 (28) 22/50 (44) 27/50 (54)
	0 ppm 50 ppm 200 ppm 600 ppm	Female	10/50 (20) 17/50 (34) 16/50 (32) 19/50 (38)

[a] Reflects the number of animals with MCL reported under "multiple organs," spleen, or liver.
[b] Gavage doses listed were adjusted several times during the course of the study. Male rats received the listed TWA daily doses through Week 78, and surviving animals were observed up to study termination in Week 110.

To evaluate whether the increased MCL incidence contributed to the increase in early deaths observed with increasing tetrachloroethylene exposure, NTP (1986) conducted supplemental analyses according to their standard methods of data evaluation. These analyses considered 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 notable 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, statistical analyses of 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.

Thomas et al. (2007) reanalyzed the NTP (1986) dose-response data comparing results with four statistical methods. In their analysis of MCL incidence in rats exposed to 500 chemicals, tetrachloroethylene was one of five chemicals shown by the authors to produce "definitive" leukemia effects in both sexes of rats. MCL effects were more often than not confined to one sex, while tetrachloroethylene induced statistically significant increases in both sexes of the F344 rat. The findings in Thomas et al. (2007) described in more detail later, are addressed in the context of other considerations in Section 4.6.2.2.2.

In the JISA (1993) study, F344/DuCrj rats were exposed via inhalation for 104 weeks to tetrachloroethylene at concentrations of 0, 50, 200, and 600 ppm. As in the NTP (1986) study, there was a higher control incidence of MCL (22% in males and 20% in females) than the reported historical rate of MCL for the Japanese laboratory of 147/1,149 [13%] in males and 147/1,048 [14.0%] in females (refer to Table 5-16, Section 5). The incidence of MCL in male and female rats exposed to tetrachloroethylene at 50, 200, and 600 ppm was 28, 44, and 54% and 34, 32, and 38%, respectively. Both male and female rats displayed a significant dose-dependent increase in MCL, at $p < 0.01$ and $p = 0.046$ (poly-3 test, conducted for this assessment), respectively. There was decreased latency in MCLs in female rats of the JISA (1993) study, with first appearance in Week 100 in controls and Weeks 66−70 in treated rats.

4.6.2.2.2. Additional considerations regarding rodent leukemia findings

Under the conditions of the NTP (1986) and JISA (1993) bioassays, a carcinogenic effect of tetrachloroethylene in male and female rats was evidenced by significant increases of MCL in both sexes. The pathology of rat MCL is well characterized and has been well described (Thomas et al., 2007; Ward et al., 1990; Stromberg, 1985). MCL is among the most common causes of death in the aging F344 rat and is readily and unequivocally diagnosed by standard histopathological techniques. However, the utility of observed increases in MCL in the chemically exposed rat for human carcinogenic risk assessment has been questioned for several reasons. In particular, the spontaneous background incidence is both high and variable, and, thus, can obscure chemical-induced increases. As noted in reviews by Caldwell (1999) and Ishmael and Dugard (2006), the high background rate of MCL in control (untreated) rats can limit the ability to separate the background response from possible chemically induced responses, particularly when the chemically induced response above background is low. Additionally, because high-incidence MCL occurs only in the F344 rat strain and not in mice, Caldwell (1999) has stated that marginal increases in incidences are of questionable biological significance. Supplemental analyses, such as have been conducted by NTP for tetrachloroethylene and summarized in the preceding section, have been endorsed as a means to aid in data interpretation for these commonly occurring tumors. In the paragraphs that follow, issues pertinent to the interpretation of evidence that tetrachloroethylene induces MCL in male and female rats for the purposes of human health risk assessment are addressed. The discussion summarizes the findings of a recent analysis by Thomas et al. (2007) and considers the available evidence for tetrachloroethylene in the context of the approach put forth by those authors. Other considerations identified by NRC (2010) are also addressed, particularly with respect to uncertainties surrounding the causes of F344 rat MCL, the biology of the disease, including the cell type of origin, as well as the mechanisms by which tetrachloroethylene may advance development of this rodent leukemia.

The significance of MCL findings in multiple NTP bioassays that used the F344 rat was the subject of a recent reanalysis by Thomas et al. (2007). They examined the incidence of leukemia in 2-yr bioassays that included untreated male and female F344 rats from 1971 to 1998. They found that background tumor incidence increased substantially, from 7.9 to 52.5% in males and from 2.1 to 24.2% in females, over that period. The reanalysis also found that MCL responses are highly variable and subject to substantial modulation by dietary as well as other, as yet unidentified, factors.

Their review of the disease pathobiology described MCL as a large granular lymphocytic (LGL) leukemia that is a rapidly progressing and fatal neoplasm, with death typically occurring within 2 weeks of onset (Thomas et al., 2007). The disease is characterized by splenomegaly

upon gross pathological examination. Leukemic cell infiltration of the splenic red pulp with variable lymphoid cell depletion is consistently seen. The tumor is transplantable; its etiological factor is unknown. The cell of origin appears to reside in and/or require the splenic microenvironment, and splenectomy dramatically reduces spontaneous MCL incidence (Moloney and King, 1973).

Thomas et al. (2007) concluded that the exact cell of origin of F344 rat MCL is unknown. The pathological characteristics of rat MCL are similar in some respects to one of the human T-cell leukemias (Caldwell, 1999), and some investigators have proposed that MCL can serve as an experimental model for human T-cell leukemia (Stromberg, 1985). However, MCLs have been shown to be heterogeneous with respect to cell phenotype and function (e.g., surface antigen expression, esterase activity, and cytotoxic activity). For example, a study of 10 primary and 10 transplanted MCLs of aging rats found that natural killer (NK) cell activity was variable and lacked correlation with surface antigens, with poorly differentiated MCL cells exhibiting less cytotoxic (i.e., NK-cell) activity (Ward and Reynolds, 1983). These and other investigations [e.g., Stromberg et al. (1983)] have provided evidence that MCLs represent a heterogeneous group of leukemias. Thomas et al. note that the use of specific monoclonal anti-rat NK-cell antibodies and other rat leukocyte specific markers would aid in establishing the cell type of origin. The lack of assessment of the rodent tumors according to current classification criteria [e.g., as specified by Swerdlow et al. (2008)] hinders ability to identify cell lineage. In particular, the lack of immunophenotyping data for MCL occurring spontaneously or as the result of chemical exposure, and the observed heterogeneity in cell phenotype and function of the spontaneously occurring tumors studied thus far, greatly limit classification of MCL. Based on the reported heterogeneity in cell phenotype and function, Thomas et al. (2007) stated that MCL may arise from either mature LGLs or from a variety of individual LGL subpopulations; alternatively, a pluripotent LGL precursor may be the cell type of origin.

Acknowledging the limitations that arise from the lack of knowledge about the cell type of origin for MCL, and the observed heterogeneity in phenotype and function among MCL, Thomas et al. (2007) characterize MCL as having an NK-cell phenotype based on functional NK-cell activity in most (but not all) MCL cells. They note that human NK-LGL and F344 rat MCL have "some characteristics in common" and conclude that F344 rat MCL "is comparable to the aggressive human NK-LGL leukemia on a morphological, functional, and clinical basis." However, current criteria to identify cell phenotype (e.g., by use of specific monoclonal antibodies and genomic analysis) were not adopted in this study, and many of the comparison criteria identify by Thomas et al. (2007) are nonspecific and common to other human leukemia or lymphoma phenotypes. Although contrary to prior reports that the F344 MCL does not have a human counterpart [e.g., Caldwell (1999)], a comparable conclusion regarding similarity of F344

rat MCL to human NK-LGL was reached by Stromberg (1985) and Ishmael and Dugard (2006). Human NK-LGL is a rare form of LGL. NK-LGL usually occurs in younger patients (median age: 39), has an aggressive clinical course, and is usually fatal within months of diagnosis despite multiagent therapy. Epstein Barr virus has been implicated in many of the reported NK-LGL cases, although the mechanism is unknown. In contrast, the majority of other human LGLs (i.e., T-cell LGL leukemias) follow a chronic indolent course. Due to the paucity of available data, mechanisms or modes of action contributing to the MCLs arising in untreated or chemically exposed F344 rats have not been identified.

Thomas et al. (2007) also evaluated MCL incidence in male and female rats exposed to 500 chemicals. On the basis of 34 NTP studies that yielded evidence of a chemically related increase in the incidence of leukemia, which included the NTP (1986) study of tetrachloroethylene, the authors conducted a reanalysis of dose-response data by comparing results with four statistical methods: Fisher's exact test for pair-wise comparison of leukemia incidence between a dose group and a control group, the Cochran-Armitage test for incidence trend, logistic regression for incidence, and life tables for survival-adjusted incidence. Tetrachloroethylene was one of five chemicals shown by the authors to produce "definitive" leukemia effects in both sexes of rats. MCL effects were more often than not confined to one sex, while tetrachloroethylene induced statistically significant increases in both sexes of the F344 rat.

In their analysis, Thomas et al. (2007) employed the rigid statistical criteria suggested in Food and Drug Administration (FDA) guidance for testing dose-related cancer incidences of common tumors ($p < 0.01$ for pairwise comparison; $p < 0.005$ for trend test). They noted that leukemia is generally considered a fatal neoplasm, thus supporting the life table test as more likely reflecting the true statistical significance of the carcinogenic effect. Life-table analysis (log-rank test) accounts for time-to-event information, is capable of testing nonlinear dose-response relationships of arbitrary shapes, and is, therefore, more flexible than the Cochran-Armitage trend test. The NTP (1986) results in male rats exposed to tetrachloroethylene revealed a significant dose-response trend when analyzed with a life table analysis ($p = 0.004$) assuming that MCL is lethal (a nonsignificant trend with logistic regression ($p = 0.097$) resulted if MCL was assumed nonlethal). Pairwise comparisons revealed dose-related incidences ($p = 0.046$; Fisher exact test) for both dose groups, and the Cochran-Armitage trend test yielded a p-value of 0.034; neither met the FDA criteria for statistical significance. The borderline significance of the trend test and nonsignificance of logistic regression for the latter two comparisons could be explained, in part, by the fact that the incidences did not follow an incrementally increasing relationship with dose. In female rats in the NTP (1986) study, use of a life table ($p = 0.053$), logistic regression ($p = 0.012$), a trend test ($p = 0.018$), and Fisher

exact test (p = 0.014 and 0.022, respectively, for two doses) all revealed dose-related increases in incidence that were of borderline significance according to the suggested FDA criteria.

Thomas et al. (2007) note that NTP does not use a rigid statistical rule in interpreting experimental results, instead relying on consideration of other factors in a weight-of-evidence approach. These factors include historical control incidences, and whether chemically induced tumors were sex-specific, dose-responsive, of shorter latency, or of more advanced stage. While encouraging stringent statistical analysis to reduce false positives, Thomas et al. (2007) characterized the NTP weight-of-evidence approach as "appropriate" and "rigorous." They proposed a similar evaluation of the pertinent data, to also include consideration of such factors as reproducibility of effect across bioassays, and other information to inform biological plausibility (i.e., evidence of toxic or carcinogenic effects on LGLs or their precursors). An assessment of the considerations identified by Thomas et al. (2007) and NRC (2010) for tetrachloroethylene is provided below:

Nature of the dose-response curve in terms of incidence and severity. The NTP (1986) study found that tetrachloroethylene increased the incidence and severity of MCL in male and female rats. The JISA (1993) study reported an increasing trend incidence of MCL in both male and female rats, and overall the number of early deaths attributed to MCL increased with increasing exposure.

Appropriate historical control data. Historical control data are available from the laboratory that performed the NTP (1986) study, the NTP program, and from the Japanese laboratory. A comparison with historical data revealed a higher MCL rate in concurrent controls in the NTP and Japanese tetrachloroethylene bioassays. Concurrent controls in the NTP studies were higher than 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%]). As in the NTP (1986) study, there was a higher control incidence of MCL (22% in males and 20% in females) than the reported historical rate of MCL for the Japanese laboratory of 147/1,149 [13%] in males and 147/1,048 [14.0%] in females (refer to Table 5-16, Section 5).

Reduction in latency time. The NTP (1986) study found that tetrachloroethylene reduced tumor latency in female rats. In the JISA (1993) study, there was also decreased latency in MCLs in female rats, with the first appearance in Week 100 in controls and Weeks 66−70 in treated rats.

Reproducibility in another species and routes of exposure. Tetrachloroethylene has reproducibly been found to be carcinogenic in rats and mice. Tetrachloroethylene was carcinogenic when tested in mice in an oral gavage study (NCI, 1977) and in two inhalation

studies [NTP (1986) and JISA (1993)], inducing hepatic neoplasms. Tetrachloroethylene also caused other types of tumors in the F344 rat. However, tetrachloroethylene has only been found to be leukemogenic in F344 rat studies. In the JISA (1993) study, deaths in female mice due to malignant lymphomas/total dead (or moribund) mice were 6/18, 4/20, 13/27, and 10/33 in the 0, 10, 50, and 250 ppm groups, respectively. Tetrachloroethylene exposure did not affect the incidence at study termination of malignant lymphomas in the lymph nodes or spleen. The NTP (1986) study also did not find an effect of tetrachloroethylene on malignant lymphoma incidence in female mice.

A similar lack of site concordance across rodent bioassays was also observed among many of the NTP chemicals causing MCL in F344 rats reviewed by Thomas et al. (2007). Tetrachloroethylene was among six chemicals (the others were allyl isovalerate, bisphenol A, pyridine, 2,4,6-trichlorophenol, and the benzene metabolite hydroquinone) for which leukemia was the only neoplastic change for either male or female rats, but for which other sex-species groups showed evidence of carcinogenicity (Thomas et al., 2007). [Note that, as discussed in Section 4.10, elevated incidences of other tumors—specifically, brain gliomas and kidney tubule adenomas and adenocarcinomas—were observed in male F344/N rats in the tetrachloroethylene NTP (1986) study but were not included in the Thomas et al. (2007) analysis.] For eight other chemicals evaluated by Thomas et al. (2007), F344 rat MCL was the only carcinogenic effect in rats or mice. For twenty chemicals, MCL was one of multiple neoplastic changes in F344 rats of one or both sexes.

Involvement of both sexes. Tetrachloroethylene induced MCL in both sexes of F344 rats in the NTP (1986) and JISA (1993) inhalation bioassays. In fact, tetrachloroethylene was one of only 5 chemicals identified in a review of 500 chemicals by Thomas et al. (2007) that were shown to produce "definitive" leukemia effects in both sexes of rats. Tetrachloroethylene was also hepatocarcinogenic in both sexes of mice in the available oral (NCI, 1977) and inhalation bioassays [NTP (1986) and JISA (1993)]. Hence, the carcinogenic effects of tetrachloroethylene are evident in both male and female rodents across multiple data sets and with tumor sites.

Comparative species metabolism. Species differences in metabolism of tetrachloroethylene have been noted, as reviewed in Section 3. Although thought to be qualitatively similar, there are clear differences among species in the quantitative aspects of tetrachloroethylene metabolism (Lash and Parker, 2001; Völkel et al., 1998; Schumann et al., 1980; Ikeda and Ohtsuji, 1972). These differences are in the relative yields and kinetic behavior of metabolites (Völkel et al., 1998; Green et al., 1990; Ohtsuki et al., 1983). Because metabolites are thought to contribute to the carcinogenicity of tetrachloroethylene, these differences in metabolism are likely to contribute to species differences in carcinogenic response, including the types of tumors observed across rodent bioassays.

The metabolite(s) contributing to the development of MCL from tetrachloroethylene have not been defined. A role for GSH-derived metabolites was posited based on early reports of fatal hemorrhagic disease in cattle fed trichloroethylene-extracted soybean oil meal, and the subsequent finding that the trichloroethylene metabolite *S*-(1,2,-dichlorovinyl)-*L*-cysteine (generated through the GSH pathway) induces renal toxicity, aplastic anemia, and marked DNA alteration in bone marrow, lymph nodes, and thymus in calves (Bhattacharya and Schultze, 1972, 1971). However, similar effects were not found in a study that administered TCVC, a GSH-derived metabolite of tetrachloroethylene, to two calves as a single dose (Lock et al., 1996). The first calf received 10 mg/kg i.v. (40 µmol/kg) and was observed for 25 days and then given a second dose of 8 mg/kg (36 µmol/kg) and observed for a further week. A second calf was given 18 mg/kg (72 µmol/kg) and observed for 20 days. An initial neutropenia was observed in the first calf during the first few days after dosing. However, no decline in platelet or neutrophil count, nor elevation in blood urea nitrogen, was observed. Based on clinical and histopathological evaluation, TCVC was concluded to lack bone marrow or kidney toxicity. The authors characterized the lack of toxicity in the kidney as "puzzling" given their prior work demonstrating the nephrotoxicity of comparable TCVC exposures in the rat (Ishmael and Lock, 1986), and their concurrent in vitro studies showing that TCVC, like DCVC, was toxic to renal transport mechanisms in cortical slices (Lock et al., 1996). Toxicokinetic differences among species were postulated as an explanation for the observed species differences in TCVC sensitivity, and the unique sensitivity of the calf to DCVC compared with TCVC and other haloalkene conjugates. Aside from the Lock et al. (1996) evaluation of bone marrow toxicity of TCVC in the juvenile cow, a species of unknown sensitivity to tetrachloroethylene-induced leukemia, other studies aimed at elucidating the active metabolites contributing to leukemic effects have not been reported. In particular, no such studies are available in the F344 rat, the species and strain in which leukemic effects have been consistently observed in both sexes.

Analyses of how differences in metabolism may lead to differences in the leukemogenicity of tetrachloroethylene across species are limited by this lack of knowledge regarding the putative leukemogenic metabolites. As reviewed in Section 3, tetrachloroethylene is metabolized by two main pathways, oxidation and GSH conjugation. Species differences in the extent of metabolism, and in the profile of resultant metabolites, have been observed in both pathways. Metabolism is higher in mice than in rats, predominantly owing to more extensive metabolism via the oxidative pathway thought to contribute to hepatic toxicity and carcinogenicity. Rats, in turn, have higher metabolic rates than do larger animals, including humans. The half-life of tetrachloroethylene is much longer in humans (>100 hours) than in rodents (<10 hours). Interindividual differences in metabolism, for instance arising from variability in activity of GSTs and other metabolic enzymes, may also contribute to interspecies

differences in metabolism. Overall, the database is insufficient to characterize how these metabolic differences may impact species sensitivity to the leukemogenic activity of tetrachloroethylene.

Genotoxicity, cytotoxicity, and any other relevant information. Thomas et al. (2007) note "little evidence to support a mode of action" for F344 rat MCL induced either spontaneously or by the 34 leukemogens they reviewed, including tetrachloroethylene. However, they propose a review of evidence that may aid in assessing the biological plausibility for tumor induction. The genotoxicity of tetrachloroethylene is reviewed in Section 4.8. None of the reviewed studies have specifically investigated the genotoxicity of tetrachloroethylene in the potential target tissue (bone marrow or spleen) of the F344 rat of either sex. A study in Sprague-Dawley rats found only marginal effects on chromosomal aberrations and aneuploidy with tetrachloroethylene exposure by inhalation (100 and 500 ppm) (Beliles et al., 1980). However, the overall conclusion for tetrachloroethylene genotoxicity supports the view that the contribution of mutagenicity to one or more carcinogenic outcomes cannot be ruled out.

No studies are available that evaluate the toxicity of tetrachloroethylene in the putative target tissues (bone marrow and/or spleen) or target cells of MCL in the F344 rat. However, as reviewed in Section 4.6.2.1.2, several studies by Marth (1987) or Marth et al. (1989; 1985a; 1985b), Seidel et al. (1992), and Ebrahim (2001) have demonstrated hematopoietic toxicity of tetrachloroethylene in mice. Ebrahim et al. (2001) found that tetrachloroethylene in sesame oil (3,000 mg/kg-day for 15 days) significantly decreased hemoglobin, RBC counts, decreased HCT (packed cell volume) and platelet counts, and significantly increased WBC count. These findings are similar to those observed in studies of tetrachloroethylene-exposed humans (Emara et al., 2010). In the Marth (1987) and Marth et al. (1989; 1987; 1985a; 1985b) studies, female NMRI mice exhibited a reversible hemolytic anemia and had microscopic evidence of splenic involvement following exposure to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age. Seidel et al. (1992) also found evidence of a reduction in red cells, supported by decreases in erythroid colony-forming units and erythroid burst-forming units and evidence of reticulocytosis in female hybrid mice (C57/BL/6 × DBA/2) to tetrachloroethylene at 270 ppm (11.5 weeks) and 135 ppm (7.5 weeks), 6 hours/day, 5 days/week. Reversible reductions in the numbers of lymphocytes/monocytes and neutrophils were also observed. The slight CFU-C depression, which persisted in the exposure-free period, could indicate the beginning of a disturbance at all progenitor cell levels. These data suggest a reversible bone marrow depression.

A number of leukemogens (e.g., benzene) have been reported to inhibit production of both red cells and various forms of white cells. A decrease in CFU-Ss, an effect not observed with tetrachloroethylene exposure (Seidel et al., 1992), has commonly been reported.

Leukemogens also cause a decrease in bone marrow myeloid progenitors CFU-GEMM, CFU-GM, and CFU-E/BFU-E, the latter of which was also decreased by tetrachloroethylene (Seidel et al., 1992). Thus, Seidel et al. (1992) provides indirect evidence that tetrachloroethylene induces effects associated with leukemogens (NRC, 2010).

Other studies that may be relevant to leukemia induction in the F344 rat include those of the immunotoxicity of tetrachloroethylene. However, the available database of such studies, as summarized in Section 4.6.2.1.1, is limited for establishing whether tetrachloroethylene affects immune parameters in a manner indicative of potential for inducing leukemia development. Immunosuppression was observed in female $B6C3F_1$ mice administered tetrachloroethylene (maximum concentration: 6.8 ppm) with a mixture of 24 frequent contaminants of ground water near Superfund sites (Germolec et al., 1989). No changes were evident in lymphocyte number, T-cell subpopulations, NK cell activity, or with challenge by *Listeria monocytgens* or PYB6 tumor cells. In a separate inhalation study in mice, exposure to 170 mg/m^3 (50 ppm) tetrachloroethylene for 3 hours increased susceptibility to respiratory streptococcus infection and significantly decreased pulmonary bactericidal activity (Aranyi et al., 1986).

As reviewed by Thomas et al. (2007), corn oil gavage has been shown to significantly ($p < 0.001$) decrease the incidence of MCL in F344 rats, particularly males, by an unknown mechanism. This complicates interpretation of the few short-term studies in rats administering tetrachloroethylene in corn oil gavage. These include a finding of atrophy of the spleen and thymus in rats receiving 2,000 (but not 1,000) mg/kg-day tetrachloroethylene via corn oil gavage for 5 days (Hanioka et al., 1995a). In a separate 14-day corn oil gavage study, tetrachloroethylene did not affect thymus and spleen weights of adult rats at a hepatotoxic dose (1,000 mg/kg-day) (Berman et al., 1995).

Summary. This assessment of considerations proposed in Thomas et al. (2007) and by NRC (2010) highlights several findings that add support to the conclusion that tetrachloroethylene is a leukemogen in the F344 rat. Particularly pertinent are findings of the evaluation by NTP (1986) of the inhalation bioassay of tetrachloroethylene, demonstrating dose-related increases in the incidence of MCL in both sexes and in the severity of MCL in both sexes, as well as a shortened time to onset of MCL in female rats, and an increased incidence of advanced MCL in female rats that died before the scheduled termination of the study. These factors are considered the most important in evaluating the significance of the MCL findings for tetrachloroethylene.

Additional factors supporting the carcinogenicity of tetrachloroethylene include the observation that tetrachloroethylene has also been found to induce other rare tumors besides MCL in the F344 rat, as well as tumors at other sites in both sexes of the mouse, in both inhalation and oral gavage bioassays. As noted by Thomas et al. (2007), chemically induced

MCL has typically been found in only one sex of the F344 rat, and tetrachloroethylene was one of only 5 chemicals identified in their review of 500 chemicals in the NTP database to definitively cause the tumor in both males and females. These findings add support to the conclusion that tetrachloroethylene is a rodent carcinogen. Although limited, studies demonstrating hemolysis and bone marrow toxicity in mice add some support to the biologic plausibility of the observed leukemic effects (NRC, 2010). The pharmacokinetics (metabolites) and pharmacodynamics (biological mechanisms) that contribute to the development of MCL in the F344 rat, both spontaneously and with chemical exposure, have not been elucidated.

Uncertainties remain regarding the causes of F344 rat MCL, the biology of the disease including the cell type of origin, as well as the mechanisms by which tetrachloroethylene may advance development of this rodent leukemia. Further research to clarify the factors that affect inherent and chemically induced susceptibility to F344 rat MCL is warranted. As proposed by Stromberg (1985), the F344 rat MCL could serve as a rodent model for human T-cell leukemias, in which research could be conducted to identify causative factors and disease mechanisms, and to test and develop novel chemotherapies. Thomas et al. (2007) similarly endorsed additional research and analyses of F344 leukemogens, such as tetrachloroethylene, to advance understanding of the mechanisms contributing to the rodent—and by inference, the related human—diseases.

In summary, although uncertainties remain regarding the pathobiology of MCL and the mechanisms by which tetrachloroethylene may contribute to disease development and/or progression, this assessment of additional factors bolsters the support for the finding of tetrachloroethylene-induced MCL in the F344 rat.

4.6.3. Summary and Conclusions

4.6.3.1. Immunotoxicity, Hematologic Toxicity, and Cancers of the Immune System in Humans

The strongest epidemiological study examining immunologic and hematopoietic effects of tetrachloroethylene exposure in terms of sample size and use of an appropriately matched control group is of 40 male dry-cleaning workers (mean exposure levels <140 ppm; mean duration: 7 years; mean blood tetrachloroethylene levels: 1,685 µg/L) by Emara et al. (2010). Statistically significant decreases in red blood cell count and hemoglobin levels and increases in total white cell counts and lymphocyte counts were observed in the exposed workers compared to age- and smoking-matched controls. Similar effects were observed in mice (Ebrahim et al., 2001). In addition, increases in several other immunological parameters, including T-lymphocyte and natural killer cell subpopulations, IgE, and interleukin-4 levels were observed

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in tetrachloroethylene-exposed dry-cleaning workers (Emara et al., 2010). These immunologic effects suggest an augmentation of Th2 responsiveness. However, the limited available data from studies in children (Delfino et al., 2003a; Delfino et al., 2003b; Lehmann et al., 2002; Lehmann et al., 2001) do not provide substantial evidence of an effect of tetrachloroethylene exposure during childhood on allergic sensitization or exacerbation of asthma symptomology. 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. The available data pertaining to risk of autoimmune disease in relation to tetrachloroethylene exposure are limited by issues regarding ascertainment of disease incidence and exposure-assessment difficulties in population-based studies. In summary, there is considerable variation in the extent and quality of the epidemiologic literature (e.g., number of studies, study design, and quality of the exposure assessment) for lymphopoeitic cancers. In general, studies with relatively strong exposure assessments are based on a small number of observed deaths or incident cases, with a relatively low statistical power. For non-Hodgkin lymphoma and multiple myeloma, the available studies are considered supportive of a role of tetrachloroethylene as a likely carcinogen. This is based on the presence of higher effect estimates in studies with better exposure-assessment methodologies and evidence of an exposure-response trend in one or more studies.

Among the specific types of lymphopoeitic cancers, there is considerable variation in the extent and quality of the epidemiologic literature (e.g., number of studies, study design, and quality of the exposure assessment). In general, studies with relatively strong exposure assessments are based on a small number of observed deaths or incident cases, with a relatively low statistical power. For non-Hodgkin lymphoma and multiple myeloma, the presence of higher relative risk estimates in studies with better exposure-assessment methodologies and evidence of an exposure-response trend in one or more studies provide the basis for considering the collection of studies as supportive of a role of tetrachloroethylene as a likely carcinogen.

For non-Hodgkin lymphoma, there is little evidence of an association in the large cohort studies examining risk in relation to the broad occupational category of work in laundry or dry cleaning [i.e., relative risk estimates ranging from 0.95 to 1.05 in females in Andersen et al. (1999), females and males in Ji and Hemminki (2006b), and Pukkala et al. (2009)]. The results from the four cohort studies that used a relatively higher quality exposure-assessment methodology, however, reported relative risks between 1.7 and 3.8 (Radican et al., 2008; Boice et al., 1999; Anttila et al., 1995). There is also some evidence of exposure-response gradients in studies with tetrachloroethylene-specific exposure measures based on intensity, duration, or cumulative exposure (Seidler et al., 2007; Miligi et al., 2006; Boice et al., 1999). Higher

non-Hodgkin lymphoma risks were observed in these studies in the highest exposure categories, with the strongest evidence from the large case-control study in Germany in which a relative risk of 3.4 (95% CI: 0.7, 17.3) was observed in the highest cumulative exposure category (trend *p*-value = 0.12) (Seidler et al., 2007). Effect estimates in studies with broader exposure assessments showed a more variable pattern (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji and Hemminki, 2006b; Blair et al., 2003; Travier et al., 2002; Cano and Pollán, 2001; Lynge and Thygesen, 1990). Confounding by lifestyle factors are unlikely explanations for the observed results because common behaviors, such as smoking and alcohol use, are not strong risk factors for non-Hodgkin lymphoma (Besson et al., 2006; Morton and Marjanovic, 1984).

Results from the multiple myeloma studies are based on a smaller set of studies than those of non-Hodgkin lymphoma, but results are similar. The larger cohort studies that use a relatively nonspecific exposure measure (broad occupational title of launderers and dry cleaners, based on census data) do not report an increased risk of multiple myeloma, with effect estimates ranging from 0.99 to 1.07 (Pukkala et al., 2009; Ji and Hemminki, 2006b; Andersen et al., 1999). Some uncertainty in these estimates arises from these studies' broader exposure-assessment methodology. Results from the cohort and case-control studies with a higher quality exposure-assessment methodology, with an exposure measure developed specifically for tetrachloroethylene, do provide evidence of an association, however, with relative risks of 7.84 (95% CI: 1.43, 43.1) in women and 1.71 (95% CI: 0.42, 6.91) in men in the cohort of aircraft maintenance workers (Radican et al., 2008) and 1.5 (95% CI: 0.8, 2.9) in a large case-control study in Washington [Gold et al. (2010b); tetrachloroethylene exposure]. Gold et al. (2010b) also reported increasing risks with increasing exposure duration (based on job titles) (Gold et al., 2010b) and based on a cumulative tetrachloroethylene exposure metric (Gold et al., 2010b). A smaller case-control study (n = 76 cases) with tetrachloroethylene-specific exposure measures based on intensity, duration, or cumulative exposure, Seidler et al. (2007), observed no cases among the highest exposure groups. A small cohort study by Boice et al. (1999) of aerospace workers observed one death among routinely exposed subjects and six deaths among subjects with a broader definition of routine or intermittent exposure.

4.6.3.2. Immunological and Hematological Toxicity and Mononuclear Cell Leukemias in Rodents

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. The data from Aranyi et al. (1986) suggest that short-term exposures may result in decreased immunological competence (immunosuppression) in CD-1 mice. The relative lack of data taken together with

the concern that other structurally related solvents (Cooper et al., 2009) have been associated with immunotoxicity contributes to uncertainty in the database for tetrachloroethylene.

The limited laboratory animal studies of hematological toxicity demonstrated an effect of tetrachloroethylene exposure on RBC [decreased RBC (Ebrahim et al., 2001), or decreased erythrocyte colony forming units (Seidel et al., 1992)] with reversible hemolytic anemia observed in female mice exposed to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age in one series of studies (Marth et al., 1989; Marth, 1987; Marth et al., 1985a; Marth et al., 1985b). Ebrahim et al. (2001) also observed decreased hemoglobin, platelet counts and packed cell volume, and increased WBC counts.

Cancer findings of primary concern are the statistically significant increases in MCL in both sexes in the NTP (1986) and JISA (1993) inhalation bioassays. Section 4.6.2.2.2 addresses issues pertinent to the interpretation of evidence that tetrachloroethylene induces MCL in male and female rats for the purposes of human health risk assessment. That discussion summarizes the findings of a recent analysis by Thomas et al. (2007) and considers the available evidence for tetrachloroethylene in the context of the approach put forth by those authors and by NRC (2010). This included a summary of the available noncancer studies that may inform the biologic plausibility of the leukemia findings. In the paragraphs that follow, the findings in and statistical analyses of the rodent bioassays are presented, and the other factors and data considered in the analysis presented in Section 4.6.2.2.2 are then summarized. Together, these analyses informed the conclusions provided concerning the application of the F344 rat leukemia data to human health risk assessment.

Statistical analysis of the NTP bioassay revealed a statistically significant trend for males ($p = 0.004$), and a marginally significant trend for females ($p = 0.053$). Life table analysis disclosed statistically significant increases in both the low- and high-dose groups in males. A significant increase in the low-dose group ($p = 0.023$) and a marginally significant increase in the high-dose group ($p = 0.053$) was observed in females. Additional statistical analyses reported by Thomas et al. (2007) of the female rat data from the NTP (1986) study found the results significant by logistic regression ($p = 0.012$), the Cochran-Armitage trend test ($p = 0.018$), and Fisher exact test ($p = 0.014$ and 0.022, respectively, for the lower and higher doses). Similarly, additional analyses reported by Thomas et al. (2007) supported the statistical significance of the male rat NTP data [logistic regression ($p = 0.097$), the Cochran-Armitage trend test ($p = 0.034$), and Fisher exact test ($p = 0.046$ for the lower and higher doses)]. Notably, these statistical analyses supported the authors' classification of tetrachloroethylene as one of only five chemicals of the 500 examined to produce "definitive" leukemia effects in both sexes of rats. While MCL effects were more often than not confined to one sex, tetrachloroethylene induced statistically significant increases in both sexes of the F344 rat.

In the JISA (1993) bioassay, MCL showed a statistically significant increasing trends with dose in both males ($p = 0.002$) and females ($p = 0.049$) by poly-3 test. Because MCL is a rapidly progressing and fatal neoplasm, Thomas et al. (2007) and NRC (2010) supported the life table test as more accurately reflecting the statistical significance of the carcinogenic effect. However, the poly-3 test for trend also addresses the time and cause of death and is comparable to the life-table test.

Other factors besides statistical analyses can inform interpretation of bioassay data and the observed effects of chemical exposures. According to NTP practices, as reviewed in Thomas et al. (2007), bioassay evaluation includes consideration of factors such as historical control tumor incidences, and whether chemically induced tumors were sex-specific, dose-responsive, of shorter latency, or of more advanced stage. NTP analyses of the tetrachloroethylene bioassay results revealed a dose-related increase in the incidence of MCL in both sexes, in the severity of MCL in both sexes, a shortened time to onset of MCL in female rats, and an increased incidence of advanced MCL in female rats that died before the scheduled termination of the study. All of these findings elevate concern that the MCL findings are related to chemical exposure, and among factors considered, add significant support to the conclusion that tetrachloroethylene is a leukemogen in F344 rats. An additional consideration in evaluation of the NTP (1986) and JISA (1993) studies is that a higher MCL incidence was observed in concurrent controls compared with historical controls. The reason for the reportedly higher MCL incidence in concurrent controls in these bioassays is not known. However, the finding of a chemically induced effect in a bioassay with a high background rate, which is more likely to obscure chemically induced findings, supports the conclusion that the observed tumors are due to tetrachloroethylene exposure. The independent findings of MCL induction in two bioassays conducted by separate laboratories also strengthen the conclusions.

Available pharmacokinetic data are insufficient to identify the active metabolite(s) of tetrachloroethylene that contribute(s) to MCL development. Such data are also insufficient to inform analyses of how interspecies differences in metabolism may affect leukemic outcomes in other species. In addition, available mechanistic data are insufficient to characterize the mechanisms or modes of action contributing to either spontaneously occurring or chemically induced MCL in the F344 rat (Thomas et al., 2007), including such tumors induced in tetrachloroethylene-exposed animals. However, the albeit limited studies demonstrating that tetrachloroethylene induces hemolysis and affects bone marrow function in mice provide indirect evidence that tetrachloroethylene induces effects associated with MCL and with known leukemogens (NRC, 2010). These studies support the biological plausibility of tetrachloroethylene as a leukemogen in rodent species, in general, and provide a basis for generating hypotheses on how these tumors may be induced. Nonetheless, the paucity of data on

contributing metabolites and mechanisms, and the lack of similar findings in other species, contribute to uncertainty in interpreting the MCL data in the F344 rat (NRC, 2010).

Knowledge gaps persist regarding the causes of F344 rat MCL, the biology of the disease including the cell type of origin, as well as the mechanisms by which tetrachloroethylene may advance development of this rodent leukemia. 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 indolent (chronic) or aggressive diseases (Sokol and Loughran, 2006). The indolent form of LGL leukemia is a disease of older adults, with a median age at diagnosis of 60 years. A number of clinical conditions have been observed in patients with LGL leukemia. These include the following: red cell aplasia and aplastic anemia; other lymphoproliferative disorders such as NHL, Hodgkin lymphoma, multiple myeloma, hairy cell leukemia, and B-cell lymphoproliferative disorders; and autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (Rose and Berliner, 2004). The etiology of LGL disorders is not known (Sokol and Loughran, 2006; Rose and Berliner, 2004). 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).

Lymphoid tumor pathobiology in rats and humans, its historical and current classification, and epidemiology, including observations in tetrachloroethylene-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 hinder 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).

Further research to clarify the factors that affect inherent and chemically induced susceptibility to F344 rat MCL is warranted, particularly given the morphological, functional, and clinical similarities of this rodent leukemia to human T-cell leukemias. As proposed by Stromberg (1985), the F344 rat MCL could serve as a rodent model for the human disease, in which research could be conducted to identify causative factors and disease mechanisms, and to

test and develop novel chemotherapies. Thomas et al. (2007) similarly endorsed additional research and analyses of F344 leukemogens, such as tetrachloroethylene, to advance understanding of the mechanisms contributing to the rodent—and by inference, the related human—diseases.

In summary, the available bioassay evidence and statistical analyses, together with a limited database of studies that characterize the biologic plausibility of tetrachloroethylene as a leukemogen, provide sufficient support of the conclusion that tetrachloroethylene causes MCL in the F344 rat. No mechanistic or other data are available that would rule out the relevance of the F344 MCL for assessing potential carcinogenic hazard to humans. The NRC (2010) peer review panel agreed that there was little information on the mode of action of tetrachloroethylene-induced rat MCL incidence. The panel, however, had differing opinions about the human relevance of rat MCL. Some of the reviewers judged that more research was needed to establish the relevance of the rat MCL to assessing human cancer hazard or risk. Some reviewers believed that available data were adequate to establish the human relevance of the rat MCL. In the context of quantitative assessment, a majority of the NRC (2010) panel judged that uncertainties associated with MCL were too great to support their selection over other tumor types.

4.7. DEVELOPMENTAL AND REPRODUCTIVE TOXICITY AND REPRODUCTIVE CANCERS

4.7.1. Development

4.7.1.1. Human Developmental Toxicity Data

Epidemiology studies of tetrachloroethylene exposure and effects on reproduction and development include occupational studies of employment at dry-cleaning establishments in the Netherlands, Scandinavia, Italy, Canada, and the United States (California) and population-based studies of exposure through drinking water in the United States (North Carolina, Massachusetts, and New Jersey). Tetrachloroethylene has been the predominant solvent used in the dry-cleaning industry in the United States and Europe since the 1970s (Gold et al., 2008; Raisanen et al., 2001). Other chemical exposures in dry-cleaning establishments are not widespread; individuals engaged in spot cleaning may use small amounts of trichloroethylene, acetic acid, ketone, and acetone solvents, petroleum naphthas, or hydrogen fluoride and hydrofluoric acid (Ruder et al., 2001). Short-term exposure to tetrachloroethylene is highest for dry-cleaning machine operators, particularly for machines requiring manual transfer of solvent-saturated clothing from a washing machine to a drying machine. The industry in the United States has gradually switched to dry-to-dry machines, associated with lower emissions, and in 1993, EPA ruled that all new

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establishments must use these machines. However, existing facilities were required to switch to dry-to-dry machines only if the older machines became inoperable. Other workplace characteristics influence exposure levels including adequacy of exhaust systems, level of equipment maintenance, occurrence of tetrachloroethylene spills, and presence of open containers (Gold et al., 2008).

Studies of occupational exposure primarily evaluated employees in dry-cleaning establishments, but a few studied reproductive and developmental outcomes by occupational groupings more broadly (Lindbohm et al., 1991; Windham et al., 1991; Taskinen et al., 1989). Although some studies identified exposed workers based on the industry they worked in, several developed more precise classifications for tetrachloroethylene exposure levels based on detailed information on reported job titles, tasks, and work histories obtained through interviews or questionnaires. Exposure classification using more detailed information is expected to reduce error in the assessment of exposure and increase confidence in the reported associations with health outcomes.

Epidemiology studies also have evaluated reproductive and developmental health effects stemming from incidents of tetrachloroethylene contamination of drinking water in the United States (Aschengrau et al., 2009a; Aschengrau et al., 2009b; Aschengrau et al., 2008; Janulewicz et al., 2008; Sonnenfeld et al., 2001; ATSDR, 1998b; Bove et al., 1995; Lagakos et al., 1986). In general, drinking water exposures were to multiple pollutants, and most studies were not able to determine the relative contribution to adverse health effects made by individual substances. In one incident in Massachusetts, however, investigators were able to evaluate a "natural experiment" that resulted from scattered water pipe replacements to the water distribution system in communities and tetrachloroethylene-contaminated water delivered to specific groups of households (Aschengrau et al., 2009b; Aschengrau et al., 2008; Janulewicz et al., 2008). The studies of exposure through drinking water are complicated by the occurrence of other water pollutants, but this literature can provide information about the consistency of health outcomes reported with those found in the occupational studies.

Studies of developmental effects evaluated low birth weight (Olsen et al., 1990; Bosco et al., 1987; McDonald et al., 1987), intrauterine growth restriction (IUGR; also known as small for gestation age [SGA]) (Sonnenfeld et al., 2001; Bove et al., 1995), birth defects (Ahlborg, 1990a; Olsen et al., 1990; Bosco et al., 1987; McDonald et al., 1987), and stillbirth (Olsen et al., 1990; McDonald et al., 1987). A brief summary of each study follows, grouped by health outcome, population (occupational, population-based), and exposure route (inhalation, drinking water). Table 4-34 summarizes these studies. Two studies evaluated effects on postnatal development including learning and behavior, and schizophrenia (Janulewicz et al., 2008; Perrin et al., 2007).

These studies are described in the section on neurotoxicological effects (refer to Section 4.1). Studies of effects on immunological development and childhood cancer are found in Section 4.6.

Overall, no associations were noted in several studies that assessed maternal or paternal occupational exposure to tetrachloroethylene and increased incidence of stillbirths, congenital anomalies, or decreased birth weight (Lindbohm, 1995; Windham et al., 1991; Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989; Bosco et al., 1987). However, the number of exposed cases for specific types of anomalies was not sufficient to evaluate risk with statistical precision. When data for adverse birth outcomes identified in Sweden, Norway, and Denmark were analyzed in relation to low or high tetrachloroethylene exposure among dry cleaners during their pregnancies, odds ratios for congenital malformation, still birth, and low birthweight (defined as <1,500 g) were 1.72 (95% CI: 0.40−7.12, 9 cases) for low exposure and 0.87 (95% CI: 0.20−3.69, 3 cases) for high exposure (Olsen et al., 1990). Kyyronen et al. (1989) reported an odds ratio for all congenital malformations of 0.8 (95% CI: 0.2−3.5) among 24 cases and 93 controls. The sample size was not large enough to evaluate specific anomalies or conduct multivariate analyses. A case-control study by Windham et al. (1991) identified one case of IUGR with prenatal exposure to both tetrachloroethylene and trichloroethylene among their sample of women with live births. The studies of occupational exposure also evaluated associations with spontaneous abortion. More detailed descriptions of these studies and analyses of spontaneous abortions are provided in Section 4.7.2. A study of parental occupational exposure has also examined schizophrenia in offspring (Perrin et al., 2007) and observed an increased incidence in offspring of parents who worked in dry-cleaning establishments (RR: 3.4, 95% CI: 1.3−9.2), as discussed in Section 4.1.

Table 4-34. Epidemiology studies on reproduction and development

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Zielhuis et al. (1989) (letter to editor) The Netherlands Cross sectional study of menstrual disorders among dry-cleaners and laundry workers (471 of 592, 80% response). Sampling frame was not described. After excluding 72 because of current pregnancy, lactation, chronic illness, or gynecological surgery, and 125 exposed and 199 unexposed because they used oral contraceptives, final data set included 68 exposed and 76 unexposed	Questionnaire responses Prevalence in referent group (%) Amenorrhea 0 Oligiomenorrhea 10 Polymenorrhea 17 Irregular cycle 38 Unusual cycle length 30 Intermenstrual blood loss 17 Menorrhagia 22 Dysmenorrhea 29 Premenstrual syndrome 10	Employment in dry cleaning compared to employment in laundries	Linear logistic regression Dry cleaning vs. laundry OR (95% CI) Oligiomenorrhea 2.1 (0.9−5.3) Polymenorrhea 0.8 (0.4−1.7) Irregular cycle 1.2 (0.7−2.2) Unusual cycle length 2.3 (1.2−4.4) Intermenstrual blood loss 1.3 (0.6−2.7) Menorrhagia 3.0 (1.6−5.6) Dysmenorrhea 1.9 (1.1−3.5) Premenstrual syndrome 3.6 (1.5−8.6)	Details concerning study design and analysis were not provided.
Eskenazi et al. (1991a) United States Men in the dry-cleaning industry compared to men working in laundries recruited from membership lists of two union locals in San Francisco Bay area and Greater Los Angeles. Included all dry cleaners (n = 85) and all laundry workers 20−50 yr in Local 3 (n = 119) and random selection of Local 52 (n = 206). Laundry workers were frequency matched by age to dry cleaners from same union local. Eligible were 20−50 yr of age, current workers, spoke English or Spanish, no vasectomy and located by telephone or mail. Participation: 20 exposed (38% of 53 eligible) and 56 unexposed (34% of 166 eligible	Semen quality Semen samples obtained from 34 exposed and 48 unexposed Brief physical exam by physician blind to occupational status to identify any medical conditions that might affect semen quality	Direct (expired air levels) and indirect (index) measure of PCE exposure Exhaled air collected 16−19 h after the end of a workweek (except 11, which were corrected to 16 hours using an elimination model) LOD: 2.67 μg/m^3, assuming 4 L breath sample Exposed: Workers at dry cleaners or laundries where dry cleaning was conducted on premises. Unexposed: Workers at laundries with no dry cleaning Confirmed by industrial hygienists	Analyzed associations with 17 measures of semen quality Difference in means and number with abnormal sperm (<20 million sperm, >40% abnormal forms, and < 60% motile sperm) Oligospermia (<20 million/mL) approx 25% in both groups Average percentage motile sperm "barely fell within normal limits" in both groups Less than 60% motile Exposed: 44% Unexposed: 31%, p = 0.23	Breath samples reflect exposure in the last week Laundry workers averaged less years education and had higher proportion Hispanic (90 vs. 41%). Smoking and alcohol use were comparable. Laundry workers reported a higher # days >80°F

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Eskenazi et al. (1991a) (continued)		In person interviews Work history, including job tasks and exposures in preceding week and past 3 mo Exposure score (0−11): estimate of exposure during 3 mo period of spermatogenesis Exhaled PCE (mean, μg/m^3) Exposed (n = 34) 7,892.9 (1.5−54,949.3) Unexposed (n = 48) 76.9 (0.6−1,562.4)	Multiple linear regression (13 sperm measures) within 34 exposed, and all 82 men, adjusted for several potential confounders No association within all 82 men for the 3 exposure measures and clinical quality measures: sperm concentration, total count, percentage motility, or percentage abnormal forms Associations, adjusted for confounding (p < 0.05) for ALH, sperm linearity, percentage round sperm; and # narrow sperm and at least one measure of exposure.	ALH and linearity measure pattern of sperm motion. Authors stated clinical interpretation is not yet "fully established" Result do not represent experience of nonunion workers (>85% of dry-cleaning industry)
Eskenazi et al. (1991a; 1991b) United States Wives of dry-cleaners and laundry workers [extension of Eskenazi et al. (1991a)] 17 of 20 dry cleaners with wives and 32 of 36 laundry workers with wives participated # with index pregnancies or trying to conceive: 14 dry cleaners, 26 laundry workers	Reproductive outcomes: * Rate of miscarriage: # of miscarriages during husband's employment in industry/total # of pregnancies during same period * Standardized fertility ratio (SFR): ratio of O/E based on U.S. national birth probabilities for race, birth cohort, parity, and age of wives for each person-year	Dates of employment in the industry and exposure to PCE from interviews (index pregnancies ended on average 2 yr before interviews) Exposure estimates: * Expired PCE for husband * Index of exposure * Occupation: dry-cleaner vs. laundry worker	SFR: Comparable between dry-cleaners and laundry workers Risk ratio: 1.01, 95% CI: 0.71−2.01 Time to conception (Cox Proportional Hazard adjusted for ethnicity and smoking): Dry cleaners vs. Laundry: Rate ratio = 0.54 (95% CI: 0.23−1.27)	# pregnancies and live births similar between dry-cleaners and laundry workers Power to detect doubling of SA rate from 12 to 24% was 0.28

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Eskenazi et al. (1991b) (continued)	Calculated SFR for periods when the men were employed and not employed in the industry * Time to conception—self report from wife—number of months to become pregnant with index pregnancy	PCE in expired air was higher among dry cleaners whose wives were interviewed (10,245.6 vs. 7,892 μg/m^3)		
Rachootin and Olsen (1983) Denmark Case-control study of couples examined or treated for infertility at Odense University Hospital, Denmark, 1977−1980. Controls selected from couples with healthy child conceived within 1 yr born at same hospital, 1977−1979. Eligible couples, residents of the island of Funen, Denmark, identified through hospital inpatient register (1,069 infertile, 4,305 fertile). Response 87% for both cases (n = 927) and controls (n = 3,728)	Infertility Data on reproductive history, SES and behaviors from questionnaire, medical records of infertile couples reviewed by collaborating physician blind to questionnaire responses	Self-report by women through mailed questionnaire sent Nov 1980−May 1981. Occupation held in year prior to hospital admission and longest held job. Classified based on job title, type of workplace and description of duties. Coded using a 5-digit Danish Occupational Code and a 5-digit industry code Exposure defined as contact with one of 15 specific chemical or physical agents (included dry-cleaning chemicals) or performance of one of 3 work processes a minimum of one time per week for at least 1 yr	1. Cases infertile for at least 1 yr compared to controls, all residing within catchment area Dry-cleaning chemicals OR (95% CI) * Sperm abnormalities: 1.0 (0.5−2.0) * Women with hormonal disturbances 1.3 (0.5−3.3) * Women with idiopathic infertility 3.0 (1.2−7.4) 2.7 (1.0−7.1) adjusted for women's age, education, residence and parity. *Men with idiopathic infertility 0.2 (0.0−1.4)	A higher percentage of case couples lived outside the hospital's catchment area Analyzed associations with 15 chemical or physical agents, 3 work processes, noise and heat Number of controls aged >20 yr: <20 Numbers of exposed cases and controls in dry cleaning was not reported

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Rachootin and Olsen (1983) (continued)			2. Within control group comparison; couples who gave birth after 1 yr compared to other controls Delayed conception Dry-cleaning chemicals OR (95% CI) Men 1.2 (0.7−1.9) Women 1.6 (0.9−2.9) Adjusted for women's age, women's education, residence, parity, women's smoking and drinking, and past use of oral contraceptives	

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Sallmen et al. (1995) Retrospective study, an extension to Lindbohm et al. (1990) Finland Case-control study of women recruited from Institute of Occupational Health database of workers biologically monitored for one or more of 6 solvents linked to national registry of medically recognized pregnancies, 1965−1983 (*n* = 3,265) 235 of 355 women responded to questionnaire (66%); after exclusions final study population was 197 women (median age 27, range 17−40 yr)	Time-to-pregnancy (TTP) (number of menstrual cycles required to become pregnant) via self report in questionnaire Identified pregnancies from nationwide database on medically diagnosed pregnancies, treated in hospital from 1973−1983, and from Finnish Register of Congenital Malformations. Used same pregnancies as for Lindbohm et al. (1990): SA (*n* = 80) or live births (*n* = 286), plus 30 referents from malformation study	Same approach as Lindbohm study et al. (1990) Exposure classification based on self-reported work description and solvent usage, and on biological exposure measurements during year before pregnancy, checked by independent industrial hygienist. Each work task classified by likelihood and level of exposure with no knowledge of TTP Not exposed—no handling of solvents, not reported by worker and no measurements. Potentially exposed: work tasks may have involved use of solvents, no or undefined solvent exposure reported and no measurements	Analysis combined workers in potential and low categories Discrete proportional hazards regression IDR: ratio of average incidence densities of clinically recognized pregnancies for exposed compared to unexposed in each menstrual cycle class All solvents Among women employed at beginning of TTP (*n* = 152) IDR (95% CI) Not exposed 1.0 Low 0.74 (0.49−1.11) High 0.44 (0.28−0.70)	Models adjusted for age, alcohol, smoking, partner's smoking, coffee, recent contraceptive use, regular menstruation, length of menstrual cycle, age at menarche, previous induced abortion or extrauterine pregnancy, previous SA, parity, SA case, unplanned pregnancy, frequency of intercourse Adjustment did not change risk estimates for organic solvents.

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Sallmen et al. (1995) (continued)		Exposed: Measurements made when holding same job and work tasks implied solvent exposure or solvent exposure was reported. High: Handled solvents daily, or 1−4 d/wk and measurements indicate clear exposure (*n* = 46) Low: Handled solvents 1−4 d/wk, no measurements or low levels, or handled solvents <1 d/wk (*n* = 59) None (*n* = 92)	PCE Low (*n* = 13) 0.63 (0.34−1.17) High (*n* = 7) 0.69 (0.31−1.52) Worked in dry-cleaning shop Low or high (*n* = 11) 0.44 (0.22−0.86) High (*n* = 6) 0.57 (0.24−1.34) Adjusted for low and high exposure to solvents in other industries, recent use of IUD/spermicides, and age at menarche	TTP info collected 8−18 yr after pregnancy

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Sallmen et al. (1998), extension of Taskinen et al. (1989) Finland Retrospective time-to-pregnancy study of paternal exposure to organic solvents. Wives of workers ever monitored for organic solvents by Finnish Institute of Occupational Health, 1965−1983. Linked ids to identify wives (*n* = 1,667) through Finnish Population Register Centre and pregnancies (*n* = 2,687) through national database of medically diagnosed pregnancies, treated in hospital, 1973−1983. Included men in their first marriage during 1985 with wives aged 18−40 yr at the end of the 1st trimester of pregnancy.	Self reported by mothers: Time-to-pregnancy (TTP) Included pregnancies begun during the marriage or up to 9 mo before Only included pregnancies identified in register and reported by participants	Self-reported paternal exposure to solvents at time attempt at pregnancy began Paternal exposure via mailed questionnaires (January 1986) to both spouses re: occupational exposure related to study pregnancy—employment, occupation including work tasks, and workplace during year of conception Use and frequency of any of the monitored solvents and any other materials Biological measurements available for 60% of men (during TTP *n* = 33, same job but not during TTP *n* = 161)	141/282 (50%) of men were highly or frequently exposed to organic solvents during TTP, 24.4% (*n* = 80) were low or intermediate exposed Discrete proportional hazards regression Paternal exposure to organic solvents; adj FDR OR (95% CI) Low/intermediate (*n* = 80) 0.74 (0.51−1.06) High/frequent (*n* = 141) 0.80 (0.57−1.11)	Evaluated several potential confounders: maternal age, maternal and paternal alcohol, maternal and paternal smoking, maternal coffee, recent contraceptive use, irregular menstruation, duration of menstrual cycle, age at menarche, previous induced abortion or extrauterine pregnancy, previous SA, parity, year of pregnancy, SA case,

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Sallmen et al. (1998), extension of Taskinen et al. (1989) (continued) Restricted to cases (n = 110) and controls (n = 332) who participated in study on pregnancy outcome. Excluded 1 case and 3 controls. 316 (72%) of wives participated. After exclusions (n = 21) and inability to give TTP (n = 13), final population was 282 couples		Exposure assessment for 80 calendar days preceding study pregnancy (spermatogenesis) blind to outcome status. Based on occupation, job description, reported solvent or other chemical use, and biological monitoring data. New assessment for TTP needed for 9 men whose job tasks had changed since last study Not exposed: Work tasks did not include handling solvents, worker did not report exposure and no biological measurement Potentially exposed: Work tasks might have involved solvent use, but not reported by worker, no biological measurements Exposed: Biological measurement taken while at same job, or tasks implied solvent exposure, or solvent exposure reported Level of Exposure High: handled solvents daily or level of biological measurements above reference value for general population Intermediate: Solvent use 1−4 d/wk and biological measurements indicate intermediate or low exposure Low: Handled solvents <1 d/wk None: all other	Adjusted for paternal and maternal smoking, maternal age, age at menarche >15, duration of menstrual cycle, frequency of intercourse, maternal exposure to organic solvents, year of pregnancy and variable for missing information Paternal exposure to PCE; adj FDR; OR (95% CI) Low (n = 9) 0.86 (0.40−1.84) Intermediate/High (n = 8) 0.68 (0.30−1.53) Adjusted for short menstrual cycle, long or irregular menstrual cycle, older age at menarche, frequency of intercourse, maternal age, maternal exposure to organic solvents, and variable for missing information	unplanned pregnancy, frequency of intercourse, maternal exposure to organic solvents Recall: Data collection on TTP occurred 8−18 yr after pregnancy Participation: Lower among women with ≥2 previous births

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
McDonald et al. (1987; 1986) Canada Hospital-based survey of maternity departments, 1982−1984. 56,012 women interviewed in 11 obstetrical units in Montreal (90% of births); 51,885 with term delivery (90% interviewed) and 4,127 SA (75% of those admitted)	Treatment in hospital of SA (4,127 women) plus self report of previous SA (before Week 28 of pregnancy) (10,910 pregnancies) Stillbirth without defect: fetal deaths after the 27th wk of gestation Congenital defects: Information extracted from medical records at time of discharge, previous births obtained from mothers report at interview and later review of physician or hospital records LBW ≤2,500 g	Self-reported occupation at time of conception for current and previous pregnancies 2nd analysis defined employment for ≥30 h/wk at beginning of pregnancy	Expected numbers calculated for each occupational category from effect of individual factors on probability of SA using logistic regression: maternal age, parity, history of previous abortion, smoking habit, and education Laundry and dry cleaning: # current pregnancies: 100 # SA: 8 O/E: 1.18 # previous pregnancies: 123 # SA: 31 O/E: 1.02 2nd analysis combined current and previous pregnancies: # pregnancies: 202 # SA: 36 O/E: 1.05 2nd analysis used maternal age, gravidity, previous spontaneous abortion, smoking, alcohol, education, and ethnicity Stillbirth (*n* = 3) O/E: 1.86 Congenital defects (*n* = 9) O/E: 1.41 LBW (*n* = 15) O/E: 1.17 *p*-value >0.05	Potential bias: * interviewers were informed of outcome status * recall time to first wk of pregnancy different for women with SA vs. term birth Dry-cleaning and laundry workers likely included many not exposed to PCE

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Taskinen et al. (1989) Finland Case-referent study Workers ever monitored for organic solvents by Finnish Institute of Occupational Health, 1965−1983. Linked IDs to identify wives through Finnish Population Register Centre and pregnancy outcomes through national registers. Included men in their first marriage during 1985 with wives aged 18−40 yr at the end of the 1st trimester of pregnancy. Included pregnancies begun during the marriage or up to 9 mo before Cases defined as wives with SA (if multiple, one randomly selected) or congenitally malformed child. Referents selected from wives with healthy birth 1973−1983 (1:3 for SA, 1:5 malformations), age matched within 30 mo Only included pregnancies identified in register and reported by participants Response rate of SA: cases 136 of 172, 79.1%; referents 370 of 505, 73.3% Final data set including eligible pregnancies for SA case-referent sets: 120 cases and 251 referents	Medically diagnosed pregnancies from Hospital Discharge Register (National Board of Health) or data on SA treated in hospital polyclinics, 1973−1983 Congenital malformations recorded in Finnish Register of Congenital Malformations SA rate among all recognized pregnancies in the cohort (including induced abortions) 8.8%	Paternal exposure via mailed questionnaires (January 1986) to both spouses re: occupational exposure related to study pregnancy—employment, occupation including work tasks, and workplace during year of conception Use and frequency of any of the monitored solvents and any other materials Exposure assessment for 80 calendar days preceding study pregnancy (spermatogenesis) blind to outcome status. Based on occupation, job description, reported solvent or other chemical use, and biological monitoring data Not exposed: Work tasks did not included handling solvents, worker did not report exposure and no biological measurement Potentially exposed: Work tasks might have involved solvent use, but not reported by worker, no biological measurements Exposed: Biological measurement taken while at same job, or tasks implied solvent exposure, or solvent exposure reported Categorized into none, low, or high	Conditional logistic regression OR for likely exposure to PCE only presented for unadjusted model (controlling for potential exposure to PCE) OR (95% CI) Likely exposed: 4 cases, 17 referents 0.5 (0.2−1.5) Trichloroethylene Likely exposed 17 cases, 35 referents 1.0 (0.6−2.0)	Potential misclassification of exposure but nondifferential: Among men with no monitoring data, 21.5% of cases and 24.2% of referents reported exposure to solvents and were categorized as exposure likely

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Taskinen et al. (1989) (continued)		High: handled solvents daily or level of biological measurements above reference value for general population Intermediate: Solvent use 1−4 d/wk and biological measurements indicate intermediate or low exposure Low: Handled solvents <1 d/wk None: all other		
Lindbohm et al. (1991) Finland All pregnancies and outcomes recorded in nationwide Hospital Discharge Register and data requested from outpatient hospital clinics, 1973−1982. Pregnancies 1973−1978 linked to 1975 Census and 1979−1982 to the 1980 Census. Central Statistical Office of Finland (1975 and 1980) Census data used for occupation and industry, SES	For exposure to any mutagenic agents, evaluated pregnancies terminated in 1976 for exposure in 1975 (to approximate 80 d prior to conception) and May 1, 1980−April 20, 1981 for 1980 Census 99,186 pregnancies among women, 12−49 yr old, with information on occupation, industry and woman's SES. For exposure to specific agents, included a 2-yr period close to the census (Jan 1, 1976−Dec 31, 1977 and May 1, 1980−April 30, 1982)	Paternal exposure classified using job-exposure matrix developed in cooperation with 2 industrial hygienists. Based on occupation and industry. Assign prevalence of chemical exposure to job groups based on monitoring data from Institute of Occupational Health Classified into 3 levels for exposure to mutagens: Moderate/high: 139 Potential/low: 820 None: 7,772	Prevalence of SA: 8.8% (Similar to national rate in Finland: 8.9%) PCE: 3 SA and 45 pregnancies defined as moderate/high exposure Linear logistic regression controlling for age only OR (95% CI) 0.7 (0.2−2.4)	Focus of exposure assessment was on "mutagens"

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Lindbohm et al. (1990) Finland Case-control study of women recruited from Institute of Occupational Health database of women biologically monitored for one or more of 6 solvents linked to national registry of medically recognized pregnancies; 80 cases (78.4% of 102 respondents) and 286 controls (99.3% of 288) (age matched 1:3) confirmed pregnancy of interest 73 cases and 167 controls with complete information for both cases and controls	Cases were women with a spontaneous abortion recorded in the national register of pregnancies in Finland and the Finnish Register of Congenital Malformations that was confirmed by the women	Self-report of employment, occupation, workplace and exposure to solvents during first trimester by mailed questionnaire Exposure assigned by 2 investigators blind to outcome status using responses and biological measurements when available Not exposed: Work tasks did not included handling solvents, worker did not report exposure and no biological measurement Potentially exposed: Work tasks might have involved solvent use, but not reported by worker, no biological measurements Exposed: Biological measurement taken while at same job, or tasks implied solvent exposure, or solvent exposure reported Level of Exposure: High: handled solvents daily or 1−4 d/wk and level of biological or available industrial hygiene measurements were high Low: Handled solvents 1−4 d/wk, and level of exposure low, or handled solvents <1 d/wk None: all other	Conditional logistic regression controlling for previous SA, parity, smoking, use of alcohol, and exposure to other solvents OR (95% CI) All solvents 2.2 (1.2−4.1) PCE (8/15 exposed cases/controls) Overall 1.4 (0.5−4.2) Low 0.5 (0.1−2.9) High 2.5 (0.6−10.5) Use of PCE in dry cleaning (4 cases/5controls) 2.7 (0.7−11.2) Other dry-cleaning work (1/6) 0.6 (0.1−5.5)	Biological measurements were available for only 5% of sample Blood PCE (mean) at time nearest pregnancy Dry cleaners (n = 6) 2.11 µmol/liter Other workers (n = 7) 0.43 µmol/liter

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Windham et al. (1991) United States Hospital-based case-control study 697 women ± 18 yr, June 1986−Feb 1987 (81.8% of 852) 1,359 controls (2 per case) randomly selected from among residents of Santa Clara County, California with a live birth, frequency matched by last menstrual period (± 1 wk) and hospital (84% of 1,485) Analysis limited to 1,361 women who were employed during pregnancy (70%)	Medically diagnosed SA defined as 20 wk gestation with pathology specimen submitted to one of 11 hospital laboratories in Santa Clara County, California; verified by review of medical charts	Computer-assisted telephone interview—exposure during pregnancy (cases) or first 20 wk (controls) Asked whether they used or worked around any of 10 solvents (including PCE) once per week or more, plus asked to name any other solvents or degreasers. For each product, number hours per week, weeks of exposure, skin contact, smelled odors, or experience symptoms Unexposed referent did not use any of the named solvents (n = 847) Exposure metric: average hours used/week of pregnancy 249 of 1,361 working women were exposed to solvents	5 PCE exposed cases, 2 exposed controls 9 TCE exposed cases, 15 exposed controls Crude OR (95% CI) PCE 4.7 (1.1−21.1) TCE 3.1 (0.55−2.9) Paint Thinners 2.3 (1.0−5.1) Paint Strippers 2.1 (0.64−6.9) PCE and/or TCE 3.4 (1.0−12.0) Adjusted OR PCE adj for hours worked 4.2 (0.86−20.2) PCE adj for age 6.0 (1.4−25.8) Intensity: respondents reported skin contact, odor, or symptoms (headaches, dizziness, or forgetfulness) Yes: ORc: 6.3, p = 0.04 None: ORc: 2.1, p = 0.54	Adjustment for confounders: Mantel-Haenszel stratification of dichotomized covariates one at a time: maternal age, race, education, prior fetal loss, smoking, and hours worked Cases and controls worked similar hours and schedules 4 of 7 women reporting use of PCE also used TCE Adjustment did not alter OR for other solvents (TCE, thinners and strippers) No consistent trend by # hours used per week

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Bosco et al. (1987) Italy 67 women working in 53 of 66 dry-cleaning shops in 2 neighborhoods in Rome, Italy (40 dry cleaners and ironing, 13 ironing service only) Average age 43 yr employed on average 20 yr	Self report by standardized Interview SA not defined Self-report by standardized interview LBW ≤2,500 g/live birth Birth defects/live births Still births/live births	Self report by standardized Interview—work activity prior to and during pregnancy (dry cleaning, housewife, other) Presence of trichloroacetic acid in 24-h urine (53 of 67) Mean (µg/L) Dry Cleaners 5.01* (*n* = 40) Ironers only 1.35 (*n* = 13) Controls 1.56 (*n* = 5) **p* = 0.06 compared dry cleaners with ironers and controls combined	5 SA of 56 pregnancies reported while employed as dry cleaner (8.9%) 1 SA of 46 pregnancies reported while house-wife Fourfold greater risk, standardized for age, not statistically significant Dry cleaners 51 live births Housewives 44 live births *n* (%) LBW Dry Cl Housewives 2 (3.9) 9 (6.8) Birth Defects/LB 2 (3.9) 1 (2.3) Still births/LB 0 (0) 1 (2.3)	Ascertainment of exposure and outcome was not independent Asked about pregnancies occurring 1≥20 yr previous

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Olsen et al. (1990) Scandinavia (Sweden, Finland, and Denmark) Nested case-control studies combining country-specific odds ratios, 1973−1983 Sweden and Denmark: All women selected from company records of all active dry-cleaning plants and laundries (dry cleaners only in Denmark) working for ≥1 mo during 1973−1983. Finland: Dry-cleaning and laundry workers identified from union registers and payroll data requested from all facilities in country, 1973−1983 (% response not provided). Asked for names of women employed for at least 3 mo during 1973−1983 Sweden: 169 women with a registered pregnancy who worked at laundry or dry cleaner during all or part of the year before delivery or 6 mo before SA, 2 matched controls per case; 84% respondents of 201 contacted. 61.7% of identified plants participated Finland: 720 pregnancies (1 randomly selected per woman) reported in hospital discharge register and reported by woman, 3 age-matched controls per case; 77.2% respondents of 932 contacted	Medically recognized SA recorded in centralized birth registries and linked to participants Sweden: Central medical birth register (n = 31) Finland: Nationwide hospital discharge registry and polyclinic data on SA (n = 118) Denmark: Central birth register and standard hospital register (n = 10) Low birth wt: <1,500 g (Sweden (n = 5), Norway (n = 7) and Denmark (n = 1) Congenital malformations (excluding certain minor malformations) Sweden: n = 6 Norway: n = 7 Denmark: n = 1 Finland: n = 24 Perinatal death (Sweden and Norway) Sweden: n = 5	Exposure during 1st trimester; Self report from questionnaires or interview Sweden and Denmark: classification by industrial hygienist blind to pregnancy outcome Finland: classification by study investigators based on work history and exposure frequency Classification: Unexposed—No exposure to PCE as defined Low—worked in dry-cleaning facility but not high exposure. High—Conducting dry cleaning or spot removal ≥1 h/d	Spontaneous abortion OR (95% CI) Combined (weighting by inverse variance of OR): Low 1.17 (0.74−1.85) High 2.88 (0.98−8.44) Sweden: Low 1.15 (0.43−3.09) High 0.82 (0.07−9.86) Denmark: Low 0.00 High 2.52 (0.26−24.1) Finland: Low 1.18 (0.71−1.97) High 4.53 (1.11−18.5) Combined outcomes (LBW, malformations and perinatal death), All countries combined, all trimesters (combined variance calculated using inverse variance of the OR) OR (95% CI) Low 1.72 (0.4−7.12) High 0.87 (0.2−3.69)	In Sweden and Denmark only 1 exposed case in high exposure group, in Finland 6 exposed cases in high category Models adjusted for parity, smoking and drinking habits (Danish model only for parity and smoking) Analyses using exposure information from employers (55% of sample) stated to have similar results

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Olsen et al. (1990) (continued) Denmark: 143 registered pregnancies of all women employed at least 1 mo at listed registered dry cleaners, 1979−1984, 77.3% respondents of 185 in cohort. 74.3% of identified plants participated				
Kyyronen et al. (1989) [Also reported in Olsen et al. (1990)] Finland 679 women confirmed the pregnancy contained in the register and provided exposure information for the 1st trimester; 25.9% of SA cases did not report the pregnancy in the register and were not included along with matched controls	130 SA reported 289 controls (women with healthy pregnancy and no SA during study period), matched by age within ±2 yr 24 cases of congenital malformation 93 controls	Unexposed—No exposure to PCE as defined Low—work tasks included pressing at a dry cleaners' or spot removing, or reported handling PCE less than once per week High—work tasks included dry cleaning for at least 1 h daily on average, or reported handling PCE at least once per week	Spontaneous abortion: Multivariate logistic regression model: High—3.4 (1−11.2) $p < 0.05$ Low exposure was not included in multivariate model: unadjusted OR: 0.7 (95% CI not reported) Model adjusted for frequent use of solvents other then PCE, frequent heavy lifting at work, frequent use of alcohol Congenital malformation: Univariate, matched logistic regression PCE (any level) 1st trimester OR (95% CI) 0.8 (0.2−3.5) 2 exposed cases	6 cases and 6 controls reported exposure to other solvents: petroleum benzene, toluene, acetone, thinner, and spot remover mixtures Other covariates (including smoking, temperature, parity, febrile disease) were not associated in univariate models so not included

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Ahlborg (1990a) [Complementary study to Olsen et al. (1990)] Sweden Case-referent study: Two cohorts of women working for ≥1 mo during 1973−1983 in dry-cleaning or laundry work Primary: 2,181 eligible women selected from company records of 475 active dry-cleaning plants and laundries, 263 used PCE and had women as employees Linked to Medical Birth Registry and Inpatient Registry for Somatic Care; identified 2,438 births and 143 SA 955 pregnancies including 66 cases of SA, perinatal death, congenital malformation, or low birth weight involved employment (at least one week) during year before delivery or 6 mo before SA. Referents matched to cases (1:2) by mother's age (± 2 yr), year of pregnancy, and parity (for deliveries only) Responses for 158 pregnancies (48 cases (75%, 110 referents (88%) Complementary: 5,176 female laundry and dry-cleaning workers registered as washers/cleaners in the national census of 1975 and 1980; linked with medical registers for 2-yr period following each census—1,136 pregnancies identified	Pregnancies and hospitalized SA identified through national registries occurring 1974−1983 Identified 2,438 births Cases defined as spontaneous abortion, perinatal death, congenital malformation, or low birth weight	Exposure during 1st trimester; Self reports through mailed questionnaires; questions re: type of production (laundry only, laundry and dry cleaning, or dry cleaning only), use of specific agents in dry-cleaning process (including PCE) Information obtained from employers on type of production, amount of dry cleaning, and use of specific cleaning agents during 1973−1983, and dates use of PCE started and ended Use of PCE: 22 of 48 cases said "don't know," 19 categorized as exposed by employer 41 of 110 referents said "don't know," 30 categorized as exposed by employer Exposure classified by 2 investigators blind to case/referent status High: Operating dry-cleaning machines or spot removing with PCE ± 2 h/wk, or ironing/pressing dry cleaned cloth >20 h/wk, or cleaning and filling the machines ≥3 times Low: Other work at workplaces where dry cleaning with PCE was performed	Multivariate conditional logistic regression model Primary study: Dry cleaning (Y/N) Referents did not work in dry cleaning or were not working during 1st trimester All outcomes combined: OR (95% CI): 1.1 (0.6−2.0) Self-report 1.02 (0.47−2.2) (Ahlborg, 1990a) Employer 1.27 (0.60−2.71) Use of PCE (Y/N) OR (95% CI): Self-report 0.92 (0.36−2.33) Employer 0.82 (0.32−2.07) Adding response from employer to data self-reported as "don't know": 1.24 (0.59−2.61) Highly exposed pregnancies Primary study: 10 of 55 cases, 27 of 106 referents Complementary: 9 of 67 cases, 17 of 126 referents For SA only: Low 1.0 (0.4−2.2) High 0.9 (0.4−2.1)	Few highly exposed pregnancies, few cases Validity of self-reports: Questionnaire data compared to employers response:. Dry cleaning Y/N: sensitivity among cases 0.97 and controls 0.96 Specificity among cases 0.75 and controls 0.69 PCE Use Y/N: Sensitivity among cases: 1.0 and controls: 0.93; Specificity among cases: 1.0 and controls: 0.94 Large plants participated in the primary study (dry cleaning accounted for <10% of total production)—air concentrations likely to be lower than for smaller plants

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Ahlborg (1990a) [Complementary study to Olsen et al. (1990)] (continued) 755 pregnancies not found in primary study, including 55 SA and 28 other adverse outcomes, response for 68 of 77 cases (88%) and 131 of 150 referents (87%)		Unexposed: Dry cleaning with PCE was not performed at workplace		Models adjusted for smoking, alcohol consumption, medical complications, and history of adverse pregnancy outcome
Doyle et al. (1997) United Kingdom 7,305 women, 16−45 yr, currently or previously employed in dry cleaning or laundry units managed by 4 companies in the UK, 1980−1995 54.5% of 5,712 questionnaires successfully delivered were returned completed Response rate for current dry-cleaning and laundry workers: 78 and 65% Previous workers 46 and 40%	Self report via mailed questionnaire, self reports verified with general practitioner (all women (114) reporting SA who worked during pregnancy and random sample of 58 who reported not working, comparison for 59). Distribution of reported exposures during pregnancy was similar for validated vs. not validated; SA defined as any fetal loss before 28 wk gestation in a confirmed pregnancy	Self report via mailed questionnaire; For each pregnancy: Work in dry cleaning or laundry during pregnancy or 3 mo prior to conception Exposure defined as machine operator during pregnancy or 3 mo prior to conception, unexposed as nonoperator	Unit of analysis: pregnancy SA rate: # reported SA/# liveborn, SAs, and stillbirths 408 pregnancies among operators # SA: Operator: 65 Nonoperator: 29 Laundry: 18 Dry cleaning vs. laundry Pregnancy completed 1980−1995: Adjusted OR (95% CI): 0.97 (0.55−1.69) Operator vs. Nonoperator: 1.63 (1.01−2.66) Compared to unexposed pregnancies before 1st exposed pregnancy: Laundry: 1.49 (0.87−2.58) Nonoperators: 1.02 (0.65−1.6) Operators: 1.67 (1.17−2.36)	Models adjusted for maternal age, pregnancy order, and year of event Separate analyses also restricted to 1st and last pregnancies Were dry cleaners more likely to report fetal death or ectopic pregnancy? No. Current workers: dry cleaners vs. laundry 11 vs. 12.9%; Previous workers: 13.9 vs. 14%

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Perrin et al. (2007) Israel Jerusalem Perinatal Study, a longitudinal study Examined risk for schizophrenia in a prospective population-based cohort of 88,829 offspring born in Jerusalem, 1964−1976, followed from birth to age 21−33 yr (January 1, 1998). Included all births to mothers in a defined geographic area and linked to Israel's national Psychiatric Registry 88,060 with complete information	The Psychiatric Registry contains diagnoses from multiple sources, including inpatient wards in psychiatric and general hospitals and psychiatric day-care facilities. Definition of schizophrenia-related discharge diagnostic codes F20-F29. Date of onset—first psychiatric admission 4 offspring of parent dry cleaners with schizophrenia (2 male, 2 female); 3 cases had exposed fathers	Occupation and demographic information from birth certificate Dry cleaning = 1 if mother or father occupation listed on birth certificate, otherwise 0 144 offspring with one or both parents a dry cleaner (63 female, 81 male)	Time to schizophrenia using proportional hazards methods Evaluated potential confounders: 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. Variables included if changed risk estimate by >10%. Results presented as crude because confounding was minimal 637 diagnosed with schizophrenia-related diagnosis; cumulative incidence = 1% RR: 3.4 (95% CI: 1.3−9.2)	Models did not adjust for family history of mental illness

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Drinking Water				
Lagakos et al. (1986) United States Retrospective population-based study of adverse pregnancy outcomes and childhood disorders in Woburn, Massachusetts in relation to drinking water from two municipal wells contaminated with chlorinated organics, 1960−1982. 7,134 of 8,109 possible interviews were completed (80%). 6,219 distinct residences were reached and 5,010 interviews were completed (57% of the towns' residences with listed telephone numbers)	235 volunteer interviewers (approx half were Woburn residents) conducted a telephone sample survey of current and former family members living in Woburn household between 1960−1982 using telephone numbers from the 1982 directory. Interviews were anonymous and residence address was not identifiable For any residents prior to 1979, self-reports on all pregnancies ending between 1960 and 1982 for women born since 1920 SA: loss in the first 6 mo of pregnancy Perinatal death: Stillbirth or livebirth surviving fewer than 7 d Low birth weight (LBW): 6 lbs (2,722 g)	Exposure estimates for water from Wells G and H using information on space—time distribution. Residence history obtained from 1982 telephone directory and self-reported residence history. 2 of 8 municipal wells (Wells G and H in eastern Woburn) were tested in May 1979 and found to contain volatile organics and the wells were shut down. TCE 267 ppb PCE 21 ppb Chloroform 12 ppb Trichlorotrifluoroethane 23 ppb Dichloroethylene 28 ppb Groundwater sampling in 1979, 61 test wells identified 48 EP*A priori*ty pollutants and 22 metals MA Dept Environmental Quality and Engineering estimated regional temporal distribution of water from Wells G and H during October 1964−May 1979 using a model of the Woburn water distribution system creating 5 zones of graduated exposure before and after 1970.	Maximum likelihood logistic regression model adjusting for maternal age, smoking status during pregnancy, year of pregnancy, SES, sex, and mother's pregnancy history 4,396 pregnancies, 1960−1982 16% were exposed during year the pregnancy ended SA: 12% (n = 520) Perinatal death: 1.5% (46 stillbirths and 21 deaths before 7 d) LBW among live births >7 d: 6.4% (220/3,462) Congenital anomalies: 4.6% (n = 177) Adjusted OR not presented SA (p = 0.66) LBW (p = 0.77) Perinatal deaths before 1970 (p = 0.55) After 1970: OR (p-value) 10 (0.003) (Based on 3 deaths out of 88 births in highest exposure quartile, 1970−1982	Rates of adverse health effects in East and West Woburn among unexposed (during years when Well G and H were not operating) were not statistically significantly different Authors explored differences between East and West Woburn for possible selection bias, and completed calls and refusals. Checked accuracy of interviewers (recontacting) and respondents (verified with medical records) Did not ask about perception of exposure to Wells G and H in survey

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Lagakos et al. (1986) (continued)	Medically diagnosed congenital anomalies grouped by involved organ or system (ICD): musculoskeletal, cardiovascular, and eye/ear defects. Grouped other organs/systems with few cases into a group with potential environmental links (CNS, chromosomal, and oral cleft anomalies) and "other." Grouped prior to exposure evaluation Childhood disorders grouped into 9 categories with ≥20 cases	These data used to estimate the percentage of annual water supply from Wells G and H at each household Calculated an annual exposure score corresponding to the mother's residence in the year the pregnancy ended For each child: sum of annual exposure scores during residence history in Woburn	Anomalies: Musculoskeletal (p = 0.78) Cardiovascular (p = 0.91) Eye/Ear OR (p-value) 14.9 (<0.0001) CNS/chromosomal/oral cleft OR (p-value) 4.5 (0.01) Other (p = 0.62) Childhood disorders: Observed vs. expected cumulative Wells G and H exposure by disorder Kidney/urinary tract (p = 0.02) Lung/respiratory disorders (p = 0.05)	Study could not associate effects with specific contaminants
Bove et al. (1995) United States Cross-sectional study of birth outcomes and fetal deaths in relation to total trihalomethanes (TTHM) and chlorinated organics in public water supplies in a 4-county area in northern New Jersey, 1985−1988. 80,938 singleton live births and 594 singleton fetal deaths (after excluding plural births, therapeutic abortions and chromosomal anomalies) from 75 out of 146 towns primarily served by public water systems	Live births and fetal deaths (plus birth weights and gestational age) identified through birth or death certificates occurring during 1/1/85−12/31/88 LBW <2,500 g among term births (≥37 wk) SGA: live births below race-, sex-, and gestational week-specific 5th percentile weight using NJ data for 1985−1988	Estimated monthly levels of individual contaminants in each of 75 towns using tap water sample data collected by the New Jersey Dept. of Environmental Protection and Energy and the water companies. At least 2 samples per year. Monthly estimates were assigned to each gestational month for each live birth and fetal death. Estimated independently of birth outcome data	Linear regression for birth weight, Logistic regression for categorical outcomes Adjusted for maternal age, maternal race, maternal education, primipara, previous stillbirth or miscarriage, sex of the birth, adequacy of prenatal care. PCE model also adjusted for TTHM Results reported with nested CI (50, 90, and 99%)	During study period, birth and death certificates did not record maternal occupation, smoking, and alcohol consumption

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Bove et al. (1995) (continued)	Preterm birth (<37 wk) Very low birth wt <1,500 g Birth weight among "term births" (≥37 wk and <42 wk) Birth defects ascertained using NJ Birth Defects Registry—a population-based, passive system—plus fetal death certificates (>20 wk) Comparison group (n = 52,334): all live births from study population that were not low birth weight, SGA, or preterm, and with no birth defects	Birth defects and fetal deaths in relation to average exposure during 1st trimester PCE Average 1st trimester: 26 ppb Average entire pregnancy: 14 ppb 55.6% of study population with surface water as source of drinking water, 11.6% had a mixture of surface and ground water 82% of comparison group had PCE concentration in public water supply ≤1 ppb, 11.5% >1−5 ppb, 5.1% >5−10 ppb and 1.4% >10 ppb	Adjusted mean decrease in birth weight among term births: 27.2 g (50% CI: −13.4−−41.0) for PCE >10 ppb No association with fetal deaths, LBW, SGA, or preterm birth Very LBW: OR, 50% CI: 1.49, 1.13−1.97 All surveillance birth defects: OR (50% CI): 1.14, >10 ppb CNS defects: no association Neural tube defects: PCE >5 ppb: 1.16 (0.69−1.83), association disappeared when TTHM included in model Oral cleft defects: PCE # OR 50% CI ≤1 67 ref >1−5 11 1.17 0.89−1.53 >5−10 1 0.24 0.05−0.63 >10 4 3.54 2.12−5.57 No monotonic trend Major cardiac defects: PCE >5 ppm: OR: 1.13	Information on these risk factors was obtained for a small number of mothers by phone interview. For these women, adjustment for these risk factors did not change the contaminant specific ORs by >15% Authors noted that nondifferential misclassification could result in underestimate or overestimates of the true effect for middle exposure categories

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Sonnenfeld et al. (2001) United States Retrospective study of birth outcome among singleton liveborn and stillborn infants of ≥20 wk gestation, and exposure to volatile organic compounds in drinking water at the U.S. Marine Corps Base at Camp Lejeune, North Carolina, 1968−1985. Included births to mothers living in base family housing at delivery and for at least 1 wk prior. Excluded 2 groups of residents exposed to TCE through a different water system and residents in trailer parks because housing records were incomplete	Outcome data obtained from birth and fetal death certificates: * Mean birth weight * Small for gestational age: gestational age calculated from last menstrual period. Weight less than the 10th percentile based on sex-specific growth curves * Preterm birth: live births <37-wk gestation (12 weighing ≥3,600 g were recorded as full term) Birth certificate data were matched to Camp Lejeune housing records to confirm address and that pregnancy occurred during occupancy	Well, dug in 1958, supplying residents at Tarawa Terrace Housing Areas I and II was contaminated with PCE and other volatile organic compounds from a dry-cleaning business that opened in 1954. Business practices did not change between 1960 and 1985, when 3 contaminated wells were disconnected from the TT water distribution system (February 8). Data on concentrations available for 1982 and later. One well (TT26) of 6 had detectable contamination and proportion of water from TT26 varied daily. Water from all wells was mixed prior to distribution Concentration (ppb) in finished water samples, 1982−85 May−June 1982 PCE 76−1,580 TCE ND−57 Exposed: TT residents Unexposed: Remaining base family housing units (minus exclusions)—based on water samples from supply wells and finished water in 1984 and 1985	Potential confounders: infant's sex and year of birth, mother's race, age, educational level, parity, adequacy of prenatal care, marital status, and history of fetal death, father's age, educational level, and military pay grade. Variable selection by backward elimination Exposed vs. unexposed Difference in mean birth wt: −26 g (90% CI: −43, −9) SGA OR (90% CI): 1.2 (1.0, 1.3) Preterm birth 1.0 (0.9, 1.1) No discernable pattern with duration of exposure estimated by length of residence at TT prior to giving birth	Adjustment for confounders did not alter risk estimates for exposure Did not control for maternal smoking, alcohol and height No data on concentration at tap in individual homes, water consumption or showering Exposure misclassification: Unexposed group was exposed to PCE prior to 1972

Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Aschengrau et al. (2009a; 2009b; 2008) United States Population-based retrospective cohort study of exposure to PCE in drinking water after installation of water distribution pipes lined with PCE-impregnated vinyl liners (VL), selected all births (index birth), 1969−1983, from birth certificates with addresses in one of 8 Cape Cod towns with some VL/asbestos cement (AC) water distribution pipes at the birth. Selected 1,492 with addresses with exposure to VL/AC pipe and 1,704 frequency matched to "exposed" by month and year of birth. 959 (64.3% of selected, 70.5% of located) of exposed and 1,087 of referents (63.8% of selected, 69.3% of located) were enrolled Included only pregnancies with completely geocoded residential histories (94.2% of reported pregnancies)	Clinically recognized pregnancy outcomes: * miscarriages, stillbirths up to Dec 1990 by self report, self-administered questionnaire Final analysis included 5,567 pregnancies from 1,891 women prevalence of loss among eligible pregnancies: 11.8% * Birthweight and gestational age among single healthy infants from birth certificates * Low birth weight (<2,500 g) * Premature birth (gestation <37 wk) * Intrauterine growth retardation (IGR) (Birth weight <10th percentile) Congenital anomalies from questionnaires	Residential history (1969−1983) by questionnaire during 2002−2003 Could not obtain information on water consumption and bathing habits by residence Estimated annual mass of PCE delivered to each address before and during the pregnancy using EPANET water distribution system modeling software with algorithm for tetrachloroethylene leaching and transport, and GIS maps of residences and a town's water distribution system Estimated water concentration: 1−5,197 µg/L Exposure: Cumulative: up to month and year of last menstrual period (LMP) Peak: up to LMP year of pregnancy Monthly average during the LMP year Before the LMP: 283 losses, 2,112 live births with some exposure; 376 losses, 2,796 live births with no exposure	Outcomes among exposed and unexposed pregnancies compared for each exposure period of interest: Cumulative, peak and average monthly Generalized estimating equation models to account for lack of independence of outcomes Considered several risk factors for pregnancy loss, associated with PCE exposure or nondrinking water sources of solvent exposure No associations or patterns observed for the 3 exposure measures and pregnancy loss, birth weight or duration of gestation All congenital anomalies 61 exposed, 95 unexposed OR adjusted for maternal and paternal age: 1.2 (95% CI: 0.8−1.7)	Nonparticipants were slightly younger (26 vs. 27.5 yr) and less educated 11.3% less than high school vs. 3.6%) but no difference by exposure Reproductive history in medical records for index pregnancy compared to self reports for 60 women: 92% of clinically recognized miscarriages and 100% of live births in record were reported by participants Compared reproductive history in birth certificates (n = 2,490) to self reports: good to excellent agreement

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Aschengrau et al. (2009a; 2009b; 2008) (continued)		During the LMP year: 213 losses and 1,743 live births with some exposure; 446 losses, 3,165 live births with no exposure	Increased odds ratios for any exposure and neural tube defects (3.5, 95% CI: 0.8−14.0), oral clefts (3.2, 95% CI: 0.7−15.0), gastrointestinal (1.8, 95% CI: 0.7−4.4), and genitourinary malformations (1.6, 95% CI: 0.6−3.8) No increased odds ratios for cardiac and musculoskeletal malformations	
Janulewicz et al. (2008) United States Population-based retrospective cohort study of exposure to PCE in drinking water after installation of water distribution pipes lined with PCE impregnated vinyl liners, selected all births (index birth), 1969−1983, from birth certificates with addresses in one of 8 Cape Cod towns with some VL/AC water distribution pipes at the birth. Selected 1910 with addresses from birth certificate with exposure to VL/AC pipe from a database of all street locations with VL/AC pipes and 1928 frequency matched to "exposed" by month and year of birth. 1,240 (64.9% of selected, 70.9% of located) of exposed and 1,250 of referents (64.8% of selected, 70.2% of located) were enrolled and returned self-administered questionnaire	Learning and behavioral disorders. Data collection from mother by self-administered questionnaire, 2002−2003. Diagnosis of attention deficit disorder (ADD) or hyperactivity disorder (HD), tutoring for math or reading, a special class placement for academic or behavioral problems, an Individual Education Plan from the school system, and if the child ever repeated a grade	Residential history (1969−1983) by questionnaire Estimated cumulative mass of PCE delivered to each address during prenatal and postnatal periods using EPANET water distribution system modeling software with algorithm for PCE leaching and transport, and GIS maps of residences and a town's water distribution system PCE exposure calculated for 94.8% of study children with completely geocoded residential histories and date of last menstrual period Estimated water concentration: Main streets: ND−80 µg/L Dead-end streets: 1,600−7,750 µg/L	Prenatal and postnatal periods analyzed separately Multivariate GEE analyses with identity or logit link function to account for siblings Final model for BW: gestational age, maternal education, race, history of LBW child, occupational exposure to solvents, use of self-service dry cleaning, and proximity of any residences to dry-cleaning establishments	Nonparticipants compared to participants: Similar for distribution of births, child's sex, race, and prevalence of children born with LBW or premature; Nonparticipants were younger, less educated, and had more prior births. Differences did not vary by exposure status

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Table 4-34. Epidemiology studies on reproduction and development (continued)

Reference, population, study design	Outcomes	Exposure assessment	Key results	Notes
Janulewicz et al. (2008) (continued) Included only pregnancies with completely geocoded residential histories (94.2% of reported pregnancies) 2,125 subjects in final data set		Exposure: • Cumulative prenatal: from month and year of last menstrual period to the month and year of birth. • Cumulative postnatal: from month and year of birth through month and year of the child's 5th birthday Final data set using refined exposure assessment Exposed: 1,349 Nonexposed: 737 Exposure variables divided into quartiles	Final model for gestational age: maternal education, race, prior preterm delivery, obstetric complications in the current pregnancy, occupational exposure to solvents, use of self-service dry cleaning, and proximity of any residences to dry-cleaning establishments No associations withprenatal or postnatal exposure and outcomes; some increased OR in low exposure groups. For example, ADD (OR [95% CI]): Low: 1.4 [0.9−2.0] High: 1.0 [0.7−1.6]	Did not use information on water consumption and bathing habits by residence—estimates are not a direct measure of PCE intake by individuals

Several studies in the United States of tetrachloroethylene in drinking water have evaluated developmental risks (Aschengrau et al., 2009b; Aschengrau et al., 2008; Janulewicz et al., 2008; Sonnenfeld et al., 2001; Bove et al., 1995; Lagakos et al., 1986). Lagakos et al. (1986) reported the results of a population-based study in Woburn, Massachusetts, among residents whose drinking water source was two wells contaminated with chlorinated organic substances from 1960 to 1982 (refer to previous study description in discussion of spontaneous abortion). Of the 3,809 infants that survived more than 7 days, 220 had low birth weights defined as 6 pounds (not the typical definition of 2,500 g). The 177 medically diagnosed congenital anomalies (4.6%) were grouped by the involved organ or system using ICD codes. Sufficient cases existed for musculoskeletal (n = 55), cardiovascular (n = 43), and eye/ear defects (n = 18) for separate analyses. CNS, chromosomal, and oral cleft anomalies were grouped together because they contained few cases. The authors felt there was evidence from previous studies to suggest that these anomalies may be associated with exposure to environmental contaminants. The rest of the anomalies were grouped into a category called "other." Childhood disorders were compiled into nine categories. Incidence of childhood leukemia in relation to exposure also was assessed and is described in the Section 4.6.1.2.5.

Logistic regression analyses, controlling for other risk factors, found no statistically significant associations between the annual exposure score for the year a pregnancy ended and musculoskeletal, cardiovascular, or "other" birth anomalies. However, an association was observed for eye/ear anomalies (OR: 14.9, $p < 0.0001$) and CNS/chromosomal/oral cleft anomalies (OR: 4.5, $p = 0.01$). In an effort to evaluate potential recall bias, the authors checked 66 of 96 disorders (perinatal death post-1970, eye/ear, or CNS/chromosomal/oral cleft anomaly, other childhood disorders) that had been confirmed in a second interview with medical records. Of the 66 events, the authors were able to verify 62 using medical records. No relation of reporting accuracy with exposure was found, thus, there was no evidence of recall bias, although the authors did not attempt to check birth records among controls.

A prevalence study in four counties in New Jersey evaluated organic contaminants monitored in the public water supply in relation to birth outcomes (Bove et al., 1995). All live births and fetal deaths reported on birth or death certificates between January 1, 1985, and December 31, 1988, among residents of 75 out of 146 towns were ascertained. The final data set included 80,938 singleton live births and 594 fetal deaths that were not therapeutic abortions or chromosomal anomalies. Birth weights and gestational age were obtained from birth or death certificates. Birth defects for live births were obtained from the New Jersey Birth Defects Registry, a population-based, passive reporting system. Additional birth defects were ascertained from fetal death certificates (>20 weeks gestation). Categorical outcomes were

compared to all live full-term births in the study population that were normal weight and had no birth defects (n = 52,334).

Monthly levels of the contaminants of interest in each town were estimated from sampling data (at least one sample per 6-month period) obtained from the New Jersey Department of Environmental Protection and Energy and the 49 water companies that served the communities. The monthly estimates were assigned to each gestational month for each live birth and fetal death. Fetal death and birth defects were evaluated in relation to levels averaged over the first trimester. Other birth outcomes were analyzed in relation to levels averaged over the entire pregnancy. Average tetrachloroethylene concentrations during the first trimester for all live births and fetal deaths were 26 ppb.

Tetrachloroethylene concentrations during the first trimester were ≤1 ppb among 82% of the comparison group. Concentrations were >1−5 ppb, >5−10 ppb, and >10 ppb for 11.5, 5.1, and 1.4% of the comparison group, respectively. Infants in the >10 ppb group were 27.2 g lighter (50% CI: −13.4−41.0). The regression models were adjusted for maternal age, race and education, primipara, previous stillbirth or miscarriage, sex of the birth, and adequacy of prenatal care, plus total trihalomethane levels. The odds ratio for very low birth weight was 1.49 (50% CI: 1.13−1.97) among term births in the >10 ppb group. An odds ratio of 1.16 (50% CI: 0.69−1.83) was observed for neural tube defects among singleton live births and fetal deaths in the >5 ppb group. The odds ratio for oral clefts in the >10 ppb group was 3.54 (50% CI: 2.12−5.57). There were 67, 11, 1, and 4 oral cleft cases in the ≤1 ppb (referent), >1−5 ppb (OR: 1.17, 50% CI: 0.89−1.53), >5−10 ppb (OR: 0.24, 50% CI: 0.05−0.63), and >10 ppb tetrachloroethylene exposure groups, respectively. The authors also reported 90 and 99% CIs for odds ratios over 1.5. For oral clefts, the 90 and 99% CIs for the odds ratio in the >10 ppb group were 1.28−8.78 and 0.82−12.15, respectively. When multipollutant models including all contaminants with associations were evaluated, the authors stated that tetrachloroethylene was no longer associated with neural tube defects, and the odds ratio for oral cleft defects was reduced to 2.0 (CIs were not presented). In the multipollutant model, the odds ratios for trichloroethylene and total trihalomethanes increased to 3.5. Therefore, while tetrachloroethylene appeared to increase risk for very low birth weight, neural tube defects, and oral clefts, other monitored drinking water contaminants also were associated with increased risk, and the contribution of individual substances cannot be determined.

A study of birth outcomes among singleton liveborn and stillborn infants, ≥20 weeks, was conducted at the U.S. Marine Corps Base at Camp Lejeune in North Carolina for the period 1968−1985 (Sonnenfeld et al., 2001; ATSDR, 1998b). Tetrachloroethylene and other volatile organic compounds used by a nearby dry-cleaning business contaminated drinking water supplied to two housing areas on the base (Tarawa Terrace I and II) until the contaminated wells

were disconnected in 1985. Water concentrations measured in samples taken between 1982 and 1985 ranged from 76 to 1,580 ppb for tetrachloroethylene and from not detected (≤10 ppb) to 57 ppb for trichloroethylene. The study population included births to mothers living in base family housing at delivery and for at least 1 week prior. Residents of Tarawa Terrace I and II were defined as exposed (n = 6,117 births). On the basis of water samples collected from wells and finished water during 1984 and 1985, residents of the remaining base family housing units were defined as unexposed (n = 5,681 births). Information on birth weight, gestational age, and preterm birth (live births less than 37 weeks gestation) was obtained from North Carolina birth records. To define small for gestational age, a gestational age specific birth weight distribution for a Caucasian population in California (Williams et al., 1982) was found to best describe the distribution of live births among the nonexposed group. Because standard birth weight distributions for military populations were not available, the California reference was used to identify a weight that classified 10% of births as small for gestational age in the nonexposed group. In models including a term for gestational age, mean birth weight among exposed infants was 26 g lower than the nonexposed infants (95% CI: −43, −9). The odds ratios for small for gestational age and preterm birth were 1.2 (95% CI: 1.0−1.3) and 1.0 (95% CI: 0.9−1.1), respectively. Regression models included several covariates to evaluate confounding, which were retained after backward elimination; however, some known factors associated with birth were not evaluated (maternal smoking, alcohol consumption, or height). Because exposure status was associated with mother and father's education, father's military pay grade, and mother's age, the unexamined risk factors also may have been associated with exposure and may have acted as confounders. Final models for mean birth weight included mother's age, history of one previous fetal loss, history of two or more fetal losses, gestational age, mother's race, living in an officer's or warrant officer's household, year of birth, and sex of the infant. Final models for small for gestational age included mother's age, mother's history of one previous fetal loss, history of two or more previous fetal losses, primiparity, living in an officer's or warrant officer's household, year of birth, and mother's education. The authors also reported the results of regression models containing cross-product terms for exposure and maternal age (<35 years, ≥35 years) or number of previous fetal losses (none, 1, ≥2). Among mothers 35 years of age or older, infants of exposed mothers weighed 104 g less than infants of unexposed mothers (90% CI: −236, −23). Birth weights of infants born to women less than 35 years of age were not different between exposure groups. In addition, among women with ≥2 previous fetal losses, exposed infants were 104 g lighter than unexposed infants (90% CI: −174, −34). Mother's age and history of previous fetal loss also appeared to modify the tetrachloroethylene risk for small for gestational age. The odds ratios for small for gestational age were 1.1 (90% CI: 0.9−1.2) and 2.1 (90% CI: 0.9−4.9) among women <35 and ≥35 years of age, respectively. There were only

11 exposed and 8 unexposed small for gestational age infants among mothers older than 35 resulting in effect estimates with lower precision. Odds ratios were 1.1 (90% CI:0.9−1.2), 1.5 (90% CI: 1.1−2.0), and 2.5 (90% CI: 1.5−4.3) among women with none, 1, and ≥2 previous fetal losses, respectively. There were 43 exposed and 14 unexposed small for gestational age infants among mothers with ≥2 previous fetal losses. The authors did not present tests for interaction.

The study found small differences in birth weight and a small increased risk of small for gestational age among live births to mothers living in two housing areas at the military base with exposure to tetrachloroethylene and other volatile organic compounds in their drinking water. Although the impact of residual confounding by unmeasured covariates is not known, a possibly larger problem may be exposure misclassification. Samples were collected over the last 3 years of the 17-year study period, although the dry-cleaning business operated during the entire period, and no operational changes occurred. Water pumped from the contaminated well was mixed with water from five other wells, but the proportion of water provided from the individual wells varied from day to day. Variation in concentrations delivered to the tap, as well as individual consumption and exposure through bathing, could not be evaluated in this study. Further, any movement on the base prior to delivery was not accounted for. During the course of an exposure reconstruction study, ATSDR learned that some of the cohort initially considered to be unexposed were in fact supplied with contaminated water from the Hadnot distribution system between 1968 and 1972 and for a 2-week period in the winter of 1985 [(NRC, 2009); www.atsdr.cdc.gov/HS/lejeune/erratum.html]. Exposed pregnancies during 1968−1972 were erroneously classified as unexposed. This calls into question the findings in Sonnenfeld et al. (2001); however, it is likely that as a result of the misclassification, any associations with birth outcome, if they exist, would have been biased toward the null. Aschengrau et al. (2008) did not observe an association of tetrachloroethylene in drinking water with either birth weight or gestational duration. This study, described previously in the discussion of spontaneous abortion, evaluated effects on pregnancy and development from tetrachloroethylene in drinking water delivered to homes in the Cape Cod region in Massachusetts between 1968 and 1980. A group of 1,910 children (1,862 singleton, 24 sets of twins) were born between 1969 and 1983 to mothers living in one of several Cape Cod towns where tetrachloroethylene leached into drinking water from vinyl-lined pipes in the water distribution system. Children initially designated as unexposed (1,853 singleton, 37 sets of twins) were randomly chosen from the remaining resident births and were frequency matched to the exposed group by month and year of birth. Response among mothers who were successfully located was comparable between the exposed and unexposed groups (70%); in the end, 56.4% of selected births designated as exposed were included, and 54.4% of selected births designated as unexposed were included. After exposure modeling, 1,353 exposed and 772 unexposed healthy, singleton births were identified.

The prevalence of prior low birth weight infants in the cohort was low: 5% (n = 68) among the exposed and 3.4% (n = 26) among the unexposed group. No differences in mean birth weight or odds ratios for low birth weight (<2,500 g) or intrauterine growth retardation (<10th percentile based on U.S. age-, sex-, and race-specific cut-offs, 1970−1976) were observed by exposure status. Generalized estimating equation regression models for birth weight differences adjusted for gestational age, maternal race, educational level, history of a low-birth-weight child, occupational exposure to solvents, use of self-service dry cleaning, and proximity of any residences to dry-cleaning establishments. Mean birth weights were slightly greater among exposed infants in almost all quartiles for all of the three exposure measures, but the estimates were statistically imprecise, and no pattern by exposure amount was observed. Average monthly maternal exposure during the year of the last menstrual period in quartiles was associated with increases in birth weight of 20.9, 6.2, 30.1, and 15.2 g compared to no exposure. Models of gestational age were adjusted for maternal race, educational level, prior preterm delivery, obstetric complications in the current pregnancy, occupational exposure to solvents, use of self-service dry cleaning, and the proximity of any residences to dry-cleaning establishments. Estimates of the difference in duration of gestation with increasing quartiles of exposure during the year of the last menstrual period were −0.2, 0.1, −0.1, and −0.2 weeks. CIs were wide, included the null, and did not indicate a pattern by exposure amount.

The study of exposure from leaching tetrachloroethylene in water distribution pipes installed between 1968 and 1980 in the Cape Cod region in Massachusetts also assessed the risk of congenital anomalies reported by participants (Aschengrau et al., 2009b). Congenital anomalies were coded by two study investigators, blind to exposure status, in consultation with a pediatrician using guidelines from the Metropolitan Atlanta Congenital Defects Program. Of the total of 4,657 children reported by the mothers, 643 were excluded because they were born after 1990, were missing prenatal information, were from multiple pregnancies, were exposed to known teratogens, mothers smoked marijuana daily or weekly, or drank 7 or more alcoholic drinks during pregnancy. There were 61 children with congenital anomalies among the 1,658 with prenatal exposure, and 95 children with congenital anomalies among the 2,999 with no prenatal exposure. The unadjusted odds ratio (generalized estimating equation regression) for all congenital anomalies was 1.1 (95% CI: 0.8−1.6) for any prenatal exposure to tetrachloroethylene. Simultaneous control for maternal and paternal age did not change the odds ratio. This also was true when other potential confounders were included one at a time (calendar year of birth, mother's educational level, cigarette smoking, alcoholic beverage consumption, prior pregnancy loss, and child's gender). Among children with an average monthly prenatal exposure greater than or equal to the 75th percentile (2.3 g), the odds ratio was 1.5 (95% CI: 0.9−2.5). Although case numbers were low, increased odds ratios were observed for several

organ systems, diagnostic groups, and any prenatal exposure compared to none. These included neural tube defects (3.5, 95% CI: 0.8−14.0, *n* = 6 exposed cases), oral clefts (3.2, 95% CI: 0.7−15.0, *n* = 5 exposed cases), gastrointestinal malformations (1.8, 95% CI: 0.7−4.4, *n* = 11 exposed cases), and genitourinary malformations (1.6, 95% CI: 0.6−3.8, *n* = 11 exposed cases). Odds ratios for cardiac (0.9, 95% CI: 0.4−2.0, *n* = 9 exposed cases) and musculoskeletal malformations (0.9, 95% CI: 0.5−1.6, *n* = 19 exposed cases) were not increased, and risk was not estimated for eye, ear, respiratory, and other malformations because the number of cases was too low.

As discussed previously, nondifferential exposure misclassification was likely given the lack of individual level exposure information, which may have resulted in lower observed risk estimates. In addition, the authors stated that the prevalence of anomalies, particularly minor ones, may have been underreported by the mothers because it was lower in the study population than reported by other monitoring programs. This would affect the statistical power of the study. The authors did not believe that recall was differential with respect to exposure status because most of the respondents did not know whether or not they were exposed.

Risk of learning and behavioral disorders was evaluated in relation to prenatal and postnatal exposure to tetrachloroethylene in the Cape Cod towns with a contaminated water distribution system (Janulewicz et al., 2008). The authors did not observe an association with increasing amount of exposure among children born between 1969−1983 whose mothers lived in one of the towns with vinyl-lined asbestos-cement pipes at the time of birth. The study is discussed in detail in Section 4.1.

In summary, some studies of tetrachloroethylene in drinking water suggest that exposure during pregnancy is associated with low birth weight (Bove et al., 1995; Lagakos et al., 1986), eye/ear anomalies (Lagakos et al., 1986), and oral clefts (Aschengrau et al., 2009b; Bove et al., 1995; Lagakos et al., 1986). No associations with tetrachloroethylene exposure were reported for small for gestational age (Bove et al., 1995) or other classifications of congenital anomalies (e.g., musculoskeletal, cardiovascular) (Aschengrau et al., 2009b; Lagakos et al., 1986). Although a small increase in risk of small for gestational age was reported for infants exposed prenatally to tetrachloroethylene at the Camp Lejeune military base, the finding remains inconclusive until ATSDR completes its reanalysis (Sonnenfeld et al., 2001). Aschengrau et al. (2008) did not observe associations with birth weight or gestational age in a Cape Cod population exposed to a wide range of tetrachloroethylene concentrations in drinking water. Occupational studies of dry-cleaning and laundry workers in Scandinavia could not evaluate specific congenital anomalies because few cases were identified (Lindbohm, 1995; Ahlborg, 1990a; Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989). The number of cases with birth anomalies in specific diagnostic groups was very small in all of the studies, and CIs

often included one. In addition, imprecise exposure estimates likely resulted in nondifferential misclassification and a bias of risk estimates toward the null. Participants in the studies were exposed to multiple contaminants, and it was not possible to analyze substance-specific risks. Finally, a more than threefold risk of schizophrenia was associated with dry cleaning as a surrogate for prenatal tetrachloroethylene exposure [Perrin et al. (2007), discussed in Section 4.1]. The longitudinal design and use of a national registry to identify psychiatric diagnoses were strengths of the study, but tetrachloroethylene exposure was not directly analyzed.

4.7.1.2. Animal Developmental Toxicity Studies

Evaluation of the developmental effects of tetrachloroethylene exposure in mammalian animal models is based on several studies of in utero exposures to maternal animals during specific periods of pregnancy. Additionally, evaluations of the developmental neurotoxic potential of tetrachloroethylene have been conducted in rats. These studies are described below.

4.7.1.2.1. In vitro developmental toxicity assay

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 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.

4.7.1.2.2. Nonmammalian developmental toxicity assay

Spencer et al. (2002) evaluated the effects of tetrachloroethylene on the embryonic development of Japanese medaka. In this study, 1-day-old in ovo embryos were exposed to concentrations of 0, 20, 40, 60, or 80 mg/L for 96 hours or to concentrations of 0, 1.5, 3, 6, 12, or 25 mg/L for 10 days. Viability, hatchability, and morphological/developmental abnormalities were evaluated. A 96-hour LC_{50} of 27.0 mg/L was identified for egg viability. Following 10 days of exposure, hatchability and larval survival were significantly decreased, and developmental abnormalities were significantly increased in a concentration-dependent manner. At the lowest concentration tested (1.5 mg/L), developmental findings included abnormalities of the circulatory system, yolk-sac edema, pericardial edema, scoliosis, hemorrhaging, blood pooling, and cardiac morphological defects. The study authors concluded that tetrachloroethylene is teratogenic to the Japanese medaka.

4.7.1.2.3. In vivo mammalian screening study

In a developmental toxicity screening study, timed-pregnant F344 rats were treated by gavage with tetrachloroethylene at doses of 900 or 1,200 mg/kg-day in corn oil vehicle on GDs 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 PNDs 1, 3, and 6. At 1,200 mg/kg, no live pups were delivered on GD 22. 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 GD 22. On PND 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.

4.7.1.2.4. In vivo prenatal developmental toxicity studies

Schwetz et al. (1975) conducted an inhalation developmental toxicity study, in which 25−30 Sprague-Dawley rats and 30−40 Swiss-Webster mice were exposed to airborne tetrachloroethylene at 300 ppm, 7 hours/day, on GDs 6−15. Following laparohysterectomy on GDs 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 percentage of litters with delayed ossification of the skull bones, delayed ossification of the sternebra, and subcutaneous edema was significantly increased. Due to the single exposure level used in this study, a dose response could not be determined.

Szakmáry et al. (1997) exposed CFY rats to tetrachloroethylene via inhalation throughout gestation (i.e., GDs 1−20) for 8 hours/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 GDs 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 (GDs 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. Percentage preimplantation loss was significantly increased from controls by 133 and 117% at these exposure levels, while percentage postimplantation loss was increased nonsignificantly 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 percentage 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 nonsignificant decreases in maternal body-weight gain (13% lower than control), and increases in pre- and postimplantation loss (49 and 38% greater than control, respectively). The percentage 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, 2/16 does aborted, total resorption of four litters was reported, and the percentage of postimplantation loss was significantly increased. The percentage of rabbit fetuses with malformations (details not provided in the report) was also increased, although not significantly.

Hardin et al. (1981) [also refer to Beliles et al. (1980)] exposed Sprague-Dawley rats (30/group) and New Zealand white rabbits (20/group) via inhalation to 500 ppm of tetrachloroethylene for 7 hours/day, 5 days/week. Tetrachloroethylene was administered with and without 3-week pregestation exposures and with both full-term and terminal two-thirds-term exposure. No maternal or developmental toxicity was identified.

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 hours/day, 7 days/week on GDs 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.

4.7.1.2.5. Developmental neurotoxicity

Developmental neurotoxicity data are also discussed in Section 4.1.2.

A cohort of rats from the Szakmáry et al. (1997) 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 PND 100. It was not clearly specified in the report whether the daily inhalation exposures continued throughout the postnatal period. Preweaning 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), minimally decreased exploratory activity and muscular strength in treated offspring (presumably at both exposure levels) that normalized by PND 51, and significantly increased motor activity on PND 100 of females exposed to 4,500 mg/m^3 of tetrachloroethylene.

Nelson et al. (1979) investigated developmental neurotoxicity in Sprague-Dawley rats by exposing pregnant dams (13−21/group) to tetrachloroethylene at concentrations of 100 ppm or 900 ppm during either early pregnancy (GDs 7 to 13) or late pregnancy (GDs 14 to 20). Morphological examination of the fetuses (gross, visceral, and skeletal) was performed, and behavioral testing and neurochemical analyses of the offspring were conducted. 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 approximately 20% at postnatal Weeks 3−5. Developmental delays were observed in both the groups exposed during early and 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 PND 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 did not identify treatment-related 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 GDs 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.

Additional evidence of potential developmental neurotoxicity was reported by Fredriksson et al. (1993). In this study (refer to Section 4.1.2.2), tetrachloroethylene was administered to male NMRI mice by gavage at dose levels of 0, 5, or 320 mg/kg-day on PNDs 10−16. At PND 17 and 60, spontaneous activity (locomotion, rearing, and total activity) was measured over three, 20 minute periods. No treatment-related alterations in activity were observed at 17 days of age; however, at 60 days of age, all three measures of spontaneous activity were altered.

4.7.2. Reproduction

4.7.2.1. Human Reproduction Data

Studies of tetrachloroethylene exposure have evaluated several outcomes including effects on menstrual disorders (Zielhuis et al., 1989), semen quality (Eskenazi et al., 1991a), fertility (Eskenazi et al., 1991b; Rachootin and Olsen, 1983), time to pregnancy (Sallmen et al., 1998; Sallmen et al., 1995), and spontaneous abortion (Doyle et al., 1997; Lindbohm et al., 1991; Windham et al., 1991; Ahlborg, 1990a; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989; Bosco et al., 1987; McDonald et al., 1987; McDonald et al., 1986). Many of the studies evaluated exposure during a specific critical window for development, usually the first trimester.

In a letter to the editor, Zielhuis et al. (1989) described the results of a cross sectional study of menstrual disorders among dry-cleaners and laundry workers in the Netherlands. A total of 471 of 592 women returned a mailed questionnaire (80%). The sampling frame for recruitment was not described. After excluding 72 respondents because the woman was currently pregnant or lactating at the time of administering the questionnaire or reported a chronic illness or gynecological surgery, and excluding another 324 respondents because the woman reported use of oral contraceptives, the final data set included 68 exposed and 76 unexposed women. Exposure was defined on the basis of occupation (dry cleaners versus laundry workers). The authors reported that the exposed and unexposed groups were similar with respect to age, lifestyle, work conditions, and personal characteristics (body mass index, number of children, and use of contraceptives). Risk of specific menstrual characteristics by occupation was evaluated using linear logistic regression adjusting for age, body mass index, substantive weight changes, number of children, history of diseases, sporting activities, life events, smoking, alcohol consumption, medical drugs, and work conditions other than exposure to tetrachloroethylene. Prevalence of menstrual conditions in the population varied between 10% (oligomenorrhea, premenstrual syndrome) to 30% (unusual cycle length) and occurred with greater frequency among dry cleaners compared to laundry workers for all symptoms except for one (polymenorrhea). There were no reports of amenorrhea. Elevated odds ratios were observed for several of the symptoms including oligomenorrhea (2.1, 90% CI: 0.9−5.3), unusual cycle length (2.3, 90% CI: 1.2−4.4), menorrhagia (3.0, 90% CI: 1.6−5.6), dysmenorrhea (1.9, 90% CI: 1.1−3.5), and premenstrual syndrome (3.6, 90% CI: 1.5−8.6). This study indicates that working in dry cleaning may adversely affect menstruation, but the lack of detail in reporting precludes a thorough assessment of selection bias or confounding. In addition, the assignment of exposure status by industry also precludes a definitive conclusion regarding a potential association with tetrachloroethylene.

Semen quality was evaluated among men who worked in the dry-cleaning industry compared to men working in laundries in California (Eskenazi et al., 1991a). The population, recruited from membership lists of the Laundry and Dry Cleaners Union Locals 3 (San Francisco Bay area) and 52 (Greater Los Angeles), included all dry cleaners (n = 85) and all laundry workers, 20−50 years of age, in Local 3 (n = 119) and a randomly selected sample of Local 52 members (n = 206). Laundry workers were frequency matched by age to dry cleaners from the same Local. Dry cleaners also were recruited from nonunion shops in the San Francisco area. Eligible individuals were 20−50 years of age, current workers in the industry, spoke English or Spanish, had not had a vasectomy, and were located by telephone or mail. Respondents included 20 union drycleaners (38% of 53 eligible) and 56 union laundry workers (34% of 166 eligible), plus 13 nonunion dry cleaners. Men were considered exposed if they worked in the dry-cleaning industry or a laundry where dry cleaning was performed. The unexposed group included laundry workers at businesses where dry cleaning was not conducted. After exposure was assessed, the final data set included 34 exposed workers and 48 unexposed workers with adequate semen samples and confirmed type of establishment. Information on sociodemographic characteristics, reproductive and medical history, and personal habits was collected by interview. In addition, a detailed work history including job tasks and exposures during the previous week and the past 3 months was obtained. A physical exam was conducted by a study physician blind to exposure status, and participants returned a semen sample collected after at least 2 days of abstinence.

The semen was analyzed for sperm concentration, morphology, and motility. Each sperm measure was evaluated in relation to three measures of exposure: dry cleaning versus laundry, tetrachloroethylene in exhaled breath (limit of quantitation: 2.67 μg/m^3), and an exposure index encompassing the entire period of spermatogenesis (approximately 3 months). Exhaled air was measured 16−19 hours after the end of the workweek or was corrected to 16 hours using an elimination model (11 samples). An industrial hygienist assigned an exposure score using responses to the questionnaire concerning job task (e.g., machine operator, presser, etc.), the type of dry-cleaning machine used (e.g., wet to dry transfer, dry-to-dry) and other tasks and attributes known to influence the level of exposure to tetrachloroethylene. The exposure score ranged from 0 among unexposed men to 11 among the exposed group. The association of semen parameters with tetrachloroethylene exposure was analyzed using multiple linear regression with adjustment for potential confounding variables that were associated with both the semen parameter and any of the exposure measures. Models of three clinically relevant measures of semen quality, oligospermia (<20 million/mL), >40% abnormal forms, and <60% motile sperm, were not associated with any exposure measure among the entire cohort. Of four measures of sperm motility, Ln median amplitude of lateral head displacement was associated with Ln tetrachloroethylene in exhaled air among all 82 participants (t = 2.0, p = 0.05), adjusting for

ethnicity, education, religion, and physical abnormalities found on exam. Exposure scores and industry group were not statistically significant predictors of this semen parameter. However, Ln tetrachloroethylene levels ($t = 2.14$, $p = 0.04$) and exposure score ($t = 3.07$, $p = 0.005$) were predictors of amplitude of lateral head displacement among the 34 participants in the exposed group. Sperm linearity was inversely associated with exposure score in both analytic groups ($t = -2.57$, $p = 0.02$). Percentage of round sperm was statistically significantly associated with all three exposure measures, controlling for history of STD and working in temperatures over 100°F among all participants but not in the dry-cleaning group alone. Percentage of narrow sperm was inversely associated with all three exposure measures controlling for ethnicity, number of days working in temperatures greater than 80°F, and use of marijuana among all participants. Among the dry cleaners, Ln percentage narrow sperm was inversely related to Ln tetrachloroethylene levels ($t = -2.29$, $p = 0.03$) but not by exposure score ($t = 0.92$, $p = 0.36$).

Tetrachloroethylene exposure appeared to alter sperm quality in this population of unionized dry cleaners. However, the effects were subtle, and the clinical significance of the semen parameters associated with tetrachloroethylene exposure is not clear. The low response rate in the primarily unionized cohort limits generalizations to the industry as a whole. Reproductive outcomes also were evaluated among the wives of the men who participated in the study of semen quality (Eskenazi et al., 1991a). Telephone interviews were conducted with 17 wives of the 20 married dry cleaners (85%) and 32 wives of the 36 married laundry workers (89%) in the original cohort. Pregnancies and miscarriages during the years of their husbands' employment in the industry were identified among 14 wives of dry cleaners and 26 wives of laundry workers. Standardized fertility ratios were calculated using the U.S. national birth rates during periods of employment in the industries and periods when the men were not employed in the industries as a comparison. Investigators also analyzed the number of months to conception for the last pregnancy during the period of employment in the industries. The wives of laundry workers were more likely to be Hispanic, Catholic, to have smoked during the year of the index pregnancy, and to have a history of reproductive disease or surgery. They had fewer years of education, and a greater proportion weighed more. The wives also were more likely to work in dry cleaning and laundries, confounding the source of exposure.

Fertility rates among the wives of dry-cleaners and laundry workers were higher than the national average for women of the same race, parity, birth cohort, and age. The standardized fertility ratios were comparable in both industry groups. However, it took longer for the wives of dry cleaners to achieve the index pregnancy compared to the wives of laundry workers (8.2 ± 10.2 months versus 4.1 ± 5.8 months, respectively, $p = 0.08$). In Cox Proportional Hazards Models with adjustments for ethnicity (Hispanic vs. non-Hispanic) and smoking, the per-cycle pregnancy rate of wives of dry cleaners was approximately one-half that of the wives

of laundry workers (rate ratio = 0.54, 95% CI: 0.23−1.27). A rate ratio of less than 1 also was indicated in models using husbands' exhaled tetrachloroethylene (rate ratio = 0.94, 95% CI: 0.85−1.04) and husbands' exposure index (rate ratio = 0.90, 95% CI: 0.78−1.03). The latter two exposure indices may not have estimated exposure during the sensitive window for the index pregnancy, however. The small sample size resulted in CIs that included the null hypothesis. The authors noted that to detect a halving of risk for pregnancy with 80% power ($\alpha = 0.05$), over 50 women per group would have been required.

A Danish case-control study of couples examined or treated for infertility during 1977−1980 reported evidence of idiopathic infertility among women reporting exposure to dry-cleaning chemicals (Rachootin and Olsen, 1983). Controls were couples with a healthy child born at the same hospital during 1977−1979. Information about occupational and reproductive history was obtained from 87% of both cases and controls who returned a mailed, self-administered questionnaire during November 1980 to May 1981. Participants were defined as exposed if they reported contact with any of 15 types of chemicals and physical agents (including dry cleaning) and three specific work processes a minimum of once per week for at least 1 year in the period prior to hospital admission. The medical records of infertile couples were reviewed by a collaborating physician who had no knowledge of exposures. Three analytic approaches were used to evaluate subgroups of couples with a medical history anticipated to be related to occupational exposures. Reported exposure to dry-cleaning chemicals was associated with idiopathic infertility among women compared to fertile couples with a healthy child conceived within 1 year (OR: 2.7, 95% CI: 1.0−7.1). The statistical method was not described, but the authors stated that the odds ratio was adjusted for the women's age, education, residence, and parity. Cases and controls lived within the catchment area of the hospital. Exposure to dry-cleaning chemicals was not associated with sperm abnormalities or idiopathic infertility among male partners or with hormonal disturbances among women. The odds ratio for idiopathic infertility among women with exposure to dry-cleaning chemicals also was increased when couples who had been infertile for at least 1 year were compared to other infertile couples with conditions believed to be unrelated to occupational exposures (crude odds ratio [ORc] = 1.8, 95% CI: 0.5−5.8). A third analysis involved comparison within the control group; couples who experienced a delay in conception of more than 1 year but who gave birth to a healthy child were compared to couples who conceived a healthy child in less than 1 year. Again, women reporting exposure to dry-cleaning chemicals had an increased odds ratio for delayed conception (ORc: 1.6, 95% CI: 0.9−2.9). Although two of the risk estimates did not reach statistical significance, all three were greater than 1.5. The consistent increased odds ratios observed using three different comparison groups suggest an effect of exposure to dry-cleaning chemicals on conception among women. The study evaluated a large number of chemicals and physical

exposures. The authors did not present the number of cases by subgroup, or the number of controls who reported exposure to dry-cleaning chemicals, so it is difficult to assess the impact of sample size on the precision of the effect estimates. Other chemical exposures, as well as noise, also were associated with idiopathic infertility among the women. In addition, the statistical analyses for dry-cleaning chemicals did not control for exposure to other chemical or physical agents.

Sallmén et al. (1995) conducted a retrospective time-to-pregnancy study among Finnish women biologically monitored at the Institute of Occupational Health in 1965−1983 for one or more of six solvents (styrene, toluene, xylene, tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane). This study was an extension of an investigation of the risk of spontaneous abortion in the same study population. That study is described later in this section (Lindbohm et al., 1990). Pregnancies and their outcomes (live birth, spontaneous abortion, or fetal loss) between 1973 and 1983 among the women had been identified using a national register of pregnancies in Finland and the Finnish Register of Congenital Malformations. Time-to-pregnancy information was obtained through questionnaires mailed to 355 women who were the cases and controls in the previous study. Information about exposure during the preceding 12 months before each woman's pregnancy began was collected. The response rate was 66%, and the final data set contained 197 women who had been attempting to become pregnant, had no other risk factors for infertility, for whom complete information was available on exposure and time-to-pregnancy. Time-to-pregnancy was defined as the number of menstrual cycles required to become pregnant and is a measure of fertility, the per cycle probability of conceiving a clinically detectable pregnancy. Increased time-to-pregnancy can indicate a loss during pregnancy during any stage from gametogenesis to fertilization to the clinical stage of pregnancy, including early stage spontaneous abortions.

The same exposure-assessment procedure as was used in the previous study was adopted for this study, and if the subject reported working in the same job, their previous exposure classification was used. Self-reported work tasks during the 12 months prior to conception were assigned to an exposure classification by likelihood and level of exposure for 84 women whose jobs or exposures were different than reported previously for the first trimester. Classifications were made without knowledge of reproductive history and were checked by an independent, experienced industrial hygienist. The three categories for likelihood of exposure were not exposed, potentially exposed, and exposed. Subjects were grouped according to high (n = 46), low (n = 59), and none (n = 92) for level of exposure [refer to description of Lindbohm et al. (1990)].

Exposure to organic solvents during their time-to-pregnancy was reported by more than one-half of the women (105 out of 197). Incidence density ratios, indicating the likelihood that

exposed women will achieve a clinical pregnancy during the fertile period in each menstrual cycle class (e.g., 1st menstrual cycle, 2nd, 3rd and 4th, 5th, 6th, etc.) compared to an unexposed woman, were estimated using discrete proportional hazards regression. Incidence density ratios (IDRs) were reported for women exposed to tetrachloroethylene (n = 20) or working in dry cleaning (n = 17). Compared to women with no exposure, the IDRs for low and high exposure were 0.63 (95% CI: 0.34−1.17) and 0.69 (95% CI: 0.31−1.52), respectively. The statistical models controlled for exposure to other solvents, recent contraceptive use, and age at menarche. For workers in dry cleaning, the IDR for 11 women with low or high exposure combined was 0.44 (95% CI: 0.22−0.86) and for 6 women with high exposure was 0.57 (95% CI: 0.24−1.34). These models controlled for low and high exposure to solvents in other industries, recent use of IUD/spermicides, and age at menarche. The model for high exposure also adjusted for low exposure to organic solvents. The authors noted that only 1 of the 11 women who worked in dry cleaning reported exposure to other solvents in addition to tetrachloroethylene. These results suggest that exposure to tetrachloroethylene may affect fecundability, however, because the focus was on a broad range of solvent exposures and industries, the sample size for assessing tetrachloroethylene was small, and statistical precision was low. However, the study had several strengths, including collection of detailed work histories. Exposure classifications were based on the frequency of solvent use, not just reported use ever or job title. In addition, several potential confounders were assessed, and statistical models controlled for exposure to other solvents. It was not clear if the models for individual solvents were assessed for confounding by case status (i.e., pregnancy ended in a spontaneous abortion). However, reduced fecundability was associated with exposure to organic solvents combined in separate analyses of cases and controls. The low response rate overall, and evidence that response was higher among cases and exposed controls, particularly those with lower parity, raises the possibility of selection bias.

Time-to-pregnancy also was evaluated among the wives of men exposed to organic solvents and monitored by the Finnish Institute of Occupational Health during 1965−1983 (Sallmen et al., 1998). This was an extension of an earlier case-referent study of risk of spontaneous abortion [refer to description later in this section of Taskinen et al. (1989)]. The investigators used a similar approach as that used in Sallmén et al. (1995), described above. Cases (n = 110) and referents (n = 332) that participated in Taskinen et al. (1989) were recruited. Time-to-pregnancy information was obtained through questionnaires mailed to 355 women who were the cases and controls in the previous study. A detailed history of occupation and work tasks during the year the pregnancy started had been obtained from the husbands in the previous study. A similar history was now requested of the wives, focusing on the preceding 12 months before the pregnancy. The response rate was 72%, and the final data set contained 282 women who had been attempting to become pregnant, had no other risk factors for infertility, for whom

complete information was available on exposure and time-to-pregnancy. The same exposure-assessment procedure as was used in the previous study was adopted for this study, and if the subject reported working in the same job at the beginning of the pregnancy, their previous exposure classification was used. A new exposure classification was required only for nine men whose jobs or exposures were different than reported previously for the first 3 months before the pregnancy began. Classifications were made without knowledge of reproductive history and were checked by an independent, experienced industrial hygienist (Taskinen et al., 1989). The three categories for likelihood of exposure were, not exposed, potentially exposed, and exposed. Subjects were grouped according to high/frequent (n = 141), intermediate/low (n = 80), and unexposed (n = 61) for level of exposure to organic solvents during the time-to-pregnancy period.

Incidence density ratios (IDRs) were reported for exposure to all organic solvents combined and for specific solvents. The IDRs for low (n = 9) and combined intermediate/high (n = 8) exposure to tetrachloroethylene were 0.86 (95% CI: 0.4−1.84) and 0.68 (95% CI: 0.30−1.53). The discrete proportional hazards regression models were adjusted for short menstrual cycle, long or irregular menstrual cycle, older age at menarche, frequency of intercourse, maternal age, maternal exposure to organic solvents, and a variable for missing information. Fecundity appeared most reduced among the wives whose husbands had a high level and/or frequency of tetrachloroethylene exposure compared to low or no exposure. However, the study was limited by low statistical precision because of small sample size. Time-to-pregnancy information and exposures were collected 8 to 18 years after the pregnancy of interest, which likely resulted in some misclassification. It is less likely that recall bias affected the risk estimates because the exposures were assigned based on information collected for the earlier study of spontaneous abortion.

Among studies evaluating effects of tetrachloroethylene on reproduction and development, the majority of studies assessed effects on risk of spontaneous abortion. These studies defined spontaneous abortion as a fetal loss prior to 20−28 weeks gestation, although one study included all fetal loss during the first 6 months of pregnancy (Lagakos et al., 1986). Several studies included only clinically recognized spontaneous abortions reported in birth registers (Lindbohm et al., 1991; Windham et al., 1991; Ahlborg, 1990a; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989; McDonald et al., 1987; McDonald et al., 1986), while some included spontaneous abortions reported by participants (Aschengrau et al., 2009a; Aschengrau et al., 2008; Doyle et al., 1997; Eskenazi et al., 1991a; Bosco et al., 1987; Lagakos et al., 1986). It should be noted that it is not possible to identify all spontaneous abortions that occur in populations because a woman may not recognize very early events and/or may not seek treatment.

McDonald et al. (1987; 1986) conducted a large survey of occupation and reproductive outcomes among 56,012 women in 11 large obstetrical units in Montreal, Canada, over a 2-year period from May 11, 1982 to May 10, 1984. Interviews were conducted with 51,885 women with a term delivery and 4,127 women treated in the hospital for a spontaneous abortion, defined in this study as a fetal loss <28 weeks of gestation. The 11 hospitals included in the survey treated approximately 90% of all births in Montreal. As part of the interview, women were asked to describe all previous pregnancies that ended in a spontaneous abortion, and 10,910 were identified. Interviews were completed for 90% of the women with term births, and 75% of women admitted for a spontaneous abortion. Information also was collected about occupation at the time of conception for the current and any previous pregnancies. Nine occupational groups in the Canadian Classification and Dictionary of Occupations were reduced to six major groupings and included 42 categories that the investigators concluded were homogenous. Logistic regression was used to evaluate risk of spontaneous abortion for five nonoccupational factors: maternal age, parity, history of a previous abortion, smoking habit, and highest educational level reached, and the expected number of spontaneous abortions for each occupational group was calculated. The ratio of observed to expected numbers was evaluated for each occupational group. Among women in the laundry and dry-cleaning occupational grouping, there were 8 spontaneous abortions out of 100 recent pregnancies (O/E: 1.18; $p > 0.1$ [CI not reported]) and 31 out of 123 previous pregnancies (O/E: 1.02). Subsequent analysis of the data included women who worked at their jobs for at least 30 hours weekly at the beginning of pregnancy (McDonald et al., 1987). In this analysis, 36 combined current and previous spontaneous abortions were observed out of 202 pregnancies. An O/E ratio of 1.05 ($p > 0.1$; CI not reported) was reported. The expected number was determined from a logistic regression model of spontaneous abortion risk including maternal age, parity, history of a previous abortion, smoking habit, and alcohol consumption. This study is not very informative regarding tetrachloroethylene risk because the group of dry-cleaners and laundry workers likely included individuals with no exposure to the solvent.

A case-referent study of adverse pregnancy outcome was conducted among the wives of male workers who had been monitored for organic solvents by the Finnish Institute of Occupational Health between 1965 and 1983 (Taskinen et al., 1989). The cohort included men in their first marriage during 1985 with wives who were 18−40 years old at the end of the 1st trimester of pregnancy. Pregnancies and outcomes were identified through national registers. Eligible pregnancies began during the marriage or up to 9 months before. Cases were defined as wives with a spontaneous abortion (if multiple, one randomly selected) or a congenitally malformed child. Referents were selected from wives with a healthy birth between 1973 and 1983 (1:3 for spontaneous abortions, 1:5 for malformations), age matched within 30 months. A

total of 136 of 172 selected cases (79.1%) and 370 of 505 selected referents (73.3%) responded to a questionnaire mailed in January 1986. Only pregnancies that were identified in the register and reported by participants were included. Because of this, and because a matched response was required, the final data set included 120 cases and 251 referents. Information on occupation and exposure to solvents during the year of conception was requested of the men. Information on occupational and other exposures during the first trimester of pregnancy was solicited from the wives. Exposure classifications were made blind to pregnancy outcome. Solvent exposure for the men was assessed for an 80-day period that preceded the pregnancy, the relevant period of spermatogenesis, using information on occupation, job description, reported solvent or other chemical use, and biological monitoring data. Workers were classified as not exposed if work tasks did not include handling solvents and no exposure was reported, and no biological measurement for a particular solvent was made. Workers were classified as potentially exposed if work tasks might have involved solvent use, but use was not reported by the worker, and no biological measurements for a particular solvent were made. Workers were classified as exposed if biological measurements for a solvent were taken while at the same job for the reported pregnancy, reported tasks implied solvent exposure, or solvent exposure was reported. Exposure was categorized into none, low, intermediate, or high. Workers with high exposure handled solvents daily, or their biological measurements were above the reference value for the general population. Workers with intermediate exposure used solvents 1−4 days per week, and biological measurements indicated intermediate or low exposure. Workers with low exposure handled solvents <1 day per week. All other scenarios were classified as no exposure.

A spontaneous abortion rate of 8.8% was observed among all recognized pregnancies, a rate within the range reported for Finland between 1973 and 1983 (Lindbohm and Hemminki, 1988). The unadjusted odds ratio for risk of spontaneous abortion in relation to likely paternal exposure to tetrachloroethylene was 0.5 (95% CI: 0.2−1.5) using conditional logistic regression. Likely exposure was assigned to 4 cases and 17 referents. Adjusted odds ratios controlling for potential paternal exposure to the solvent, likely paternal exposure to other organic solvents and dusts, maternal exposure to solvents, maternal heavy lifting, and history of previous spontaneous abortion were presented only for likely exposure to all halogenated hydrocarbons. In addition to exposure to tetrachloroethylene, this group included exposure to trichloroethylene and 1,1,1-trichloroethane. The adjusted odds ratios for low/rare, intermediate, and high/frequent exposure were 1.1 (95% CI: 0.5−2.6), 1.3 (95% CI: 0.5−3.1), and 0.8 (95% CI: 0.3−2.2), respectively. The exposure assessment encompassed a broad range of solvents, and only a small number reported exposure to tetrachloroethylene. In addition, exposure to multiple chemicals was possible for much of the cohort, and this was not controlled for in the chemical-specific models.

A subsequent study of paternal occupational exposure and spontaneous abortions attempted to identify all medically recognized pregnancies (spontaneous abortion, induced abortion, and healthy births) between 1973 and 1982 through the Finnish nationwide Hospital Discharge Register and from outpatient hospital clinics (Lindbohm et al., 1991). Information on occupation was obtained from 1975 and 1980 national census records. Pregnancies during 1973 to 1978 were linked to the 1975 Census, and pregnancies during 1979 to 1982 were linked to the 1980 Census. A job-exposure classification, developed in cooperation with two industrial hygienists, assigned chemical exposures commonly used by job groups within industries. Exposures were assigned to job groupings using a list of 78 exposures, including specific substances, mixtures, and nonspecific exposures, plus industrial hygiene measurements made by the Institute of Occupational Health and the Finnish register of employees occupationally exposed to carcinogens. Exposure assessment focused on mutagens, and three levels were defined: moderate/high, potential/low, and none.

The susceptible exposure period of interest was an 80-day period prior to conception corresponding to spermatogenesis. Because the investigators did not have temporally resolved exposure information, pregnancies that were terminated during a 2-year period close to the census were selected (January 1, 1976−December 31, 1977 for the 1975 Census, and May 1, 1980−April 30, 1982 for the 1980 Census). A total of 99,186 pregnancies to women aged 12−50 years with complete information about occupation, industry, and socioeconomic status occurred during these time periods. There were three spontaneous abortions among the wives of men with moderate or high exposure to tetrachloroethylene (out of 45 pregnancies). The odds ratio was 0.7 (95% CI: 0.2−2.4) in a linear logistic regression model adjusting only for age. This large occupational survey was meant to evaluate reproductive risks associated with paternal exposures to a wide array of substances and mixtures believed to be mutagens. While the focus was on exposure to mutagens as a whole, specific exposures also were analyzed, and a broad 2-year time period was used to identify pregnancies related to occupation listed in the 1975 or 1980 censuses. The nonspecific exposure window and use of a crude exposure assignment method based on occupational title in a census, along with the small number of cases, limit the ability to draw conclusions concerning paternal tetrachloroethylene exposure and risk of spontaneous abortion.

A case-control study in Finland evaluated the association of medically diagnosed spontaneous abortions and maternal occupational exposure to specific solvents (Lindbohm et al., 1990). The sampling frame was a database of women biologically monitored at the Institute of Occupational Health in 1965−1983 for one or more of six solvents (styrene, toluene, xylene, tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane). Pregnancies and their outcomes between 1973 and 1983 among the women were identified using a national register of

pregnancies in Finland and the Finnish Register of Congenital Malformations. Cases were women with a spontaneous abortion recorded in the database. One to three controls per case were selected from among women with a live birth (congenital malformations were not included) matched for age (± 2.5 years). Among the 456 women, overall response to a mailed questionnaire was 85% for both cases and controls. A lower proportion of cases (78%) than controls (99%) confirmed the pregnancy selected from the register. The final data set contained 73 cases and 167 controls with complete information about their occupational history and solvent exposures during the first trimester of pregnancy.

Likelihood and level of exposure to specific solvents was determined by two investigators blind to pregnancy outcome using responses to the questionnaires and biological measurements when available. Women were defined as not exposed if work tasks did not include handling solvents, the worker did not report exposure, and no biological measurements were available. Women were defined as potentially exposed if work tasks might have involved solvent use, but exposures were not reported by the worker, and no biological measurements were available. Women were defined as exposed if biological measurements were taken while at the same job, reported tasks implied solvent exposure, or solvent exposure was reported. The level of exposure was categorized into none, low, or high. High exposure involved handling solvents daily or 1−4 days per week and high-recorded concentrations for biological or available industrial hygiene measurements. Low exposure involved handling solvents 1−4 days per week with low biological concentrations, or solvents were handled <1 day per week. All other exposure scenarios were defined as none. Biological measurements during the first trimester were available for only 5% of the population, and, therefore, exposure assignments were based primarily on reports of work tasks and reported solvent use. Exposure classifications were checked by an experienced industrial hygienist.

Among the exposed women, there were 8 cases and 15 controls with exposure to tetrachloroethylene. An odds ratio of 1.4 (95% CI: 0.5−4.2) was observed using conditional logistic regression with adjustment for previous spontaneous abortions, parity, smoking, use of alcohol, and exposure to other solvents. The adjusted odds ratios for low and high exposure were 0.5 (95% CI: 0.1−2.9) and 2.5 (95% CI: 0.6−10.5), respectively. Among four cases and five controls who reported tetrachloroethylene exposure and whose work tasks involved dry cleaning, the odds ratio for spontaneous abortion, controlling for exposure to other solvents, was 2.7 (95% CI: 0.7−11.2). The odds ratio for women who reported tetrachloroethylene exposure but who conducted other work in dry cleaners (1 case and 6 controls) was 0.6 (95% CI: 0.1−5.5). Blood tetrachloroethylene measurements taken closest to the pregnancy were available for six women who worked in dry cleaning and seven women in other occupations. The mean concentration was higher among dry cleaners (2.11 μmol/L versus 0.43 μmol/L). The authors

reported that the proportion of study subjects who did not report exposure to a specific solvent in contrast to a biological measurement that indicated that they were exposed was 18% among cases and 20% among controls, suggesting that recall was not different by exposure. The study, which is limited by small sample size and a low prevalence of exposure to tetrachloroethylene, suggests that exposure during the first trimester may increase risk of spontaneous abortion. Moreover, odds ratios increased in size when the analysis was restricted to more homogenous exposure groups representing high exposures.

A case-control study in Santa Clara County, California, also focused on occupational exposure to solvents, including tetrachloroethylene (Windham et al., 1991). Selection of cases was hospital based; spontaneous abortions, defined in this study as <20 weeks gestation, among women 18 years of age or older that occurred between June 1986 to February 1987 were identified through records of pathology specimens submitted to the 11 hospital laboratories located in the county. Investigators reviewed medical charts to differentiate spontaneous abortions from induced abortions. Controls, two per case, were randomly selected from women with live births, frequency matched by last menstrual period and hospital. A total of 697 of 772 eligible cases (90.3%) and 1,359 of 1,485 controls (91.5%) participated. The analysis was limited to 1,361 women who were employed during their pregnancy. A higher proportion of cases was over 35 years of age, reported a prior fetal loss, and consumed more alcohol per week. Information on exposure during the first 20 weeks of pregnancy or for the duration of the pregnancy for cases was obtained through a computer-assisted telephone interview. The women provided detailed information about industry and occupation, job tasks and use of 10 solvents, plus reported exposure to any other solvents or degreasers. Among the women who reported that they used tetrachloroethylene during the first weeks of pregnancy, 5 were cases, and 2 were controls (ORc: 4.7, 95% CI: 1.1−21.1, calculated using Haldane's method for small samples). Unexposed participants reported no use of any named solvents and did not work in the microelectronics industry (n = 847). Four of the women exposed to tetrachloroethylene also reported use of trichloroethylene. The unadjusted odds ratio for use of tetrachloroethylene and/or trichloroethylene was 3.4 (95% CI: 1.0−12.0). Odds ratios also were calculated in stratified analyses using Mantel-Haenszel estimation for each of six dichotomous variables individually (age, race, education, prior fetal loss, smoking, and hours worked). This limited evaluation of potential confounding does not appear to have resulted in a large decrease of the summary odds ratios compared to the crude odds ratio, although the adjusted odds ratios were presented only as a range (e.g., 4.2 [95% CI: 0.86−20.2] controlling for hours worked to 6.0 [95% CI: 1.4−25.8] controlling for age). Estimated risk increased with a higher level or intensity of exposure when the analyses were stratified by whether exposed participants reported symptoms, skin contact, or odor versus none (6.3, p-value for Fisher exact test (1-tail) = 0.04

compared to 2.1, *p*-value = 0.54). Despite the small numbers with tetrachloroethylene exposure, the results suggest an elevated risk for spontaneous abortion. However, several of the exposed women also were exposed to other solvents, including trichloroethylene, and a detailed evaluation of potential confounding was precluded by small numbers.

One of the first studies to evaluate adverse reproductive outcomes, including spontaneous abortions, stillbirths, birth defects, and low birth weight, among female dry cleaners evaluated 53 of 66 small establishments (40 dry cleaning and ironing and 13 ironing only) in two neighborhoods in Rome, Italy (Bosco et al., 1987). The study population included all of the 67 women who worked in the participating shops. The women averaged 43 years of age and had been employed an average of 20 years. Information on the work setting and operations and reproductive histories were collected through a standardized interview. Participants reported if they had worked in dry cleaning, as a housewife, or other job prior to and during their pregnancies. In addition, a 24-hour urine sample was collected on a Friday at the end of the workweek from 53 of the women. Trichloroacetic acid concentrations were higher among 40 dry cleaners (5.01 µg/L) compared to 13 ironers (1.35 µg/L) and 5 controls (1.56 µg/L). Of 56 pregnancies reported during employment as a dry cleaner, 5 ended in a spontaneous abortion (8.9%). One spontaneous abortion was reported among the 46 pregnancies that occurred while the women were working at home. The fourfold higher incidence of spontaneous abortion suggests a tetrachloroethylene-related risk among the dry cleaners. However, individual characteristics and behaviors that may pose a risk of spontaneous abortion were not presented by exposure status during pregnancy, and potential confounding was not assessed in this very small study.

A study that used a common protocol to evaluate reproductive outcomes among dry cleaners in Denmark, Finland, Norway, and Sweden employed a more precise definition of tetrachloroethylene exposure (Olsen et al., 1990). All women who had worked at identified laundries and dry-cleaning plants for at least 1 month during 1973−1983 were included, and a nested case-referent study was conducted in each country. Identification numbers were linked to national birth registers and hospital discharge registers to obtain information on births and outcomes, including spontaneous abortions, in the cohort. In Denmark, all women in the cohort and every pregnancy that occurred during the study period were included. In Sweden and Finland, two and three controls per case, matched on maternal age (±2 years), year of pregnancy, and parity (for Denmark and Sweden), were selected from women with a healthy newborn. In Norway, information on spontaneous abortions was not available. Women were identified through company records of active dry-cleaning plants (Sweden and Denmark) and laundries (Sweden). Approximately 62 and 74% of dry-cleaning plants in Sweden and Norway participated, respectively. The final study sample consisted of 31 spontaneous abortions and 53

referents in Sweden (84% response) and 10 spontaneous abortions and 119 referents in Denmark (77.3% response). In Finland, laundry and dry-cleaning workers on the rolls of the Union of Chemical Workers and the Municipal Workers of Finland and or included in payroll data from employers for 1973−1983 were identified and linked with the nationwide hospital discharge register and polyclinic data for information on pregnancies. One pregnancy for each woman was randomly selected for analysis. The final data set included 118 spontaneous abortions and 264 referents (77.2% response). Information on exposure to tetrachloroethylene was obtained from the interviews and questionnaires and was classified by an industrial hygienist blinded to pregnancy status (Sweden and Denmark). The Finnish investigators had more detailed information and used reported work history and exposure frequency to classify exposure status. Exposure was categorized into three groups: unexposed (no dry cleaning), low (worked in dry cleaning but not high exposures), and high (workers who conducted dry cleaning or spot removal for at least 1 hour per day). Risk of spontaneous abortion in relation to exposure during the first trimester was analyzed using conditional logistic regression for matched Swedish and Finnish data, and unconditional logistic regression for the Danish data set. Models were adjusted for parity, smoking, and alcohol consumption (Sweden and Finland only).

Odds ratios greater than 1 were observed for the high exposure group in Denmark (OR: 2.52, 95% CI: 0.26−24.1) and Finland (OR: 4.53, 95% CI: 1.11−18.5). The high exposure group contained small numbers of cases and controls with one case each in Sweden and Denmark, and six cases in Finland. The odds ratios were combined using the inverse variance of the odds ratio. The odds ratios for low and high exposure (95% CI) were 1.17 (0.74−1.85) and 2.88 (0.98−8.44), respectively. The authors stated that similar results were obtained when exposure information provided by the employers (55% of sample) was used instead of responses from the participants.

A separate report of the Finnish study population was published, evaluating 130 cases of spontaneous abortions and 289 controls matched for maternal age (Kyyronen et al., 1989). Slightly different categorizations were used to define exposure. High exposure included women whose tasks included dry cleaning at least 1 hour daily, and who handled tetrachloroethylene at least once a week (n = 15). Low exposure included women whose work tasks involved pressing at a dry cleaners or spot removing, or who handled tetrachloroethylene less than once a week (n = 31). Blood tetrachloroethylene measurements, taken within 10 months of the first trimester of pregnancy, were available for seven of the participants (except for one more distant measurement). These data corresponded well to their reported exposure. Exposure to other solvents, including petroleum, benzene, toluene, acetone, thinner, and spot remover mixtures, was reported by six cases (5.9% of women who worked during their pregnancy) and six controls (2.9%). The odds ratio for high exposure to tetrachloroethylene was 3.4 (95% CI: 1.0−11.2,

$p < 0.05$) in a multivariate model adjusted for frequent use of solvents other than tetrachloroethylene (OR: 1.5, 95% CI: 0.4−5.4), frequent heavy lifting at work (OR: 1.9; 95% CI: 1.0−2.8), and frequent use of alcohol (OR: 2.0, 95% CI: 1.0−4.0). Selection bias did not appear to be a major factor; when exposure information obtained from employers was used to classify eight cases and six controls instead of self-reports, the proportion returning the questionnaire was similar (0.25 and 0.17, respectively).

Ahlborg et al. (1990a) published the Swedish results separately along with a complementary study designed to be more representative of the entire dry-cleaning and laundry sector. Laundry and dry-cleaning establishments, identified from the Swedish Post Address Register in 1984, were mailed a questionnaire to obtain names and contact information for all women employees who had worked for at least 1 month during 1974 and 1983. Cases of spontaneous abortion (defined in this study as fetal death at <28 weeks gestation), perinatal death, congenital malformation, or low birth weight (<1,500 g) were identified among deliveries during 1974−1983 recorded in the Medical Birth Registry, the Swedish Registry of Congenital Malformations, and the Inpatient Registry for Somatic Care (spontaneous abortion treated in a hospital). Dates of delivery or spontaneous abortion were used to identify women who had been working while they were pregnant (at least 1 week of the year before delivery or 6 months before a spontaneous abortion). A total of 67 cases were identified among 955 pregnancies, and two referents per case were selected, matched on mother's age, year of pregnancy, and parity (only for deliveries). Responses were received from 48 cases (75%) and 110 referents (88%). Recruitment for the complementary study involved the identification of women registered as washers/cleaners via an occupational code in the 1975 and 1980 Censuses. A total of 755 additional pregnancies were identified via linkage with the medical registers for the 2-year period after each census. Responses to the mailed questionnaire were received from 68 cases (88%) and 131 referents (87%). Exposure to tetrachloroethylene during the first trimester was classified independently by two investigators who were unaware of the worker's case/control status. High exposure included operating a dry-cleaning machine or conducting spot removing using tetrachloroethylene at least 2 hours per week, or ironing/pressing dry-cleaned cloth for over 20 hours per week, or cleaning and filling the machines at least three times. Low exposure included other work in dry-cleaning businesses where tetrachloroethylene was used. Unexposed workers were employed in companies that did not dry clean using tetrachloroethylene. In the combined data set, 31 and 19 cases (all outcomes) were classified as having low and high exposure, respectively. The numbers of spontaneous abortions by exposure category were not reported. Odds ratios for spontaneous abortion among workers with low and high exposure using conditional logistic regression were 1.0 (95% CI: 0.4−2.2) and 0.9 (95% CI: 0.4−2.1), respectively. The models adjusted for smoking, alcohol consumption, medical complications,

and history of adverse pregnancy outcome. This study did not find an increased risk of spontaneous abortion among workers reporting tetrachloroethylene exposure during the first trimester.

A relatively large study in the United Kingdom evaluated the risk of spontaneous abortions among current and former employees of dry-cleaning and laundry establishments managed by four companies between 1980 and 1995 (Doyle et al., 1997). Information about workplace exposure and reproductive history were obtained in 1995−1996 via mailed questionnaires sent to 7,301 women, aged 16−45 years, who were identified by the employers. Of the 5,712 questionnaires successfully delivered, 54.5% were completed (n = 3,110). The responses by current dry-cleaners and laundry workers were 78 and 65%, respectively, but were lower among former workers (46.1 and 39.7%, respectively). The authors reported that the age distribution of responders was comparable to that of nonresponders. The final data set included 3,092 respondents with complete information about 3,517 total pregnancies. Pregnancies were included in the analysis if the women reported that it had been confirmed by a doctor, hospital treatment was required, or it ended in a live birth. The rate of spontaneous abortions was evaluated in relation to the woman's employment during her pregnancy or the 3 months prior. Work at a dry cleaner and as a dry-cleaning machine operator was used as an exposure surrogate for tetrachloroethylene. This was compared to work at a laundry or no employment at a dry cleaners or laundry during the pregnancy or 3 months prior.

Spontaneous abortions were compared to total pregnancies (spontaneous abortions, stillbirths, and live births) excluding ectopic and molar pregnancies and induced abortions. For the 325 reported spontaneous abortions between 1980−1995, the odds ratio for dry cleaning compared to laundry work was 0.97 (0.55−1.69). However, among 93 spontaneous abortions to women employed in dry cleaning, machine operators had a 63% higher risk of spontaneous abortion compared to nonoperators (OR: 1.63, 95% CI: 1.01−2.66). The unconditional logistic regression models controlled for maternal age, pregnancy order, and year of birth. A similar pattern of risk was observed when the analyses were restricted to the women's first or last pregnancies. These latter analyses were meant as a check to address the lack of independence of multiple pregnancies reported by the same woman. For example, among dry-cleaning machine operators, when the last exposed pregnancy was compared to pregnancies that occurred later during periods with no exposure to tetrachloroethylene, risk of spontaneous abortion was 82% higher (OR: 1.82, 95% CI: 1.09−3.05). An elevated risk also was observed when pregnancies during work as a dry-cleaning machine operator were compared to unexposed pregnancies before the first exposed pregnancy. Laundry workers also experienced more spontaneous abortions when employed in laundries compared to periods when they had other employment or were not employed; however, the CIs included one. The investigators were not able to compare risks

between dry cleaning generally and laundry work because the number of spontaneous abortions reported for pregnancies while working in a laundry was low (n = 19). Doyle et al. (1997) found an elevated risk of spontaneous abortion for work as a dry-cleaning machine operator during or 3 months before a pregnancy compared to work in other dry-cleaning jobs or work in other industries or in the home during this sensitive period.

The rate of self-reported spontaneous abortions was comparable among the wives of dry cleaners (n = 14) and laundry workers (n = 26) in a cohort of primarily unionized men in northern and southern California who participated in a study of semen quality (Eskenazi et al., 1991a). Rates of spontaneous abortion during the time periods when their husbands worked in the industry were 11.1 and 15.2% among the wives of dry-cleaners and laundry workers, respectively (X^2 = 0.32, p = 0.57). Although the authors presented the rates as spontaneous abortion rates, it does not appear that the fetal deaths reported were limited to <28 weeks of gestation. The rate was calculated as the total number of miscarriages during the husband's employment in the industry divided by the total number of pregnancies during the same time period, multiplied by 100. It was not stated how many years the women, whose average age was mid-thirties, had to recall previous miscarriages.

A population-based study in Woburn, Massachusetts, evaluated outcomes during pregnancy and effects in children among residents whose drinking water source was two wells contaminated with chlorinated organic substances from 1960 to 1982 (Lagakos et al., 1986). The two wells were operated as a single water source. The contamination of the two wells, located in eastern Woburn, was discovered in May, 1979. Levels of trichloroethylene (267 ppb), tetrachloroethylene (21 ppb), and chloroform (12 ppb) were detected, and the wells were shut down. The other six wells that supplied Woburn were located in the southwest part of town, and testing did not find levels above state and federal standards. Information was collected through a telephone survey of former and current family members residing in Woburn from 1960−1982 and listed in the 1982 town directory. The survey was conducted by 235 volunteers trained in interview techniques who successfully contacted 6,219 residences. In the end, 5,010 completed interviews were obtained, approximately 57% of the town's residences with listed telephone numbers. All pregnancies ending between 1960 and 1982 to women born since 1920 were ascertained, and information was collected on pregnancy outcomes and the health of offspring, maternal characteristics, and residence history. Regional and temporal distribution of the water from the two contaminated wells was determined by the Massachusetts Department of Environmental Quality and Engineering during October 1964 to May 1979. The town was partitioned into five zones of graduated exposure to water from the wells. The study investigators estimated the proportion of each household's annual water supply that came from the two wells. Each pregnancy was assigned an annual exposure score using the mother's

residence during the year the pregnancy ended. An exposure history was constructed for each child consisting of the sum of annual scores accumulated during their residence in Woburn.

Of the 4,396 pregnancies that occurred during 1960 to 1982, 16% were exposed during the year the pregnancy ended. There were 520 spontaneous abortions (12%), defined in this study as a fetal loss in the first 6 months, and 67 perinatal deaths (1.5%), defined as a stillbirth or a live birth that survived fewer than 7 days. Logistic regression analyses, controlling for other risk factors, found no statistically significant associations between the annual exposure score for the year a pregnancy ended and spontaneous abortion, or perinatal deaths before 1970. An odds ratio of 10 ($p = 0.003$) was observed for perinatal deaths after 1970, when changes in industrial water demand occurred, and a different set of five zones representing exposure to water from the contaminated wells was constructed. This was due to 3 perinatal deaths that occurred in households with the highest exposure score category of 0.51−1.0.

A population-based retrospective study of tetrachloroethylene in drinking water evaluated effects on pregnancy and development from exposure resulting from leaching of tetrachloroethylene from vinyl linings in water distribution pipes installed between 1968 and 1980 in the Cape Cod region in Massachusetts (Aschengrau et al., 2009a; Aschengrau et al., 2009b; Aschengrau et al., 2008). Because the pipes were used to replace existing pipes or to extend the distribution system to serve a growing population, population exposure was irregularly distributed, and a wide range of tetrachloroethylene concentrations were detected in samples collected in 1980. In addition, only one town used a chlorinated surface water supply, resulting in a low probability that drinking water was contaminated with chlorinated byproducts. Water concentrations ranged from 1.5 to 80 µg/L along main streets, and from 1,600 to 7,750 µg/L along dead end streets where water flow was low. All births between 1969 and 1983 were identified from birth certificates, and women residing in one of eight Cape Cod towns with vinyl-lined water distribution pipes at the time of the index birth were eligible for the study. A total of 1,492 women with addresses along streets where the pipes had been installed or with connections to such pipes were initially defined as exposed. A comparison group of 1,704 births, frequency matched to the exposed group by month and year of birth, was selected. Follow-up of the selected individuals occurred during 2002−2003. The final data set contained 959 women with potential exposure and 1,087 potentially unexposed women who returned a self-administered questionnaire, comprising 64% of the selected sample and 69% of those who were located. Response did not vary by potential exposure status. The study population was primarily Caucasian, with an average age of 27 years, and most had adequate prenatal care (72−73%). The annual mass of tetrachloroethylene delivered to each address before and during pregnancy was estimated using self-reported residential histories mapped using GIS (94% of reported pregnancies), a leaching and transport model developed for the study, and EPA's EPANET

modeling software estimating water flow and direction. Estimated water concentrations of tetrachloroethylene ranged between 1 and 5,197 µg/L.

Self-reported clinically recognized pregnancy loss (659 spontaneous abortions and stillbirths) and 4,908 live births up to December 1990 were eligible for analysis. Pregnancy outcomes were analyzed in relation to three measures of exposure: cumulative exposure up to the month and year of the last menstrual period (prepregnancy window), peak exposure up to the last menstrual period year of the pregnancy (prepregnancy window), and average monthly exposure during the year containing the last menstrual period (time of conception). Risk of pregnancy loss associated with exposure measures, divided into quartiles, was evaluated using generalized estimating equations to account for lack of independence of multiple pregnancies by the same woman. Risk estimates for pregnancy loss by increasing quartiles of exposure were similar across the three exposure measures. For example, the multivariate GEE odds ratios for average monthly exposure in increasing quartiles during the year of the last menstrual period were 1.1 (95% CI: 0.8−1.6), 0.7 (95% CI: 0.5−1.1), 0.8 (95% CI: 0.6−1.2), and 0.7 (95% CI: 0.5−1.0), respectively. Several covariates were evaluated for potential confounding, including risk factors for pregnancy loss, those associated with tetrachloroethylene exposure, and nondrinking water sources of solvent exposure. Maternal age, year of pregnancy, paternal age, maternal history of gynecologic infections, and the number of prior live births were included in the final models. The authors checked the validity of self-reported birth outcomes by comparing the reproductive histories reported by the women for all of the index pregnancies with information from birth certificates. Further, information from medical records about pregnancies reported by 60 women also was compared to self-reported histories. The authors reported good-to-excellent agreement including for gestational duration and birth weight, prenatal cigarette smoking, number of prior live births, and spontaneous and induced abortions. The study evaluated a large number of pregnancy losses using a detailed exposure model and carefully assessed potential confounding. It is important to note, however, that exposure estimates were not based on household measurements, and individual consumption was not known. Therefore, exposure misclassification may not have allowed detection of a small increase in risk. Finally, use of exposure prior to the last menstrual period or during that year may not have had the required precision to identify a risk associated with a particular susceptible window for pregnancy loss (e.g., the first trimester).

In summary, the literature contains few studies of effects on spermatogenesis or menstruation among subjects with exposure to tetrachloroethylene. One study of primarily unionized workers in the dry-cleaning and laundry industries in California observed subtle deficits in sperm quality in relation to tetrachloroethylene in exhaled breath, an exposure index, and occupational group (dry-cleaning or laundry worker) (Eskenazi et al., 1991a). However,

three clinically recognized measures of sperm quality were not associated with exposure in the study population. The results of Eskenazi et al. (1991a) are compelling, but more studies are needed to understand the spectrum of effects on sperm and their impact on fecundity. Two other studies that evaluated effects on sperm, hormonal disturbances, or menstruation among men and women with occupational exposure were not adequate to draw conclusions concerning the association (Zielhuis et al., 1989; Rachootin and Olsen, 1983). Some studies that relied on detailed work histories and monitoring data to classify exposure suggested that maternal or paternal exposure to tetrachloroethylene or work in dry cleaning reduces fertility or delays conception (Sallmen et al., 1998; Sallmen et al., 1995; Eskenazi et al., 1991b). However, the risk estimates were imprecise because the number of participants reporting exposure to tetrachloroethylene was small. As a consequence, the existing literature is inconclusive concerning effects of tetrachloroethylene on reproduction and fertility.

A number of studies have evaluated the risk of spontaneous abortions in relation to maternal and paternal occupational exposure to tetrachloroethylene. Results of several studies of maternal occupational exposure to tetrachloroethylene suggest an increased risk of spontaneous abortion, particularly at higher levels (Doyle et al., 1997; Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989). Most of the studies evaluated exposure during the first trimester of pregnancy. Some of the studies observed an increased odds ratio ranging between 1.4 to 4.7, but had low statistical power because the cohort contained small numbers of exposed cases and controls, and were limited in their ability to evaluate potential confounding (Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Bosco et al., 1987). In general, the studies that used a more precise definition of exposure, or categorized exposure into levels of increasing dose or intensity, observed higher risk estimates (Doyle et al., 1997; Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989). Increased risks were not found among dry cleaners in Sweden (Ahlborg, 1990a; Olsen et al., 1990). Three studies of paternal occupational exposure prior to the beginning of the pregnancy did not observe an association (Eskenazi et al., 1991a; Lindbohm et al., 1991; Taskinen et al., 1989). Two of these surveyed occupational exposure to a broad array of substances and, consequently, had low statistical power for chemical-specific analyses (Lindbohm et al., 1991; Taskinen et al., 1989). Although there is no evidence of an increased risk associated with paternal exposure, the studies were not of sufficient size or detail in exposure estimates to draw conclusions. No associations with incidence of spontaneous abortion were observed among two populations exposed to tetrachlorethylene in drinking water (Aschengrau et al., 2009a; Aschengrau et al., 2008; Lagakos et al., 1986). The populations were likely exposed to lower levels compared to the occupational populations. In addition, the window of exposure used to assess risk in both studies may not have had been precise enough to detect a small elevation in risk for spontaneous abortion.

4.7.2.2. Animal Reproductive Toxicity Studies

Evaluation of the reproductive effects of tetrachloroethylene exposure in mammalian animal models is based on a two-generation reproduction studies in rats, an in vivo sperm assay, and an in vitro oocyte fertilization assay following in vivo exposure of adult female rats. These studies are described below.

4.7.2.2.1. In vitro fertilization assay

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 hours/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.

4.7.2.2.2. In vivo reproductive toxicity studies

Beliles et al. (1980) described an experiment in which male rats and mice (12/group) were exposed via inhalation to tetrachloroethylene concentrations of 100 and 500 ppm, for 7 hours/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. In mice, at 4 weeks, but not at 1 or 10 weeks after exposure, there was a significant increase ($p < 0.05$) in the percentage of males with abnormal sperm heads (19.7%) in the 500 ppm exposure group. 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.

A multigeneration study of the effects on rats of exposure to airborne concentrations of tetrachloroethylene was performed by Tinston (1994). It was conducted under GLP standards and received frequent quality assurance audits. In this study, weanling male and female (Alpk:APfSD) rats (F0) (24/sex/group) were exposed to airborne tetrachloroethylene concentrations of 0, 100, 300, or 1,000 ppm, 6 hours/day, 5 days/week, for 11 weeks prior to mating and then for 6 hours/day during mating and through GD 20. There were no exposures from GD 21 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 PND 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 premating 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 PND 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 4-35 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, transient statistically significant reductions of mean body weights (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 PND 29. These PND 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 premating period, mean body weights were similar to those of controls for both 100 and 300 ppm-animals.

Table 4-35. 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 (percentage)		1,000[b]		1,000[c]	1,000[c]	
Decreased mean percentage pup survival Days 1−5		1,000		1,000[c]		1,000[c]
Decreased mean percentage 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).

Mean litter size was decreased at 1,000 ppm for F2A and F2B litters. Statistically significant decreases in the number of live pups on PND 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 PND 1 and between PNDs 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. It is additionally noted that this study was conducted according to the pre-1998 EPA harmonized two-generation reproduction study guideline and, thus, did not assess a number of sensitive endpoints such as estrous cyclicity, sperm measures, age of sexual maturation, and enhanced reproductive organ pathology.

A summary of the doses at which treatment-related effects were observed in the Tinston (1994) study is presented in Table 4-35. Overall, the parental systemic toxicity was observed at 300 and 1,000 ppm, with a NOAEL of 100 ppm. For offspring, the LOAEL of 100 ppm was based upon decreased body weight in F1A pups at PND 21; no NOAEL was established. There was no evidence of treatment-related effects on reproductive function at any exposure level tested.

4.7.2.3. Reproductive Cancers in Humans

Thirteen epidemiologic studies reporting data on breast cancer and tetrachloroethylene exposure and 12 epidemiologic studies reporting data on cervical cancer and tetrachloroethylene exposure were identified. This set of studies includes 10 cohort studies on breast and cervical cancers (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Sung et al., 2007; Chang et al., 2005; Blair et al., 2003; Ruder et al., 2001; Andersen et al., 1999; Boice et al., 1999; Lynge and Thygesen, 1990), one study reporting on breast cancer but not cervical cancer (Radican et al., 2008), two studies reporting on cervical cancer but not breast cancer (Travier et

al., 2002; Anttila et al., 1995), two breast cancer case-control studies of occupational exposures (Peplonska et al., 2007; Band et al., 2000), one cervical cancer nested case-control study of occupational exposure (Lynge et al., 2006), and one breast cancer case-control study of residential exposure through contaminated drinking water (Aschengrau et al., 2003). Aschengrau et al. (2003) extended Aschengrau et al. (1998), adding additional breast cancer cases from 1987−1993, and presenting odds ratios for the combined 10-year study period, 1983−1993. Most breast cancer studies examined females (Radican et al., 2008; Peplonska et al., 2007; Sung et al., 2007; Aschengrau et al., 2003; Blair et al., 2003; Band et al., 2000) or males and females combined (Calvert et al., 2011; Boice et al., 1999). Five studies, mostly of Nordic subjects, presented risk estimates for male subjects separately (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Chang et al., 2005; Andersen et al., 1999; Lynge and Thygesen, 1990). These studies represent the core studies evaluated by EPA, as described in more detail below. Appendix B reviews the design, exposure-assessment approach, and statistical methodology for each study. Most studies were of the inhalation route of exposure, of occupational exposure, and lacked quantitative exposure information. Nine studies reporting risk estimates for breast or cervical cancer examine occupational titles such as dry cleaner, launderer, and presser as surrogates for tetrachloroethylene, given its widespread use from 1960 onward in the United States and Europe (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Peplonska et al., 2007; Lynge et al., 2006; Blair et al., 2003; Ruder et al., 2001; Band et al., 2000; Andersen et al., 1999; Lynge and Thygesen, 1990). Five studies conducted in Nordic countries are either based on either the entire Swedish population or on combined populations of several Nordic countries; strengths of these studies are their use of job title as recorded in census databases and ascertainment of cancer incidence using national cancer registries (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Lynge et al., 2006; Andersen et al., 1999; Lynge and Thygesen, 1990). Subjects in the multi-Nordic country study of Pukkala et al. (2009) overlapped those of Lynge and Thygesen (1990), Andersen et al. (1999), Lynge et al. (2006), and Sélden and Ahlborg (2011). Studies examining mortality among U.S. dry-cleaner and laundry workers (Blair et al., 2003; Ruder et al., 2001) are of smaller cohorts than the Nordic studies, with fewer observed lung cancer events.

The exposure surrogate in studies of dry-cleaners and laundry workers is a broad category containing jobs of differing potential for tetrachloroethylene exposure. Thus, these studies have a greater potential for exposure misclassification bias compared to studies with exposure potential to tetrachloroethylene assigned by exposure matrix approaches applied to individual subjects. Calvert et al. (2011) studied unionized dry cleaners in the United States in California, Illinois, Michigan, and New York who worked for one or more years before 1960 in one or more shops known to use tetrachloroethylene as the primary solvent (Calvert et al., 2011;

Ruder et al., 2001, 1994). The cohort was stratified into two groups based on the level of certainty that the worker was employed only in facilities using tetrachloroethylene as the primary solvent; tetrachloroethylene-only and tetrachloroethylene plus. Lynge et al. (2006), using job titles reported in the 1970 Census, identified subjects as dry cleaners (defined as dry cleaners and supporting staff if employed in a business of <10 workers), other job titles in dry cleaning (launderers and pressers), unexposed (job title reported on 1970 Census was other than in dry cleaning), or unclassifiable (information was lacking to identify job title of subject). Sélden and Ahlborg (2011) identified subjects as either dry cleaners or laundry workers and presented risk estimates separately by job title.

Four other cohorts with potential tetrachloroethylene exposure in industrial settings have been examined. These studies include aerospace or aircraft maintenance workers in the United States (Radican et al., 2008; Boice et al., 1999), workers, in Finland, primarily in the metal industry (Anttila et al., 1995) and electronic factory workers in Taiwan (Sung et al., 2007; Chang et al., 2005). Boice et al. (1999) and Radican et al. (2008) used an exposure assessment based on a job-exposure matrix, and Anttila et al. (1995) used biological monitoring in blood to assign potential tetrachloroethylene exposure to individual subjects. In contrast and less sensitive, the exposures in the Taiwan studies included multiple solvents and tetrachloroethylene exposure was not linked to individual workers. Additionally, cohorts included white-collar workers, who had an expected lower potential for exposure (Sung et al., 2007; Chang et al., 2005).

Aschengrau et al. (2003) is a case-control study that examined residential proximity to drinking water sources contaminated with tetrachloroethylene in Cape Cod, MA, and used an exposure model incorporating leaching and characteristics of the community water distribution system to assign quantitative estimates of a household relative dose of tetrachloroethylene.

In summary, with respect to exposure-assessment methodologies, four studies with breast or cervical cancer data assigned tetrachloroethylene exposure to individuals within the study using a job exposure matrix (Boice et al., 1999; Anttila et al., 1995), an exposure model (Aschengrau et al., 2003), a classification of the cohort by certainty of tetrachloroethylene exposure (Calvert et al., 2011), or restricting analyses to subjects identified as dry cleaners (Seldén and Ahlborg, 2011; Lynge et al., 2006). The relative specificity of these exposure-assessment approaches strengthens their ability to identify cancer hazards compared to studies with broader and less sensitive exposure-assessment approaches. The least sensitive exposure assessments are those using very broad definitions such as working in a plant or a factory (Sung et al., 2007; Chang et al., 2003).

Five[26] of the nine breast cancer studies evaluated by EPA with exposure assessment to tetrachloroethylene or employment as dry-cleaner or laundry worker reported estimated relative risks based on 50 or more observed events (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Aschengrau et al., 2003; Blair et al., 2003; Lynge and Thygesen, 1990); the observed number of breast cancer cases or deaths ranged from 56 (Blair et al., 2003) to 1,757 (Pukkala et al., 2009). The largest cohort of breast cancer cases in female dry-cleaners and laundry workers (n = 1,757) observed a standardized incidence ratio of 0.89 (95% CI: 0.85, 0.94) (Pukkala et al., 2010). Three other studies of dry-cleaners and laundry workers with findings based on between 68 and 219 cases or deaths observed a standardized incidence ratio or SMR estimate of 0.88 (95% CI: 0.77, 1.01) (Seldén and Ahlborg, 2011), 1.0 (95% CI: 0.8, 1.3) (Blair et al., 2003), and 1.11 (95% CI: 0.90, 1.34) (Lynge and Thygesen, 1990) for the association between breast cancer risk and ever having a job title of dry-cleaner or laundry worker (refer to Table 4-36). A case-control study with findings based on 50 or more exposed cases observed an odds ratio of 1.2 (95% CI: 0.9, 1.7) for living in a residence receiving contaminated water with a relative delivered dose of tetrachloroethylene above the median value (median: 2.1, range: 0.001−243.8) compared to controls (Aschengrau et al., 2003). SMRs or standardized incidence ratios for breast cancer were similar for subjects identified as dry cleaners compared to laundry workers or for the subcohort of females whose starting date of employment was after 1960 compared to the larger cohort (Seldén and Ahlborg, 2011).

In addition to the evidence from the large cohort and case-control studies, evidence is found in five other studies whose effect estimates for breast cancer are based on fewer observed events and that carry lesser weight in the analysis. As expected, the magnitude of the point estimate of the association reported in these studies is more variable than in the larger studies: 0.48 (Radican et al., 2008), 1.1 to 1.5 (Calvert et al., 2011; Peplonska et al., 2007; Boice et al., 1999), and >2.0 (Band et al., 2000). Of these five studies, only risk estimates of Band et al. (2000) excluded 1.0. Chang et al. (2005) and Sung et al. (2008), a follow-up study of the same population, reported standardized incidence ratios of 1.19 (95% CI: 1.03, 1.36) and 1.09 (95% CI: 0.96, 1.22). Both studies observed over 200 breast cancer incident cases; however, these studies carry lesser weight in the analysis, given their low level of detail of the exposure assessment.

[26] Andersen et al. (1999) is not included in this summary of the data from the individual studies because it was updated and expanded in the analysis by Pukkala et al. (2009).

Table 4-36. Summary of human studies on tetrachloroethylene exposure and breast cancer

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort Studies				
Biologically monitored workers				Anttila et al. (1995)
	All subjects	Not reported		849 Finnish men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, external referents (SIR)
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure to PCE	1.16 (0.32, 2.97)	4	77,965 (*n* = 2,631 with routine PCE exposure and *n* = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, job exposure matrix without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm [mean] and 9.5 ppm [median], external reference for routine exposure (SMR) and internal references (workers with no chemical exposures) for routine-intermittent PCE exposure (RR), male (ICD-9, 175) and female breast cancer (ICD-9, 174)
	Routine-Intermittent exposure duration to PCE	Not reported		
Electronic factory workers (Taiwan)				Chang et al. (2005); Sung et al. (2007)
	All Subjects			86,868 (*n* = 70,735 female), follow-up 1979−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, cancer mortality, external referents (SIR) (Chang et al., 2005); 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, multiple solvents exposures and PCE not identified to individual subjects, cancer incidence, external referents, analyses lagged 15 yr (SIR) (Sung et al., 2007)
	Males	0.90 (0.48, 1.53)	0 0.11 exp	
	Females	1. 19 (1.03, 1.36)	215	
	Females	1.09 (0.96, 1.22)	286	

Table 4-36. Summary of human studies on tetrachloroethylene exposure and breast cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aircraft maintenance workers from Hill Air Force Base				Radican et al. (2008)
	Any PCE exposure	0.48 (0.07, 3.50)	1	10,461 men and 3,605 women (total *n* = 14,066, *n* = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952 to 1956, follow-up 1973−2000, job exposure matrix (intensity), internal referent (workers with no chemical exposures [RR]), female breast cancer (ICD-A8, -9, 174; ICD-10, C50)
Dry-cleaner and laundry workers				Andersen et al. (1999)
	All laundry worker and dry cleaners			29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway), follow-up 1971−1987 or 1991, PCE not identified to individual subjects, external referents (SIR), ICD-7, 170
	Males	(0, 3.41)	0	
	Females	0.89 (0.83, 0.97)	634	
				Blair et al. (2003)
	All subjects	1.0 (0.8, 1.3)	68	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR), female breast (ICDA-8, 174).
	Semiquantitative exposure score			
	Little to no exposure	0.8 (0.6, 1.2)	30	
	Medium to high exposure	1.2 (0.8, 1.7)	29	
				Ji et al. (2005b)
	Laundry workers and dry cleaners in 1960 Census			9,255 Swedish men and 14,974 Swedish women employed in 1960 (men) or 1970 (women) as laundry worker or dry cleaner, follow-up 1961/1970−2000, PCE not identified to individual subjects, external referent (SIR) and adjusted for age, period and socioeconomic status.
	Males	Not reported		
	Females	Not reported		
				Lynge and Thygsen (1990)
	All laundry worker and dry cleaners			10,600 Danish men and women, 20−64 yr old, employed in 1970 as laundry worker, dry cleaners and textile dye workers, follow-up 1970−1980, external referents (SIR), ICD-7, 170.
	Males		0 0.2 exp	
	Females	1.11(0.90, 1.34)	94	

Table 4-36. Summary of human studies on tetrachloroethylene exposure and breast cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Pukkala et al. (2009)
Launderer and dry cleaner			Men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30−64 yr, follow-up 2005, occupational title of launderer and dry cleaner in any census, external referents (SIR), ICD-7, 170.
Male	0.86 (0.18, 2.50)	3	
Female	0.89 (0.85, 0.94)	1,757	
			Calvert et al. (2011)
All subjects	1.05 (0.70, 1.52)	28	1,704 U.S. men and women dry-cleaning union member in CA, IL, MI, NY follow-up 1940−2004 (618 subjects worked for one or more years prior to 1960 only at shops where PCE was the primary cleaning solvent, identified as PCE-only exposure), cancer mortality (SMR), female and male breast cancer (ICD-9, 174, 175)
Exposure duration/time since 1st employment	Not reported		
PCE-only subjects	1.06 (0.51, 1.94)	10	
			Seldén and Ahlborg (2011)
Dry-cleaners and laundry workers			9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−2000, exposure assigned using company self-reported information on PCE usage—PCE (dry cleaners and laundries with a proportion of PCE dry cleaning), laundry (no PCE use), and other (mixed exposures to PCE, CFCs, TCE, etc.), external referents (SIR), ICD-7, 170
Males	(0.00, 7.68)	0	
Females	0.88 (0.77, 1.01)	219	
PCE			
Males		0	
Females	0.85 (0.72, 1.00)	140	
Laundry			
Males		0	
Females	0.96 (0.76, 1.21)	76	

Table 4-36. Summary of human studies on tetrachloroethylene exposure and breast cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Travier et al. (2002)
All subjects, 1960 or 1970 Census in laundry and dry cleaner or related occupation and industry	Not reported		Swedish men and women identified as laundry worker, dry cleaner, or presser (occupational title), in the laundry, ironing, or dyeing industry or related industry in 1960 or 1970 (543,036 person-years); or, as laundry worker, dry cleaner, or presser (occupational and job title) (46,933 person-years) in both censuses, follow-up 1971−1989, external referents (SIR)
All subjects in 1960 and 1970 in laundry and dry cleaner occupation and industry	Not reported		
Case-control studies			
British Columbia, Canada			Band et al. (2000)
Laundry and dry cleaning occupation			995 breast cancer cases, females,75 yr, 1988−1989, identified from British Columbia Cancer Registry, Canadian citizens and British Columbia residents, English speaking, 1,020 population controls matched on age and sex, self-administered questionnaire, job title and industry coded to Canadian SOC and Canadian SIC as exposure surrogate, OR for postmenopausal subjects, adjusted for body weight in 1986, family history of breast cancer, history of benign breast disease, cumulative alcohol score. OR for pre- and postmenopausal subjects also adjusted for smoking pack-years
Pre- and postmenopausal			
Usual occupation	5.24 (1.41, 19.5)	9	
Postmenopausal			
Usual occupation	4.85 (1.26, 18.7)	8	
Power laundries and dry cleaners industry			
Pre- and postmenopausal			
Usual occupation	2.00 (0.78, 5.13)	9	
Postmenopausal			
Usual occupation	1.57 (0.68, 3.61)	10	

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Table 4-36. Summary of human studies on tetrachloroethylene exposure and breast cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Poland, 2 regions (Warsaw and Lódź)				Peplonska et al. (2007)
	Laundry, cleaning and garment services industry	1.2 (0.7, 1.9)	28	2,275 histologically confirmed in situ or invasive breast cancers in female residents of Warsaw and Lódź, 20−74 yr, 2000−2003, population controls, identified from the Polish Electronic System of Population Evidence and matched to cases by city of residence and age within 5-yr age groups, in-person interview, structured questionnaire, lifetime occupational history, employed ≥6 mo in relevant industry exposure surrogate, OR adjusted for age, education, age of menarche, menopausal status, age at menopause, number of full-time births, MBI, family breast cancer history, and previous screening mammography
	Exposure duration			
	≤10 yr	1.5 (0.8, 2.8)	23	
	>10 yr	0.5 (0.2, 16)	5	
Geographic-based studies				
Cape Cod, MA				Aschengrau et al. (2003; 1998)
	PCC RDD ≤ median	1.0 (0.7, 1.3)[a] 0.9 (0.6, 1.3)[b]	91 59	334 histologically confirmed breast cancer cases in males and females, 1983−1986, 2,236 population controls identified by random digit dialing, vital records for deceased controls, and HCFA records if >65 yr (Aschengrau et al., 1998); 672 histologically confirmed primary or recurrent breast cancer cases in females, 1987−1993, 616 population controls identified by random digit dialing, vital records for deceased controls, and HCFA records if >65 yr (Aschengrau et al., 2003); MA Cancer Registry, telephone interview, algorithm of (1993) to estimate mass of PCE in drinking water entering residence was surrogate exposure metric [90th percentile, 53.4], OR adjusted for age of diagnosis or index year, vital status at interview, family history of breast cancer, age at first live birth, personal history of prior breast cancer and benign breast disease, and occupational exposure to solvents (PCE, benzene, other solvents), statistically analyses also explored effect of different latent periods (0,5,7,9,11,13, and 15 yr)
	PCE RDD > median	1.2 (0.9, 1.7)[a] 1.3 (0.9, 1.9)[b]	100 69	
	PCE RDD >90th percentile	1.3 (0.7, 2.6)[a] 1.7 (0.8, 4.4)[b]	4 16	

[a] In Aschengrau et al. (2003), odds ratios for breast cancer are presented for combined data from Aschengrau et al. (1998).
[b]Odds ratios considering a 7-yr latent period.

HCFA = Health Care Financing Administration, ISCO = International Standard Classification of Occupation, ISIC = International Standard Industry Classification, JEM = job-exposure-matrix, RDD = relative delivered dose, TWA = time-weighted-average.

No male breast cancer cases were observed in four of the five studies reporting risk estimates for males separately from that of females (Seldén and Ahlborg, 2011; Chang et al., 2005; Anderson et al., 1990; Lynge and Thygesen, 1990). Not surprising given the low background rate of male breast cancer, less than one case was expected in each study. Pukkala et al. (2010) reported three observed cases among a cohort of 8,744 male dry-cleaners and laundry workers.

Two[27] of the eight cervical cancer studies evaluated by EPA with exposure assessment to tetrachloroethylene or employment as dry-cleaner or laundry worker reported estimated relative risks based on 50 or more observed events. Estimates of the standardized incidence ratio or SMR in these studies were 1.34 (95% CI: 1.12, 1.60) and 1.20 (95% CI: 1.08, 1.34) in Travier et al. (2002) and Pukkala et al. (2009), respectively. In addition to the evidence from the two large cohort studies, additional evidence is found in six other studies whose effect estimates are based on fewer observed events and that carry lesser weight in the analysis. As expected, the magnitude of the point estimate of the association reported in these studies is more variable than in the larger studies: 0.40 to 0.98 (Lynge et al., 2006; Lynge and Thygesen, 1990), 1.1 to 1.5 (Seldén and Ahlborg, 2011), 1.6 to 2.0 (Calvert et al., 2011; Blair et al., 2003; Ruder et al., 2001), and >3.0 (Anttila et al., 1995). Chang et al. (2005) and Sung et al. (2008), a follow-up study of the same population, observed over 200 cervical cancer incident cases and reported standardized incidence ratios of 1.06 (95% CI: 0.95, 1.18) and 0.69 (95% CI: 0.87, 1.06). Although based on a large number of observed events, these studies carry lesser weight in the analysis given their lower level exposure-assessment approach. SMRs or standardized incidence ratios for cervical cancer were lower for subjects identified as dry cleaners compared to laundry workers or for the subcohort of females whose starting date of employment was after 1960 compared to the larger cohort (Seldén and Ahlborg, 2011; Lynge et al., 2006) (refer to Table 4-37).

[27] In addition to Andersen et al. (1999), Boice et al. (1999) is not counted because no cervical deaths are observed among tetrachloroethylene-exposed female subjects.

Table 4-37. Summary of human studies on tetrachloroethylene exposure and cervical cancer

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort studies				
Biologically monitored workers				Anttila et al. (1995)
	All subjects	3.20 (0.39, 11.6)	2	849 Finnish men and women, blood PCE [0.4 µmol/L in females and 0.7 µmol/L in males (median)], follow-up 1974−1992, external referents (SIR)
Aerospace workers (Lockheed)				Boice et al. (1999)
	Routine exposure to PCE	(0.00, 7.77)	0 0.47 exp	77,965 (n = 2,631 with routine PCE exposure and n = 3,199 with intermittent-routine PCE exposure), began work during or after 1960, worked at least 1 yr, follow-up 1960−1996, job exposure matrix without quantitative estimate of PCE intensity, 1987−1988 8-h TWA PCE concentration (atmospheric monitoring) 3 ppm [mean] and 9.5 ppm [median], external reference for routine exposure (SMR) and internal references (workers with no chemical exposures) for routine-intermittent PCE exposure (RR)
	Routine-Intermittent exposure duration to PCE	Not reported		
Electronic factory workers (Taiwan)				Chang et al. (2005); Sung et al. (2007)
	All Subjects			86,868 (n = 70,735 female), follow-up 1979−1997, multiple solvents exposure, does not identify PCE exposure to individual subjects, cancer mortality, external referents (SIR); female genital organs (Chang et al., 2005); 63,982 females, follow-up 1979−2001, factory employment proxy for exposure, multiple solvents exposures and PCE not identified to individual subjects, cancer incidence, external referents, analyses lagged 15 yr (SIR) (Sung et al., 2007)
	Females	1.06 (0.95, 1.18)	337	
	Females	0.96 (0.87, 1.06)	337	
Aircraft maintenance workers from Hill Air Force Base				Radican et al. (2008)
	Any PCE exposure	Not reported		10,461 men and 3,605 women (total n = 14,066, n = 10,256 ever exposed to mixed solvents, 851 ever-exposed to PCE), employed at least 1 yr from 1952 to 1956, follow-up 1973−2000, job exposure matrix (intensity), internal referent (workers with no chemical exposures) (RR)

Table 4-37. Summary of human studies on tetrachloroethylene exposure and cervical cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Dry-cleaner and laundry workers				Andersen et al. (1999)
	All laundry worker and dry cleaners	1.18 (1.01, 1.38)	155	29,333 men and women identified in 1960 Census (Sweden) or 1970 Census (Denmark, Finland, Norway), follow-up 1971−1987 or 1991, PCE not identified to individual subjects, external referents (SIR)
				Blair et al. (2003)
	All subjects	1.6 (1.0, 2.3)	27	5,369 U.S. men and women laundry and dry-cleaning union members (1945−1978), follow-up 1979−1993, semiquantitative cumulative exposure surrogate to dry clean solvents, cancer mortality, external referents (SMR)
	Semiquantitative exposure score			
	Little to no exposure	1.5 (0.8, 2.7)	12	
	Medium to high exposure	1.4 (0.7, 1.7)	11	
				Ji et al. (2005a, b); Ji and Hemminki (2005a, b, c)
	Laundry workers and dry cleaners in 1960 Census	Not reported		9,255 Swedish men and 14,974 Swedish women employed in 1960 (men) or 1970 (women) as laundry worker or dry cleaner, follow-up 1961/1970−2000, PCE not identified to individual subjects, external referent (SIR) and adjusted for age, period and socioeconomic status
				Lynge and Thygesen (1990)
	Laundry worker and dry cleaners	0.40 (0.28, 0.52)	34	10,600 Danish men and women, 20−64 yr old, employed in 1970 as laundry worker, dry cleaners and textile dye workers, follow-up 1970−1980, external referents (SIR)
				Pukkala et al. (2009)
	Launderer and dry cleaner	1.20 (1.08, 1.34)	332	Men and women participating in national census on or before 1990, 5 Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), 30−64 yr, follow-up 2005, occupational title of launderer and dry cleaner in any census, external referents (SIR)

Table 4-37. Summary of human studies on tetrachloroethylene exposure and cervical cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
			Calvert et al. (2011)
All subjects	1.84 (0.98, 3.14)	13	1,704 U.S. men and women dry-cleaning union member in CA, IL, MI, NY follow-up 1940−2004 (618 subjects worked for one or more years prior to 1960 only at shops where PCE was the primary cleaning solvent, identified as PCE-only exposure), cancer mortality (SMR), female and male breast cancer (ICD-9, 174, 175)
Exposure duration/time since 1st employment	Not reported		
<5 yr/<20 yr	0.84 (0.15, 2.66)	2	
≥5 yr/<20 yr	2.63 (0.90, 6.03)	4	
<5 yr/>20 yr	2.75 (0.94, 6.30)	4	
≥5 yr/>20 yr	2.08 (0.57, 5.38)	3	
PCE subcohort	2.10 (0.68, 4.90)	5	
			Seldén and Ahlborg (2011)
Dry-cleaners and laundry workers	1.25 (0.81, 1.85)	25	9,440 Swedish men (n = 2,810) and women (n = 9,440) in 461 washing and dry-cleaning establishments, identified by employer in mid-1980s, employed 1973−1983, follow-up 1985−200, exposure assigned using company self-reported information on PCE usage—PCE (dry cleaners and laundries with a proportion of PCE dry cleaning), laundry (no PCE use), and other (mixed exposures to PCE, CFCs, TCE, etc.), external referents (SIR)
PCE	1.19 (0.64, 1.93)	16	
Duration of employment			
<1 yr	0.32 (0.01, 1.78)	1	
1−4 yr	1,72 (0.7, 3.40)	8	
5−11 yr	1.24 (0.50, 2.56)	7	
Laundry	1.45 (0.66, 2.75)	9	
			Travier et al. (2002)
All subjects, 1960 or 1970 Census in laundry and dry cleaner or related occupation and industry	1.34 (1.12, 1.60)	129	Swedish men and women identified as laundry worker, dry cleaner, or presser (occupational title), in the laundry, ironing, or dyeing industry or related industry in 1960 or 1970 (543,036 person-years); or, as laundry worker, dry cleaner, or presser (occupational and job title) (46,933 person-years) in both censuses, follow-up 1971−1989, external referents (SIR)
All subjects in 1960 and 1970 in laundry and dry cleaner occupation and industry	1.09 (0.57, 2.09)	9	

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Table 4-37. Summary of human studies on tetrachloroethylene exposure and cervical cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Case-control studies				
Nordic Countries (Denmark, Finland, Norway, Sweden)				Lynge et al. (2006)
	Unexposed	1.00	105	Case-control study among 46,768 Danish, Finnish, Norwegian, and Swedish men and women employed in 1960 as laundry worker or dry cleaner, follow-up 1970−1971 to 1997−2001, 102 cervical cancer cases, 3 controls per case randomly selected from cohort matched on country, sex, age, calendar period at diagnosis time, occupational task at 1970 Census proxy for exposure, cervical cancer incidence, RR adjusted for matching criteria
	Dry cleaner	0.98 (0.65, 1.47)	36	
	Other in dry-cleaning	1.72 (1.00, 2.97)	22	
	Unclassifiable	1.11 (0.72, 1.71)	44	
	Dry cleaner, employment duration, 1964−1979			
	≤1 yr	2.68 (0.89, 8.11)	7	
	2−4 yr	0.78 (0.31, 1.94)	6	
	5−9 yr	0.47 (0.20, 1.13)	6	
	≥10 yr	1.18 (0.64, 2.15)	16	
	Unknown	1.14 (0.12, 11.00)	1	

HCFA = Health Care Financing Administration, ISCO = International Standard Classification of Occupation, ISIC = International Standard Industry Classification, JEM = job-exposure-matrix, TWA = time-weighted-average.

Establishment of an exposure or concentration-response relationship can add to the weight of evidence for identifying a cancer hazard, but only limited data pertaining to exposure-response relationships for lung cancer and tetrachloroethylene exposure are available. Three studies of breast cancer presented risk estimates for increasing exposure categories; one study using exposure duration as a proxy (Peplonska et al., 2007) and two studies with a semiquantitative or quantitative exposure surrogate (Aschengrau et al., 2003; Blair et al., 2003). Risk estimates are larger for highest exposure groups compared to overall exposure or to a no or low exposed group in one cohort study that use a semiquantitative or quantitative exposure-assessment approach (Blair et al., 2003), and in one study when latent periods are considered (Aschengrau et al., 2003). One other study with an exposure assessment based on exposure duration reported a lower risk estimate with >10 years longer exposure duration than the risk estimate for ≤10 years (Peplonska et al., 2007).

With respect to cervical cancer, five studies presented risk estimates for increasing exposure categories using exposure duration (Calvert et al., 2011; Seldén and Ahlborg, 2011; Lynge et al., 2006; Blair et al., 2003; Travier et al., 2002). Calvert et al. (2011) was the only study to report a higher risk estimate for cervical cancer for the group with longest exposure duration (<5 years versus 5+ years).

All three case-control studies of breast cancer controlled for associated risk factors (Peplonska et al., 2007; Aschengrau et al., 2003; Band et al., 2000). Direct examination of possible confounders is less common in cohort studies examining breast cancer compared to case-control studies where information is obtained from study subjects or their proxies. None of the cohort studies of cervical cancer considered socioeconomic or lifestyle factors such as smoking or exposure to the human papilloma virus (HPV), a known risk factor for cervical cancer and correlated with socioeconomic status, particularly with the squamous cell subtype (NCI, 2010; Pukkala et al., 2010). The case-control study of Lynge et al. (2006) included controls similar in socioeconomic status as cases, and the odds ratio estimate in this study for dry cleaners did not support an association with tetrachloroethylene.

In conclusion, most studies examined breast cancer in females (Radican et al., 2008; Peplonska et al., 2007; Sung et al., 2007; Aschengrau et al., 2003; Blair et al., 2003; Band et al., 2000); or males and females combined (Calvert et al., 2011; Ruder et al., 2001; Boice et al., 1999). Five studies, mostly of Nordic subjects, presented risk estimates for male subjects separately (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Chang et al., 2005; Anderson et al., 1990; Lynge and Thygesen, 1990). The results from the large studies of breast cancer risk in women in relation to tetrachloroethylene exposure are mixed. The largest, based on 1,757 breast cancer cases in female dry-cleaners and laundry workers, reported a statistically significant deficit in the risk of breast cancer incidence compared to the populations of Nordic countries

(Pukkala et al., 2009). Findings in the other six studies were based on fewer events or exposed cases; two of four studies with nonspecific exposure-assessment methodology provided evidence for association between breast cancer in females and tetrachloroethylene exposure (Sung et al., 2007; Chang et al., 2005; Aschengrau et al., 2003; Anderson et al., 1990; Lynge and Thygesen, 1990) but effects were not observed in two other large cohort studies with a relatively high quality exposure-assessment methodology to tetrachloroethylene (Seldén and Ahlborg, 2011; Blair et al., 2003). Small studies observed mixed findings (Calvert et al., 2011; Radican et al., 2008; Peplonska et al., 2007; Sung et al., 2007; Chang et al., 2005; Aschengrau et al., 2003; Band et al., 2000; Boice et al., 1999). Band et al. (2000), but not other less-weighted studies, excluded chance as an alternative explanation. Although cohort studies were unable to control for potential confounding from reproductive history or menopausal status, observations in case-control studies controlled for these potential confounders in statistical analyses and provided support of an association between female breast cancer and tetrachloroethylene compared to controls (Peplonska et al., 2007; Aschengrau et al., 2003; Band et al., 2000). Three studies examined exposure response, with risk estimates in females monotonically increased in higher exposure groups in two studies with semiquantitative or quantitative exposure-assessment approaches (Aschengrau et al., 2003; Blair et al., 2003). A third study examining exposure duration observed an inverse relation (Peplonska et al., 2007). Exposure duration is more uncertain than use of a semiquantitative surrogate given increased potential for bias associated with exposure misclassification. Because of the limitation in statistical power, none of the five studies reporting on male breast cancer is adequate to examine tetrachloroethylene exposure. All studies of male breast cancer are sufficiently underpowered; no male breast cancer cases were observed in four of the five studies (Seldén and Ahlborg, 2011; Pukkala et al., 2010; Chang et al., 2005; Anderson et al., 1990; Lynge and Thygesen, 1990).

For cervical cancer, the results from the two large cohort studies of dry cleaners are consistent with an elevated cervical cancer risk of 20−30% (Pukkala et al., 2009; Travier et al., 2002). Results from four smaller cohort and case-control studies with a relatively high quality exposure-assessment methodology presented a pattern of more variable results, with relative risks of 0.98 (95% CI: 0.65, 1.47), 1.19 (95% CI: 0.64, 1.93), 2.10 (95% CI: 0.68, 4.90), and 3.20 (95% CI: 0.39, 11.6) in Lynge et al. (2006), Sélden and Ahlborg (2011), Calvert et al. (2011), and Anttila et al. (1995), respectively. A fourth study with higher quality exposure-assessment specific to tetrachloroethylene did not observe any cervical cancer deaths among women, but less than one death was expected (Boice et al., 1999). Calvert et al. (2011) was the only study to report an exposure response gradient with employment duration. Dry cleaning workers did not have higher cervical cancer risks compared with laundry workers or other categories of dry cleaning workers (Seldén and Ahlborg, 2011; Lynge et al., 2006). 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. Potential confounding by socioeconomic status is an alternative explanation, with some support provided by Lynge et al. (2006), a case-control study with controls of similar socioeconomic status as cases, and who did not observe an association between cervical cancer and dry cleaning.

4.7.3. Summary of Human and Animal Developmental/Reproductive Studies

4.7.3.1. Summary of Human Data

Studies of tetrachloroethylene exposure have evaluated several reproductive outcomes including effects on menstrual disorders (Zielhuis et al., 1989), semen quality (Eskenazi et al., 1991a; Eskenazi et al., 1991b), fertility (Eskenazi et al., 1991a; Rachootin and Olsen, 1983), time to pregnancy (Sallmen et al., 1998; Sallmen et al., 1995), and risk of adverse pregnancy outcomes including spontaneous abortion (Aschengrau et al., 2009a; Doyle et al., 1997; Lindbohm et al., 1991; Windham et al., 1991; Ahlborg, 1990b; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989; Bosco et al., 1987; McDonald et al., 1987; McDonald et al., 1986), low birth weight or gestational age (Aschengrau et al., 2008; Olsen et al., 1990; Bosco et al., 1987; McDonald et al., 1987), birth anomalies (Aschengrau et al., 2009b; Ahlborg, 1990b; Olsen et al., 1990; Bosco et al., 1987; McDonald et al., 1987), and stillbirth (Olsen et al., 1990; McDonald et al., 1987). A few studies evaluated effects of prenatal exposure to tetrachloroethylene on postnatal development including learning and behavior, and schizophrenia (Janulewicz et al., 2008; Perrin et al., 2007). Many of the studies evaluated exposure during a specific critical window relevant to the health endpoint under study, for example, the period before conception or during the first trimester.

Some studies that relied on detailed work histories and monitoring data to classify exposure were suggestive that maternal or paternal exposure to tetrachloroethylene or work in dry cleaning reduces fertility or delays conception (Sallmen et al., 1998; Sallmen et al., 1995; Eskenazi et al., 1988). However, the risk estimates were imprecise because the number of participants reporting exposure to tetrachloroethylene was small. One small study of primarily unionized workers in the dry-cleaning and laundry industries in California observed subtle deficits in sperm quality in relation to tetrachloroethylene exposure (Eskenazi et al., 1988). However, three clinically recognized measures of sperm quality were not associated with exposure in the study population. A study of occupational exposures among a group of infertile couples who sought treatment found no association between either a diagnosis of sperm

abnormalities among male partners, or a diagnosis of hormonal disturbances among female partners with self-reported exposure to dry-cleaning chemicals (Rachootin and Olsen, 1983).

The results of Eskenazi et al. (1988) are compelling, but more studies are needed to conclude if exposure to tetrachloroethylene is associated with adverse effects on male and female reproduction.

Results of several studies of maternal occupational exposure to tetrachloroethylene suggest an increased risk of spontaneous abortion, particularly at higher levels (Doyle et al., 1997; Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989). Most of the studies evaluated exposure during the first trimester of pregnancy. Some of the studies observed an increased odds ratio ranging between 1.4 to 4.7, but had low statistical power because the cohort contained small numbers of exposed cases and controls, and were limited in their ability to evaluate potential confounding (Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Bosco et al., 1987). In general, the studies that used a more precise definition of exposure, or categorized exposure into levels of increasing dose or intensity, observed higher risk estimates. For example, two reports of occupational exposure in the dry-cleaning and laundry industries in Finland observed a dose-related increase in risk among employees classified into risk levels based on whether or not their work tasks involved dry cleaning (Olsen et al., 1990; Kyyronen et al., 1989). Odds ratios for low and high exposure compared to no exposure were 1.18 (95% CI: 0.71−1.97) and 4.53 (95% CI: 1.11−18.5), respectively. The Finnish studies controlled for reported exposure to other substances in the workplace as well as for several potential confounders. They also found agreement between self-reported exposures and biological measurements taken close to the time of pregnancy for a small subset of the cohorts. A relatively large study of workers in the United Kingdom classified exposure among current and former employees at dry-cleaning and laundry establishments by job tasks (machine operator versus other tasks) and analyzed risk of spontaneous abortions among all pregnancies reported between 1980 and 1995 (Doyle et al., 1997). Machine operators had a 63% higher risk of spontaneous abortion compared to nonoperators adjusting for several potential confounders (OR: 1.63, 95% CI: 1.09−3.05). These findings are consistent with breathing zone measurements of tetrachloroethylene in dry-cleaning establishments, indicating that machine operators have the highest exposures (Gold et al., 2008).

Increased risks were not found among dry cleaners in Sweden using a comparable study design (Ahlborg, 1990a; Olsen et al., 1990). Further, three studies of paternal occupational exposure prior to the beginning of the pregnancy did not observe an association (Eskenazi et al., 1991a; Lindbohm et al., 1991; Taskinen et al., 1989). Two of these surveyed occupational exposure to a broad array of substances and, consequently, had low statistical power for chemical-specific analyses (Lindbohm et al., 1991; Taskinen et al., 1989). Although there is no

evidence of an increased risk associated with paternal exposure, the studies were not of sufficient size, nor did they provide adequate detail regarding exposure estimates to allow definitive conclusions. Finally, no associations with incidence of spontaneous abortion were observed among two populations exposed to tetrachlorethylene in drinking water (Aschengrau et al., 2009a; Aschengrau et al., 2008; Lagakos et al., 1986). The studies of drinking water contamination evaluated populations with much lower exposures compared to the occupational cohorts.

Studies of tetrachloroethylene in drinking water have reported that exposure during pregnancy is associated with low birth weight (Sonnenfeld et al., 2001; Bove et al., 1995; Lagakos et al., 1986), eye/ear anomalies (Lagakos et al., 1986), and oral clefts (Aschengrau et al., 2009b; Bove et al., 1995; Lagakos et al., 1986). However, the number of cases with birth anomalies in specific diagnostic groups was very small, and CIs often included one. In addition, imprecise exposure estimates likely resulted in nondifferential misclassification, biasing risk estimates toward the null. Participants in the studies were exposed to multiple contaminants, and it was not possible to disentangle substance-specific risks.

Aschengrau et al. (2008) evaluated a unique exposure event in a population in eight Cape Cod towns exposed to a wide range of tetrachloroethylene concentrations in an irregular pattern throughout the region (1.5−7,750 µg/L). It is less likely that the population was exposed to sizable concentrations of other halogenated substances. A detailed exposure model was used to estimate the distribution of contaminated water to the homes of residents. Birth weight and gestational age were not associated with exposure to tetrachlorethylene. Effect estimates for some congenital anomalies were increased, although the number of infants with anomalies was very small, and statistical power was low. The small increased risk is consistent with the other studies of drinking water exposure to mixtures of halogenated pollutants. Diagnoses of attention deficit disorder, hyperactive disorder or educational histories reported by the mothers about their children were not increased in relation to the amount of tetrachloroethylene delivered to the homes during pregnancy or childhood (Janulewicz et al., 2008). On the other hand, a more than threefold risk of schizophrenia was associated with dry cleaning as a surrogate for prenatal tetrachloroethylene exposure (Perrin et al., 2007). The longitudinal design and use of a national registry to identify psychiatric diagnoses were strengths of the study, but tetrachloroethylene exposure was not directly analyzed. In conclusion, the literature is insufficient to draw conclusions regarding effects of tetrachloroethylene exposure on development in infants and children.

Most epidemiologic studies examined breast cancer in females (Radican et al., 2008; Peplonska et al., 2007; Sung et al., 2007; Aschengrau et al., 2003; Blair et al., 2003; Band et al., 2000) or males and females combined (Calvert et al., 2011; Ruder et al., 2001; Boice et al.,

1999); five studies presented risk estimates for male subjects separately (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Chang et al., 2005; Anderson et al., 1990; Lynge and Thygesen, 1990). The largest study, based on 1,757 breast cancer cases in female dry-cleaners and laundry workers, reported a statistically significant deficit in the risk of breast cancer incidence compared to the populations of Nordic countries. Findings in the other four large studies were based on fewer events or exposed cases with mixed findings (Seldén and Ahlborg, 2011; Aschengrau et al., 2003; Blair et al., 2003; Lynge and Thygesen, 1990). Additional studies carrying less weight also observed mixed findings (Radican et al., 2008; Peplonska et al., 2007; Sung et al., 2007; Chang et al., 2005; Ruder et al., 2001; Band et al., 2000; Boice et al., 1999). Three studies examined exposure-response, with risk estimates in females monotonically increased in higher exposure groups in two studies with semiquantitative or quantitative exposure-assessment approaches (Aschengrau et al., 2003; Blair et al., 2003). A third study examining exposure duration observed an inverse direction (Peplonska et al., 2007). Exposure duration is more uncertain than use of a semiquantitative approach because there is increased potential for bias associated with exposure misclassification. None of the five studies reporting on male breast cancer is adequate to examine tetrachloroethylene exposure. All studies of male breast cancer are statistically underpowered; no male breast cancer cases were observed in four of the five studies (Seldén and Ahlborg, 2011; Chang et al., 2005; Anderson et al., 1990; Lynge and Thygesen, 1990), less than one case was expected in each study, and Pukkala et al. (2009) observed three cases among a cohort of 8,744 male dry-cleaners and laundry workers.

For cervical cancer, the results from the two large cohort studies with broad exposure assessment is consistent with an elevated cervical cancer risk of 20−30% (Pukkala et al., 2009; Travier et al., 2002). Results from four smaller cohort and case-control studies with a higher quality exposure-assessment methodology presented a pattern of more variable results, with relative risks of 0.98 (95% CI: 0.65, 1.47), 1.19 (95% CI: 0.64, 1.93), 2.10 (95% CI: 0.68, 4.90), and 3.20 (95% CI: 0.39, 11.6) in Lynge et al. (2006), Sélden and Ahlborg (2011), Calvert et al. (2011), and Anttila et al. (1995), respectively. A fourth study with high quality exposure assessment specific to tetrachloroethylene did not observe any cervical cancer deaths among women and was insensitive, as less than one death was expected (Boice et al., 1999). Calvert et al. (2011) was the only study to report an exposure response gradient. Dry cleaning workers did not have higher cervical cancer risks compared with laundry workers or other categories of dry cleaning workers (Seldén and Ahlborg, 2011; Lynge et al., 2006). 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. Potential confounding by socioeconomic status is an alternative explanation with some support provided by Lynge et al. (2006), a case-control study with

controls of similar socioeconomic status as cases, and who did not observe an association between cervical cancer and dry cleaning.

4.7.3.2. Summary of Animal Data

Table 4-38 summarizes the findings of the animal developmental and reproductive toxicity studies described in Sections 4.7.2.1 to 4.7.2.3. The inhalation study database includes assessments of developmental toxicity in rats, mice, and rabbits following exposures during gestation, assessments of developmental neurotoxicity in rats following pre- and/or postnatal exposures of the offspring, and evaluation of reproductive and fertility outcomes in rats and mice. Additional supportive studies include in vitro assays of embryo development and oocyte fertilizability, a developmental assay in Japanese medaka, and two oral gavage studies that assessed developmental toxicity in rats and developmental neurotoxicity in mice.

Limitations of the inhalation developmental and reproductive toxicity studies are described in the individual study summaries above. These limitations include the lack of dose-response information due to the use of a single treatment level in the prenatal developmental toxicity assessment by Schwetz et al. (1975); the lack of either maternal or developmental toxicity in Hardin et al. (1981); absence of methodological details in study reporting (Szakmáry et al., 1997); and a concern about a short peri-parturition exposure gap in Tinston (1994). Additionally, the studies were conducted in accordance with standard EPA and OECD toxicological study guidelines in place at the time but did not assess endpoints that are included in the guidelines that were revised and harmonized in 1998 [e.g., refer to Tinston (1994)]. Maternal toxicity, when observed, did not compromise the evaluation or interpretation of treatment-related findings in the offspring.

Table 4-38. Summary of mammalian developmental and reproductive toxicity studies for tetrachloroethylene

Subjects	Effects	Concentration	Authors
Developmental toxicity studies			
Rat (whole embryo culture)	Mortality, malformations, delayed growth and differentiation	No effect at 2.5 mM, effects at 3.5 mM and higher	Saillenfait et al. (1995)
Japanese medaka	Decreased egg viability at 96-h (LC_{50} = 27 mg/L); at 10 d: decreased hatchability and larval survival, increased developmental abnormalities	10 d: 0, 1.5, 3, 6, 12, 25 mg/L LOAEL = 1.5 mg/L	Spencer et al. (2002)
SW Mice	Maternal toxicity (statistically significant, 21% increase in mean relative liver weight), decreased fetal weight, delayed ossification, 9% decrease in birth weight	Inhalation: 0, 300 ppm on GDs 6−15	Schwetz et al. (1975)
S-D Rats	Maternal toxicity (slight, but statistically significant, 4-5% reductions in maternal body weight gains), increased resorptions (fetal death)	Inhalation: 0, 300 ppm on Days 6−15	Schwetz et al. (1975)
S-D Rats, NZW Rabbits	No developmental toxicity	Inhalation: Exposures throughout gestation NOAEL = 500 ppm	Hardin et al. (1981)
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, 0, 900, 1,200 mg/kg-day on GDs 6−19	Narotsky and Kavlock (1995)
CFY Rats	Maternal toxicity (statistically significant, 37-40%; decreased body weight gain; slight, but statistically significant, 6-10% increased liver weight; and increased serum enzymes); increased pre- and postimplantation loss, skeletal retardation, and total malformations; decreased fetal weight	Inhalation: 0, 1,500, 4,500, 8,500 mg/m^3 on GDs 1−20 LOAEL = 1,500 mg/m^3	Szakmáry et al. (1997)[a]
C57Bl Mice	Maternal toxicity (statistically significant increased liver weight); visceral malformations	Inhalation: 0, 1,500 mg/m^3 on GDs 7−15 LOAEL = 1,500 mg/m^3	Szakmáry et al. (1997)

Table 4-38. Summary of mammalian developmental and reproductive toxicity studies for tetrachloroethylene (continued)

Subjects	Effects	Concentration	Authors
NZW Rabbits	Maternal toxicity (decreased body weight gain, increased liver weight); abortions, total litter resorptions, increased postimplantation loss, malformations	Inhalation: 0, 4,500 mg/m^3 on GDs 7−20 LOAEL = 4,500 mg/m^3	Szakmáry et al. (1997)
S-D Rats	Maternal toxicity (slight, but statistically significant, decreased body weight gain; decreased gravid uterine weight); fetal body weight and placental weight decrements, increased delays in thoracic vertebral ossification	Inhalation: 0, 75, 250, or 600 ppm (actual concentrations: 0, 66, 249, 600 ppm), 6 h/d, 7 d/wk, on GDs 0−19 Maternal LOAEL = 600 ppm Fetal LOAEL = 250 ppm	Carney et al. (2006)
Developmental neurotoxicity assessments			
CFY Rats	Decreased postnatal survival, minimal transient decreases in exploratory activity and muscular strength, and increased motor activity in females on PND 100	Inhalation: 0, 1,500, 4,500 mg/m^3 on GDs 1−20 (and perhaps postnatally to PND 100) LOAEL = 1,500 mg/m^3	Szakmáry et al. (1997)[a]
S-D Rats	Decreased weight gain, behavioral changes (more extensive for late pregnancy exposure), decreased brain acetylcholine	Inhalation: 0, 100, 900 ppm on Days 7−13 or on Days 14−20 NOAEL = 100 ppm LOAEL = 900 ppm	Nelson et al. (1979)
S-D Rats, two-generation study	Behavioral effects (decreased activity; reduced response to sound) in F1 pups	Inhalation: 0, 100, 300, 1,000 ppm NOAEL = 300 ppm LOAEL = 1,000 ppm	Tinston (1994)[b]
NMRI Mice	Alterations in spontaneous motor activity (locomotion, rearing, and total activity) at PND 60	Gavage: 0, 5, 320 mg/kg-day on PNDs 10−16 LOAEL = 5 mg/kg-day	Fredriksson et al. (1993)
Reproductive toxicity studies			
Rat (in vitro)	Reduced fertilizability of extracted oocytes	12,000 mg/m^3, 2 hours/d, 5 d/wk for 2 wk	Berger and Horner (2003)
CD-1 Mice	Abnormal sperm heads at 500 ppm but not at 100 ppm, spermatogonia or spermatocyte stage affected	Inhalation: 0, 100, 500 ppm for 5 d LOAEL = 500 ppm	Beliles et al. (1980)

Table 4-38. Summary of mammalian developmental and reproductive toxicity studies for tetrachloroethylene (continued)

Subjects	Effects	Concentration	Authors
S-D Rats, two-generation study	Increased death of F1A and F2A and F2B pups, decreased body weight	Inhalation: 0, 100, 300, 1,000 ppm NOAEL = 100 ppm for body weight reduction	Tinston (1994)[b]

[a] The Szakmáry et al. (1997) study in CFY rats assessed both developmental toxicity and developmental neurotoxicity outcomes.
[b] The Tinston (1994) study in S-D rats demonstrated both developmental neurotoxicity and reproductive toxicity outcomes.

The tetrachloroethylene database included assessments of the various potential manifestations of developmental toxicity, i.e., alterations in survival, growth, morphology, and functional development. Indications of effects on prenatal survival following in utero exposure included increased pre- and/or postimplantation loss in rats, mice, and rabbits (Szakmáry et al., 1997; Schwetz et al., 1975). These findings were supported by evidence of embryo mortality in a rat whole embryo culture (WEC) assay (Saillenfait et al., 1995) and decreased viability in a Japanese medaka assay (Spencer et al., 2002). Decreased prenatal growth was observed in mice (Schwetz et al., 1975) and rats (Szakmáry et al., 1997). Morphological alterations associated with prenatal exposures to tetrachloroethylene included delays in skeletal ossification in mice (Schwetz et al., 1975) and rats (Carney et al., 2006; Szakmáry et al., 1997), which were often associated with fetal weight decrements, and increased incidences of malformations in mice, rats, and rabbits (Szakmáry et al., 1997). Evidence of tetrachloroethylene exposure-related malformations was also observed in the rat WEC and Japanese medaka assays (Spencer et al., 2002; Saillenfait et al., 1995) and in a gavage prenatal developmental toxicity screening study in rats (Narotsky and Kavlock, 1995). Alterations in neurological function following pre- and/or postnatal inhalation exposures to tetrachloroethylene were observed in rats by Szakmáry et al. (1997), Nelson et al. (1979), and Tinston (1994). These findings were supported by a study that found altered spontaneous motor activity in young adult rats that had been treated orally with tetrachloroethylene postnatally during a critical period of nervous system development (Fredriksson et al., 1993). Additionally, reductions in brain acetylcholine and dopamine were observed in rat offspring following gestational tetrachloroethylene exposures (Nelson et al., 1979).

An assessment of fertility and reproductive function in rats exposed to tetrachloroethylene via inhalation over the course of two generations was conducted by Tinston (1994). Effects on offspring included decreased pup weights and postnatal survival in both

generations, as well as behavioral alterations in the F1 pups. Decreased mean testes weight was observed in F1a males; however, no effects on male or female fertility or other evidence of alterations in reproductive function were observed. For males, this finding is supported by the results of a study by Beliles et al. (1980), who found no sperm abnormalities in rats following up to 10 weeks of tetrachloroethylene inhalation exposures. While the Beliles et al. (1980) study identified an increase in abnormal sperm heads in mice after 4 weeks of exposure, no other reproductive toxicity data in mice were available to aid in the interpretation of this finding.

In conclusion, based upon a consideration of the entire available database of animal developmental and reproductive toxicity studies for tetrachloroethylene, the overall inhalation NOAEL is 100 ppm, based on Tinston (1994). The overall inhalation 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.

Overall, the developmental and reproductive toxicity database for tetrachloroethylene was judged to include a range of data from appropriate well-conducted studies in several laboratory animal species plus limited human data and was considered sufficient for hazard characterization and dose-response assessment, based upon EPA risk assessment guidelines (U.S. EPA, 1996a, 1991b).

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 a causative agent or contribute to developmental toxicity expressed as morphological changes, lethality, or growth reductions. 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 GDs 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.

Arguments counter to the involvement of TCA in the MOA for tetrachloroethylene developmental toxicity include that the types of malformations associated with TCA [i.e., cardiac malformations reported by Smith et al. (1989) and Johnson et al. (1998)] or other weak acid exposures [e.g., valproic acid and ethylhexanoic acid (Scott et al., 1994)] are not consistent with those observed in tetrachloroethylene studies. Additionally, relatively high concentrations of TCA are required to cause developmental toxicity compared with the concentration expected to result from metabolism of tetrachloroethylene in vivo, which may account for the differences in the type of developmental effects resulting from tetrachloroethylene exposure. There is also a lack of information on the availability of metabolized TCA to the developing fetus and the potential differences related to oral-versus-inhalation exposure in tetrachloroethylene studies.

4.8. GENOTOXICITY

Tetrachloroethylene and its metabolites have been extensively studied for genotoxic activity in a variety of in vitro assay systems such as bacteria, yeast, and mammalian cells [Refer to reviews by IARC (1995), WHO (2006), and ATSDR (1997a)]. This section discusses the genotoxic potential of tetrachloroethylene and its known or postulated metabolites (TCA, DCA, CH, TCVC, TCVG, NAcTCVC, tetrachloroethylene epoxide), with a summary provided at the end of each section for tetrachloroethylene or its metabolite for their mutagenic potential, in addition to an overall synthesis summary at the end of this section. TCVC sulfoxide does not appear to have been investigated for genotoxicity.

The application of genotoxicity data to predict potential carcinogenicity is based on the principle that genetic alterations are found in all cancers. Genotoxicity is the ability of chemicals to alter genetic material in a manner that permits changes to be transmitted during cell division. Although most tests for mutagenicity detect changes in DNA or chromosomes, some specific

modifications of the epigenome, which includes proteins associated with DNA or RNA, can also cause transmissible changes. Genetic alterations can occur through a variety of mechanisms including gene mutations, deletions, translocations, or amplifications; evidence of mutagenesis provides mechanistic support for the inference of potential for carcinogenicity in humans.

Evaluation of genotoxicity data entails a weight-of-evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity tests are, by design, complementary evaluations of different mechanisms of genotoxicity, a recent IPCS publication (Eastmond et al., 2009) notes that "multiple negative results may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay." These considerations inform the present approach. In addition, consistent with EPA's *Guidelines on Carcinogenic Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005a), the approach does not address relative potency (e.g., among tetrachloroethylene metabolites, or of such metabolites with other known genotoxic carcinogens) per se, nor does it consider quantitative issues related to the probable production of these metabolites in vivo. Instead, the analysis of genetic toxicity data presented here focuses on the identification of a genotoxic hazard of these metabolites; a quantitative analysis of tetrachloroethylene metabolism to reactive intermediates, via PBPK modeling, is presented in Section 3.

Below, the genotoxicity data for tetrachloroethylene and its metabolites are briefly reviewed, with detailed study information in the corresponding tables. The contributions of these data are twofold. First, to the extent that these metabolites may be formed in the in vitro and in vivo test systems for tetrachloroethylene, these data provide insight into what agent or agents may contribute to the limited activity observed with tetrachloroethylene in these genotoxicity assays. Second, because the in vitro systems do not necessarily fully recapitulate in vivo metabolism, the demonstration of in vitro genotoxicity by the known in vivo metabolites themselves provides information regarding the expected genotoxicity of tetrachloroethylene following in vivo exposure.

4.8.1. Tetrachloroethylene (PCE)

Limited studies have been performed examining tetrachloroethylene genotoxicity in vivo. These and in vitro genotoxicity studies of tetrachloroethylene are described below and listed in Tables 4-39 and 4-40.

4.8.1.1. Mammalian Systems (Including Human Studies)

4.8.1.1.1. Gene mutation

Tetrachloroethylene was negative for increased frequency of mutations of thymidine kinase locus in L5178Y/TK +/- mouse lymphoma cells both with and without S9 activation (F344 rat liver) (NTP, 1986). Experiments were performed twice, with replicates of all doses. L5178Y/TK +/- mouse lymphoma cells were exposed to tetrachloroethylene in 1% dimethylsufoxide for 4 hours at 37°C in medium; cells were then washed and resuspended in fresh medium for 48 hours at 37°C. TK mutation frequency was determined by plating cells in medium supplemented with trifluorothymidine. Overall cell viability was determined by plating cells in nonselective medium. Mutation frequency was not above background for any dose tested (6.25, 12.50, 25, 50, 100 nL/mL in the presence of S9; 12.5, 25, 50, 75, and 150 nL/mL in the absence of S9). Positive controls in both the presence and absence of S9 activation [3-methylcholanthrene (2.5 µg/mL) and ethyl methanesulfonate (250 µg/mL), respectively] showed significant increases in mutation frequencies ($p < 0.001$, *t*-test) (NTP, 1986).

Gene mutations were induced in a host-mediated assay, using *S. typhimurium* strain TA98 implanted into the peritoneal cavity of male and female CD-1 mice that were previously exposed to tetrachloroethylene by inhalation (100 or 500 ppm, 7 hours/day, for 5 days) (Beliles et al., 1980). Positive results were observed in male mice at 100 (but not 500) ppm, and in female mice at 500 (but not 100) ppm. Although no explanation was given for the variability in the dose response, the authors conclude that tetrachloroethylene is an active frameshift mutagen using in vivo activation.

In summary, the in vitro thymidine kinase gene mutation assay in mammalian cells was negative for gene mutations in the presence and absence of S9 (F344 rat liver) metabolic activation (NTP, 1986). Positive results for frameshift mutagenicity were observed in a host-mediated assay by implanting *S. typhimurium* into mice exposed to tetrachloroethylene, but without a clear dose-response effect (Beliles et al., 1980).

Table 4-39. Genotoxicity of tetrachloroethylene—mammalian systems (in vitro and in vivo)[a]

Test system/endpoint		Doses (LED or HID)[b]	Results[c]		Reference
			With activation	Without activation	
Unscheduled DNA synthesis, rat primary hepatocytes in vitro		166 (vapor)	NT	–[d]	Shimada et al. (1985)
Unscheduled DNA synthesis, Osborne Mendel rat primary hepatocytes in vitro		NA	NT	–	Milman et al. (1988)
Unscheduled DNA synthesis, $B6C3F_1$ mouse primary hepatocytes in vitro		NA	NT	–	Milman et al. (1988)
Gene mutation, mouse lymphoma L5178Y cells, tk locus		245	–	–	NTP (1986)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells in vitro		164	–	–	Galloway et al. (1987)
Chromosomal aberrations, Chinese hamster lung (CHL) cells in vitro		500	–	–	Sofuni et al. (1985)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells in vitro		136	–	–	Galloway et al. (1987)
Cell transformation, RLV/Fischer rat embryo F1706 cells in vitro		16	NT	+	Price et al. (1978)
BALB/c-3T3 mouse cells, cell transformation in vitro		250	NT	–	Tu et al. (1985)
Rat and mouse hepatocyte, DNA damage (unscheduled DNA synthesis)		2.5mM	NT	–	Costa and Ivanetich (1984)
Human fibroblast cells, DNA damage (unscheduled DNA synthesis)		0.1 nL/mL	(+/–)	(+/–)	Beliles et al. (1980)
Host mediated assay—*S. typhimurium* implanted in CD-1 mice		100 ppm (male mice;500 ppm (female mice)	+	NT	Beliles et al. (1980)
Chinese hamster ovary cells, sister chromatid exchange		164 µg/mL	–	–	NTP (1986)
Chinese hamster ovary (CHO-K1) cells, increased frequency of micronuclei		~63 ppm	NT	+	Wang et al. (2001)
Cytochalasin B-blocked micronucleus assay using human lymphoblastoid cell lines with enhanced metabolic activity, increased frequency of micronuclei	AHH-1	5 mM	NT	+	Doherty et al. (1996)
	H2E1	1 mM	NT	+	Doherty et al. (1996)
	MCL-5	1 mM	NT	+	Doherty et al. (1996)
Human white blood cells, length of DNA migration Human lymphocytes, sister chromatid exchange		5×10^{-3} M	– –	– –	Hartmann and Speit (1995)

Table 4-39. Genotoxicity of tetrachloroethylene—mammalian systems (in vitro and in vivo)[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
Gene conversion and reverse mutation in *S. cerevisiae* D7 recovered from liver, lungs, and kidneys of CD-1 mice	11,000 p.o. × 1	NT	−	Bronzetti et al. (1983)
Gene conversion and reverse mutation in *S. cerevisiae* D7 recovered from liver, lungs, and kidneys of CD-1 mice	2,000 p.o. × 12	−	NT	Bronzetti et al. (1983)
DNA single-strand breaks (alkaline unwinding) in liver and kidney of male NMRI mice in vivo	660 i.p. × 1	NT	+[e]	Walles (1986)
Sister chromatid exchange, human lymphocytes in vivo	1,500 mg/m^3 inhaled	NT	−	Ikeda et al. (1980)
Chromosomal aberrations, human lymphocytes in vivo	92 ppm inhaled	NT	−	Ikeda et al. (1980)
Binding (covalent) to calf thymus DNA in vitro	2.5 µCi ^{14}C-PCE	+	Data not shown	Mazzullo et al. (1987)
Binding (covalent) to DNA in male B6C3F$_1$ mouse liver in vivo	1,400 inhaled 6 h 600 ppm	NT	−	Schumann et al. (1980)
Binding (covalent) to DNA in male B6C3F$_1$ mouse liver in vivo	500 p.o. × 1	NT	−	Schumann et al. (1980)
Binding (covalent) to DNA in male BALB/c mouse and Wistar rat liver, kidney, lung, and stomach in vivo	1.4 i.p. × 1 22 h	NT	+	Mazzullo et al. (1987)
Binding (covalent) to RNA and protein in male BALB/c mouse and Wistar rat liver, kidney, lung, and stomach in vivo	1.4 i.p. × 1 22 h	NT	+	Mazzullo et al. (1987)
Human lymphocytes, sister chromatid exchange	10 ppm (geometric mean)	NT	−	Seiji et al. (1990)
Mouse, reticulocytes, micronucleus	2,000 mg/kg	NT	−	Murakami and Horikawa (1995)
Mouse, hepatocytes, micronucleus Before partial hepatectomy After partial hepatectomy	1,000 mg/kg	NT	 − +	Murakami and Horikawa (1995)
Mouse, induction of DNA damage in hepatocytes (alkaline Comet assay)	1,000 mg/kg-day 2,000 mg/kg-day	NT	+/− +/−	Cederberg et al. (2010a)
Mouse, induction of DNA damage in kidney (alkaline Comet assay)	1,000 mg/kg-day 2,000 mg/kg-day	NT	− −	Cederberg et al. (2010a)
Rat bone marrow cells, chromosomal aberrations	100 and 500 ppm	NT	−	Beliles et al. (1980)

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Table 4-39. Genotoxicity of tetrachloroethylene—mammalian systems (in vitro and in vivo)[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	With activation	
Enzyme-altered foci in male Osborne Mendel rat liver in vivo, promotion protocol, with or without N-nitrosodiethylamine as an initiator	1,000, 5 d/wk for 7 wk	NT	+	Milman et al. (1988)
Enzyme-altered foci in male Osborne Mendel rat liver in vivo, initiation protocol, phenobarbital as a promoter	1,000	NT	−	Milman et al. (1988)
Micronucleus induction (Chinese hamster lung cell line)	250 µg/mL	−	−	Matsushima et al. (1999)
Gap Junction Intercellular Communication (rat liver cells)	0.1 mM	NT	+	Benane et al. (1996)
DNA damage (8-OHdG) in urine and leukocytes of dry cleaners (female only)	3.8 ± 5.3 ppm (TWA)	NT	−	Toraason et al. (2003)
DNA damage (8-OHdG) in Fischer rats measured in urine, lymphocytes, and liver	100−1,000 mg/kg	NT	− (Substantial morbidity at all doses limits interpretation.)	Toraason et al. (1999)
Human lymphocytes in vitro (unscheduled DNA synthesis)	1 mM	−	−	Perocco et al. (1983)
Human lymphocytes in vivo (Chromosomal aberrations)	144 mg/m^3 (but contaminated with trichloroethylene)	NT	+	Fender (1993)
DNA single-strand breaks	1,000 mg/kg p.o.	NT	−	Potter et al. (1996)

[a]Table adapted from ATSDR (1997a) and IARC monograph (1995) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests; mg/kg for in vivo tests unless otherwise specified; i.p. = intraperitoneal; p.o. = oral; NA = not available.
[c]Results: + = positive; (+) = weakly positive; (+/−) = mixed results; − = negative; NT = not tested.
[d]PCE with stabilizers was positive with and without metabolic activation.
[e]Negative in lung.

Table 4-40. Genotoxicity of tetrachloroethylene—bacterial, yeast, and fungal systems[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
SOS chromotest, *E. coli* PQ37	8,150	–	–	Mersch-Sundermann et al. (1994)
SOS chromotest, *E. coli* PQ37	NA	–	–	von der Hude et al. (1988)
λ Prophage induction, *E. coli* WP2	10,000	–	–	DeMarini et al. (1994)
S. typhimurium BAL13, forward mutation *(ara* test)	76	–	–	Roldán-Arjona et al. (1991)
S. typhimurium TA100, reverse mutation	660	–	–	Bartsch et al. (1979)
S. typhimurium TA100, reverse mutation	167	–	–	Haworth et al. (1983)
S. typhimurium TA100, reverse mutation	1,000	–	–	Connor et al. (1985)
S. typhimurium TA100, reverse mutation	166 (vapor)	–	–[d]	Shimada et al. (1985)
S. typhimurium TA100, reverse mutation	NA	–	–	Milman et al. (1988)
S. typhimurium TA100, reverse mutation	332	+[e]	–	Vamvakas et al. (1989d)
S. typhimurium TA100, reverse mutation	1.3 (vapor)	–	–	DeMarini et al. (1994)
S. typhimurium TA1535, reverse mutation	50	NT	–	Kringstad et al. (1981)
S. typhimurium TA1535, reverse mutation	167	–	–	Haworth et al. (1983)
S. typhimurium TA1535, reverse mutation	66 (vapor)	(+)	–[d]	Shimada et al. (1985)
S. typhimurium TA1535, reverse mutation	NA	–	–	Milman et al. (1988)
S. typhimurium TA1537, reverse mutation	167	–	–	Haworth et al. (1983)
S. typhimurium TA1537, reverse mutation	NA	–	–	Milman et al. (1988)
S. typhimurium, gene mutation TA100, TA1535, TA1537, TA98	333 µg/plate	–	–	NTP (1986)
S. typhimurium TA98, reverse mutation	167	–	–	Haworth et al. (1983)
S. typhimurium TA98, reverse mutation	1,000	–	–	Connor et al. (1985)
S. typhimurium TA98, reverse mutation	NA	–	–	Milman et al. (1988)
S. typhimurium UTH8413, reverse mutation	1,000	–	–	Connor et al. (1985)
S. typhimurium UTH8414, reverse mutation	1,000	–	–	Connor et al. (1985)
S. typhimurium TA102, TA2638 *E. coli* WP2/pKM101, WP2 *uvr*A/pKM101, gene mutation	1,250 µg/plate	–	NT	Watanabe et al. (1998)

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Table 4-40. Genotoxicity of tetrachloroethylene—bacterial, yeast, and fungal systems[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
S. typhimurium, YG7108pin3ERb$_5$, gene mutation (strain is methyltransferase deficient and stably expresses complete electron transport chain including P450 reductase, cytochrome b5 and CYP2E1)	200 µg/plate	NT	-	Emmert et al. (2006)
E. coli K12, forward mutation	150	–	–	Greim et al. (1975)
E. coli K12, reverse mutation *(arg*)*	150	–	–	Greim et al. (1975)
E. coli K12, reverse mutation *(gal*)*	150	–	–	Greim et al. (1975)
E. coli K12, reverse mutation *(nad*)*	150	–	–	Greim et al. (1975)
S. cerevisiae D7, log-phase cultures, gene conversion	1,100	NT	+	Callen et al. (1980)
S. cerevisiae D7, gene conversion	9,960	–	–	Bronzetti et al. (1983)
S. cerevisiae D7, log-phase and stationary cultures, gene conversion	2,440	–	–	Koch et al. (1988)
S. cerevisiae D7, log-phase cultures, mitotic recombination or other genetic alterations *(ade2)*	1,100	NT	+	Callen et al. (1980)
S. cerevisiae D7, mitotic recombination	9,960	–	–	Bronzetti et al. (1983)
S. cerevisiae D7, log-phase cultures, reverse mutation	810	NT	(+)	Callen et al. (1980)
S. cerevisiae D7, reverse mutation	9,960	–	–	Bronzetti et al. (1983)
S. cerevisiae D7, log-phase and stationary cultures, reverse mutation	2,440	–	–	Koch et al. (1988)
S. cerevisiae D61.M, growing cells, aneuploidy	810	(+)	(+)	Koch et al. (1988)
D. melanogaster, sex-linked recessive lethal mutation	4,000 ppm p.o. 1,000 ppm injection	NT	–	NTP (1986)
D. melanogaster, sex-linked recessive lethal mutation	3,400 mg/m^3, 7 h	NT	–	Beliles et al. (1980)

[a]Table adapted from ATSDR (1997a) and IARC monograph (1995) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests unless otherwise specified; NA = not available.
[c]Results: + = positive; (+) = weakly positive; – = negative; NT = not tested.
[d]PCE with stabilizers was positive with and without metabolic activation.
[e]Weak increase in activity with rat liver S9, rat kidney microsomes and glutathione (GSH): fourfold increase with rat kidney microsomes, GSH and GSH *S*-transferase.

4.8.1.1.2. DNA binding

Schumann et al. (1980) assessed hepatic macromolecular binding in both rats and mice exposed to radiolabeled tetrachloroethylene by inhalation (10 or 600 ppm, 6 hours; binding measured at 6, 24, 48, and 72 hours postexposure) or a single oral gavage (500 mg/kg in corn oil; binding measured at 1, 6, 12, 24, 48, and 72 hours). In mice, tetrachloroethylene binding to macromolecules in liver peaked at the termination of the inhalation exposure or 6 hours postoral exposure. In rats, hepatic macromolecular binding peaked 24 hours after either oral or inhalation exposure. At these peak times, no DNA binding was observed in the mouse (rat data not reported). Using a more sensitive assay, Mazzullo et al. (1987) reported low levels of DNA binding (2.9 pmol/mg) in mouse liver 22 hours after i.p. injection (1.4 mg/kg bw). Levels of DNA binding were 6- to 10-fold lower in rat liver and in the kidney, lung, and stomach of mice and rats. Binding to RNA or protein was considerably higher than binding to DNA in both mice and rats. This raises the concern that possible contamination with RNA or protein might have contributed to the DNA results. Protein binding levels were highest in mouse liver and rat kidney. In a companion in vitro study, binding to calf thymus DNA was increased by microsomal fractions from rat or mouse liver, but not kidney, lung, or stomach. Cytosolic fractions from rat or mouse liver, kidney, lung, or stomach also enhanced DNA binding in vitro, with mouse and rat liver and mouse lung fractions being the most efficient. Cytosolic and microsomal fractions, when combined, enhanced DNA binding to a comparable extent as cytosolic fractions alone. Phenobarbital pretreatment of animals increased cytosol-mediated binding but minimally affected microsomal-mediated binding. DNA binding by rat liver microsomal fraction was enhanced 17-fold by GSH but decreased by superoxide dismutase or mannitol (Mazzullo et al., 1987).

In summary, DNA binding was not observed in one assay in mice exposed to tetrachloroethylene by inhalation and oral routes, while protein and RNA binding was observed (Schumann et al., 1980). Low levels of DNA binding in mouse liver, and yet lower levels in mouse kidney or rat and mouse stomach, were observed after i.p. injection using a more sensitive assay (Mazzullo et al., 1987). In vitro binding to calf thymus DNA was enhanced by microsomal and cytosolic fractions from various mouse and rat tissues. These results suggest a role for metabolic activation of the parent compound in DNA binding in vitro.

4.8.1.1.3. Chromosomal aberrations

Beliles et al. (1980) assessed bone marrow chromosomal aberrations and aneuploidy in male and female Sprague-Dawley rats after acute (sacrificed 6, 24, or 48 hours after dosing) and subchronic (7 hours a day, for 5 days; sacrificed 6 hours after last exposure) exposures to tetrachloroethylene by inhalation (100 and 500 ppm). The only effect reported with acute

exposure was a slight increase in the percentage of cells with aberrations and aneuploidy (peak of 3.3% compared to 0.7% in controls with 500 ppm tetrachloroethylene) in male, but not female, rats. No significant effects were observed in any subchronically exposed groups, but female rats showed a nonsignificant increase in cells with aberrations (Beliles et al., 1980). NTP (1986) did not observe chromosomal aberrations in Chinese hamster ovary cells exposed to tetrachloroethylene (17, 34.1, 68.1, and 136.3 µg/mL without activation or 17, 34.1, and 68.1 µg/mL with activation by Sprague-Dawley rat liver S9).

4.8.1.1.3.1. Micronucleus induction

Tetrachloroethylene exposure increased the frequency of micronuclei in hepatocytes, but not peripheral blood reticulocytes, of ddY mice given single i.p. injections of 1,000 or 2,000 mg/kg tetrachloroethylene after, but not prior to, partial hepatectomy (Murakami and Horikawa, 1995). This twofold increase in micronuclei in hepatocytes after partial hepatectomy was statistically significant but was not evident at the lower dose of 500 mg/kg. Conflicting results of other studies of tetrachloroethylene micronuclei induction have also been reported in cultured Chinese hamster cells (Wang et al., 2001; Matsushima et al., 1999) and in human cells (White et al., 2001; Doherty et al., 1996). Micronucleus induction was not observed in a Chinese hamster lung cell line (CHL/IU) following exposure to high doses of tetrachloroethylene (125−250 µg/mL) as part of a test validation assay, but some induction (not statistically significant) was observed at the lower dose (75 µg/mL) in the presence of S9 fraction (Matsushima et al., 1999). Details from this study are limited. Wang et al. (2001) examined micronuclei induction following in vitro exposure to tetrachloroethylene (~63 ppm in culture medium at peak) in a closed system. Chinese hamster ovary (CHO-K1) cells were plated in a petri dish surrounding a glass dish of tetrachloroethylene and incubated for 24 hours. Tetrachloroethylene exposure led to a dose-dependent significant increase in micronuclei induction ($p < 0.001$) (Wang et al., 2001). Similar results were also observed in human cell lines in other studies.

Micronucleus induction was enhanced by tetrachloroethylene exposure in AHH-1 parental human lymphoblastoid cells, and in two daughter cell lines (h2E1 and MCL-5) stably expressing human metabolic enzymes lines (Doherty et al., 1996). Parental AHH-1 cells possess native, albeit low, CYP1A1 activity but considerable glutathione-*S*-transferase activity; h2E1 cells stably express human CYP2E1; and MCL-5 cells stably express human CYP1A2, 2A6, 3A4, 2E1, and microsomal epoxide hydrolase. Tetrachloroethylene (5 mM) induced a threefold increase in micronuclei in AHH-1 cells and ninefold increases in h2E1 and MCL-5 cells, respectively (Doherty et al., 1996). White et al. (2001) similarly observed dose-dependent

increases in micronuclei induction after 24 hours incubation ($p < 0.05$) with tetrachloroethylene (0, 0.01, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0 mM) in the MCL-5 cell line.

4.8.1.1.3.2. Sister chromatid exchanges (SCEs)

Limited studies of sister chromatid exchanges demonstrate conflicting results. No differences were observed in the frequency of chromosomal aberrations and SCE between unexposed workers, workers exposed to moderate levels of tetrachloroethylene (70−280mg/m^3), and those exposed to high doses (200−1,500 mg/m^3) (Ikeda et al., 1980). Although an exposure assessment was performed in this study, the results are limited by the small number of subjects (total $n = 19$). Another study from this group had similar limitations (total $n = 10$) and also found no sister chromatid exchanges in lymphocytes in workers occupationally exposed to either high-dose tetrachloroethylene (92 ppm, geometric mean) or low-dose tetrachloroethylene (10−40 ppm range) (Ikeda et al., 1980). Similarly, no differences were observed between exposed and controls in a larger Japanese study, which examined SCE in 27 occupationally exposed workers (Seiji et al., 1990), or a German study on dry-cleaning workers (Böttger and Elstermeier, 1989). Increased chromosomal aberrations were observed in another occupational study following exposure to tetrachloroethylene (144−348 mg/m^3); however, exposure also included a small amount of trichloroethylene (0.11−0.43% by wt), so interpretation of the results relative to tetrachloroethylene alone may be limited (Fender, 1993)

Tetrachloroethylene-induced damage was also not observed in the sister chromatid exchange (SCE) assay or in the single-cell gel test (i.e., the Comet assay) in cultured human blood exposed to up to 5 mM (~830 mg/L) tetrachloroethylene, a dose that reduced viability by 40% due to cytotoxicity (Hartmann and Speit, 1995). Neither chromosome aberrations nor SCE were induced in Chinese hamster ovary cells following in vitro exposure to tetrachloroethylene (Galloway et al., 1987; Sofuni et al., 1985) as summarized in NRC (2010). Chinese hamster ovary cells exposed to tetrachloroethylene (16.4, 54.5, or 164 µg/mL) in the presence and absence of S9 activation (Sprague-Dawley rat livers) showed no increase in frequency of sister chromatid exchanges following exposure to tetrachloroethylene (NTP, 1986).

In summary, the majority of studies of chromosomal aberrations, micronuclei induction, and sister chromatid exchange following exposure to tetrachloroethylene are negative. Positive micronuclei induction was observed following partial hepatectomy at high doses (2,000 mg/kg-day i.p.) in ddY mice (Murakami and Horikawa, 1995). Increased micronuclei induction was observed in CHO cells in vitro when exposed to tetrachloroethylene in a closed system (Wang et al., 2001) but not in CHL cells when exposed in an open system (Matsushima et al., 1999), suggesting the need to control for loss of tetrachloroethylene via vaporization in in vitro assays. Dose-dependent increases in micronuclei were observed in human lymphoblastoid

cell lines, an effect enhanced by stable expression of CYP450 enzymes (White et al., 2001; Doherty et al., 1996); however, these cell lines are not generally considered part of the standard genotoxicity testing battery. No in vitro studies of tetrachloroethylene (Hartmann and Speit, 1995; Galloway et al., 1987; NTP, 1986) and only one occupational exposure study of exposures to tetrachloroethylene and trichloroethylene (Fender, 1993) reported sister chromatid exchanges.

4.8.1.1.4. Unscheduled DNA synthesis

Human fibroblasts (WI-38 cells) were assayed for unscheduled DNA synthesis following exposure to tetrachloroethylene (0.1 to 5.0 μL/mL), but the results were equivocal, with results at low doses similar to the positive controls and negative results at high doses, but it is noted that the high doses yielded considerable cytotoxicity (Beliles et al., 1980). The positive controls were only weakly positive, as described based on the laboratory criteria (criteria details not given). No evidence of unscheduled DNA synthesis was observed in human lymphocytes, human fibroblasts, or rat and mouse hepatocytes (Milman et al., 1988; Shimada et al., 1985; Costa and Ivanetich, 1984; Perocco et al., 1983). In summary, UDS was not statistically significantly increased in any published studies, although some increases were observed in one study (Beliles et al., 1980).

4.8.1.1.5. DNA strand breaks

An increased level of DNA single-strand breaks (SSB), as assessed by a DNA unwinding technique, was observed in liver and kidney tissues but not in the lung tissue of male NMRI mice 1 hour after single i.p. injections in Tween 80 of 4−8 mmol/kg (663−1,326 mg/kg) of tetrachloroethylene (Walles, 1986). This effect was reversible as early as 24 hours postexposure, presumably by DNA repair. Limitations of i.p. injection include the potential inflammatory effect at the site of injection, which could, in turn, lead to production of reactive oxygen species and other inflammatory mediators. These could lead to an increase in DNA damage unrelated to the specific exposure. Potter et al. (1996) found no increases in DNA strand breaks, when assessed by an alkaline unwinding procedure, in kidneys of male F344 rats assessed after daily oral gavage treatment with 1,000 mg/kg tetrachloroethylene for 7 days. A more recent study (Cederberg et al., 2010a) found oral gavage exposure to tetrachloroethylene (1,000 or 2,000 mg/kg-day given as two administrations, 24 hours apart, in corn oil) led to slight increases (1.3- and 1.4-fold as compared to control) in DNA damage in liver (but not kidney) of CD1 mice as measured by the alkaline Comet assay when tissues were sampled 3 hours after the last administration. Others have interpreted these data to demonstrate a lack of DNA damage in the liver and kidney of CD1 mice after oral tetrachloroethylene exposure [presented in Dreessen (2003) and in Lillford (2010)]. Cederberg et al. (2010a) reported a statistically significant dose-related increase in tail intensity ($p = 0.041$; one-sided Jonckheere-Terpstra test using exact

permutation) in the liver following exposure to PCE. The authors note that 8 of 12 tetrachloroethylene-exposed animals had higher tail intensity values than the highest value in the controls, a finding significant by the Fisher exact probability test ($p = 0.013$). No statistically significant effects were observed for tail moment in the liver, or for either tail intensity or moment in the kidney. The alternative interpretation is that the variability between mice in the treatment groups and the low magnitude of the response in the tetrachloroethylene-dosed animals does not support the conclusion that tetrachloroethylene induced DNA damage in this study. This interpretation is supported by the lack of statistical significance when the results are analyzed by Dunnett's test for pairwise comparisons. Cederberg et al. (2010a) argue that the interindividual animal variability is not exceptionally large, and that the Dunnett's pairwise test has less power than the trend test of Jonckheere-Terpstra (Cederberg et al., 2010a). Further discussion of this publication in the literature is ongoing (Lillford et al., 2010; Lovell, 2010). Lillford et al. (2010) give additional details on the alternative interpretation described in the original paper, stating also that the limited biological significance of these slight increases in tail intensity needs to be taken into account. This paper states that the results described in the original study are within the range of historical controls in the study laboratory. Lillford et al. (2010) endorse the use of the parametric test for statistical analysis (Dunnett's), which showed no statistical significance for the results reported in Cederberg. The third publication discusses the use of various statistical analyses used in the two interpretations (Lovell, 2010). Overall, Lovell (2010) states that it is not a question of one statistical analysis being right and the other wrong; it is more a question of using the best statistical analysis for the hypothesis being tested. The different approaches show a contrast between a powerful trend test and a more conservative pairwise comparison. Lovell (2010) also commented on the magnitude of the response as it relates to biological relevance. Further studies, as suggested by Cederberg et al. (2010b), may or may not address this issue if carried out the same way as the original study. Finally, both Lillford et al. (2010) and Lovell (2010) agree that the statistical analysis utilized should not be used as the sole determinant of how the results of this, or any study, are interpreted.

In summary, the results of the limited DNA strand break assays following exposure to tetrachloroethylene are equivocal. Walles (1986) demonstrated DNA single-strand breaks in the liver and kidney of male mice exposed by i.p. injection, but this was reversible within 24 hours. A second study examined DNA strand breaks after 1 week oral exposure to tetrachloroethylene and demonstrated no DNA damage (Potter et al., 1996). A recently published report on DNA strand breaks showed a marginal increase in only one parameter from the Comet assay (tail length) following oral exposure to tetrachloroethylene in mice (Cederberg et al., 2010a), but the statistical and biological significance of this result has been disputed (Cederberg et al., 2010b; Lillford et al., 2010; Lovell, 2010).

4.8.1.1.6. DNA damage related to oxidative stress

Toraason et al. (2003) reported no increase in leukocyte 8-OHdG in 18 dry-cleaner workers compared with 20 launderers, and reported no increase in urinary 8-OHdG among the dry-cleaner workers sampled pre- and postshift work (time-weighted average [TWA] concentration of tetrachloroethylene was 3.8 ± 5.3 ppm). Under the conditions of this study, no evidence of oxidative DNA damage was found. Toraason et al. (1999) measured 8-OHdG and a "free radical-catalyzed isomer of arachidonic acid and marker of oxidative damage to cell membranes, 8-Epi-prostaglandin F2α (8-epiPGF)," excretion in the urine, and TBARS (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats exposed to single i.p. injections of tetrachloroethylene in Alkamuls vehicle. Male Fischer rats sacrificed 24 hours after a single i.p. injection of tetrachloroethylene (0, 100, 500, or 1,000 mg/kg) showed no significant increases in 8-OHdG in liver, lymphocytes, or urine (Toraason et al., 1999). Lipid peroxidation of the liver (as measured by TBARS) was also not observed following a single exposure to tetrachloroethylene. However, the authors reported morbidity and mortality with a single 500 mg/kg tetrachloroethylene exposure inducing Stage II anesthesia (loss of righting reflex but maintained reflex response) and a single 1,000 mg/kg tetrachloroethylene exposure inducing Level III or IV (absence of reflex response) anesthesia and burgundy-colored urine during the first 12 hours of collection. Although none of the rats exposed to 1,000 mg/kg tetrachloroethylene died from treatment, the authors state that some in this high-dose group would not have survived another 24 hours. Thus, using this paradigm, there was significant toxicity and additional issues related to route of exposure. Urine volume declined significantly during the first 12 hours of treatment, and while water consumption was not measured, it was suggested by the authors to be decreased due to the moribundity of the rats. Although the authors suggest that evidence of oxidative damage was equivocal, the effects on urine volume and water consumption, as well as the profound toxicity induced by this exposure paradigm, limit interpretation of these data. In summary, the limited studies examining DNA adduct formation related to oxidative stress are inconclusive, with no results in the urine or leukocytes of occupationally exposed individuals and limited utility of the animal study due to significant toxicity in the exposed animals.

4.8.1.1.7. Cell transformation

Tetrachloroethylene exposure did not lead to cell transformation in BALB/c-3T3 cells after 3-day exposure (0, 1, 10, 100, and 250 µg/mL) followed by a 30-day incubation period (Tu et al., 1985). Exposure to tetrachloroethylene (study details not given) was also negative for cell transformation in BALB/c-3T3 cells (Milman et al., 1988). However, Fischer rat embryo cells were transformed in the absence of metabolic activation (Price et al., 1978).

4.8.1.1.8. Gap junction intercellular communication

One assay examined gap junction intercellular communication following exposure to tetrachloroethylene in rat liver cells (0, 0.01, 0.1, and 1 mM at 0, 1, 4, 6, 24, 48, and 168 hours) (Benane et al., 1996). Communication was inhibited following exposure to 0.1-mM tetrachloroethylene at 48 hours and continued at the final time point tested (168 hours). This study also examined tetrachloroethylene metabolites, including DCA, TCA, CH, and trichloroethanol. These metabolites also led to decreases in intercellular communication, but to varying levels.

4.8.1.1.9. Tumor initiation

Milman et al. (1988) reported a statistically significant increase ($p < 0.01$) in γ-glutamyltranspeptidase-positive liver foci in a promotion, but not in an initiation, test protocol in male Osborne-Mendel rats. Initiation capacity was tested by exposing 10 rats to 1,000 mg/kg tetrachloroethylene after partial hepatectomy, followed by phenobarbital promotion for 7 weeks. In the promotion test, rats were initiated with DEN after partial hepatectomy, followed by promotion with tetrachloroethylene for 7 weeks. In a separate initiation study of neonatal female Wistar rats exposed to 2,000 ppm, 8 hours/day, 5 days/week, for 10 weeks [described in Bolt et al. (1982), as reported in NRC (2010)], preneoplastic liver foci were reportedly not observed.

4.8.1.2. *Drosophila Melanogaster*

Limited tetrachloroethylene genotoxicity studies have been performed in *Drosophila melanogaster*. One study was negative for both the induction of sex-linked recessive lethal mutations and chromosomal aberrations following inhalation exposure to tetrachloroethylene in *D. melanogaster* (up to 3,400 mg/m^3 for 7 hours) (Beliles et al., 1980). The frequencies of the sex-linked recessive lethal mutations were 0 and 0.10% for the low- and high-dose exposures, respectively, which was not significantly different from the negative control (0.11%). This study also showed no chromosomal aberrations, as there were no significant loses of the long arm of the Y chromosome for either the low (0.11%) or high (0.02%) doses as compare to the negative control (0.02%). A second study, also negative for sex-linked recessive lethal mutations, exposed male *Drosophila* by feeding tetrachloroethylene (4,000 ppm) or by injection (1,000 ppm) before successive mating with untreated females for 3 days (NTP, 1986; Valencia et al., 1985). F1 heterozygous daughters were mated to their siblings. Analysis of the data after 17 days demonstrated no significant increase in sex-linked recessive lethal mutations following exposure to tetrachloroethylene.

4.8.1.3. Bacterial and Fungal Systems

Cells of *Saccharomyces cerevisiae* contain cytochrome P450 monooxygenase system and are capable of metabolizing promutagens to genetically active products. Tetrachloroethylene alone was positive for mitotic recombination in yeast following 1 hour exposure to 6.6 mM tetrachloroethylene (Callen et al., 1980) but negative in yeast exposed in suspension with metabolic activation or in the intrasinguineous hose-mediated assay (Koch et al., 1988; Bronzetti et al., 1983). Results were negative in the same assay for tetrachloroethylene, but the high level of cytotoxicity in this assay at the dose used (9.8 mM) limits the interpretation of these results (Koch et al., 1988). Bronzetti et al. (1983) also demonstrated negative results both in vitro (0, 5, 10, 20, 60, and 85 mM) with and without S9 activation. There also appeared to be high cytotoxicity in yeast cells exposed to high dose tetrachloroethylene based on decreasing percentage survival in this study, which may also limit the interpretation of these data.

A number of in vitro genotoxicity assays have been performed using prokaryotic cells. Studies of mutagenicity on *Escherichia coli* have been negative (Greim et al., 1975; also reported in Henschler, 1977). Most Ames tests using *S. typhimurium* have indicated that tetrachloroethylene in the absence of metabolic activation or in the presence of the standard S9 fraction is not a mutagen (Watanabe et al., 1998; DeMarini et al., 1994; Roldán-Arjona et al., 1991; Milman et al., 1988; Warner et al., 1988; NTP, 1986; Connor et al., 1985; Shimada et al., 1985; Haworth et al., 1983; Hardin et al., 1981; Kringstad et al., 1981; Bartsch et al., 1979; Greim et al., 1975). However when incubated with rat liver GST, GSH, and a rat kidney fraction, tetrachloroethylene exhibited a clear dose response (Vamvakas et al., 1989d). Specifically, this study demonstrated the mutagenicity in *S. typhimurium* (primarily strain TA100) of tetrachloroethylene that had been preincubated with rat liver GST, GSH, and rat kidney microsomes, and of TCVG that had been preincubated with rat kidney microsomes. Additionally, the bacterial mutagenicity of bile from liver perfusate following tetrachloroethylene exposure in rats was demonstrated (Vamvakas et al., 1989d). These results support a role for GSH conjugation in the genotoxicity of tetrachloroethylene.

A more recent study examined genotoxicity of tetrachloroethylene in an *S. typhimurium* strain (YG7108pin3ERb5) with enhanced metabolic activity (transformed with CYP2E1, cytochrome P450 reductase, and cytochrome b5),which led to microcolony formation believed to be from toxicity of tetrachloroethylene metabolites formed at 200 and 1,000 µg doses (but not at the higher doses of 2,000 or 3,000 µg) (Emmert et al., 2006). Tetrachloroethylene was negative in the parent strain (YG7108) at all doses in the presence of S9. These results support a role for CYP2E1-derived metabolites in the toxicity of tetrachloroethylene, but not the mutagenicity of tetrachloroethylene.

In summary, gene mutations were not observed following exposure to tetrachloroethylene in *E. coli* or *S. typhimurium* cells in the absence of metabolic activation. Addition of standard S9 fraction also did not lead to mutagenicity, but exposure to bacterial cells with enhanced metabolic activity (CYP2E1 GSH) led to positive Ames test results. These support a role of metabolic activation of tetrachloroethylene in its genotoxicity. Results in yeast cells are conflicting, with one positive study (Callen et al., 1980) and two negative studies (Koch et al., 1988; Bronzetti et al., 1983). However, tetrachloroethylene led to cytotoxicity of *S. cerevisiae* at the doses tested, making interpretation of these results difficult. These results, although limited, suggest tetrachloroethylene exposure can lead to genotoxicity in the presence of appropriate metabolic activation.

4.8.1.4. Summary

The in vitro thymidine kinase gene mutation assay in mammalian cells was negative in the presence and absence of S9 (F344 rat liver) metabolic activation (NTP, 1986). Positive results for frameshift mutation were observed in a host-mediated assay by implanting *S. typhimurium* into mice exposed to tetrachloroethylene, but without a clear dose-response effect (Beliles et al., 1980). Studies of mutagenicity on *E. coli* have been negative (Greim et al., 1975) and also reported in Henschler (1977). A number of mutagenicity studies in *S. typhimurium* indicate that, in the absence of metabolic activation or in the presence of the standard S9 fraction, tetrachloroethylene is not a mutagen (Emmert et al., 2006; Watanabe et al., 1998; DeMarini et al., 1994; Roldán-Arjona et al., 1991; Milman et al., 1988; Warner et al., 1988; NTP, 1986; Connor et al., 1985; Shimada et al., 1985; Haworth et al., 1983; Hardin et al., 1981; Kringstad et al., 1981; Bartsch et al., 1979; Greim et al., 1975). However, when tetrachloroethylene was activated with rat liver GST, GSH, and a rat kidney fraction, tetrachloroethylene exhibited a clear dose-response (Vamvakas et al., 1989d). These findings support a role of metabolic activation of tetrachloroethylene in its in vitro genotoxicity. Results in yeast cells are conflicting, with one positive study (Callen et al., 1980) and two negative studies (Koch et al., 1988; Bronzetti et al., 1983). However, tetrachloroethylene led to cytotoxicity of *S. cerevisiae* at the doses tested, making interpretation of these results difficult. These results, although limited, suggest tetrachloroethylene exposure can lead to genotoxicity in the presence of appropriate metabolic activation.

DNA binding was not observed in one assay in mice exposed to tetrachloroethylene by inhalation and oral routes, while protein and RNA binding was observed (Schumann et al., 1980). With a more sensitive assay, low levels of DNA binding were observed in mouse liver, and even lower levels in mouse kidney and rat and mouse stomach after i.p. injection exposure (Mazzullo et al., 1987). In vitro binding to calf thymus DNA occurred in the presence of various

microsomal fractions, as well as in the presence of cytosolic fractions from mice and rats. These results suggest a role for metabolic activation of the parent compound in DNA binding.

The majority of studies of chromosomal aberrations, micronuclei induction, and sister chromatid exchange following exposure to tetrachloroethylene are negative. Positive micronuclei induction was observed following partial hepatectomy at high doses (2,000 mg/kg-day) in mice (Murakami and Horikawa, 1995). Increased micronuclei induction was observed in CHO cells in vitro when exposed to tetrachloroethylene in a closed system (Wang et al., 2001) but not in CHL cells when exposed in an open system (Matsushima et al., 1999). Dose-dependent increases were observed in human lymphoblastoid cell lines that were enhanced by stable expression of CYP450 enzymes (White et al., 2001; Doherty et al., 1996). Sister chromatid exchanges were not observed in any in vitro studies (Hartmann and Speit, 1995; Galloway et al., 1987; NTP, 1986) and were observed in only one occupational exposure study, but exposures were contaminated with trichloroethylene, so the interpretation of these results is limited (Fender, 1993).

Although some increases were observed in UDS following exposure, these were not statistically significant (NTP, 1986). The results of DNA strand break assays following exposure to tetrachloroethylene are equivocal. Walles (1986) demonstrated DNA single-strand breaks in the liver and kidney of male mice exposed by i.p. injection, but this was reversible within 24 hours. A second study examined DNA strand breaks after 1 week oral exposure to tetrachloroethylene, and demonstrated no DNA damage (Potter et al., 1996). A study of DNA strand breaks showed a marginal increase in only one parameter from the alkaline Comet assay (tail intensity) in the liver but not the kidney following oral exposure to tetrachloroethylene in mice (Cederberg et al., 2010a), but the statistical and biological significance of this result has been disputed (Cederberg et al., 2010b; Lillford et al., 2010; Lovell, 2010).

Studies examining DNA adduct formation related to oxidative stress are inconclusive, with no results in the urine or leukocytes of occupationally exposed individuals (Toraason et al., 2003) and limited utility of the animal study due to significant toxicity in the exposed animals (Toraason et al., 1999). Tumor initiation was not observed in Milman et al. (1988) or Bolt et al. (1982), but the former study reported significant increases in liver foci in a tumor promotion study. A study examining inhibition of gap junction intercellular communication was positive (Benane et al., 1996). Negative results were found for a limited number of other genotoxicity endpoints including cell transformations (Tu et al., 1985) and sex-linked recessive lethal mutation assay in *Drosophila* [(NTP, 1986; Beliles et al., 1980); also reported in Valencia et al. (1985)].

Overall, evidence from a number of different analyses with various genetic endpoints indicates that tetrachloroethylene has the potential to induce damage to the structure of the

chromosome in a number of targets but has little-to-no ability to induce mutation in bacterial systems in the absence of metabolic activation or with the standard S9 fraction. However, metabolic activation via GSH conjugation or cytochrome P450s yields positive results in bacterial mutagenicity assays.

4.8.2. Trichloroacetic Acid (TCA)

The tetrachloroethylene metabolite TCA has been studied using a variety of genotoxicity assays for its genotoxic potential [refer to International Agency for Research on Cancer (2004) and U.S. EPA (2011c) for additional information]. Evaluation of in vitro studies of TCA must consider toxicity and acidification of medium resulting in precipitation of proteins, as TCA is commonly used as a reagent to precipitate proteins. These studies are summarized in Tables 4-41 and 4-42.

4.8.2.1. Mammalian Systems (Including Human Studies)

4.8.2.1.1. Gene mutations

The mutagenicity of TCA has also been tested in cultured mammalian cells (refer to Table 4-41). Harrington-Brock et al. (1998) examined the potential of TCA to induce mutations in L5178Y/TK+/- −3.7.2C mouse lymphoma cells. In this study, mouse lymphoma cells were incubated in a culture medium treated with TCA concentrations up to 2,150 µg/mL in the presence of S9 metabolic activation and up to 3,400 µg/mL in the absence of S9 mixture. In the presence of S9, a doubling of mutant frequency was observed at concentrations of 2,250 µg/mL and higher, including several concentrations with survival >10%. In the absence of S9, TCA increased the mutant frequency by twofold or greater only at concentrations of 2,000 µg/mL or higher. These results were obtained at ≤11% survival rates. The authors noted that the mutants included both large-colony and small-colony mutants. The small-colony mutants are indicative of chromosomal damage. It should be noted that no rigorous statistical evaluation was conducted on these data.

Table 4-41. Genotoxicity of trichloroacetic acid (TCA)—mammalian systems (in vitro and in vivo)[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma L5178Y/TK+/- cells, in vitro	3,000	(+)	?	Harrington-Brock et al. (1998)
DNA strand breaks, B6C3F$_1$ mouse and Fischer 344 rat hepatocytes, in vitro	1,630	NT	−	Chang et al. (1992)
DNA strand breaks, human CCRF-CEM lymphoblastic cells, in vitro	1,630	NT	−	Chang et al. (1992)
DNA damage, Chinese hamster ovary cells, in vitro, comet assay	3 mM	NT	−	Plewa et al. (2002)
DNA strand breaks, B6C3F$_1$ mouse liver, in vivo	1.0, p.o., ×1	NT	+	Nelson and Bull (1988)
DNA strand breaks, B6C3F$_1$ mouse liver, in vivo	500, p.o., ×1	NT	+	Nelson et al. (1989)
DNA strand breaks, B6C3F$_1$ mouse liver, in vivo	500, p.o., 10 repeats	NT	−	Nelson et al. (1989)
DNA strand breaks, B6C3F$_1$ mouse liver and epithelial cells from stomach and duodenum, in vivo	1,630, p.o., ×1	NT	−	Chang et al. (1992)
DNA strand breaks, male B6C3F$_1$ mice, in vivo	500 (neutralized)	NT	−	Styles et al. (1991)
DNA strand breaks, male B6C3F$_1$ mouse liver, in vivo	300, p.o.	NT	+	Hassoun and Dey (2008)
Micronucleus formation, Swiss mice, in vivo	125, i.p., ×2	NT	+	Bhunya and Behera (1987)
Micronucleus formation, female C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, in vivo	1,300, i.p., ×2	NT	−	Mackay et al. (1995)
Micronucleus formation, male C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, in vivo	1,080, i.p., ×2	NT	−	Mackay et al. (1995)
Micronucleus formation, *Pleurodeles waltl* larvae peripheral erythrocytes, in vivo	80	NT	+	Giller et al. (1997)
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	125, i.p., ×1	NT	+	Bhunya and Behera (1987)
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	100, i.p., ×5	NT	+	Bhunya and Behera (1987)
Chromosomal aberrations, Swiss mouse bone-marrow cells in vivo	500, p.o., ×1	NT	+	Bhunya and Behera (1987)
Chromosomal aberrations, chicken *Gallus domesticus* bone marrow, in vivo	200, i.p., ×1	NT	+	Bhunya and Jena (1996)

Table 4-41. Genotoxicity of trichloroacetic acid (TCA)—mammalian systems (in vitro and in vivo)[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
Chromosomal aberrations, human lymphocytes, in vitro	5,000 (neutralized)	NT	−	Mackay et al. (1995)
Sperm morphology, Swiss mouse, in vivo	125, i.p., ×5	NT	+	Bhunya and Behera (1987)
Increased detection of M_1G and 8-OHdG adducts, $B6C3F_1$ neonatal mouse liver DNA, in vivo	2,000 nmol	NT	+	Von Tungeln et al. (2002)

[a]Table adapted from ATSDR (1997a) and IARC monograph (1995) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests; mg/kg for in vivo tests unless specified.
[c]Results: + = positive; (+) = weakly positive; − = negative; NT = not tested; ? = inconclusive.

4.8.2.1.2. Chromosomal aberrations

Mackay et al. (1995) investigated the ability of TCA to induce chromosomal damage in an in vitro chromosomal aberration assay using cultured human cells. The authors treated the cells with TCA as free acid, both in the presence and absence of metabolic activation. TCA induced chromosomal damage in cultured human peripheral lymphocytes at concentrations (2,000 and 3,500 µg/mL) that significantly reduced the pH of the medium. However, exposure of cells to neutralized TCA did not have any effect, even at a cytotoxic concentration of 5,000 µg/mL. It is possible that the reduced pH was responsible for the TCA-induced clastogenicity in this study. To further evaluate the role of pH changes in the induction of chromosome damage, the authors isolated liver-cell nuclei from $B6C3F_1$ mice and suspended the isolates in a buffer at various pH levels. The cells were stained with chromatin-reactive (fluorescein isothiocyanate) and DNA-reactive (propidium iodide) fluorescent dyes. A decrease in chromatin staining intensity was observed with the decrease in pH, suggesting that pH changes, independent of TCA exposure, can alter chromatin conformation. It was concluded by the authors that TCA-induced pH changes are likely to be responsible for the chromosomal damage induced by unneutralized TCA. In another in vitro study, Plewa et al. (2002) evaluated the induction of DNA strand breaks by TCA (1−25 mM) in CHO cells and did not observe any genotoxicity.

4.8.2.1.2.1. Micronucleus induction

Genotoxicity of TCA was tested in a mouse in vivo system using three different cytogenetic assays (bone marrow chromosomal aberrations, micronucleus and sperm-head

abnormalities) (Bhunya and Behera, 1987) and for chromosomal aberrations in chicken (Bhunya and Jena, 1996). TCA induced a variety of anomalies including micronucleus in the bone marrow of mice and chicken. A small increase in the frequency of micronucleated erythrocytes at 80 µg/mL in a newt (*Pleurodeles waltl* larvae) micronucleus test was observed in response to TCA exposure (Giller et al., 1997). Mackay et al. (1995) investigated the ability of TCA to induce chromosomal DNA damage in the in vivo bone-marrow micronucleus assay in mice. C57BL mice were given TCA i.p. at doses of 0, 337, 675, or 1,080 mg/kg-day for males and 0, 405, 810, or 1,300 mg/kg-day for females for 2 consecutive days, and bone-marrow samples were collected 6 and 24 hours after the last dose. The administered doses represented 25, 50, and 80% of the median lethal dose, respectively. No treatment-related increase in micronucleated polychromatic erythrocytes was observed.

4.8.2.1.2.2. DNA damage studies

DNA unwinding assays have been used as indicators of single-strand breaks. Studies were conducted on the ability of TCA to induce DNA single-strand breaks [(Chang et al., 1992; Styles et al., 1991; Nelson et al., 1989; Nelson and Bull, 1988); Table 4-12]. Nelson and Bull (1988) evaluated the ability of TCA and other compounds to induce DNA single-strand breaks in vivo in Sprague-Dawley rats and $B6C3F_1$ mice. Single oral doses were administered to three groups of three animals, with an additional group as a vehicle control. Animals were sacrificed after 4 hours, and 10% liver suspensions were analyzed for DNA single-strand breaks by the alkaline unwinding assay. Dose-dependent increases in DNA single-strand breaks were induced in both rats and mice, with mice being more susceptible than rats. The lowest dose of TCA that produced significant SSBs was 0.6 mmol/kg (98 mg/kg) in rats but 0.006 mmol/kg (0.98 mg/kg) in mice.

Table 4-42. Genotoxicity of trichloroacetic acid (TCA)—bacterial systems[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
λ Prophage induction, *E. coli* WP2s	10,000	–	–	DeMarini et al. (1994)
SOS chromotest, *E. coli* PQ37	10,000	–	–	Giller et al. (1997)
S. typhimurium TA1535, 1536, 1537, 1538, reverse mutation	20 µg/plate	NT	–	Shirasu et al. (1976)
S. typhimurium TA100, 98, reverse mutation	450 µg/plate	–	–	Waskell (1978)
S. typhimurium TA100, 1535, reverse mutation	4,000 µg/plate	–	–	Nestmann et al. (1980)
S. typhimurium TA1537, 1538, 98, reverse mutation	2,000 µg/plate	–	–	Nestmann et al. (1980)
S. typhimurium TA100, reverse mutation	520 µg/plate	NT	–	Rapson et al. (1980)
S. typhimurium TA100, 98, reverse mutation	5,000 µg/plate	–	–	Moriya et al. (1983)
S. typhimurium TA100, reverse mutation	600 ppm	–	–	DeMarini et al. (1994)
S. typhimurium TA100, reverse mutation, liquid medium	1,750	+	+	Giller et al. (1997)
S. typhimurium TA104, reverse mutation, microsuspension	250 µg/plate	–	–	Nelson et al. (2001b)
S. typhimurium TA100, RSJ100, reverse mutation	16,300	–	–	Kargalioglu et al. (2002)
S. typhimurium TA98, reverse mutation	13,100	–	–	Kargalioglu et al. (2002)
S. typhimurium TA1535, SOS DNA repair	NA	+	–	Ono et al. (1991)

[a]Table adapted from IARC monograph (2004) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests, unless otherwise specified.
[c]Results: + = positive; – = negative; NT = not tested.

However, in a follow-up study (Nelson et al., 1989), no significant differences from controls in DNA single-strand breaks in whole liver homogenates were observed in male $B6C3F_1$ mice exposed to 500 mg/kg TCA. Moreover, DCA increased single-strand breaks but with no dose response between 10 and 500 mg/kg, raising concerns about the reliability of the DNA unwinding assay used in these studies. In an additional follow-up experiment with a similar experimental paradigm, Styles et al. (1991) tested TCA for its ability to induce strand

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breaks in male B6C3F$_1$ mice in the presence and absence of liver growth induction. The test animals were given 1, 2, or 3 daily doses of neutralized TCA (500 mg/kg) by gavage and killed 1 hour after the final dose. Additional mice were given a single 500 mg/kg gavage dose and sacrificed 24 hours after treatment. Liver nuclei DNA were isolated, and the induction of single-strand breaks was evaluated using the alkaline unwinding assay. Exposure to TCA did not induce strand breaks under the conditions tested in this assay. In a study by Chang et al. (1992), administration of single oral doses of TCA (1 to 10 mmol/kg) to B6C3F$_1$ mice did not induce DNA strand breaks in a dose-related manner as determined by the alkaline unwinding assay. No genotoxic activity (evidence for strand breakage) was detected in F344 rats administered by gavage up to 5 mmol/kg (817 mg/kg).

In summary, Nelson and Bull (1988) reported that DCA and TCA enhance DNA unwinding in mice, with DCA having the highest activity and TCA the lowest. However, Nelson et al. (1989) reported no effect for TCA and a lack of dose response for the effect of DCA (with 10 and 500 mg/kg DCA inducing the same magnitude of effect). Moreover, Styles et al. (1991) did not report a positive result for TCA using the same paradigm as Nelson and Bull (1988) and Nelson et al. (1989). Furthermore, Chang et al. (1992) also did not find increased DNA single-strand breaks for TCA exposure in rats.

4.8.2.2. Bacterial Systems

4.8.2.2.1. Gene mutations

TCA has been evaluated in a number of in vitro test systems including the bacterial assays (Ames) using different *S. typhimurium* strains such as TA98, TA100, TA104, TA1535, and RSJ100 (refer to Table 4-42). The majority of these studies did not report positive findings for genotoxicity (Kargalioglu et al., 2002; Nelson et al., 2001b; DeMarini et al., 1994; Moriya et al., 1983; Nestmann et al., 1980; Rapson et al., 1980; Waskell, 1978; Shirasu et al., 1976). Waskell (1978) studied the effect of TCA (0.45 mg/plate) on bacterial strains TA98 and TA100 both in the presence and absence of S9. The author did not find any revertants at the maximum nontoxic dose tested. Following exposure to TCA, Rapson et al. (1980) reported no change in mutagenic activity in strain TA100 in the absence of S9. DeMarini et al. (1994) performed different studies to evaluate the genotoxicity of TCA, including the Microscreen prophage-induction assay (TCA concentrations: 0 to 10 mg/mL) and use of the *S. typhimurium* TA100 strain using bag vaporization technique (TCA concentrations: 0−100 ppm), neither of which yielded positive results. Nelson et al. (2001b) reported no positive findings with TCA using a *S. typhimurium* microsuspension bioassay (*S. typhimurium* strain TA104) following incubation of TCA for various lengths of time, with or without rat cecal microbiota. Similarly, no activity was observed in a study conducted by Kargalioglu et al. (2002) where *S. typhimurium* strains TA98,

TA100, and RSJ100 were exposed to TCA (0.1−100 mM) either in the presence or absence of S9 (Kargalioglu et al., 2002).

TCA was also negative in other bacterial systems. The SOS chromotest (which measures DNA damage and induction of the SOS repair system) in *E. coli* PQ37, with and without S9 (Giller et al., 1997), evaluated the genotoxic activity of TCA ranging from 10 to 10,000 µg/mL, and no response was reported. Similarly, TCA was not genotoxic in the Microscreen prophage-induction assay in *E. coli* with TCA concentrations ranging from 0 to 10,000 µg/mL, with and without S9 activation (DeMarini et al., 1994).

However, TCA induced a small increase in SOS DNA repair (an inducible error-prone repair system) in *S. typhimurium* strain TA1535 in the presence of S9 (Ono et al., 1991). Furthermore, Giller et al. (1997) reported that TCA demonstrated genotoxic activity in an Ames fluctuation test in *S. typhimurium* TA100 in the absence of S9 at noncytotoxic concentrations ranging from 1,750 to 2,250 µg/mL. The addition of S9 decreased the genotoxic response, with effects observed at 3,000−7,500 µg/mL. Cytotoxic concentrations in the Ames fluctuation assay were 2,500 and 10,000 µg/mL, without and with microsomal activation, respectively.

4.8.2.3. Summary

TCA, an oxidative metabolite of tetrachloroethylene, exhibits little, if any genotoxic activity in vitro. TCA did not induce mutations in *S. typhimurium* strains in the absence of metabolic activation or in an alternative protocol using a closed system (Kargalioglu et al., 2002; Nelson et al., 2001b; Giller et al., 1997; DeMarini et al., 1994; Rapson et al., 1980; Waskell, 1978), but a mutagenic response was induced in TA100 in the Ames fluctuation test (Giller et al., 1997). However, in vitro experiments with TCA should be interpreted with caution if steps have not been taken to neutralize pH changes caused by the compound (Mackay et al., 1995). Measures of DNA-repair responses in bacterial systems have shown induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations (Harrington-Brock et al., 1998). TCA was positive in some genotoxicity studies in vivo mouse, newt, and chick test systems (Giller et al., 1997; Bhunya and Jena, 1996; Birner et al., 1994; Bhunya and Behera, 1987). DNA unwinding assays have either shown TCA to be much less potent than DCA (Nelson and Bull, 1988) or negative (Styles et al., 1991; Nelson et al., 1989). Due to limitations in the genotoxicity database, the possible contribution of TCA to tetrachloroethylene genotoxicity is unclear.

4.8.3. Dichloroacetic Acid (DCA)

DCA is another metabolite of tetrachloroethylene that has been studied using a variety of genotoxicity assays for its genotoxic potential [refer to Tables 4-43 and 4-44; refer to IARC (2004) for additional information].

4.8.3.1. Mammalian Systems

4.8.3.1.1. Gene mutations

The mutagenicity of DCA has been tested in mammalian systems, particularly, mouse lymphoma cell lines in vitro (Harrington-Brock et al., 1998; Fox et al., 1996) and lacI transgenic mice in vivo (Leavitt et al., 1997). Harrington-Brock et al. (1998) evaluated DCA for mutagenic activity in L5178Y/TK +/- (-) 3.7.2C mouse lymphoma cells. A dose-related increase in mutation (and cytotoxic) frequency was observed at concentrations between 100 and 800 µg/mL. Most mutagenic activity of DCA at the Tk locus was due to the production of small-colony Tk mutants (indicating chromosomal mutations). Different pH levels were tested in induction of mutant frequencies, and it was determined that the mutagenic effect observed was due to the chemical and not pH effects.

Mutation frequencies were studied in male transgenic $B6C3F_1$ mice harboring the bacterial lacI gene administered DCA at either 1.0 or 3.5 g/L in drinking water (Leavitt et al., 1997). No significant difference in mutant frequency was observed after 4 or 10 weeks of treatment in both the doses tested as compared to control. However, at 60 weeks, mice treated with 1.0 g/L DCA showed a slight increase (1.3-fold) in the mutant frequency over the control, but mice treated with 3.5 g/L DCA had a 2.3-fold increase in the mutant frequency. Mutational spectra analysis revealed that ~33% had G:C-A:T transitions and 21% had G:C−T:A transversions, and this mutation spectra was different than that was observed in the untreated animals, indicating that the mutations were likely induced by the DCA treatment. The authors conclude that these results are consistent with the previous observation that the proportion of mutations at T:A sites in Codon 61 of the H-ras gene was increased in DCA-induced liver tumors in $B6C3F_1$ mice (Leavitt et al., 1997).

4.8.3.1.2. Chromosomal aberrations and micronucleus induction

Harrington-Brock et al. (1998) evaluated DCA for its potential to induce chromosomal aberrations in DCA-treated (0, 600, and 800 µg/mL) mouse lymphoma cells. A clearly positive induction of aberrations was observed at both concentrations tested. No significant increase in micronucleus was observed in DCA-treated (0, 600, and 800 µg/mL) mouse lymphoma cells (Harrington-Brock et al., 1998). However, no chromosomal aberrations were found in Chinese hamster ovary cells exposed to DCA (Fox et al., 1996).

Fuscoe et al. (1996) investigated in vivo genotoxic potential of DCA in bone marrow and blood leukocytes using the peripheral-blood-erythrocyte micronucleus assay (to detect chromosome breakage and/or malsegregation) and the alkaline single cell gel electrophoresis (comet) assay, respectively. Mice were exposed to DCA in drinking water, available ad libitum, for up to 31 weeks. A statistically significant dose-related increase in the frequency of micronucleated PCEs was observed following subchronic exposure to DCA for 9 days. Similarly, a significant increase was also observed when mice were exposed for ≥10 weeks, particularly at the highest dose of DCA tested (3.5 g/L). DNA cross-linking was observed in blood leukocytes in mice exposed to 3.5 g/L DCA for 28 days. These data provide evidence that DCA may have some potential to induce chromosome damage when animals are exposed to concentrations similar to those used in the rodent bioassay.

4.8.3.1.3. DNA damage studies

Nelson and Bull (1988) and Nelson et al. (1989) have been described above in Sections 4.2.2.4 and 4.2.3. Nelson and Bull (1988) reported positive results for DNA unwinding for DCA, although Nelson et al. (1989) reported the same response at 10 and 500 mg/kg in mice, raising concerns about the reliability of the assay in these studies. Chang et al. (1992) conducted both in vitro and in vivo studies to determine the ability of DCA to cause DNA damage. Primary rat (Fischer 344) hepatocytes and primary mouse hepatocytes treated with DCA for 4 hours did not induce DNA single-strand breaks as detected by the alkaline DNA unwinding assay. No DNA single-strand breaks were observed in human CCRF-CEM lymphoblastoid cells in vitro exposed to DCA. Similarly, analysis of the DNA single-strand breaks in mice killed 1 hour after a single dose of 1, 5, or 10 mM/kg DCA did not cause DNA damage. None of the Fischer 344 rats killed 4 hours after a single gavage treatment (1−10 mM/kg) produced any detectable DNA damage.

Table 4-43. Genotoxicity of dichloroacetic acid (DCA)—mammalian systems (in vitro and in vivo)[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma cell line L5178Y/TK+/- in vitro	5,000	–	–	Fox et al. (1996)
Gene mutation, mouse lymphoma cell line L5178Y/TK+/- -3.7.2C in vitro	400	NT	+	Harrington-Brock et al. (1998)
DNA strand breaks and alkali-labile damage, Chinese hamster ovary cells in vitro (single-cell gel electrophoresis assay)	3,225 µg/mL	NT	–	Plewa et al. (2002)
DNA strand breaks, $B6C3F_1$ mouse hepatocytes in vitro	2,580	NT	–	Chang et al. (1992)
DNA strand breaks, Fischer 344 rat hepatocytes in vitro	1,290	NT	–	Chang et al. (1992)
Micronucleus formation, mouse lymphoma L5178Y/TK+/- -3.7.2C cell line in vitro	800	NT	–	Harrington-Brock et al. (1998)
Micronucleus induction, peripheral blood erythrocytes, Tg.AC hemizygous mouse, dermal application in vivo	500 mg/kg	NT	–	NTP (2007)
Micronucleus induction, peripheral blood erythrocytes, Tg.AC hemizygous mouse, drinking water, in vivo	2,000 mg/L	NT	–	NTP (2007)
Micronucleus induction, peripheral blood erythrocytes, p53 haploinsufficient mouse, drinking water, in vivo	2,000 mg/L	NT	–	NTP (2007)
Micronucleus induction, peripheral blood erythrocytes, $B6C3F_1$ mouse, drinking water, in vivo	67 mg/L	NT	– (male) equivocal (female)	NTP (2007)
Chromosomal aberrations, Chinese hamster ovary in vitro	5,000	–	–	Fox et al. (1996)
Chromosomal aberrations, mouse lymphoma L5178Y/Tk+/- -3.7.2C cell line in vitro	600	NT	+	Harrington-Brock et al. (1998)
Aneuploidy, mouse lymphoma L5178Y/Tk+/- -3.7.2C cell line in vitro	800	NT	–	Harrington-Brock et al. (1998)
DNA strand breaks, human CCRF-CEM lymphoblastoid cells in vitro	1,290	NT	–	Chang et al. (1992)
DNA strand breaks, male $B6C3F_1$ mouse liver in vivo	13, p.o., ×1	NT	+	Nelson and Bull (1988)
DNA strand breaks, male $B6C3F_1$ mouse liver in vivo	10, p.o., ×1	NT	+	Nelson et al. (1989)
DNA strand breaks, male $B6C3F_1$ mouse liver in vivo	1,290, p.o., ×1	NT	–	Chang et al. (1992)

Table 4-43. Genotoxicity of dichloroacetic acid (DCA)—mammalian systems (in vitro and in vivo)[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
DNA strand breaks, male B6C3F$_1$ mouse splenocytes in vivo	1,290, p.o., ×1	NT	−	Chang et al. (1992)
DNA strand breaks, male B6C3F$_1$ mouse epithelial cells from stomach and duodenum in vivo	1,290, p.o., ×1	NT	−	Chang et al. (1992)
DNA strand breaks, male B6C3F$_1$ mouse liver in vivo	5,000, dw, ×7−14 d	NT	−	Chang et al. (1992)
DNA strand breaks, male B6C3F$_1$ mouse liver, in vivo	300, p.o.	NT	+	Hassoun and Dey (2008)
DNA strand breaks, alkali-labile sites, cross linking, male B6C3F$_1$ mouse blood leukocytes in vivo (single-cell gel electrophoresis assay)	3,500, dw, ×28 d	NT	+	Fuscoe et al. (1996)
DNA strand breaks, male Sprague-Dawley rat liver in vivo	30, p.o., ×1	NT	+	Nelson and Bull (1988)
DNA strand breaks, male Fischer 344 rat liver in vivo	645, p.o., ×1	NT	−	Chang et al. (1992)
DNA strand breaks, male Fischer 344 rat liver in vivo	2,000, dw, ×30 wk	NT	−	Chang et al. (1992)
Gene mutation, lacI transgenic male B6C3F$_1$ mouse liver assay in vivo	1,000, dw, ×60 wk	NT	+	Leavitt et al. (1997)
Altered gene expression, male B6C3F$_1$ mouse liver assay in vivo	2,000, dw, ×4 wk	NT	+	Thai et al. (2003)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes in vivo	3,500, dw, ×9 d	NT	+	Fuscoe et al. (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes in vivo	3,500, dw, ×28 d	NT	−	Fuscoe et al. (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes in vivo	3,500, dw, ×10 wk	NT	+	Fuscoe et al. (1996)
Micronucleus formation, male and female Crl:CD (S-D) BR rat bone-marrow erythrocytes in vivo	1,100, i.v., ×3	NT	−	Fox et al. (1996)
Micronucleus formation, *Pleurodeles waltl* larvae peripheral erythrocytes in vivo	80 d	NT	−	Giller et al. (1997)

[a]Table adapted from IARC monograph (2004) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests; mg/kg for in vivo tests unless specified; dw = drinking-water (in mg/L); i.v. = intravenous.
[c]Results: + = positive; − = negative; NT = not tested.

Table 4-44. Genotoxicity of dichloroacetic acid (DCA)—bacterial systems[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
λ Prophage induction, *E. coli* WP2s	2,500	+	−	DeMarini et al. (1994)
SOS chromotest, *E. coli* PQ37	500	−	(+)	Giller et al. (1997)
S. typhimurium, DNA repair-deficient strains TS24, TA2322, TA1950	31,000	−	−	Waskell (1978)
S. typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation	NA	−	−	Herbert et al. (1980)
S. typhimurium TA100, reverse mutation	50	+	+	DeMarini et al. (1994)
S. typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	5,000	−	−	Fox et al. (1996)
S. typhimurium TA100, reverse mutation, liquid medium	100	+	+	Giller et al. (1997)
S. typhimurium RSJ100, reverse mutation	1,935	−	+	Kargalioglu et al. (2002)
S. typhimurium TA104, reverse mutation, microsuspension	150 µg/plate	−	−	Nelson et al. (2001b)
S. typhimurium TA98, reverse mutation	10 µg/plate	(+)	−	Herbert et al. (1980)
S. typhimurium TA98, reverse mutation	5,160	−	+	Kargalioglu et al. (2002)
S. typhimurium TA100, reverse mutation	1,935	+	+	Kargalioglu et al. (2002)
S. typhimurium TA98, gene mutation	3 µg/plate	−	−	NTP (2007)
S. typhimurium TA100, gene mutation	333 µg/plate	−	+	NTP (2007)
S. typhimurium TA1535, gene mutation	333 µg/plate	−	+	NTP (2007)
E. coli WP2uvrA, reverse mutation	5,000	−	−	Fox et al. (1996)

[a]Table adapted from IARC monograph (2004) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests, unless otherwise specified; NA = not available.
[c]Results: + = positive; (+) = weakly positive; − = negative.

4.8.3.2. Bacterial Systems

4.8.3.2.1. Gene mutations

Studies were conducted to evaluate mutagenicity of DCA in different *S. typhimurium* and *E. coli* strains [(Kargalioglu et al., 2002; Nelson et al., 2001b; Giller et al., 1997; Fox et al., 1996; DeMarini et al., 1994; Herbert et al., 1980; Waskell, 1978); summarized in Table 4-44]. DCA was mutagenic in three strains of *S. typhimurium*: strain TA100 in three of five studies, strain RSJ100 in a single study, and strain TA98 in two of three studies. DCA failed to induce point mutations in other strains of *S. typhimurium* (TA104, TA1535, TA1537, and TA1538) or in *E. coli* strain WP2uvrA. In one study, DCA caused a weak induction of SOS repair in *E. coli* strain PQ37 (Giller et al., 1997).

DeMarini et al. (1994), in the same study as described in the TCA section (refer to Section 4.8.2), also studied DCA as one of their compounds for analysis. In the prophage-induction assay using *E. coli*, DCA, in the presence of S9, was genotoxic, producing 6.6−7.2 plaque-forming units (PFU)/mM and slightly less than threefold increase in PFU/plate in the absence of S9. In the second set of studies, which involved the evaluation of DCA at concentrations of 0−600 ppm for mutagenicity in *S. typhimurium* TA100 strain, DCA was mutagenic both in the presence and absence of S9, producing three- to fivefold increases in the revertants/plate compared to the background. The lowest effective concentration for DCA without S9 was 100 ppm and 50 ppm in the presence of S9. In the third and most important study, mutation spectra of DCA were determined at the base-substitution allele hisG46 of *S. typhimurium* TA100. DCA-induced revertants were chosen for further molecular analysis at concentrations that produced mutant yields that were two- to fivefold greater than the background. The mutation spectra of DCA were significantly different from the background mutation spectrum. Thus, despite the modest increase in the mutant yields (3−5 times) produced by DCA, the mutation spectra confirm that DCA is mutagenic. DCA primarily induced GC-AT transitions.

Kargalioglu et al. (2002) analyzed the cytotoxicity and mutagenicity of the drinking water disinfection by-products including DCA in *S. typhimurium* strains TA98, TA100, and RSJ100 +/- S9. DCA was mutagenic in this test, although the response was low when compared to other disinfection by-products tested in strain TA100. This study was also summarized in a review by Plewa et al. (2002). Nelson et al. (2001b) investigated the mutagenicity of DCA using a *S. typhimurium* microsuspension bioassay following incubation of DCA for various lengths of time, with or without rat cecal microbiota. No mutagenic activity was detected for DCA with *S. typhimurium* strain TA104. Although the data are limited, it appears that DCA has mutagenic activity in the *S. typhimurium* strains, particularly TA100.

4.8.3.3. Summary

DCA, a chloroacid metabolite of tetrachloroethylene, has also been studied using different types of genotoxicity assays. Although studies are limited for different genetic endpoints, DCA has demonstrated mutagenicity at high doses in some strains in *S. typhimurium* assays (Kargalioglu et al., 2002; Plewa et al., 2002; DeMarini et al., 1994), a mouse lymphoma assay (Harrington-Brock et al., 1998), in vivo cytogenetic tests (Leavitt et al., 1997; Fuscoe et al., 1996), the micronucleus induction test, the Big Blue mouse system, and other tests (Harrington-Brock et al., 1998; Leavitt et al., 1997; Fuscoe et al., 1996; DeMarini et al., 1994; Chang et al., 1989; Nelson et al., 1989; Nelson and Bull, 1988). DCA can cause DNA strand breaks in mouse and rat liver cells following in vivo exposures (Fuscoe et al., 1996). Because of uncertainties as to the extent of DCA formed from tetrachloroethylene exposure, inferences as to the possible contribution from DCA genotoxicity to tetrachloroethylene toxicity are difficult to make.

4.8.4. Chloral Hydrate

Although chloral hydrate is postulated as a metabolite of tetrachloroethylene, this is not widely accepted. However, to be inclusive of all known genotoxicity information, chloral hydrate genotoxicity studies have been reviewed in the following section. Chloral hydrate has been evaluated for its genotoxic potential using a variety of genotoxicity assays (refer to Tables 4-45, 4-46, and 4-47).

4.8.4.1. Mammalian Systems (Including Human Studies)

4.8.4.1.1. Gene mutations

Harrington-Brock (1998) noted that chloral hydrate-induced concentration related cytotoxicity in TK+/- mouse lymphoma cell lines without S9 activation. A nonstatistical increase in mutant frequency was observed in cells treated with chloral hydrate. The mutants were primarily small colony TK mutants, indicating that most chloral hydrate-induced mutants resulted from chromosomal mutations rather than point mutations. It should be noted that in most concentrations tested (350−1,600 µg/mL), cytotoxicity was observed. Percentage cell survival ranged from 96 to 4%.

Table 4-45. Genotoxicity of chloral hydrate—mammalian systems (in vitro)[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
DNA-protein cross-links, rat nuclei in vitro	41,250	NT	−	Keller and Heck (1988)
DNA single-strand breaks, rat primary hepatocytes in vitro	1,650	NT	−	Chang et al. (1992)
Gene mutation, mouse lymphoma L5178Y/TK+/-, in vitro	1,000	NT	(+)	Harrington-Brock et al. (1998)
Sister chromatid exchange, CHO cells, in vitro	100	+	+	Beland (1999)
Micronucleus formation (kinetochore-positive), Chinese hamster C1 cells, in vitro	165	NT	+	Degrassi and Tanzarella (1988)
Micronucleus formation (kinetochore-negative), Chinese hamster C1 cells, in vitro	250	NT	−	Degrassi and Tanzarella (1988)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Parry et al. (1990)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Lynch and Parry (1993)
Micronucleus formation, Chinese hamster V79 cells, in vitro	316	NT	+	Seelbach et al. (1993)
Micronucleus formation, mouse lymphoma L5178Y/TK+/-, in vitro	1,300	NT	−	Harrington-Brock et al. (1998)
Micronucleus formation, mouse lymphoma L5178Y/TK+/-, in vitro	500	NT	+	Nesslany and Marzin (1999)
Chromosomal aberrations, Chinese Hamster cells, in vitro	20	NT	+	Furnus et al. (1990)
Chromosomal aberrations, Chinese Hamster ovary cells, in vitro	1,000	+	+	Beland (1999)
Chromosomal aberrations, mouse lymphoma L5178Y/TK +/- cells line, in vitro	1,250	NT	(+)	Harrington-Brock et al. (1998)
Aneuploidy, Chinese hamster CHED cells, in vitro	10	NT	+	Furnus et al. (1990)
Aneuploidy, primary Chinese hamster embryonic cells, in vitro	250	NT	+	Natarajan et al. (1993)
Aneuploidy, Chinese hamster LUC2p4 cells, in vitro	250	NT	+	Warr et al. (1993)
Aneuploidy, mouse lymphoma L5178Y/TK+/- , in vitro	1,300	NT	−	Harrington-Brock et al. (1998)
Tetraploidy and endoredupliation, Chinese hamster LUC2p4cells, in vitro	500	NT	+	Warr et al. (1993)
Cell transformation, Syrian hamster embryo cells (24-h treatment)	350	NT	+	Gibson et al. (1995)

Table 4-45. Genotoxicity of chloral hydrate—mammalian systems (in vitro)[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
Cell transformation, Syrian hamster dermal cell line (24-h treatment)	50	NT	+	Parry et al. (1996)
DNA single-strand breaks, human lymphoblastoid cells, in vitro	1,650	NT	−	Chang et al. (1992)
Gene mutation, tk and hprt locus, human lymphoblastoid	1,000	NT	+	Beland (1999)
Sister chromatid exchanges, human lymphocytes, in vitro	54	NT	(+)	Gu et al. (1981)
Micronucleus formation, human lymphocytes, in vitro	100	−	+	Van Hummelen and Kirsch-Volders (1992)
Micronucleus formation, human lymphoblastoid AHH-1 cell line, in vitro	100	NT	+	Parry et al. (1996)
Micronucleus formation, human lymphoblastoid MCL-5 cell line, in vitro	500	NT	−	Parry et al. (1996)
Micronucleus formation (kinetochore-positive), human diploid LEO fibroblasts, in vitro	120	NT	+	Bonatti et al. (1992)
Aneuploidy (double Y induction), human lymphocytes, in vitro	250	NT	+	Vagnarelli et al. (1990)
Aneuploidy (hyperdiploidy and hypodiploidy), human lymphocytes in vitro	50	NT	+	Sbrana et al. (1993)
Polyploidy, human lymphocytes, in vitro	137	NT	+	Sbrana et al. (1993)
C-Mitosis, human lymphocytes, in vitro	75	NT	+	Sbrana et al. (1993)

[a]Table adapted from IARC monograph (2004) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests unless otherwise specified.
[c]Results: + = positive; (+) = weakly positive; − = negative; NT = not tested.

Table 4-46. Genotoxicity of chloral hydrate—mammalian systems (in vivo)[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]	Reference
DNA single-strand breaks, male Sprague-Dawley rat liver	300, p.o.	+	Nelson and Bull (1988)
DNA single-strand breaks, male Fischer 344 rat liver	1,650, p.o.	−	Chang et al. (1992)
DNA single-strand breaks, male $B6C3F_1$ mouse liver	100, p.o.	+	Nelson and Bull (1988)
DNA single-strand breaks, male $B6C3F_1$ mouse liver	825, p.o.	−	Chang et al. (1992)
Increased detection of M_1G and 8-OHdG adducts, $B6C3F_1$ neonatal mouse liver DNA, in vivo, i.p. injection	2,000 nmol	+	Von Tungeln et al. (2002)
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes	500, i.p.	−	Leuschner and Leuschner (1991)
Micronucleus formation, BALB/c mouse spermatids	83, i.p.	−	Russo and Levis (1992a)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids	83, i.p.	+	Russo and Levis (1992b)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes	200, i.p.	+	Russo et al. (1992)
Micronucleus formation, male F1 mouse bone-marrow erythrocytes	400, i.p.	−	Leopardi et al. (1993)
Micronucleus formation, C57B1 mouse spermatids	41, i.p.	+	Allen et al. (1994)
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini et al. (1994)
Micronucleus formation, $B6C3F_1$ mouse spermatids after spermatogonial stem-cell treatment	165, i.p.	+	Nutley et al. (1996)
Micronucleus formation, $B6C3F_1$ mouse spermatids after meiotic cell treatment	413, i.p.	_	Nutley et al. (1996)
Micronucleus formation, male F1, BALB/c mouse peripheral-blood erythrocytes	200, i.p.	_	Grawé et al. (1997)
Micronucleus formation, male $B6C3F_1$ mouse bone-marrow erythrocytes	500, i.p., ×3	+	Beland (1999)
Micronucleus formation, infants, peripheral lymphocytes	50, p.o.	+	Ikbal et al. (2004)
Chromosomal aberrations, male and female F1 mouse bone marrow cells	600, i.p.	_	Xu and Alder (1990)
Chromosomal aberrations, male and female Sprague-Dawley rat bone-marrow cells	1,000, p.o.	_	Leuschner and Leuschner (1991)
Chromosomal aberrations, BALB/c mouse spermatogonia treated	83, i.p.	_	Russo and Levis (1992a)
Chromosomal aberrations, F1 mouse secondary spermatocytes	82.7, i.p.	+	Russo et al. (1984)
Chromosomal aberrations, male Swiss CD-1 mouse bone-marrow erythrocytes	400, i.p.	_	Marrazzini et al. (1994)

Table 4-46. Genotoxicity of chloral hydrate—mammalian systems (in vivo)[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]	Reference
Chromosomal aberrations, ICR mouse oocytes	600, i.p.	_	Mailhes et al. (1993)
Micronucleus formation, infants, peripheral lymphocytes	50, p.o.	+	Ikbal et al. (2004)
Polyploidy, male and female F1, mouse bone-marrow cells	600, i.p.	_	Xu and Adler (1990)
Aneuploidy F1 mouse secondary spermatocytes	200, i.p.	+	Miller and Adler (1992)
Aneuploidy, male F1 mouse secondary spermatocytes	400, i.p.	_	Leopardi et al. (1993)
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini et al. (1994)

[a]Table adapted from IARC monograph (2004) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in mg/kg for in vivo tests unless otherwise specified; i.p. = intraperitoneally, p.o. = orally.
[c]Results: + = positive; − = negative.

Table 4-47. Genotoxicity of chloral hydrate—bacterial, yeast, and fungal systems[a]

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
SOS chromotest, *E. coli* PQ37	10,000	–	–	Giller et al. (1995)
S. typhimurium TA100, TA1535, TA98, reverse mutation	10,000	–	–	Waskell (1978)
S. typhimurium TA100, TA1537, TA1538, TA98, reverse mutation	1,000	+	+	Haworth et al. (1983)
S. typhimurium TA100, reverse mutation	5,000 µg/plate	–	–	Leuschner and Leuschner (1991)
S. typhimurium TA100, reverse mutation	2,000 µg/plate	+	+	Ni et al. (1994)
S. typhimurium TA100, reverse mutation, liquid medium	300	+	–	Giller et al. (1995)
S. typhimurium TA100, TA104, reverse mutation	1,000 µg/plate	+	+	Beland (1999)
S. typhimurium TA104, reverse mutation	1,000 µg/plate	+	+	Ni et al. (1994)
S. typhimurium TA1535, reverse mutation	1,850	–	–	Leuschner and Leuschner (1991)
S. typhimurium TA1535, TA1537 reverse mutation	6,667	–	–	Haworth et al. (1983)
S. typhimurium TA1535, reverse mutation	10,000	–	–	Beland (1999)
S. typhimurium TA98, reverse mutation	7,500	–	–	Haworth et al. (1983)
S. typhimurium TA98, reverse mutation	10,000 µg/plate	-	+	Beland (1999)
A. nidulans, diploid strain 35X17, mitotic crossovers	1,650	NT	–	Crebelli et al. (1985)
A. nidulans, diploid strain 30, mitotic crossovers	6,600	NT	–	Kafer (1986)
A. nidulans, diploid strain NH, mitotic crossovers	1,000	NT	–	Kappas (1989)
A. nidulans, diploid strain P1, mitotic crossovers	990	NT	–	Crebelli et al. (1991)
A. nidulans, diploid strain 35X17, nondisjunctions	825	NT	+	Crebelli et al. (1985)
A. nidulans, diploid strain 30, aneuploidy	825	NT	+	Kafer (1986)
A. nidulans, haploid conidia, aneuploidy, polyploidy	1,650	NT	+	Kafer (1986)
A. nidulans, diploid strain NH, nondisjunctions	450	NT	+	Kappas (1989)
A. nidulans, diploid strain P1, nondisjunctions	660	NT	+	Crebelli et al. (1991)
A. nidulans, haploid strain 35, hyperploidy	2,640	NT	+	Crebelli et al. (1991)

Table 4-47. Genotoxicity of chloral hydrate—bacterial, yeast, and fungal systems[a] (continued)

Test system/endpoint	Doses (LED or HID)[b]	Results[c]		Reference
		With activation	Without activation	
S. cerevisiae, meiotic recombination	3,300	NT	?	Sora and Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	2,500	NT	+	Sora and Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	3,300	NT	+	Sora and Agostini Carbone (1987)
S. cerevisiae, D61.M, mitotic chr. malsegregation	1,000	NT	+	Albertini (1990)
D. melanogaster, somatic mutation wing spot test	825	NT	+	Zordan et al. (1994)
D. melanogaster, induction of sex-linked lethal mutation	37.2 feed	NT	?	Beland (1999)
D. melanogaster, induction of sex-linked lethal mutation	67.5 injection	NT	−	Beland (1999)

[a]Table adapted from IARC monograph (2004) and modified/updated for newer references.
[b]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for in vitro tests; inj = injection.
[c]Results: + = positive; − = negative; NT = not tested; ? = inconclusive.

4.8.4.1.2. DNA binding studies

Limited analysis has been performed examining the DNA binding potential of chloral hydrate (Von Tungeln et al., 2002; Ni et al., 1995; Keller and Heck, 1988). Keller and Heck (1988) conducted both in vitro and in vivo experiments using the B6C3F1 mouse strain. The mice were pretreated with 1,500 mg/kg TCE for 10 days and then given 800 mg/kg [14C] chloral. No detectable covalent binding of 14C to DNA in the liver was observed. Another study with in vivo exposures to nonradioactive chloral hydrate at a concentration of 1,000 and 2,000 nmol in B6C3F1 mice demonstrated an increase in malondialdehyde-derived and 8-oxo-2'-deoxyguanosine adducts in liver DNA (Von Tungeln et al., 2002). Ni et al. (1995) observed malondialdehyde adducts in calf thymus DNA when exposed to chloral hydrate and microsomes from male B6C3F$_1$ mouse liver.

Keller and Heck (1988) investigated the potential of chloral to form DNA-protein cross-links in rat liver nuclei using concentrations of 25, 100, or 250 mM. No statistically significant increase in DNA-protein cross-links was observed. DNA and RNA isolated from the [14C]

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chloral-treated nuclei did not have any detectable 14C bound. However, the proteins from choral-treated nuclei did have a concentration-related binding of 14C.

4.8.4.1.3. Chromosomal aberrations

Chloral hydrate induced aneuploidy in vitro in multiple Chinese hamster cell lines (Natarajan et al., 1993; Warr et al., 1993; Furnus et al., 1990) and human lymphocytes (Sbrana et al., 1993; Vagnarelli et al., 1990) but not mouse lymphoma cells (Harrington-Brock et al., 1998). In vivo studies performed in various mouse strains led to increased aneuploidy in spermatocytes (Miller and Adler, 1992; Liang and Pacchierotti, 1988; Russo et al., 1984) but not oocytes (Mailhes et al., 1988) or bone marrow cells (Leopardi et al., 1993; Xu and Adler, 1990).

The potential of chloral hydrate to induce aneuploidy in mammalian germ cells has been of particular interest since Russo et al. (1984) first demonstrated that chloral hydrate treatment of male mice results in a significant increase in frequencies of hyperploidy in metaphase II cells. This hyperploidy was thought to have arisen from chromosomal nondisjunction in premeiotic/meiotic cell division and may be a consequence of chloral hydrate interfering with spindle formation [reviewed by Russo et al. (1984) and Liang and Brinkley (1985)]. Chloral hydrate also causes meiotic delay, which may be associated with aneuploidy (Miller and Adler, 1992). Chloral hydrate has been shown to induce micronuclei but not structural chromosomal aberrations in mouse bone-marrow cells. Micronuclei induced by nonclastogenic agents are generally believed to represent intact chromosomes that failed to segregate into either daughter-cell nucleus at cell division (Russo and Levis, 1992b; Xu and Adler, 1990). Furthermore, chloral hydrate-induced micronuclei in mouse bone-marrow cells (Russo et al., 1992) and in cultured mammalian cells (Bonatti et al., 1992; Degrassi and Tanzarella, 1988) have shown to be predominantly kinetochore-positive in composition upon analysis with immunofluorescent methods. The presence of a kinetochore in a micronucleus is considered evidence that the micronucleus contains a whole chromosome lost at cell division (Eastmond and Tucker, 1989; Degrassi and Tanzarella, 1988; Hennig et al., 1988). Therefore, both TCE and chloral hydrate appear to increase the frequency of micronuclei.

Allen et al. (1994) exposed male C57B1/6J mice to a single i.p. injection of 0, 41, 83, or 165 mg/kg chloral hydrate. Spermatids were harvested at 22 hours and 11, 13.5, and 49 days following exposure (Allen et al., 1994). Harvested spermatids were processed to identify both kinetochore-positive micronucleus (aneugenicity) and kinetochore-negative micronucleus (clastogenicity). All chloral hydrate doses administered 49 days prior to cell harvest were associated with significantly increased frequencies of kinetochore-negative micronuclei in spermatids; however, dose dependence was not observed. This study is in contrast with other

studies (Bonatti et al., 1992; Degrassi and Tanzarella, 1988) that demonstrated predominantly kinetochore-positive micronucleus.

The ability of chloral hydrate to induce aneuploidy and polyploidy was tested in human lymphocyte cultures established from blood samples obtained from two healthy nonsmoking donors (Sbrana et al., 1993). Cells were exposed for 72 and 96 hours at doses between 50 and 250 µg/mL. No increase in percentage hyperdiploid, tetraploid, or endoreduplicated cells was observed when cells were exposed for 72 hours at any doses tested. However, at 96 hours of exposure, a significant increase in hyperdiploid was observed at one dose (150 µg/mL) and was not dose dependent. Tetraploidy was significantly increased at 137 mg/mL, again without dose-dependency.

Ikbal et al. (2004) assessed genotoxicity (i.e., induction of micronuclei) in cultured peripheral blood lymphocytes of 18 infants (age range of 31−55 days) before and after administration of a single dose of chloral hydrate (50 mg/kg of body weight) for sedation before a hearing test. A significant increase in micronuclei frequency was observed after administration of chloral hydrate.

Analysis of chloral hydrate treated mouse lymphoma cell lines for chromosomal aberrations resulted in a nonsignificant increase in chromosomal aberrations (Harrington-Brock et al., 1998). However, it should be noted that the concentrations tested (1,250 and 1,300 µg/mL) were cytotoxic (with a cell survival of 11 and 7%, respectively). Chinese hamster embryo cells were also exposed to 0.001, 0.002, and 0.003% chloral hydrate for 1.5 hours (Furnus et al., 1990). A nonstatistically significant increase in frequency of chromosomal aberrations was observed only at 0.002 and 0.003% concentrations, with the increase not being dose dependent. In this study, it should be noted that the cells were only exposed for 1.5 hours to chloral hydrate and cells were allowed to grow for 48 hours (two cell cycles) to obtain similar mitotic indices before analyzing for chromosomal aberrations. No information on cytotoxicity was provided except that higher doses decreased the frequency of mitotic cells at the time of fixation.

In vivo chromosome aberration studies have mostly reported negative or null results (Mailhes et al., 1993; Russo and Levis, 1992a; Leuschner and Leuschner, 1991; Xu and Adler, 1990; Liang and Pacchierotti, 1988) with the exception of one study (Russo et al., 1984) in an F1 cross of mouse strain between C57B1/Cne × C3H/Cne.

4.8.4.1.3.1. Micronucleus induction

Micronuclei induction following exposure to chloral hydrate is positive in most test systems in both in vitro and in vivo assays, although some negative tests do also exist (Ikbal et al., 2004; Beland, 1999; Nesslany and Marzin, 1999; Harrington-Brock et al., 1998; Grawé et al.,

1997; Nutley et al., 1996; Parry et al., 1996; Giller et al., 1995; Allen et al., 1994; Marrazzini et al., 1994; Leopardi et al., 1993; Lynch and Parry, 1993; Seelbach et al., 1993; Bonatti et al., 1992; Russo and Levis, 1992a, b; Van Hummelen and Kirsch-Volders, 1992; Leuschner and Leuschner, 1991; Degrassi and Tanzarella, 1988). Some studies have attempted to make inferences regarding aneuploidy induction or clastogenicity as an effect of chloral hydrate. Aneuploidy results from defects in chromosome segregation during mitosis and is a common cytogenetic feature of cancer cells. Giller et al. (1995) studied chloral hydrate genotoxicity in three short-term tests. Chloral hydrate caused a significant increase in the frequency of micronucleated erythrocytes following in vivo exposure of the amphibian *Pleurodeles waltl* larvae.

4.8.4.1.3.2. Sister chromatid exchanges (SCEs)

SCEs were assessed by Ikbal et al. (2004) in cultured peripheral blood lymphocytes of 18 infants (age range of 31−55 days) before and after administration of a single dose of chloral hydrate (50 mg/kg of body weight) for sedation before a hearing test. The authors report a significant increase in the mean number of SCEs, from before administration (7.03 ± 0.18 SCEs/cell) and after administration (7.90 ± 0.19 SCEs/cell), with each of the 18 individuals showing an increase with treatment. Micronuclei were also significantly increased. SCEs were also assessed by Gu et al. (1981) in human lymphocytes exposed in vitro with inconclusive results, although positive results were observed by Beland (1999) in Chinese hamster ovary cells exposed in vitro with and without an exogenous metabolic system.

4.8.4.1.4. Cell transformation

Chloral hydrate was positive in the two studies designed to measure cellular transformation (Parry et al., 1996; Gibson et al., 1995). Both studies exposed Syrian hamster cells (embryo and dermal) to chloral hydrate, which induced cellular transformation.

4.8.4.2. Bacterial and Fungal Systems

4.8.4.2.1. Gene mutations

Chloral hydrate induced gene mutations in *S. typhimurium* TA100 and TA104 strains but not in most other strains assayed. Four of six studies of chloral hydrate exposure in *S. typhimurium* TA100 and two of two studies in *S. typhimurium* TA104 were positive for revertants (Beland, 1999; Giller et al., 1995; Ni et al., 1994; Haworth et al., 1983). Waskell (1978) studied the effect of chloral hydrate along with TCE and its other metabolites. Chloral hydrate was tested at different doses (1.0−13 mg/plate) in different *S. typhimurium* strains (TA98, TA100, TA1535) for gene mutations using the Ames assay. No revertant colonies were observed in strains TA98 or TA1535 both in the presence and absence of S9 mix. Similar results

were obtained by Leuschner and Leuschner (1991). However, in TA100, a dose-dependent statistically significant increase in revertant colonies was obtained both in the presence and absence of S9. It should be noted that chloral hydrate that was purchased from Sigma was recrystallized from one to six times from chloroform, and the authors describe this as crude chloral hydrate. However, this positive result is consistent with other studies in this strain as noted above. Furthermore, Giller et al. (1995) studied chloral hydrate genotoxicity in three short-term tests. Chloral-induced mutations in strain TA100 of *S. typhimurium* (fluctuation test). Similar results were obtained by Haworth et al. (1983). These are consistent with several studies of TCE, in which low, but positive, responses were observed in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present.

A significant increase in mitotic segregation was observed in *Aspergillus nidulans* when exposed to 5 and 10 mM chloral hydrate (Crebelli et al., 1985). Studies of mitotic crossing-over in *A. nidulans* have been negative, while these same studies were positive for aneuploidy (Crebelli et al., 1991; Kappas, 1989; Käfer, 1986; Crebelli et al., 1985).

Two studies were conducted in *S. cerevisiae* to understand the chromosomal malsegregation as a result of exposure to chloral hydrate (Albertini, 1990; Sora and Agostini Carbone, 1987). Chloral hydrate (1−25 mM) was dissolved in sporulation medium, and the frequencies of various meiotic events such as recombination and disomy were analyzed. Chloral hydrate inhibited sporulation as a function of dose and increased diploid and disomic clones. Chloral hydrate was also tested for mitotic chromosome malsegregation using *S. cerevisiae* D61.M (Albertini, 1990). The tester strain was exposed to a dose range of 1−8 mg/mL. An increase in the frequency of chromosomal malsegregation was observed as a result of exposure to chloral hydrate.

Limited analysis of chloral hydrate mutagenicity has been performed in *Drosophila* (Beland, 1999; Zordan et al., 1994). Of these two studies, chloral hydrate was positive in the somatic mutation wing spot test (Zordan et al., 1994), equivocal in the induction of sex-linked lethal mutation when administered in feed, but negative when exposed via injection (Beland, 1999).

4.8.4.3. Summary

Chloral hydrate has been reported to induce micronuclei formation, aneuploidy, and mutations in multiple in vitro systems and in vivo. In vivo studies are limited to increased micronuclei formation mainly in mouse spermatocytes. CH is positive in some in vitro genotoxicity assays that detect point mutations, micronuclei induction, chromosomal aberrations, and/or aneuploidy. The in vivo data exhibit mixed results (Leuschner and Beuscher, 1998; Nutley et al., 1996; Allen et al., 1994; Mailhes et al., 1993; Xu and Adler, 1990). Most of the

positive studies show that chloral hydrate induces aneuploidy. Based on the existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. Some have suggested that chloral hydrate may act through a mechanism of spindle poisoning, resulting in numerical changes in the chromosomes, but some data also suggest induction of chromosomal aberrations. These results are consistent with tetrachloroethylene, albeit there are more limited data on tetrachloroethylene for these genotoxic endpoints.

4.8.5. Trichloroacetyl Chloride

Trichloroacetyl chloride results from oxidative metabolism of tetrachloroethylene. The limited genotoxicity studies of this metabolite are described below and listed in Table 4-48.

4.8.5.1. Bacterial Systems

4.8.5.1.1. Gene mutation

The genotoxicity of trichloroacetyl chloride has been studied in *S. typhimurium* with inconsistent results. Reichert et al. (1983) found no mutagenicity of trichloroacetyl chloride exposed in a liquid suspension to *S. typhimurium* TA98 and TA100 strains with and without S9 activation. A second study (DeMarini et al., 1994) evaluated genotoxicity in *S. typhimurium* TA100 in the vapor state and found trichloroacetyl chloride to be positive in the presence and absence of S9 activation, but inducing predominantly GC-to-TA transversions (the predominant background mutation). Trichloroacetyl chloride was negative for prophage induction in *E. coli* in the same study (DeMarini et al., 1994).

4.8.6. Tetrachloroethylene (PCE) Epoxide

Tetrachloroethylene epoxide, a hypothesized intermediate in tetrachloroethylene P450 oxidative metabolism (Henschler and Bonse, 1977; Henschler, 1977), has been investigated in only one published study. This study is described below and listed in Table 4-48.

4.8.6.1. Bacterial Systems

4.8.6.1.1. Gene mutation

In a study examining the genotoxicity of multiple chloroepoxides, tetrachloroethylene epoxide (0, 0.5, 1.3, 2.5, 5.0, 25.0 mM, closed system) was mutagenic in *S. typhimurium* TA1535 but not in *E. coli* WP2 uvrA (Kline et al., 1982). Mutagenicity was observed at the lower doses in *S. typhimurium,* but not at higher doses, most likely due to cytotoxicity at the high doses.

4.8.7. Trichloroethanol (TCOH)

4.8.7.1. Bacterial Systems

4.8.7.1.1. Gene mutation

Limited studies are available on the effect of TCOH on genotoxicity (refer to Table 4-47). TCOH is negative in the *S. typhimurium* assay using the TA100 strain (DeMarini et al., 1994; Bignami et al., 1980; Waskell, 1978). A study by Beland (1999) using *S. typhimurium* strain TA104 did not induce reverse mutations without exogenous metabolic activation, however, did increase mutant frequency in the presence of exogenous metabolic activation at a dose above 2,500 µg/plate. TCOH has not been evaluated in other recommended screening assays. Therefore, the database is limited for the determination of TCOH genotoxicity (summarized in Table 4-48).

4.8.8. *S*-(1,2,2-Trichlorovinyl)-*L*-Cysteine (1,2-TCVC), *S*-Trichlorovinyl Glutathione (TCVG), *N*-Acetyl-*S*-(1,2,2-Trichlorovinyl)-*L*-Cysteine (NAcTCVC)

Limited studies have been performed examining the genotoxicity of three metabolites from the GSH-conjugation metabolic pathway of tetrachloroethylene. The results for all three are described below and summarized in Table 4-48.

4.8.8.1. Bacterial Systems

4.8.8.1.1. Gene mutation

TCVG produced from tetrachloroethylene in isolated perfused rat liver and excreted into bile, in the presence of a rat kidney fraction, was mutagenic in *Salmonella*, as was purified TCVG (Vamvakas et al., 1989d). This study performed the Ames assay in *S. typhimurium* TA100, TA98, and TA2638 with tetrachloroethylene, TCVG, and bile from liver perfusate following tetrachloroethylene exposure in rats, demonstrated that the GST-metabolites or tetrachloroethylene in the presence of bile containing GST led to gene mutations in *S. typhimurium* TA100. Dreessen et al. (2003) also demonstrated for TCVG an unequivocal dose-dependent mutagenic response in the TA100 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 (1−10 nmol/plate) was also positive in *Salmonella* strains TA98 and TA100 but not strain TA2638, and inhibition of β-lyase activity was blocked by the addition of aminooxyacetic acid (AOAA) (Dekant et al., 1986a). A subsequent study from this same group indicated that *Salmonella* also were capable of deacetylating the urinary metabolite NAcTCVC (50−100 nmol/plate) when TA100 showed a clear positive response in the Ames assay without exogenous activation (Vamvakas et al., 1987).

Addition of cytosolic protein increased this mutagenicity, while addition of a β-lyase inhibitor (AOAA) decreased it.

4.8.8.2. Mammalian Systems

4.8.8.2.1. Unscheduled DNA synthesis

Vamvakas et al. (1989a) 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 β-lyase inhibitor. This effect was observed at exposure to 5×10^{-6}–10^{-5} M TCVC for 24 hours. This study also measured LDH release to determine cytotoxicity at the same doses, and no increases in LDH were observed at these doses.

4.8.9. TCVC Sulfoxide

TCVC sulfoxide does not appear to have been investigated for genotoxicity.

4.8.10. Synthesis and Overall Summary

Tetrachloroethylene and its metabolites (TCA, DCA, CH, TCVC, TCVG, and NAcTCVC) have been evaluated to varying degrees for their genotoxic activity in several of in vitro systems such as bacteria, yeast, and mammalian cells and, also, in in vivo systems. Genotoxicity studies of other metabolites (e.g., TCVC sulfoxide, tetrachloroethylene epoxide, trichloroacetyl chloride, trichloroethanol) are limited or nonexistent but are discussed where available.

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 in the absence or presence of the standard S9 fraction [(Watanabe et al., 1998; DeMarini et al., 1994; Roldán-Arjona et al., 1991; Milman et al., 1988; Warner et al., 1988; NTP, 1986; Connor et al., 1985; Shimada et al., 1985; Haworth et al., 1983; Hardin et al., 1981; Kringstad et al., 1981; Bartsch et al., 1979; Greim et al., 1975); summarized in Table 4-40]. A more recent study demonstrated cytotoxicity but not genotoxicity of tetrachloroethylene in an *S. typhimurium* strain (YG7108pin3ERb5) with enhanced metabolic activity (transformed with CYP2E1, cytochrome P450 reductase, and cytochrome b5) (Emmert et al., 2006). PCE was negative in the parent strain (YG7108) at all doses in the presence of S9. However, when tetrachloroethylene was activated with rat liver GST, GSH, and a rat kidney fraction, tetrachloroethylene exhibited a clear dose response (Vamvakas et al., 1989d). These findings support a role of metabolic activation of tetrachloroethylene in its in vitro genotoxicity.

Table 4-48. Genotoxicity of additional tetrachloroethylene metabolites—all systems

Metabolite	Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
			With activation	Without activation	
Chloral	*S. typhimurium* TA100, increased mutation frequency	NA	+	*possible*	Sato et al. (1985)
Oxalic acid	*Sclerotinia sclerotiorum,* DNA fragmentation	10 mM	NT	+	Kim et al. (2008)
	Madin-Darby cultured canine kidney cells, renal prothrombin fragment-1 mRNA expression	0.09 mM	NT	+	Moryama et al. (2005)
	Crepis capillaris, chromosomal aberrations	1.0 mM	NT	(+)	Shevchenko et al. (1985)
Trichloroethanol (TCOH)	*S. typhimurium* TA100, 98, reverse mutation	7,500 µg/plate	−	−	Waskell (1978)
	S. typhimurium TA100, reverse mutation	0.5 µg/cm^3 vapor	−	−	DeMarini et al. (1994)
	S. typhimurium TA104, reverse mutation	2,500 µg/plate	+	−	Beland (1999)
	S. typhimurium TA100, 1535 reverse mutation	NA	−	−	Bignami et al. (1980)
	Sister chromatid exchanges	NA	NA	+	Gu et al. (1981)
Trichloroacetyl chloride	PRB, λ Prophage induction, *E. coli* WP2	10,000	−	−	DeMarini et al. (1994)
	SA0, *S. typhimurium* TA100, reverse mutation	2.6	+	+	DeMarini et al. (1994)
	S. typhimurium TA100, increased mutation frequency	5 µg/mL	−	−	Reichert et al. (1983)
Trichlorovinyl-glutathione (TCVG)	*S. typhimurium* TA100, reverse mutation	100 nmol/plate	+	−	Dreessen et al. (2003)
	S. typhimurium TA100, increased mutation frequency	25 nmol/plate (with) 250−500 nmol/plate (without)	+	(+)	Vamvakas et al. (1989c)
	Cultured porcine LLC-PK1 (kidney) cells, unscheduled DNA synthesis, in vitro	7.5×10^{-6} M	NT	+	Vamvakas et al. (1989d)

Table 4-48. Genotoxicity of additional tetrachloroethylene metabolites—all systems (continued)

Metabolite	Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
			With activation	Without activation	
Trichlorovinyl-cysteine (TCVC)	*S. typhimurium* TA100, reverse mutation	50 nmol/plate	NT	+	Dreessen et al. (2003)
	Cultured porcine LLC-PK1 (kidney) cells, unscheduled DNA synthesis, in vitro	5×10^{-6} M	NT	+	Vamvakas et al. (1989a)
NAcTCVC	*S. typhimurium* TA100, increased mutation frequency	<50 nmol[c]	+	+	Vamvakas et al. (1987)
PCE oxide	*S. typhimurium* TA1535, reverse mutation	2.5 mM	NT	+	Kline et al. (1982)
	E. coli WP2 uvrA, reverse mutation	25 mM	NT	−	Kline et al. (1982)

[a]LED, lowest effective dose; HID, highest ineffective dose; NA = not available.
[b]Results: + = positive; (+) = weakly positive; − = negative; NT = not tested.
[c]Lower-level concentrations that indicate mutagenicity are not specified in Vamvakas et al. (1987).

Limited in vivo studies of tetrachloroethylene are inconsistent, with only negative (NTP, 1986; Bronzetti et al., 1983) or equivocal (Cederberg et al., 2010a; Beliles et al., 1980) genotoxicity assay results demonstrated following inhalation or oral exposure to tetrachloroethylene in animals (refer to Table 4-39). Intraperitoneal injection assays have demonstrated both negative (NTP, 1986) as well as positive results for different genotoxicity endpoints (Walles, 1986). Assays of clastogenic effects following inhalation exposure in humans have shown inconsistent results and are suggested to be related to coexposures (Seiji et al., 1990; Ikeda et al., 1980). Studies of chromosomal aberrations following exposure to tetrachloroethylene are mostly negative (Galloway et al., 1987; NTP, 1986; Sofuni et al., 1985), but positive results have been observed in vivo (Murakami and Horikawa, 1995) and in vitro studies with enhanced metabolic activation (Doherty et al., 1996).

TCA, an oxidative metabolite of tetrachloroethylene, exhibits little, if any, genotoxic activity in vitro (refer to Tables 4-41 and 4-42). TCA did not induce mutations in *S. typhimurium* strains in the absence of metabolic activation or in an alternative protocol using a closed system (Kargalioglu et al., 2002; Nelson et al., 2001a; Giller et al., 1997; DeMarini et al., 1994; Rapson et al., 1980; Waskell, 1978), but a mutagenic response was induced in TA100 in the Ames fluctuation test (Giller et al., 1997). However, in vitro experiments with TCA should be interpreted with caution if steps have not been taken to neutralize pH changes caused by the

compound (Mackay et al., 1995). Measures of DNA-repair responses in bacterial systems have shown induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations (Harrington-Brock et al., 1998). TCA was positive in some genotoxicity studies in in vivo mouse, newt, and chick test systems (Giller et al., 1997; Bhunya and Jena, 1996; Birner et al., 1994; Bhunya and Behera, 1987). DNA unwinding assays have either shown TCA to be much less potent than DCA (Nelson and Bull, 1988) or negative (Styles et al., 1991; Nelson et al., 1989). Due to limitations in the genotoxicity database, the possible contribution of TCA to tetrachloroethylene genotoxicity is unclear.

DCA, a chloroacid metabolite of tetrachloroethylene, has also been studied using different types of genotoxicity assays (refer to Tables 4-43 and 4-44). Although limited studies are conducted for different genetic endpoints, DCA has been demonstrated to be mutagenic in the *S. typhimurium* assays, in vitro (Kargalioglu et al., 2002; Plewa et al., 2002; DeMarini et al., 1994) in some strains, in a mouse lymphoma assay (Harrington-Brock et al., 1998), in vivo cytogenetic tests (Leavitt et al., 1997; Fuscoe et al., 1996), in the micronucleus induction test, using the Big Blue mouse system, and in other tests (Harrington-Brock et al., 1998; Leavitt et al., 1997; Fuscoe et al., 1996; DeMarini et al., 1994; Chang et al., 1989; Nelson et al., 1989; Nelson and Bull, 1988; Gu et al., 1981). DCA can cause DNA strand breaks in mouse and rat liver cells following in vivo exposure in mice and rats (Fuscoe et al., 1996). Because of uncertainties as to the extent of DCA formed from tetrachloroethylene exposure, inferences as to the possible contribution from DCA genotoxicity to tetrachloroethylene toxicity are difficult to make.

Chloral hydrate is mutagenic in the standard battery of screening assays (refer to Tables 4-45, 4-46, and 4-47). Effects include positive results in bacterial mutation tests for point mutations and in the mouse lymphoma assay for mutagenicity at the Tk locus (Haworth et al., 1983). In vitro tests showed that CH also induced micronuclei and aneuploidy in human peripheral blood lymphocytes and Chinese hamster pulmonary cell lines. Micronuclei were also induced in Chinese hamster embryonic fibroblasts. Several studies demonstrate that chloral hydrate induces aneuploidy (loss or gain of whole chromosomes) in both mitotic and meiotic cells, including yeast (Gualandi, 1987; Sora and Agostini Carbone, 1987; Käfer, 1986; Singh and Sinha, 1979, 1976), cultured mammalian somatic cells (Degrassi and Tanzarella, 1988), and spermatocytes of mice (Liang and Pacchierotti, 1988; Russo et al., 1984). 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. Based on the

existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. Chloral hydrate appears to act through a mechanism of spindle poisoning, resulting in numerical changes in the chromosomes. These results are consistent with tetrachloroethylene, albeit there are limited data on tetrachloroethylene for these genotoxic endpoints.

The genotoxicity analysis of other metabolites (e.g., trichloroacetyl chloride, tetrachloroethylene epoxide, trichloroethanol) is limited (refer to Table 4-48). Trichloroacetyl chloride was found to be mutagenic in *S. typhimurium* when exposed in vapor phase (DeMarini et al., 1994) but not in liquid phase (Reichert et al., 1983); tetrachloroethylene epoxide was found to be mutagenic in *S. typhimurium* but not *E. coli* (Kline et al., 1982); and trichloroethanol was found to be negative in three (DeMarini et al., 1994; Bignami et al., 1980; Waskell, 1978) of four mutagenicity studies (Beland, 1999). These results are limited, and further studies are needed to make any conclusions on the genotoxicity of these metabolites.

Although also limited, genotoxicity tests for the GSH conjugation metabolites are positive (refer to Table 4-48). These include 1,2-TCVC, TCVG, and NAcTCVC. In the one mammalian study, unscheduled DNA synthesis in porcine kidney cells was observed to increase in a dose-dependent manner following exposure to TCVC (Vamvakas et al., 1989c). Mutagenicity assays found TCVG (Dreessen et al., 2003; Vamvakas et al., 1989d) and NAcTCVC (Vamvakas et al., 1987) to be mutagenic in the presence of activation, while TCVC was mutagenic even in the absence of activation (Dreessen et al., 2003; Dekant et al., 1986a).

In summary, tetrachloroethylene has been shown to induce some genotoxic effects (micronuclei induction following in vitro exposure, DNA binding, and SSBs in tumor tissue), but these result are inconsistent. A number of in vitro mutagenicity (Ames) tests of tetrachloroethylene have largely been negative in the absence or presence of the standard S9 fractions. Positive results have been observed in tests of conditions where metabolites of the GSH pathway are generated. These support a role of metabolic activation of tetrachloroethylene in its genotoxicity. Consistent with this view, positive results have been reported when the GSH metabolites were used as the test agent, and certain of the oxidative metabolites are also 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.

There are several challenges in interpreting the genotoxicity results obtained from tetrachloroethylene exposure. Because of the volatile nature of tetrachloroethylene, there could be false negative results if proper precautions are not taken to limit evaporation, such as the use of a closed sealed system. The adequacy of the enzyme-mediated activation of tetrachloroethylene in vitro tests is another consideration. For example, it is not clear if standard

S9 fractions can adequately recapitulate the complex in vivo metabolism of tetrachloroethylene to reactive intermediates, which, in some cases, entails multiple sequential steps involving multiple enzyme systems (e.g., CYP, GST, etc.). In addition, the relative potency of the metabolites in vitro may not necessarily inform their relative contribution to the overall mechanistic effects of the parent compound, tetrachloroethylene. Furthermore, although different assays provided data relevant to different types of genotoxic endpoints, not all effects that are relevant for carcinogenesis are encompassed. The standard battery of prokaryotic as well as mammalian genotoxicity test protocols typically specify the inclusion of significantly cytotoxic concentrations of the test compound.

In conclusion, uncertainties with regard to the characterization of tetrachloroethylene genotoxicity remain. This is primarily because in vivo tests of tetrachloroethylene have been equivocal, with at most, modest evidence of genotoxic effects in rodent tumor tissues examined (including mouse liver and rat kidney) following exposure at tumorigenic doses. However, no evidence is available regarding the potential contribution of tetrachloroethylene genotoxicity to other rodent tumor types (particularly, MCL, testes, and brain). Ames assays of tetrachloroethylene have yielded largely negative results. The tetrachloroethylene metabolites TCVG, TCVC, NAcTCVC, tetrachloroethylene oxide, and DCA are genotoxic, but not all such metabolites have been sufficiently tested in the standard screening battery to support clear conclusions about their genotoxic potential. However, the predominance of positive data for these metabolites supports their potential genotoxicity following in situ production and/or bioactivation. This, in turn, supports the view that contribution of genotoxicity to tetrachloroethylene carcinogenesis cannot be ruled out for one or more target organs. Additional testing of the genotoxicity of tetrachloroethylene and its metabolites (particularly those from the GSH conjugation pathway) using state–of–the–art methods and in a more comprehensive panel of tumor tissues is warranted.

4.9. SUSCEPTIBLE POPULATIONS

Variation in response to tetrachloroethylene may be due to age, gender, genetics, and race/ethnicity, as well as differences in lifestyle factors, nutrition, preexisting disease status, socioeconomic status, and multiple exposures. These could be potential modifying risk factors that play an important role in determining an individual's susceptibility to chemical exposures and are discussed below.

4.9.1. Life-Stages

Individuals in one life-stage are physiologically, anatomically, and biochemically unique from individuals in another life-stage. 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 toxicokinetics of parent chemicals and their metabolites from the body (Guzelian et al., 1992b). This section presents and evaluates the pertinent published literature available to assess how individuals of early life-stages (refer to Section 4.9.1.1) and later life-stages (refer to Section 4.9.1.2) may respond differently to tetrachloroethylene than adults. The limited data on tetrachloroethylene exposure suggest that these populations—particularly individuals in early life-stages—may have greater susceptibility than does the general population.

4.9.1.1. Early Life-Stages

4.9.1.1.1. Early 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 (refer to Section 2.2.1) and contaminated water (refer to Section 2.2.2). Ingestion of contaminated food or soil is also a possible exposure route (refer to Section 2.2.3), as is direct ingestion (refer to Section 2.2.6). In addition, certain exposure pathways to tetrachloroethylene are unique to early life-stages, such as through placental transfer or via breast milk ingestion (refer to Section 2.2.4), or may be increased during early or later life-stages. Other reviews of the reproductive and developmental effects of tetrachloroethylene exist (Brown Dzubow et al., 2010; Beliles, 2002; Bove et al., 2002; Danielsson, 1990; van der Gulden and Zielhuis, 1989; Tabacova, 1986).

Prenatal. In utero, lipophilic substances are known to cross the placental barrier (Herrera et al., 2006). There is biological plausibility of transfer of tetrachloroethylene across the human placental barrier as tetrachloroethylene has been measured in fetal blood and amniotic fluid in rodents (Szakmáry et al., 1997; Ghantous et al., 1986). Fetal blood concentrations have been modeled for human exposure (Gentry et al., 2003).

Inhalation. Inhalation exposures may be altered for early life-stages compared to adults, because children have increased ventilation rates (both intake and exhalation) per kg body weight compared to adults (U.S. EPA, 2008; NRC, 1993). These populations spend the majority of their time indoors (Bateson and Schwartz, 2008; U.S. EPA, 2008; NRC, 1993), where increased concentrations of tetrachloroethylene have been found compared to those measured outdoors (U.S. EPA, 2001a). Increased indoor air concentrations have been measured in places where children may spend time: inside apartments containing dry-cleaned clothing (Thomas et al., 1991; Tichenor et al., 1990), in the homes of dry-cleaning employees (ATSDR, 1997a; Aggazzotti et al., 1994a; Aggazzotti et al., 1994b), in apartments above or adjacent to dry cleaners (Storm et al., 2011 [previously reported in NYSDOH, 2010]; McDermott et al., 2005; Schreiber et al., 2002; Garetano and Gochfeld, 2000; Chien, 1997; Altmann et al., 1995; Schreiber, 1993; Verberk and Scheffers, 1980), in daycare centers adjacent to dry cleaners

(NYSDOH, 2005b), in a classroom exposed to tetrachloroethylene from an air "emission from a small chemical factory" (Monster and Smolders, 1984), and in automobiles containing dry-cleaned clothing (Park et al., 1998; Gulyas and Hemmerling, 1990). Similarly, increased ambient air concentrations have been measured in places where children may spend time: outside of a daycare center adjacent to a dry cleaner (NYSDOH, 2005c), and on a playground near a factory (Monster and Smolders, 1984). Adgate and colleagues (Adgate et al., 2004b; Adgate et al., 2004a) measured tetrachloroethylene in outside and indoor air at school, indoor air at home, and using personal samplers on children, and demonstrated that tetrachloroethylene levels are lower in homes with greater ventilation and in homes in nonurban settings (Adgate et al., 2004b; Adgate et al., 2004a). In addition, inhalation may also occur indoors during showering or bathing as dissolved tetrachloroethylene in the warm tap water is volatilized, although dermal exposure is also relevant during these scenarios (Rao and Brown, 1993).

Ingestion. Due to its lipophilicity, tetrachloroethylene has been found in human breast milk samples (Schreiber et al., 2002; U.S. EPA, 2001a; Schreiber, 1997, 1993; Sheldon et al., 1985; Pellizzari et al., 1982; Bagnell and Ellenberger, 1977), as well as in milk from cows (Wanner et al., 1982), goats (Hamada and Tanaka, 1995), and rats (Byczkowski et al., 1994; Byczkowski and Fisher, 1994). The breast milk of one woman was found to contain 10 mg/L tetrachloroethylene 1 hour following a visit to her spouse working at a dry-cleaning establishment, dropping to 3 mg/L after 24 hours (Bagnell and Ellenberger, 1977). Tetrachloroethylene has also been measured in the breast milk of two women living in apartments colocated with a dry-cleaning facility (NYSDOH, 2005c; Schreiber et al., 2002). 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; Fisher et al., 1997; Byczkowski and Fisher, 1995; Byczkowski et al., 1994; Schreiber, 1993). A PBPK model was also developed and validated for breast milk ingestion in nursing rats after maternal inhalation exposure (Fisher, 1994). Using different exposure scenarios, Schreiber (1993) 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 et al., 1993). Byczkowski and Fisher (1995) refined the approach used by Schreiber (1993) and found that with the same residential exposure conditions, the results predicted lower doses to the infant (0.0009−0.202 mg/kg-day). Using milk production and suckling variables, Fisher et al. (1997) estimated the dose that a human infant might receive after maternal occupational exposure to be 25 ppm/day. Gentry et al. (2003) modeled a rapid decline in concentration of tetrachloroethylene and TCA during lactation in humans. Although ingestion of tetrachloroethylene through breast milk may

be a significant pathway of exposure for some infants, 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 less important when compared with the dose resulting from inhalation exposure (Schreiber, 1997; McKone and Daniels, 1991).

Children ingest higher amounts of water per body weight than adults (U.S. EPA, 2008; NRC, 1993). For infants on formula, ingestion of tetrachloroethylene-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. In addition, incidental water consumption may occur for children when swimming or bathing (U.S. EPA, 2008).

Children consume a higher quantity of food per body weight compared to adults, specifically dairy and other foods with high fat content (U.S. EPA, 2008) that have been found to have elevated concentrations of tetrachloroethylene (refer to Section 2.2.3). Assuming 100 mg/kg represents the average tetrachloroethylene concentration in fatty foods such as butter, and using daily total fat intake rates by age (U.S. EPA, 2008), the daily dose would be 0.46 mg/kg-day for a 10 kg 1-year-old compared to the daily dose of 0.12 mg/kg-day for a 70 kg adult. 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.

Where contamination occurs, tetrachloroethylene can be measured in soil (U.S. EPA, 2001a). This pathway for ingestion of tetrachloroethylene has not been directly examined. A clear need exists to evaluate this pathway because children, particularly those with pica, can ingest high quantities of contaminated soil through hand-to-mouth activity, as has been shown for lead (U.S. EPA, 2008).

Rare instances of direct ingestion of tetrachloroethylene have been documented, including a 6-year-old boy who directly ingested 12−16 g of tetrachloroethylene (Koppel et al., 1985).

Dermal. Dermal exposures may be increased for both early life-stages, because infants have increased surface area-per-body weight-ratio than adults (U.S. EPA, 2008; NRC, 1993). Although an infant's skin has similar permeability to adults, a premature infant may have increased permeability (Guzelian et al., 1992a). Dermal exposure for children may occur in a residential setting from showering, bathing, or swimming in contaminated water, although inhalation exposure is also relevant during these scenarios (U.S. EPA, 2001a; Rao and Brown,

1993). 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).

4.9.1.1.2. Early life-stage-specific toxicokinetics

Section 3 describes the toxicokinetics of tetrachloroethylene. However, children may have differential exposure to tetrachloroethylene compared to adults due to age-related physiological differences. These include body composition, organ function, and many other physiological parameters that can influence the toxicokinetics of chemicals and their metabolites from the body (Renwick, 1998; Guzelian et al., 1992b). Early life-stage-specific information regarding toxicokinetics needs to be considered for a child-specific and chemical-specific PBPK model. To adequately address the risk to infants and children, age-specific parameters for these values should be used in PBPK models that can approximate the internal dose an infant or child receives based on a specific exposure level [(Clewell et al., 2004; Gentry et al., 2003; Byczkowski and Fisher, 1994; Rao and Brown, 1993); refer to Section 3.5].

Absorption. 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. For lipophilic compounds such as tetrachloroethylene, percentage adipose tissue, which varies with age (NRC, 1993), will affect absorption and retention of the absorbed dose. Absorption into the lungs via inhalation is related to the ventilation rate per body weight, which is higher in children than in adults (U.S. EPA, 2008; WHO, 2006; NRC, 1993), with an increased alveolar surface area per kg body weight for the first 2 years (NRC, 1993). Absorption into the gut from oral ingestion may be altered by gastric pH levels, which are higher in infants than in adults (WHO, 2006). Absorption during dermal exposure may be affected by the ratio of surface area, which is higher in infants than in adults (U.S. EPA, 2008; WHO, 2006).

Distribution. The distribution of tetrachloroethylene to specific organs will depend on organ blood flow and the lipid and water content of the organ, which may vary between life-stages (WHO, 2006; NRC, 1993). Due to its high lipophilicity, tetrachloroethylene has been found to distribute widely to all tissues in the body as observed in early lifestages of humans (Garnier et al., 1996; Gaillard et al., 1995; Koppel et al., 1985) and early lifestages of animals (Szakmáry et al., 1997; Dallas et al., 1994a; Ghantous et al., 1986; Schumann et al., 1980; Savolainen et al., 1977b); however, this is true for adults as well, and it is not clear whether distribution may vary differentially with life-stage. It should be noted that the total body burden of tetrachloroethylene increases with age (Clewell et al., 2004), as would be expected, given that adult body weight is generally positively correlated with age.

Rodent studies demonstrate that tetrachloroethylene crosses the placental barrier when pregnant dams are exposed (Szakmáry et al., 1997; Ghantous et al., 1986), and in humans, it has

been shown that during lactation, tetrachloroethylene distributes to breast milk (NYSDOH, 2005c; Schreiber et al., 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 or its metabolites have been measured in blood of children (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a; Popp et al., 1992). A longitudinal study of blood concentrations of 11 volatile organic chemicals (VOCs) measured in more than 150 poor, minority children in Minneapolis, MN, found the mean blood tetrachloroethylene levels to be 0.06 ng/mL (Sexton et al., 2005). When compared to adult data from NHANES III, the blood level in children was lower (Sexton et al., 2005). However, these results do not necessarily represent TK differences between lifestages because the study did not control for exposure differences between these two cohorts. Lower estimated blood concentrations of tetrachloroethylene in children compared to adults have also been described in Clewell et al. (2004), although the variability of the parameters used as well as the results have not been validated.

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 (Garnier et al., 1996; Gaillard et al., 1995).

Metabolism. Section 3.3.3 describes the enzymes involved in the metabolism of tetrachloroethylene. In general, expression of CYP enzymes changes during various stages of fetal development (Hakkola et al., 1998b; Hakkola et al., 1996a; Hakkola et al., 1996b) and during postnatal development (Shao et al., 2007; Clewell et al., 2004; Hakkola et al., 1998a; Hakkola et al., 1998b; Tateishi et al., 1997; Hakkola et al., 1996a; Hakkola et al., 1996b; George et al., 1995). In addition, production of GST enzymes varies significantly during early postnatal lifestages (Shao et al., 2007; McCarver and Hines, 2002; Dorne et al., 2001; Raijmakers et al., 2001; Nakasa et al., 1997; Mera et al., 1994).

After maternal oral exposure to tetrachloroethylene it was observed that fetus and infant blood levels were higher for TCA than for tetrachloroethylene (Gentry et al., 2003),

demonstrating that metabolism of tetrachloroethylene does occur during these lifestages. In addition, there is in vitro evidence of an age-related increase in metabolism of tetrachloroethylene as estimated in the blood (Clewell et al., 2004; Sarangapani et al., 2003), associated with age-related activation of oxidative metabolism pathways, suggesting a decreased ability to metabolize tetrachloroethylene during early lifestages compared to during adulthood. 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; Hakkola et al., 1996b), and differences between a group of 10 prenatal and infant patients showed significantly lower CYP2B6 protein expression in placental hepatic microsomes as compared with an adult group (Tateishi et al., 1997). These findings need to be validated in studies of target tissues in addition to blood to better evaluate any role of variation and heterogeneity.

Excretion. The major processes of excretion of tetrachloroethylene and its metabolites are discussed in Sections 3.3 and 3.4, respectively. Excretion profile differences in exhaled breath and urinary excretion are likely between children and adults. This is due to differences in ventilation rate, activity level, and the solubility of the compound in blood and tissue, as well as differences in amounts of water ingested per body weight (U.S. EPA, 2008; NRC, 1993).

Tetrachloroethylene or its metabolites have been measured in exhaled breath (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a; Delfino et al., 2003b; Schreiber et al., 2002; Monster and Smolders, 1984), and urine (NYSDOH, 2005c; Schreiber et al., 2002; Popp et al., 1992) of children. However, these studies do not provide clear information whether excretion levels in children differ from those of adults for a similar exposure concentration.

PBPK Models. A number of PBPK models present toxicokinetic variation between early lifestages and adulthood for tetrachloroethylene and its metabolites for both humans and animals. Early lifestage-specific exposure scenarios considered in these models include fetal exposure (Gentry et al., 2003) and breast milk exposure (Gentry et al., 2003; Fisher et al., 1997; Byczkowski and Fisher, 1995; Byczkowski et al., 1994; Schreiber et al., 1993). Other PBPK models have addressed comparisons of early lifestage toxicokinetics with those in adulthood for inhalation (Mahle et al., 2007; Rodriguez et al., 2007; Sarangapani et al., 2003; Pelekis et al., 2001), drinking water (Clewell et al., 2004), and bathing and showering (Rao and Brown, 1993). When considering inhalation exposure, Mahle et al. (2007) found no difference in the blood:air partition coefficient for tetrachloroethylene for children aged 3−10 years compared to adults (420 years old). This same study reported that rats at PND 10 and at 2 months (adult) have an

age-dependent difference in fat:air, muscle:air, and brain:air partition coefficients, but not for blood:air, liver:air, or kidney:air (Mahle et al., 2007). Another study of rats found higher peak concentrations of tetrachloroethylene in the blood at PND 10 compared to 2 months (adult) after inhalation exposure, likely due to the lower metabolic capacity of the young rats as observed in the liver (Rodriguez et al., 2007). Pelekis et al. (2001) found little difference in the suggested intraspecies uncertainty factor when including lifestage-specific pharmacokinetics. Sarangapani et al. (2003) also found no age-related difference in tetrachloroethylene blood concentration; however, this study found that metabolite concentrations were lowest in infancy and increased with age. For drinking water exposure, Clewell et al. (2004) found an age-related trend in the average daily dose and cumulative lifetime dose of tetrachloroethylene and its metabolites, with lower levels of metabolites observed in children compared to higher levels of metabolites observed in adulthood. In a showering/bathing scenario, Rao and Brown (1993) found that tetrachloroethylene accumulates in the brain at higher levels in younger versus older children. Validation and further refinement of the parameters in these PBPK models are necessary, in particular, modeling of fetal and breast milk exposure, and child-adult differences in partition coefficients after inhalation, drinking water, and bathing scenarios.

4.9.1.1.3. Early life-stage-specific 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, Section 4.4 on liver toxicity, Section 4.5 on kidney toxicity, Section 4.6 on neurotoxicity, and Section 4.8 on toxic effects in other organ systems characterize a wide array of postnatal developmental effects.

4.9.1.1.3.1. Preconception

Exposures occurring prior to conception may result in adverse reproductive outcomes. For tetrachloroethylene exposure, adverse outcomes assessed prior to conception include reduced fertility, altered sperm, and altered reproductive hormones.

Fertility. In humans, limited evidence exists on impacts to fertility. A study of couples seeking treatment for infertility found that employment in dry cleaning was significantly associated with infertility among women but not among men, although exposure to tetrachloroethylene was inferred but not documented (Rachootin and Olsen, 1983). Another study observed no impacts on the number of pregnancies or fertility ratio among wives of men employed as dry cleaners compared to wives employed as laundry workers, although wives of

dry cleaners took longer to become pregnant compared to wives of laundry workers (Eskenazi et al., 1991b). Other epidemiological studies have not shown any association between reduced fertility and working in dry cleaning or exposed to tetrachloroethylene, although these results were imprecise because the prevalence of exposure was low (Sallmen et al., 1998; Sallmen et al., 1995). A review of the data by the National Research Council regarding exposures to tetrachloroethylene, trichloroethylene, or solvent mixtures in drinking water at Camp Lejeune, NC, found limited/suggestive evidence of an association for female infertility with concurrent exposure to solvent mixtures, but inadequate/insufficient evidence to determine whether an association exists for female infertility after exposure cessation, and inadequate/insufficient evidence to determine whether an association exists for male infertility (NRC, 2009).

In experimental animals, a study found that the percentage of fertilized oocytes in vitro was reduced in tetrachloroethylene-treated female rats as compared with controls, although this study found no effect from exposure in drinking water (Berger and Horner, 2003). Other studies in rats also found no change in fertility (Carney et al., 2006; Tinston, 1994), and one earlier study reported an increase in fertility of female rats exposed to tetrachloroethylene (Carpenter, 1937).

Sperm. Few studies in either humans or animals have examined altered sperm quality, generally with no observed adverse or consistent effects. Eskenazi and colleagues found that tetrachloroethylene can have subtle effects on sperm quality (Eskenazi et al., 1991a); however, they also reported that altered sperm parameters did not appear to affect reproduction because wives did not have fewer pregnancies as compared with a national standard (Eskenazi et al., 1991b). A study of couples treated for infertility also examined sperm abnormalities among dry cleaners but did not observe an elevated prevalence of sperm alterations, suggesting that the observed reduced fertility rate among these couples was related to other reasons (Rachootin and Olsen, 1983). One rodent study demonstrated inconsistent effects (abnormal sperm at 4 weeks but not 1 or 10 weeks after exposure) in mice, but no adverse effect was observed in rats (Beliles, 2002). Additionally, reduced testes weight was observed in the offspring of rats after inhalation exposure, although these were not significant after adjusting for body weight (Tinston, 1994).

Reproductive Hormones. Few studies in either humans or animals have examined altered hormones related to reproduction, generally with no observed adverse or consistent effects. The study discussed above of couples seeking treatment for infertility examined employment in dry cleaning and found inconsistent results for "a female diagnosis indicating hormonal disturbances" among three analyses (Rachootin and Olsen, 1983). An exploratory study of menstrual disorders among dry-cleaning workers found associations with unusual cycle length, menorrhagia, dysmenorrhea, and premenstrual syndrome, but not with oligomenorrhea, polymenorrhea, irregular cycle, and intermenstrual blood loss (Zielhuis et al., 1989).

A study of rats exposed to 1,700 ppm tetrachloroethylene did not affect progesterone levels (Berger and Horner, 2003). The few studies on altered reproductive hormones suggest this as an area for further research, both in females and males.

4.9.1.1.3.2. Prenatal and birth outcomes

Prenatal and birth outcomes resulting from exposure occurring prior to conception or during fetal development include fetal death (i.e., spontaneous abortion, perinatal death), birth defects, and decreased birth weight. It is important to note that maternal toxicity (e.g., reduced maternal body-weight gain) may influence adverse outcomes in the offspring and was assessed in a number of experimental animal studies of tetrachloroethylene exposure (Szakmáry et al., 1997; Narotsky and Kavlock, 1995; Tinston, 1994; Hardin et al., 1981; Schwetz et al., 1975).

Pregnancy Loss. Human and animal studies examining pregnancy loss are discussed in detail in Section 4.7. For humans, both occupational and drinking water studies have examined fetal loss, an outcome for which there is good retrospective recall, and any bias would result in an underestimation of the true risk (Wilcox and Horney, 1984). However, the available studies may be limited by selection bias and small sample sizes.

A number of occupational studies have shown spontaneous abortion or perinatal loss among women employed as dry cleaners (Doyle et al., 1997; Olsen et al., 1990; Kyyronen et al., 1989; Bosco et al., 1987), or otherwise exposed occupationally (Lindbohm et al., 1991; Windham et al., 1991). An increased risk of spontaneous abortion was not observed in other studies of women who were dry cleaners or wives of dry cleaners (Eskenazi et al., 1991b; Lindbohm et al., 1991; Ahlborg, 1990a; Taskinen et al., 1989; McDonald et al., 1987; McDonald et al., 1986).

A few residential studies have examined spontaneous abortion or perinatal loss among women drinking contaminated water (Aschengrau et al., 2009a; ATSDR, 1998b; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986) or inhaling VOCs (ATSDR, 2008), with no conclusive results. Lagakos et al. (1986) found no association with drinking contaminated water and risk of spontaneous abortion and no association for risk of perinatal death prior to 1970; however, a positive association was observed for perinatal death since 1970. No association was observed in Aschengrau et al. (2009a), but the authors note that the differences between occupational and residential studies may be due to the exposure levels. The National Research Council determined that there is limited/suggestive evidence of an association for miscarriage with tetrachloroethylene-contaminated drinking water exposure at Camp Lejeune during pregnancy (NRC, 2009). No increased risk was observed among women living in a community concerned about vapor intrusion from VOCs including tetrachloroethylene (ATSDR, 2008).

Fetal loss in experimental animals correlates with the observation of spontaneous abortions in humans, with varying tendencies for fetal loss depending on species (rodents have a very low propensity to abort, while rabbits and primates have higher rates). There is evidence of increased preimplantation loss in rats (Szakmáry et al., 1997), increased resorption of pups after maternal inhalation in rats and rabbits (Szakmáry et al., 1997; Schwetz et al., 1975), reduction in litter size and pup survival in rats and guinea pigs (Szakmáry et al., 1997; Narotsky and Kavlock, 1995; Tinston, 1994; Kyrklund and Haglid, 1991), spontaneous abortion in rabbits (Szakmáry et al., 1997), and litters with dead pups (Tinston, 1994). However, fetal loss was not observed in other in vivo studies (Carney et al., 2006; Hardin et al., 1981). In vitro studies of exposure to tetrachloroethylene show decreased fertilized oocytes (Berger and Horner, 2003), and increased mortality, malformations, and delayed growth and differentiation of embryos (Saillenfait et al., 1995).

Birth Defects. After residential exposure to contaminated drinking water, birth defects related to in utero exposure in humans include eye/ear anomalies and CNS/chromosomal/oral cleft anomalies (Lagakos et al., 1986). A study of residents living in a community with vapor intrusion including tetrachloroethylene examined birth outcomes and observed a significantly higher prevalence of total and major cardiac defects (ATSDR, 2006); a follow-up study of this cohort noted that conotruncal heart malformations were particularly elevated (ATSDR, 2008). A recent study in Massachusetts of maternal exposure to drinking water contaminated with tetrachloroethylene reported a 20% increased risk (95% CI: 0.8−1.7) between any maternal exposure at the time of conception and congenital anomalies (oral cleft anomalies, neural tube defects, and gastrointestinal and genitourinary malformations) in the offspring after adjustment for maternal and paternal ages (Aschengrau et al., 2009b); however, this study is inconclusive due to limited adjustment for potential confounding factors and low statistical power. A hypothesis-generating ecological study found a 3.5-fold increased risk of oral cleft defects in New Jersey towns with 410 ppb tetrachloroethylene in drinking water (Bove, 1996; Bove et al., 1995), although a case-control study of oral cleft defects from a larger area in New Jersey designed to test this hypothesis did not confirm the earlier observation (Bove, 1996). Three overlapping studies similarly did not observe any association with birth defects among women who were dry cleaners or laundry workers, although the number of exposed cases was very small (Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989). While the NAS has determined that there is inadequate/insufficient evidence to determine whether an association exists between drinking water at Camp Lejeune, NC, and congenital malformations (NRC, 2009), a follow-up study is currently underway to examine the incidences of neural tube defects and oral cleft anomalies (NRC, 2009; ATSDR, 2003).

In experimental animals, an increase in microphthalmia or anophthalmia in rat offspring was observed after maternal gavage exposure, but no other evaluation of birth defects was undertaken in this study (Narotsky and Kavlock, 1995). Delayed ossification was observed in mice but not in rats exposed prenatally (Schwetz et al., 1975); for skeletal retardation, no significant differences were observed for exposed mice in another study (Szakmáry et al., 1997). Skeletal malformations were increased in mice pups after maternal inhalation exposure, but no additional details were given regarding type of malformation (Szakmáry et al., 1997), and no significant differences were observed in other studies (Carney et al., 2006; Schwetz et al., 1975). Internal organ malformations were significantly increased in mice exposed in utero (Szakmáry et al., 1997; Schwetz et al., 1975), and an in vitro study of rat embryos exposed to tetrachloroethylene showed increased malformations (Saillenfait et al., 1995). No birth defects were observed in other studies of rats (Hardin et al., 1981; Nelson et al., 1979; Schwetz et al., 1975) or rabbits (Hardin et al., 1981).

Conclusions about the association of birth defects with exposure to tetrachloroethylene cannot be drawn from the available epidemiological studies, which contain a number of deficiencies and uncertainties that may introduce a positive or negative bias on observations. A clear need exists for better studies of tetrachloroethylene exposure and birth defects. In particular, given the evidence for heart defects reported in animal studies with exposure to TCE and its metabolites, TCA (Johnson et al., 1998; Smith et al., 1989) and DCA [(Epstein et al., 1992); refer to Sections 4.6.2, 4.7.2, and 4.8.2], there is a need for additional studies of heart defects after exposure to tetrachloroethylene.

Birth Weight. The epidemiological studies reported equivocal findings on birth weight. At the military base of Camp Lejeune, NC, babies born to women living in housing that received drinking water containing VOCs including tetrachloroethylene had a slight decrease in mean birth weight (−26 g, 90% CI: −43, −9) and an increase in small for gestational age (SGA, 22 weeks gestation) (OR: 1.2, 90% CI: 1.0−1.3), most notably among women who had two or more prior fetal losses (OR: 2.5, 90% CI: 1.5−4.3), compared to unexposed women; no increase in preterm births was observed (OR: 1.0, 90% CI: 0.9−1.1) (Sonnenfeld et al., 2001; ATSDR, 1998b). The NAS determined that there is inadequate/insufficient evidence to determine whether an association exists between contaminated drinking water and decreased birth weight at Camp Lejeune, NC (NRC, 2009).

Risk of intrauterine growth restriction (IUGR) was observed in an occupational study (OR: 12.5, no CI given) based on one case exposed to tetra- and trichloroethylene (Windham et al., 1991). A second residential study of a community with VOC exposure from vapor intrusion reported that low birth weight was slightly but statistically elevated (OR: 1.26, 95% CI: 1.00−1.59), as was SGA (OR: 1.22, 95% CI: 1.02−1.45) and full-term low birth weight

(OR: 1.41, 95% CI: 1.01−1.95) (ATSDR, 2006). However, the analysis did not adjust for smoking and sociodemographic factors, which are known to also cause birth weight reductions. Other residential drinking water (Aschengrau et al., 2008; Lagakos et al., 1986) and occupational (Olsen et al., 1990) studies showed no association between exposure to tetrachloroethylene and low birth weight.

In experimental animals, exposure to tetrachloroethylene caused decreased birth weight (Tinston, 1994) and decreased fetal body weight in some studies of rats (Carney et al., 2006; Szakmáry et al., 1997) and mice (Schwetz et al., 1975). However, no effect on birth weight was found in other studies of mice (Szakmáry et al., 1997), rats (Hardin et al., 1981; Schwetz et al., 1975), and rabbits (Szakmáry et al., 1997; Hardin et al., 1981). Experimental animal studies also observed decreased weight gain after either pre- or postnatal tetrachloroethylene exposure. A study in rats demonstrated a reduction in overall pup body weight after preconception, prenatal, and postnatal inhalation exposure (0−1,000 ppm) through 29 days of age (Tinston, 1994). Another study found that the offspring of rats exposed to tetrachloroethylene (0−900 ppm) during late pregnancy (GDs 14−20) had reduced weight gain at postnatal Weeks 3−5, but the same effect was not observed in those exposed earlier in pregnancy (GDs 7−13) (Nelson et al., 1979).

4.9.1.1.3.3. Developmental neurotoxicity

Neurotoxicological effects have been reported after low exposure levels to tetrachloroethylene in children (refer to Section 4.6 and Table 4-4) and in animals after prenatal exposure (refer to Sections 4.6.2 and 4.7.2). Both human and animal evidence supports an association between neurodevelopmental effects and tetrachloroethylene exposure. While other neurotoxic effects are observed in adults (refer to Table 4-5), decreased VCS has been the main observation in children.

Visual deficits. Recent studies have examined the visual system as a target of tetrachloroethylene toxicity in both children and adults. Subjects were New York City apartment residents (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a; Schreiber et al., 2002) and employees and children at a daycare center (NYSDOH, 2005a, b, c; Schreiber et al., 2002) exposed to tetrachloroethylene by proximity to dry cleaners. Exposure was measured in indoor air, exhaled air, and blood levels, and the visual system was assessed by visual contrast sensitivity (VCS) and color confusion index (CCI).

In the day-care studies, visual tests were not conducted on children at the time of exposure due to their young age (NYSDOH, 2005c; Schreiber et al., 2002), and a follow-up evaluation 4 to 5 years after the colocated dry cleaner closed showed no residual changes in VCS or CCI (NYSDOH, 2005a, b). There is a possibility that the results of these test results for

children could be due to a learning disability or a developmental delay (Storm and Mazor, 2004), although these data were not available for the control children (Hudnell and Schreiber, 2004).

The residential studies were designed to assess vision in children and adults living in the same household colocated near dry cleaners (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a; Schreiber et al., 2002). Investigators found that children generally performed better than adults for both VCS and CCI. Children exposed to tetrachloroethylene performed worse than adults for VCS for the highest category of exposure compared to both child and adult reference subjects (NYSDOH, 2010, 2005a), indicating there may be increased susceptibility for children. Poorer CCI scores were associated with levels of tetrachloroethylene-in exhaled breath in children but not in adults (NYSDOH, 2005a), but a later study found that CCI was not associated with levels of tetrachloroethylene exposure in either children or adults (NYSDOH, 2010). The investigators noted that exposure to tetrachloroethylene was highly correlated with race and income, but small sample sizes made it difficult to fully examine this correlation (NYSDOH, 2010, 2005a).

Additionally, a case study reported reduced VCS in a 2.5-year-old boy after prenatal exposure to tetrachloroethylene (Till et al., 2003), as do reports from Till et al. (2005; 2001a; 2001b) and Laslo-Baker et al. (2004) showing visual system functioning deficits in young children of mothers exposed to multiple solvents during pregnancy, although exposure to tetrachloroethylene was not uniquely identified. An important factor to consider in the testing of visual function in children is the requirement for sustained attention and cognition (Tschopp et al., 1998; Scharre et al., 1990). For this reason, visual testing of young children, particularly, contrast sensitivity in children younger than 6 years of age, is difficult, and responses of young children are more variable than those of adults (Scharre et al., 1990). A need exists for developing methods to better evaluate contrast sensitivity effects in the very young-aged child.

Acute Neurotoxicity. Acute neurotoxicity has been observed in children exposed to tetrachloroethylene. A case study by Koppel et al. (1985) reported that a 6-year-old boy who directly ingested 12−16 g of tetrachloroethylene suffered from drowsiness, vertigo, agitation, and hallucinations before lapsing into a coma. One hour after ingestion, his blood tetrachloroethylene concentration was 21.5 mg/L. He recovered, but because follow-up testing was not conducted, any potential long-term effects of the exposure are unknown (Koppel et al., 1985). Garnier et al. (1996) reported mild CNS depression (dizziness and drowsiness were the most common symptoms, along with nausea, vomiting, headache, tinnitus, unconsciousness) after exposure to coin-operated dry-cleaned items in 5 cases of children and 24 cases of adults but did not separate the analysis by age group. Garnier et al. (1996) also described two additional reports (published in Danish) of unconsciousness in a 9-year-old boy who died after

using his dry-cleaned sleeping bag (Korn, 1977), and in a 7-year-old girl who was left in a car with dry-cleaned clothing (Larsen et al., 1977).

Brain neurochemistry. There are no studies in humans measuring brain neurochemistry after exposure to tetrachloroethylene, in either children or adults. In experimental animals, altered brain biochemistry (fatty acid composition) was observed in the offspring after gestational exposure to rats and guinea pigs (Kyrklund and Haglid, 1991; Nelson et al., 1979). These studies do not necessarily indicate effects on brain neurochemistry after gestational exposure compared to adult exposure.

Neurobehavior. Two cohorts examined behavior in children after exposure to tetrachloroethylene, with neither finding any association. In the daycare study described above, 18 children were examined for neurobehavioral deficits using a battery of tests for both neurological and behavioral function. Tests were conducted approximately 5 weeks after exposure ceased (at ages 4−5 years old) (NYSDOH, 2005c), and again in 13 children at a follow-up evaluation 4−5 years later (NYSDOH, 2005a) and reported no functional change at either examination. A large retrospective cohort study in Cape Cod, MA, examined prenatal and postnatal exposure to drinking water contaminated by tetrachloroethylene leaching into water distribution pipes (Janulewicz et al., 2008). Children born in 1969−1983 were included in the analysis (n = 2,086), and followed during 2002−2003. Data were collected from birth certificates and self-administered questionnaires including information on medical history for the mother and child, potential solvent exposure, and water use. Cumulative exposure during the prenatal period was estimated to be 4×10^{-5} to 1,328 g, and exposure during the postnatal period was estimated to be 2.9×10^{-4} to 3,310 g. No statistically significant association was observed with attention, learning, or behavioral functions.

Rats exposure to tetrachloroethylene during pregnancy resulted in developmental delay as measured by the ascent test and rotorod test (Nelson et al., 1979), although another study found no adverse effects for running wheel activity, avoidance behaviors, or operant conditioning (Nelson et al., 1979). Other effects observed include 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).

Young animals have also been directly exposed postnatally to tetrachloroethylene. Daily exposure of rats to 1,000 ppm tetrachloroethylene on PNDs 6−29 resulted in sedation and hypothermia, but the effect ceased 2 hours or less after exposure ended (Tinston, 1994). One gavage study on young 45−50 gram rats showed behavioral and locomotor effects (Chen et al., 2002a). One study of mice showed no neurobehavioral effects immediately after exposure ceased at PND 17, but the mice exhibited increased locomotion and total activity and decreased rearing at PND 60 (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).

Autism spectrum disorder: One case-control study examined the relationship between autism spectrum disorder (ASD) for births in 1994 in the San Francisco Bay Area and estimates of 19 hazardous air pollutant concentrations for the census tract of the birth residence (Windham et al., 2006). Risk estimates for the upper 3rd quartile and upper 4th quartile of tetrachloroethylene exposure were OR: 1.31 (95% CI: 0.93−1.84) and OR: 1.11 (95% CI: 0.78−1.59), respectively, with no suggestion of a linear concentration-response pattern. The low level of exposure detail for individual subjects in the study does not provide sufficient information either for or against an association between tetrachloroethylene and ASD. The causes of autism are unknown, but environmental factors have been hypothesized (Grandjean and Landrigan, 2006). Epidemiologic studies of analytical designs and with more sensitive exposure-assessment approaches are needed to more clearly define any role of tetrachloroethylene and other air pollutants.

4.9.1.1.3.4. Developmental immunotoxicity

Section 4.8.1.1.1 and Table 4-38 describe studies relating tetrachloroethylene to immune response in children. The developing immune system is an area of potential susceptibility (Dietert, 2008), although there are few published studies relating to immune response after tetrachloroethylene exposure to either children or adults. The childhood studies examined a relationship with tetrachloroethylene exposure and allergy, asthma, and infection—immunotoxic outcomes not reported in any of the studies of adults. In addition, family members of children diagnosed with leukemia from Woburn, MA, exhibited altered lymphocyte (CD3, CD4, CD8) and CD4/CD8 ratios (Byers et al., 1988), though this was a mixed exposure to other contaminants in addition to tetrachloroethylene. Other immunological conditions have been observed in adults, but these are distinct from those observed in children discussed below. This is an area for future research.

Allergy. Lehmann et al. (2002) examined cord blood samples from healthy, full-term neonates for T-cell populations and associated them with indoor exposure to VOCs measured 4 weeks after birth (likely to reflect late-prenatal exposures) and observed a significant association of tetrachloroethylene exposure with a reduction of interferon-g-producing Type 1 T-cells. 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 significant association between tetrachloroethylene exposure and allergic sensitization to egg white and milk (Lehmann et al., 2001). No studies of allergy after exposure to tetrachloroethylene were reported in adults.

However, tetrachloroethylene has been demonstrated to adversely affect IL-4 and TNF-a in rodent mast cells (Seo et al., 2008a) and passive cutaneous anaphylaxis in rats exposed i.p. (Seo et al., 2008a) and in drinking water (Seo et al., 2008b).

Asthma. In a study of inhalation exposure, Delfino et al. (2003a; 2003b) measured the concentration of ambient air pollutants, including tetrachloroethylene, and correlated it with subsequent symptoms of asthma in children in the Los Angeles, CA area. These results suggested 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).

Susceptibility to Infection. Only one report on tetrachloroethylene exposure and childhood infection was found in the published literature. Higher prevalences of kidney and urinary tract disorders (primarily infection) and lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia) in children were reported by mothers living in a community with a past history of VOC-contaminated drinking water compared to prevalences reported by mothers living in uncontaminated areas (Lagakos et al., 1986).

4.9.1.1.3.5. Hepatotoxicity

Bagnell and Ellenberger (1977) reported that a child suffered from obstructive jaundice and hepatomegaly after consuming tetrachloroethylene-contaminated breast milk (10 mg/L), with conditions improving when breastfeeding was discontinued.

4.9.1.1.3.6. Fatality

A case report found that vapors off-gassing from dry-cleaned fabrics were implicated in causing the death of a 2-year-old boy who had slept in a room with multiple curtains that had been incorrectly dry cleaned (Gaillard et al., 1995) and retained 6 kg of tetrachloroethylene as estimated by a later experiment repeating the conditions (Garnier et al., 1996). Another case reported a death in a 17-year-old employed at a plastics manufacturing plant and using tetrachloroethylene to clean the inside of a metal mold (NIOSH, 1994).

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 (refer to Section 2.2.5). Due to the rarity of these cases, there are little data to support any hypothesis regarding increased susceptibility for acute mortality in childhood compared to adulthood.

4.9.1.1.3.7. Childhood cancer

The epidemiologic and experimental animal evidence is limited regarding susceptibility to cancer from exposure to tetrachloroethylene during early life-stages. Generally speaking, there may be developmental susceptibility for early lifestage exposure to chemicals and cancer (Anderson et al., 2000; Olshan et al., 2000). The human epidemiological evidence is summarized above for cancer in the liver (refer to Section 4.4.1.2), kidney (refer to Section 4.5.1.2), and other organ systems (refer to Section 4.8.1.2). The experimental animal research is summarized above for cancer in the liver (refer to Section 4.4.2.2), kidney (refer to Section 4.5.2.2), and other organ systems (refer to Section 4.8.2). Few studies have examined cancer in children after exposure to tetrachloroethylene; those few have examined total childhood cancer, leukemia, and brain tumors. A recent review of the data related to exposure to tetrachloroethylene, trichloroethylene, or solvent mixtures found inadequate/insufficient evidence to determine whether an association exists for childhood leukemia, neuroblastoma, or brain cancer (NRC, 2009).

Total Childhood Cancer. One study examined childhood cancers in an area in Endicott, NY, for which vapor intrusion into homes was of concern. Many VOCs were identified in samples and included trichloroethylene and tetrachloroethylene (ATSDR, 2006). This study found fewer than six cases of cancer over a 20-year period, in children up to 19 years of age, which did not exceed expected cases or types.

Childhood Leukemia. Leukemia has been observed in a few studies after exposure to tetrachloroethylene in adults and children. However, the studies are limited by small sample sizes, lack of exposure measurements, exposure to multiple contaminants, and possible participation bias.

A small case-control study of children residing in Woburn, MA, found a strong but imprecise association between maternal exposure during pregnancy and drinking water contaminated with multiple solvents including tetrachloroethylene and childhood leukemia, with a positive dose-response trend, when compared with exposure prior to pregnancy or postnatal exposure to the infant via lactation [(Costas et al., 2002; MDPH, 1997); refer to Section 4.9.1.2.4]. However, it is difficult to uniquely identify tetrachloroethylene as the causative agent given the higher concentrations of trichloroethylene reported. Other population case-control

studies of childhood leukemia have not shown an increased risk from paternal (Shu et al., 1999; Lowengart et al., 1987) or maternal (Infante-Rivard et 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 metastatic progression, and the apoptotic process (Diodovich et al., 2005). In addition, a follow-up study of children from Camp Lejeune, NC, is currently being conducted to determine any association between maternal ingestion of contaminated water and the incidence of childhood leukemia and non-Hodgkin lymphoma (NRC, 2009; ATSDR, 2003). No data are available on cancer risk in animals from early lifestage tetrachloroethylene exposure.

Childhood Brain Cancer. Very few studies of tetrachloroethylene exposure have reported brain tumors, and these are generally quite limited. One study of parental occupational exposure to tetrachloroethylene (8 cases, 11 controls) found no risk of neuroblastoma in the offspring (OR: 0.5, 95% CI: 0.2−1.4) (De Roos et al., 2001). This study, like those on childhood leukemia, is quite limited for examining parental exposure to tetrachloroethylene and childhood cancer.

4.9.1.1.3.8. Age-dependent adjustment factors (ADAFs)

According to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic MOA. Although the contribution of genotoxicity to tetrachloroethylene carcinogenesis cannot be ruled out for one or more target organs, uncertainties with regard to the characterization of tetrachloroethylene genotoxicity remain. This is primarily because in vivo tests of tetrachloroethylene have been equivocal, with at most, modest evidence of genotoxic effects in rodent tumor tissues examined (including mouse liver and rat kidney) following exposure at tumorigenic doses. Additionally, no evidence is available regarding the potential contribution of tetrachloroethylene genotoxicity to other rodent tumor types (particularly, MCL, testes, and brain) or to human cancers. Ames assays of tetrachloroethylene have yielded largely negative results. Certain tetrachloroethylene metabolites (TCVG, TCVC, NAcTCVC, tetrachloroethylene oxide, and DCA) exhibit genotoxicity, the database of available studies is limited, and not all metabolites have been sufficiently tested to support clear conclusions about their genotoxic potential. Additionally, the specific active moiety(ies) that contribute to tetrachloroethylene carcinogenesis are not known.

Thus, because the specific active moiety(ies), mechanisms, or modes of action by which tetrachloroethylene induces carcinogenesis are not known, early-life susceptibility is not assumed, and the application of ADAFs is not recommended.

4.9.1.1.3.9. Early lifestage exposure and outcomes in adulthood

Many additional studies have described adverse outcomes in adults only, mainly based on the assumption of exposure occurring in adulthood; whether or not early lifestage exposures might have occurred is often not considered. Only one identified study reports an examination of early lifestage exposure to tetrachloroethylene and latent outcomes in adults. A large prospective study of the offspring of dry cleaners found a significant increased risk for schizophrenia at 21−33 years of age (Perrin et al., 2007). This is a preliminary report that did not adjust for family history of mental disease, a risk factor for schizophrenia.

4.9.1.2. Later Life-Stages

Due to changes in physiology, in the elderly, exposure levels may be distinct from those observed in younger adults. The elderly have increased ventilation rates per kg body weight compared to adults (U.S. EPA, 2006a) and spend the majority of their time indoors, where increased concentrations of tetrachloroethylene have been found compared to those measured outdoors (U.S. EPA, 2001a). The elderly also experience changes in skin permeability (U.S. EPA, 2006a), which may lead to increased exposure while showering, bathing, or swimming in contaminated water (U.S. EPA, 2001a; Rao and Brown, 1993). While dermal exposure is generally not considered a major route of exposure, this route of exposure is not well characterized for later life-stages.

Toxicokinetics in later lifestages can be distinct in younger adults (Benedetti et al., 2007; U.S. EPA, 2006b; Ginsberg et al., 2005), although there is only limited evidence showing a possible age-related difference in CYP expression (Dorne and Renwick, 2005; Parkinson et al., 2004; George et al., 1995). GST expression has been observed to decrease with age in human lymphocytes, with the lowest expression in those aged 60−80 years old (van Lieshout and Peters, 1998).

Few studies examined the exposure to tetrachloroethylene in elderly adults (>65 years old). One study found elevated blood tetrachloroethylene levels (310−1,770 µg/L) and urine trichloroacetic acid levels (22−1,650 µg/L) in an elderly couple living above a dry-cleaning facility (Popp et al., 1992).

Similarly, few studies examine the effects of tetrachloroethylene exposure in elderly adults. 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).

One PBPK modeled tetrachloroethylene in adults aged 65, 75, and 85 years old and predicted lower concentrations in all compartments for older adults compared to younger adults, and similar predictions for TCA in older and younger adults (Yokley and Evans, 2007). The authors noted that these results indicate that increased susceptibility is likely among older adults due to metabolic changes associated with aging. Another model predicted a decrease in alveolar concentration of tetrachloroethylene in 65-year-olds versus 25-year-olds, which the authors attribute to age-related decreases in cardiac output and ventilation (Guberan and Fernandez, 1974).

These very limited studies suggest that older adults may experience increased exposure to tetrachloroethylene and resulting increased VCS deficits compared to younger adults. However, there is no further evidence of effects for older adults exposed to tetrachloroethylene beyond these 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 observed in a population response to tetrachloroethylene toxicity.

4.9.2.1. Gender

Individuals of different genders are physiologically, anatomically, and biochemically different. Males and females can differ greatly in many physiological parameters such as body composition, organ function, ventilation rate, and metabolic enzyme expression, which can influence the toxicokinetics of chemicals and their metabolites in the body (Gochfeld, 2007; Gandhi et al., 2004; Parkinson et al., 2004). 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 (twofold at age 40) with regard to TCA blood concentration levels [(Clewell et al., 2004); refer to Section 3.5.2 and Figure 3-7]. 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 (refer to Figures 3-6a, b). It is not known whether gender variation of β-lyase activity (refer to 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 (Völkel 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).

In humans, there have been a few studies demonstrating sex-specific effects (refer to Section 4.7.2.3), but it has not been determined whether there is a gender difference in response to exposure to tetrachloroethylene. Among former residents of Camp Lejeune, NC, exposed to contaminated drinking water, there is limited/suggestive evidence of an association between breast cancer and tetrachloroethylene, and inadequate/insufficient evidence to determine whether an association exists for cervical, ovarian/uterine, or prostate cancer (NRC, 2009). Male breast cancer has also been reported by former residents of Camp Lejeune exposed to contaminated drinking water; however, this association has not been investigated sufficiently to draw any conclusions (NRC, 2009).

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 a test session relative to the day of menstruation [(U.S. EPA, 2004); refer to Section 4.6.1.2.5].

Some studies have observed an increased risk for NHL, Hodgkin lymphoma, chronic lymphocytic leukemia or multiple myeloma in females compared to males [(Radican et al., 2008; Ji and Hemminki, 2006b; Miligi et al., 2006; Ji and Hemminki, 2005b; Blair et al., 2003; Andersen et al., 1999; Cohn et al., 1994; Spirtas et al., 1991; Morton and Marjanovic, 1984); refer to Section 4.6.1.2], whereas other studies observed an increase in both males and females (Travier et al., 2002) or no increase in either males or females (Lynge et al., 2006; Boice et al., 1999). Other studies did not examine the outcome in both sexes. Some of these studies are limited by lack of quantitative exposure information, ecological design, or exposure to mixtures, 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 (refer to

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 the 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, 1986). In a rat gavage study, there was no gender difference for toxic nephropathy (NCI, 1977). A marked gender difference was observed 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 α2u-globulin nephropathy (refer to 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); refer to Sections 4.4.2.1 and 4.4.2.2].

4.9.2.2. Race/Ethnicity

Race/ethnicity can often be observed as an important consideration, and may be due to actual increased exposure or to variation in expression of metabolic enzymes due to genetic variability (Garte et al., 2001). In particular, ethnic variability in expression has been reported for CYP (Neafsey et al., 2009; Dorne and Renwick, 2005; Parkinson et al., 2004; McCarver et al., 1998; Shimada et al., 1994; Stephens et al., 1994) and GST (Ginsberg et al., 2009; Nelson et al., 1995).

Studies of VCS in residents in apartments colocated with dry cleaners in New York, NY, found that participants of minority status and low income (≤$60,000) were more likely to have high indoor air levels of tetrachloroethylene (>100 μg/m^3), but analyses of this small sample size of participants in this exposure category could not definitively separate minority status from VCS performance (Storm et al., 2011 [previously reported in NYSDOH, 2010]).

Oxidative damage among female dry cleaners appeared to be increased among black workers compared to female Caucasian workers, although female dry cleaners had decreased levels of oxidative damage compared to female launderers (Toraason et al., 2003). 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).

4.9.2.3. 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 known to exist: 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 50th and 99th percentiles. Opdam (1989a) found a twofold 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 (Chiu and Bois, 2006; Bois et al., 1996). 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.

4.9.2.4. Preexisting Disease

It is known that kidney and liver diseases can affect the clearance of chemicals from the body, and, therefore, poor health may lead to increased half-lives for tetrachloroethylene and its metabolites. There are limited data indicating that certain diseases may alter susceptibility to tetrachloroethylene exposure, mainly through altered metabolism. Presence of cancer likely alters tetrachloroethylene metabolism, because increased CYP2E1 expression has been observed in these individuals (Neafsey et al., 2009). Cirrhosis of the liver likely alters tetrachloroethylene metabolism, because increased CYP2E1 expression has been observed in these individuals [(Neafsey et al., 2009); also refer to Section 4.9.2.5.1]. Tetrachloroethylene is lipophilic and stored in adipose tissue (Monster and Houtkooper, 1979); therefore, obese individuals may experience altered toxicokinetics of tetrachloroethylene compared to nonobese individuals. Obesity also likely alters tetrachloroethylene metabolism, because increased CYP2E1 expression has been observed in obese individuals, compared to nonobese individuals (Neafsey et al., 2009; McCarver et al., 1998). For obese individuals, a model predicted a decrease in alveolar

concentration of tetrachloroethylene during exposure and a decrease in elimination, compared to nonobese individuals (Guberan and Fernandez, 1974).

4.9.2.5. Lifestyle Factors and Nutrition Status

4.9.2.5.1. Alcohol intake

Alcohol is generally regarded as a confounder, although the additive or interactive effects of these exposures along with tetrachloroethylene are not well characterized. Alcohol intake likely alters tetrachloroethylene metabolism and causes higher toxicity, because increased CYP2E1 expression has been observed in individuals who consume alcohol, compared to those who do not (Neafsey et al., 2009; Liangpunsakul et al., 2005; Parkinson et al., 2004; Meskar et al., 2001; McCarver et al., 1998; Lieber, 1997; Perrot et al., 1989). Those exposed to both tetrachloroethylene and TCE and consumed alcohol demonstrated an elevated color confusion index (Valic et al., 1997).

4.9.2.5.2. Tobacco smoking

Smoking, or the number of factors correlated to smoking (e.g., socioeconomic status, diet, alcohol consumption), is generally regarded as a confounder in epidemiological studies (Ruder, 2006), although the additive or interactive effects of these exposures along with tetrachloroethylene are not well characterized. Immunotoxicity and hematotoxicity were observed in tetrachloroethylene-exposed dry cleaners, particularly for those who were smokers (Emara et al., 2010). Sister chromatid exchange in peripheral lymphocytes was observed more frequently in male smokers exposed to tetrachloroethylene alone or in combination with TCE (Seiji et al., 1990). No increase in oxidative damage among tetrachloroethylene-exposed dry cleaners was observed among smokers compared to nonsmokers (Toraason et al., 2003). 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); refer to Section 4.8.1.2.2].

4.9.2.5.3. Nutritional status

Vegetable or vitamin intake may decrease susceptibility to tetrachloroethylene because CYP2E1 inhibition has been observed in individuals who consume various vegetables, herbs, and teas, and increased expression in those consuming high-fat diets (Neafsey et al., 2009). Coexposure to α-tocopherol (vitamin E) along with tetrachloroethylene resulted in decreased rat (Costa et al., 2004) and mouse (Ebrahim et al., 2001; Ebrahim et al., 1996) liver cell toxicity. A similar protective effect was also observed with coexposure to 2-deoxy-D-glucose in mice (Ebrahim et al., 2001; Ebrahim et al., 1996) and taurine in mice (Ebrahim et al., 2001). An in

vitro study of cultured normal human epidermal keratinocytes demonstrated an increase in lipid peroxidation in a dose-dependent manner after exposure to tetrachloroethylene, which was then attenuated by exposure to vitamin E (Ding et al., 2006). However, no associations were found for blood levels of vitamin E and β-carotene in rats [(Toraason et al., 2003); refer to Sections 4.3 and 4.4.4.4.3].

4.9.2.5.4. Physical activity

Studies and models have examined the effect of increased workloads on the toxicokinetics of inhaled tetrachloroethylene alone (Droz et al., 1989a; Droz et al., 1989b; Imbriani et al., 1988; Jakubowski and Wieczorek, 1988; Pezzagno et al., 1988) or with TCE (Opdam, 1989a, b). These studies are equivocal on whether an increase in pulmonary ventilation increases the amount of tetrachloroethylene taken up during exposure. A model predicted an increase in alveolar concentration of tetrachloroethylene after exercise, which the authors attribute to increased cardiac output and ventilation (Guberan and Fernandez, 1974).

4.9.2.6. Socioeconomic Status

Socioeconomic status (SES) can be an indicator for a number of coexposures, such as increased tobacco smoking, poor diet, education, income, and health care access, which may play a role in the results observed in the health effects of tetrachloroethylene exposure.

Children's exposure to tetrachloroethylene was measured in a low SES community, as characterized by income, educational level, and receipt of free or reduced cost school meals (Sexton et al., 2005); however, this study did not compare data to a higher SES community, nor examine health effects. Studies of VCS measured in child and adult residents in apartments colocated with dry cleaners in New York, NY, found that the study participants more likely to be exposed to high indoor air levels of tetrachloroethylene (>100 μg/m^3) were of minority status, low income (≤$60,000), or, for adults, had significantly lower level of education (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a). However, analyses of the small sample size in this exposure category could not definitively separate race/ethnicity or SES from VCS performance.

4.9.2.7. 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 a *Framework for Cumulative Risk Assessment* (U.S. EPA, 2003) 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, 2004). Coexposure 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 nonoccupational sources such as in ground water contamination (e.g., Bove et al., 2002; Sonnenfeld et al., 2001; ATSDR, 1998a; MDPH, 1997; Lagakos et al., 1986). However, no evidence from the available studies indicates greater-than-additive effects for liver and kidney toxicity.

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 (Costa and Ivanetich, 1980; Moslen et al., 1977; Ikeda and Imamura, 1973). 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 (refer to 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 (Hudnell et al., 1996a; Hudnell et al., 1996b; Altmann et al., 1995; Mergler et al., 1991). 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 was 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 coexposed 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).

4.9.3. Uncertainty of Database and Research Needs for Susceptible Populations

There is some evidence that certain populations may be more susceptible to exposure to tetrachloroethylene. The factors examined for tetrachloroethylene include age, gender, race/ethnicity, genetics, preexisting disease, lifestyle factors, nutritional status, socioeconomic status, and multiple exposures and cumulative risk. Areas where the database is currently insufficient for characterizing the impact of tetrachloroethylene on susceptible populations are identified below, along with research needs.

While there is more information on early life exposure to tetrachloroethylene than on other potentially susceptible populations, there remain a number of uncertainties regarding childhood susceptibility. Although inhalation is believed to be of most concern for tetrachloroethylene, pathways of exposure for children are not well characterized. It is not clear to what extent tetrachloroethylene may pass through the placenta in humans, as shown in rodent studies (Szakmáry et al., 1997; Ghantous et al., 1986); for some infants, the primary route of exposure may be through breast milk ingestion (refer to Sections 2.2.4 and 3.2), while for other infants, the dose received through ingestion of breast milk will become insignificant when compared with the inhalation exposure (Schreiber, 1997). The amount of tetrachloroethylene ingested from food is not well described; and it is not known to what extent tetrachloroethylene is absorbed by a child and to which organs tetrachloroethylene and its metabolites may be distributed. The neurological effects of tetrachloroethylene constitute the most sensitive endpoints of concern for noncancer effects, and limited data show that early life-stages may be more susceptible to visual deficits than are adults (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a; Schreiber et al., 2002), yet developmental neurotoxic effects, particularly in the developing fetus, need further evaluation using age-appropriate testing for assessment. There are a number of adverse health effects observed uniquely in early lifestages, with no comparable observations in adults to determine relative sensitivity (e.g., birth outcomes, autism, allergy); conversely, there are some adverse outcomes that have been observed only in adults.

There is suggestive evidence that there may be greater susceptibility for exposures to the elderly, but the available data are much more limited with related uncertainties. Improved PBPK modeling that contains physiologic information for infants and children (including, for example, the effects of maternal inhalation exposure and the resulting concentration in breast milk) and for older adults, and validation of these models, will aid in determining differences in life-stage toxicokinetics of tetrachloroethylene. There may be a true difference in outcome after exposure

during one life-stage compared to another, a lack of assessment of these outcomes in all life-stages, or a lack of assessment of effects of exposures in one life-stage and latent outcomes. 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.

For other susceptibility factors, the data are more limited and based mainly on nonchemical specific data that provide information on variation in physiology, exposure, and toxicokinetics. 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. In addition, further evaluation of the effects of aggregate exposure to tetrachloroethylene from multiple routes and pathways is needed. Similarly, the effects due to coexposures to other compounds with similar or different MOAs need to be evaluated.

4.10. SUMMARY OF HAZARD IDENTIFICATION

4.10.1. Overview of Noncancer and Cancer Hazard

This section summarizes the noncancer and cancer hazard findings for tetrachloroethylene. This summary is based on the analyses presented in the preceding sections, which discussed tetrachloroethylene toxicity on an organ-specific basis, in the following order of presentation: neurotoxicity (refer to Section 4.1); kidney and bladder toxicity and cancer (refer to Section 4.2); liver toxicity and cancer (refer to Section 4.3); esophageal cancer (refer to Section 4.4); lung and respiratory cancer (refer to Section 4.5); immunotoxicity, hematologic toxicity, and cancers of the immune system (refer to Section 4.6); and developmental and reproductive toxicity and reproductive cancers (refer to Section 4.7). Section 4.8 discusses genotoxicity, and susceptible populations are addressed in Section 4.9.

The noncancer hazard characterization for tetrachloroethylene is presented in Section 4.10.2. Effects that were noted in humans and in experimental animals (i.e., neurotoxicity [refer to Section 4.10.2.1], kidney toxicity [refer to Section 4.10.2.2], liver toxicity [refer to Section 4.10.2.3], immunotoxicity and hematologic toxicity [refer to Section 4.10.2.4], and reproductive and developmental toxicity [refer to Section 4.10.2.5]) are first summarized. A tabular summary of the inhalation (refer to Table 4-49) and oral (refer to Table 4-50) studies that are suitable for dose-response analysis, considering all studies across toxicologic endpoints, is then presented in Section 4.10.2.6. Neurotoxicity is identified as a sensitive endpoint following either oral or inhalation exposure to tetrachloroethylene. Section 5 presents dose-response analyses of the neurotoxicity data set as a basis for derivation of inhalation and oral reference values. Quantitative dose-response analyses of the findings for other toxicological effects (i.e., kidney, liver, reproductive and developmental toxicity) are also presented in Section 5.

The cancer hazard characterization for tetrachloroethylene is presented in Section 4.10.3. Section 4.10.3.1 presents the hazard descriptor, characterizing tetrachloroethylene as "likely to be carcinogenic to humans." Section 4.10.3.2 synthesizes the epidemiologic data pertaining to tetrachloroethylene and several cancer types, including non-Hodgkin lymphoma, multiple myeloma, bladder, esophageal, kidney, lung, cervical, and breast cancer. Section 4.10.3.3 summarizes the results from three chronic bioassays that identified tetrachloroethylene-induced rodent cancer, including mononuclear cell leukemia, kidney, and brain tumors in rats and liver tumors in mice. The available mode-of-action information for the carcinogenicity of tetrachloroethylene is presented in Section 4.10.3.4. Section 5 presents dose-response analyses of the rodent bioassay data as a basis for derivation of inhalation and oral cancer slope factors.

4.10.2. Characterization of Noncancer Effects

4.10.2.1. Neurotoxicity

Human and animal studies provide complementary evidence regarding the association of neurobehavioral deficits and tetrachloroethylene exposure. Tetrachloroethylene exposure in humans has primarily been shown to affect visual function (including color vision) and visuospatial memory and other aspects of cognition. Brain-weight changes have been measured in animal studies. A more in-depth discussion of the human neurotoxicological studies can be found in Section 4.1.1.3. The animal inhalation and oral or i.p. exposure studies are discussed in Sections 4.1.2.1 and 4.1.2.2, respectively.

Table 4-49. Inhalation studies suitable for dose-response analyses

Organ/ system	Study	Species	Duration/dosing	NOAEL/LOAEL[a] (ppm)	Effect
CNS	Schreiber et al. (2002)	Human	4 yr mean duration	0.3 (daycare workers, mean and median)	Visual contrast sensitivity
	Schreiber et al. (2002)	Human	5.8 yr (mean), continuous	0.1 (residents, median and mean), maybe as high as 0.4 (mean) and 0.3 (median) [b]	Visual contrast sensitivity[b]
	NYSDOH (2010)	Human	10 yr mean duration	0.002, 0.05 (children) 0.002, 0.07 (adults)	Visual contrast sensitivity
	Cavalleri et al. (1994); Gobba et al. (1998)	Human	8.8 yr mean duration	6, Cavalleri et al. (1994)	Dyschromatopsia
	Spinatonda et al. (1997)	Human	No information on duration	8 (median)	Reaction time
	Seeber (1989)	Human	>10 yr mean duration	12, 53	Visuospatial function, information processing speed
	Ferroni et al. (1992)	Human	10.6 yr mean duration	15	Reaction time, continuous performance
	Echeverria et al. (1995)	Human	15 (high-exposure group) yr mean duration	11[c] (operators)	Visuospatial function
	Altmann et al. (1990)	Human	4-h exposure each day for 4 d.	10, 50	Visual Evoked Potentials
	Hake and Stewart (1977)	Human	7.5 h exposure each day for 5 d.	20, 100, 150	EEGs
	Kjellstrand et al. (1985)	Mouse	Acute (1 h)	0, 90, 320, 400, 600, 800, 1,200, 1,800, 3,600	Increased motor activity
	Rosengren et al. (1986)	Gerbil	Subchronic (12 wk, with 16-wk follow-up) continuous	0, 60, 300	Brain: protein, DNA concentration
	Mattsson et al. (1998)	Rat	Subchronic (13 wk) 6 h/d, 5 d/wk	0, 50, 200, 800	Flash-evoked potential
	Wang et al. (1993)	Rat	Subchronic (12 wk) continuous	0, 300, 600	Reduced brain weight, DNA, protein
	Oshiro et al. (2008)	Rat	60 min	500, 1,000, 1,500	Reaction time
				500, 1,000, 1,500	False alarms
				500, 1,000, 1,500	Trial completions—Signal Detection Task)

Table 4-49. Inhalation studies suitable for dose-response analyses (continued)

Organ/ system	Study	Species	Duration/dosing	NOAEL/LOAEL[a] (ppm)	Effect
CNS (continued)	Boyes et al. (2009)	Rat	90 minutes	250, 500, 1,000	Impairment in steady state visual evoked potential
			120 minutes	1,000, 2,000, 3,000, 4,000	Impairment in steady state visual evoked potential
Kidney	Mutti et al. (1992)	Human	10 yr duration	15 (median)	Urine and serum markers of nephrotoxicity
	NTP (1986)	Rat	Chronic bioassay (104 wk)	0, 200, 400	Increased karyomegaly (74%), megalonuclear-cytosis
	JISA (1993)	Rat	Chronic (104 wk)	0, 50, 200, 600	Increased relative kidney weight; karyomegaly in proximal tubules
	JISA (1993)	Mouse	Chronic (104 wk)	0, 10, 50, 250	Increased relative kidney weight; karyomegaly in proximal tubules
Liver	Kjellstrand et al. (1984)	Mouse	Subchronic (4 wk) continuous	0, 9, 37, 75, 150	Increased liver weight
	NTP (1986)	Mouse	Chronic bioassay (104 wk)	0, 100, 200	Increased liver degeneration, necrosis
	JISA (1993)	Mouse	Chronic (104 wk)	0, 10, 50, 250	Increased angiectasis
Immune and hematologic toxicity	Emara et al. (2010)	Human	Mean duration 7 yr	Mean exposure levels <140 ppm; mean blood levels 1,685 µg/L	Reduced RBC count, reduced hemoglobin, increased WBC count, increased lymphocytes, increased IgE
Reproductive and develop-mental toxicity	Sallmen et al. (1995)	Human	Exposure during year before initiation of pregnancy, occupational, 1973−1983	Mean concentration for dry cleaners in Nordic countries, 1964−1979 = 24 ppm [from Lynge et al. (2006)[d]]	Time to pregnancy
	Eskenazi et al. (1991a)	Human	Wives of exposed men working as dry cleaners, 1980s	31 ppm average concentration, personal samples (n = 208), any job title, all sample durations [Table II, Gold et al. (2006)]	Time to conception

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Table 4-49. Inhalation studies suitable for dose-response analyses (continued)

Organ/ system	Study	Species	Duration/dosing	NOAEL/LOAEL[a] (ppm)	Effect
Reproductive and developmental toxicity (continued)	Olsen et al. (1990); Kyyronen et al. (1989)	Human	1st trimester, occupational, 1973−1983	4.9ppm[d]	Spontaneous abortion
	Nelson et al. (1979)	Rat	7 h/d on GDs 7−13 or 14−20	0, 100, 900	Decreased weight gain in offspring; CNS: behavior, brain acetylcholine
	Beliles et al. (1980)	Mouse	5 d exposure; 1, 4, and 10 wk follow-up	0, 100, 500	Sperm quality
	Tinston (1994)	Rat	Developmental—multigeneration; 6 h/d, 5 d/wk	0, 100, 300, 1,000	F2A pup deaths by Day 29; F1 and F2 generations: CNS depression
	Carney et al. (2006)	Rat	Developmental—6 h/d on GDs 6−19	0, 65, 250, 600	Decreased fetal and placental weight and incomplete ossification of thoracic vertebral centra

[a]Experimental/observational NOAEL is underlined; LOAEL is double-underlined.
[b]Schreiber et al. (2002) found mean PCE 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 mo 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).
[c]Echeverria et al. (1995)—the lowest exposure group is chosen to represent the LOAEL; β coefficient for lifetime or chronic PCE exposure was positive and statistically significant for pattern memory, pattern recognition, and pattern reproduction.
[d]Low group (working at dry cleaners but not operator or spot removal ≥1 h/d); Calculated from mean concentration for dry cleaners 1964−1979 [24 ppm, Lynge et al. (2006)] divided by ratio of exposure for operators versus other work in dry cleaners. Chose a ratio of 5:1 as an intermediate level between 7:1 from Gold et al. (2008) (pg. 816) that included transfer type machines in the United States and 3.5:1 from Räisänen et al. (2001) which included only dry to dry, primarily nonvented machines in Finland.

Table 4-50. NOAELs and LOAELs in selected studies involving oral exposure to tetrachloroethylene

Organ/ System	Study	Species	Duration/exposure Route	NOAEL/LOAEL[a] (mg/kg-day)	Effect
CNS	Fredriksson et al. (1993)	Mouse	PND 10−16/oral gavage	0, 5, 320	Day 60: Increased locomotion, decreased rearing
Kidney	NCI (1977)	Mouse, Rat	Chronic (78 wk)/oral gavage	0, 536, 1,072 (male mice); 0, 386, 772 (female mice); 0, 475, 950 (male and female rats)	Toxic nephropathy
Liver, kidney	Jonker et al. (1996)	Rat	4 wk/oral gavage	0, 600, 2,400	Liver weight, enzyme levels; kidney weight, kidney enzyme levels
Liver	Berman et al. (1995)	Rat	14 d/oral gavage	0, 50, 150, 500, 1,500, 5,000	Liver weight, ALT
Liver	Buben and O'Flaherty (1985)	Mouse (40 g)	6 wk/oral gavage	0, 20, 100, 200, 500, 1,000, 1,500, 2,000	Liver weight, triglycerides
Hematologic toxicity	Marth et al. (1989; 1985a; 1985b); Marth (1987)	Mouse (2 wk old, 20 g)	7 wk/drinking water	0, 0.05, 0.1	Reversible hemolytic anemia, increased serum triglycerides, decreased cholesterol
Development	Bove et al. (1995)	Human	1st trimester, drinking water, 1985−1988	≤1, 3.5, 7.5 and ≥10 µg/L[b]	Oral clefts

[a]NOAELs are underlined once; LOAELs are double-underlined.
[b]Bove et al. (1995) reported risks for categories of drinking water concentration of ≤1, >1−5, >5−10, and >10 µg/L. Exposure levels are the midpoints of these exposure categories. Supported by Aschengrau et al. (2009b) who observed an increased risk of oral clefts associated with any exposure to PCE versus no exposure (1−5, 197 µg/L).

Visual contrast sensitivity deficits as well as color discrimination deficits are commonly present prior to detectable pathology in the retina or optic nerve head. These deficits are, thus, among the earliest signs of disease and potentially more sensitive measures than evoked potentials from visual stimuli (Regan, 1989). Several independent lines of evidence can be found in the occupational and residential exposure studies to support an inference of visual deficits following chronic tetrachloroethylene exposure. The studies that observed effects on color vision using the Lanthony D-15 color vision test include cross-sectional and longitudinal

designs in dry cleaning (Gobba et al., 1998; Cavalleri et al., 1994) and residential (Schreiber et al., 2002) settings. Decrements in color confusion were reported among all workers exposed to a mean TWA of 6 ppm for an average of 8.8 years (Cavalleri et al., 1994). A significant dose-response relationship between CCI value and tetrachloroethylene concentration ($p < 0.01$) was also observed in Cavalleri et al. (1994). As noted previously, the color vision testing in this study was blinded to exposure level of the study participants, and the study participants were well matched in terms of age, smoking, and alcohol use. A follow-up of these workers 2 years later (Gobba et al., 1998) showed greater loss in color discrimination in those who were subsequently exposed to a higher concentration [increase in geometric mean from 1.7 to 4.3 ppm], with no change in those exposed to lower concentrations [decrease in geometric mean from 2.9 to 0.7 ppm]). Although Gobba et al. (1998) demonstrated persistent color confusion effects in this follow up evaluation, the study exposures are not clearly characterized over the course of the 2-year duration. Nakatsuka et al. (1992) did not observe an association with color vision among dry cleaners in China ($n = 64$, geometric mean: TWA 11 and 15 ppm in females and males, respectively), but the relative insensitivity of the specific type of color vision test used in this study (Lanthony, 1978) is a likely explanation for these results. Effects on color vision were also observed among 14 dry cleaners in the small study in Malaysia by Sharanjeet-Kaur et al. (2004), but this study provides little weight to the strength of the evidence because of the lack of exposure information (other than job title), and differences between dry cleaners and controls regarding test conditions and smoking habits. Two other small studies also reported lower scores on the Lanthony D-15 color vision test in much lower exposure settings, but the differences were not statistically significant. A study of residents living above dry cleaners (mean tetrachloroethylene exposure during active dry cleaning = 0.4 ppm), reported mean CCI scores of 1.33 and 1.20 for 17 exposed and 17 controls, respectively ($p = 0.26$). A study of workers in a daycare center located in a building with a dry-cleaning business (mean tetrachloroethylene exposure: 0.32 ppm) reported mean CCI scores of 1.22 and 1.18 in the exposed daycare workers and controls, respectively ($p = 0.39$) (Schreiber et al., 2002). Overall, the evidence reveals a high degree of consistency in this aspect of visually mediated function.

Visual contrast sensitivity changes were reported in two NYSDOH residential studies. In a small pilot study (4 children and 13 adults), mean scores for visual contrast sensitivity (using a near vision visual contrast sensitivity test) across spatial frequencies were statistically significantly lower in exposed residents than in controls, indicating poorer visual function in the exposed groups (Schreiber et al., 2002). Controls were age- and sex-matched to the exposed group, and both groups were English speaking and of predominately Caucasian ethnicity; however, they were drawn from different geographic areas. In addition, two of the four exposed children had diagnoses of learning disabilities or developmental delays, which could affect

performance on this type of test. In the larger study (NYSDOH, 2010, 2005a, b), the test (Functional Acuity Contrast Test, FACT) assessed far vision visual contrast sensitivity, and the test had a low rate of detecting visual contrast changes. For contrast vision, a number of analyses in NYSDOH (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a) suggest a vulnerability among children. However, exposure to >0.015 ppm (>100 µg/m^3) tetrachloroethylene was highly correlated with race and children's age. Additionally, the sample sizes in the highest exposure group, especially in higher income, nonminority groups, makes it difficult to fully examine possible effects of income, race, and age on vision. Therefore, while both studies report visual contrast sensitivity changes, with exposed children being more sensitive, there are concerns with the methodological and analytic approaches in these studies.

Acute human exposure studies reported increased latencies of up to 3.0 ms in visual evoked potentials (Altmann et al., 1990) and changes in EEGs (magnitude of effect was not specified) (Hake and Stewart, 1977; Stewart et al., 1970) at higher exposures ranging from 340 to 680 mg/m^3.

In rats, acute inhalation exposure to tetrachloroethylene results in significant changes to the flash-evoked potential at 800 ppm (Mattsson et al., 1998) and a decrease in F2 amplitudes of the steady state visual evoked potential at 250 ppm (Boyes et al., 2009). In a subchronic exposure study (13 weeks, up to 800 ppm tetrachloroethylene), changes in flash-evoked potential responses were not observed at tetrachloroethylene exposures up to 200 ppm. In the 800 ppm group, there was a significant increase in the amplitude and a significant increase in latency (~3.0 ms) of the mid-flash-evoked potential waveform (N3), but histopathological lesions were not observed in the examination of the visual system brain structures [e.g., visual cortex; optic nerve; Mattsson et al. (1998)].

Effects on visuospatial memory in humans were also reported in each of the studies that examined this measure (Altmann et al., 1995; Echeverria et al., 1995; Echeverria et al., 1994; Seeber, 1989). These effects (increased response times or cognition errors) were observed in occupational and residential studies, and the occupational studies were quite large, involving 101, 65, and 173 dry-cleaning workers in Seeber (1989), Echeverria et al. (1995), and Echeverria et al. (1994), respectively. Several different types of tests were used including digit reproduction (Seeber, 1989), switching, pattern memory, and pattern recognition (Echeverria et al., 1995; Echeverria et al., 1994), and the Benton test (Altmann et al., 1995). Exposure ranges for the increased reaction time observations (LOAELs) ranged from 4.99 to 102 mg/m^3 (Altmann et al., 1995; Echeverria et al., 1995; Ferroni et al., 1992). The changes in the cognitive tasks were observed at exposures (LOAELs) ranging from 53.9 to 364.22 mg/m^3 (Spinatonda et al., 1997; Echeverria et al., 1995; Seeber, 1989). All of these studies except Altmann et al. (1995) indicate that the neurobehavioral assessment was blinded to knowledge of the exposure level of the

subject, and all of the studies adjusted for potentially confounding factors. It should be noted, however, that residual confounding from education-level differences between exposed and referent subjects may still be present in Altmann et al. (1995).

Increased reaction time, increased number of false alarms, and decreased trial completions in a signal detection task (measures of decreased attention) were reported in an acute (60 minutes) exposure (6,782 mg/m^3 or higher) study in rats (Oshiro et al., 2008). Additionally, 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 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.

An occupational exposure study (n=60) (Ferroni et al., 1992) and a residential exposure study (n = 14) (Altmann et al., 1995), with mean exposure levels of 15 and 0.7 ppm, respectively, reported significant increases in simple reaction time of 24 ms (11% increase) (Ferroni et al., 1992) and 40 and 51.1 ms (15 and 20% increases`, respectively`, for two separate measurements) (Altmann et al., 1995) for the exposed subjects. A third study, Lauwerys et al. (1983), reported better performance on simple reaction time in 21 exposed workers (mean TWA: 21 ppm) compared with controls when measured before a work shift but not when measured after work.

The changes in brain weight, DNA/RNA, and neurotransmitter levels that were observed in the animal studies are highly supportive of the neurobehavioral changes observed with tetrachloroethylene exposure. Changes in brain DNA, RNA, or protein levels and lipid composition were altered following inhalation, with changes observed in cerebellum, hippocampus, and frontal cortex (Wang et al., 1993; Rosengren et al., 1986; Savolainen et al., 1977a; Savolainen et al., 1977b). The replication of these changes in biochemical parameters and effects in brain weight in both rats and gerbils is pathognomonic. Changes in neurotransmitters systems (Briving et al., 1986; Honma et al., 1980a; Honma et al., 1980b) and circadian rhythm (Motohashi et al., 1993) in animal studies are consistent with neuroendocrine alterations observed in humans (Ferroni et al., 1992).

In conclusion, the weight of evidence across the available studies of humans and animals exposed to tetrachloroethylene indicates that chronic exposure to tetrachloroethylene can result in decrements in color vision, visuospatial memory, and possibly other aspects of cognition and neuropsychological function, including reaction time.

4.10.2.2. Kidney Toxicity

The epidemiologic studies support association between inhalation tetrachloroethylene exposure and chronic kidney disease, as measured by urinary excretion of renal proteins and ESRD. The elevated urinary RBP levels observed in two studies (Verplanke et al., 1999; Mutti et al., 1992) and lysozyme or β-glucuronidase in Franchini et al. (1983) provide some evidence for effects to the proximal tubules from tetrachloroethylene exposure. Exposures in the studies that observed renal toxicity were time-weighted averages of 8, 10, and 15 ppm. None of the reviewed studies reported exposure-response relationships, and this is an important limitation of the available data. Calvert et al. (2011) supports an association between inhalation tetrachloroethylene exposure and ESRD, particularly hypertensive ESRD, and observed a twofold elevated incidence among subjects who worked only in a shop where tetrachloroethylene was the primary cleaning solvent compared to that expected based on U.S. population rates. An exposure-response pattern was further suggested because hypertensive ESRD risk was highest among those with longest employment durations. No human studies investigating drinking water or other oral tetrachloroethylene exposures on kidney toxicity have been published.

Adverse effects on the kidney have been observed in studies of rodents exposed to high concentrations of tetrachloroethylene by inhalation (JISA, 1993; NTP, 1986), oral gavage (Ebrahim et al., 2001; Ebrahim et al., 1996; Jonker et al., 1996; Green et al., 1990; Goldsworthy et al., 1988; NCI, 1977), and i.p. injection of tetrachloroethylene metabolites (Elfarra and Krause, 2007). The nephrotoxic effects include 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 male rat has been shown to be more sensitive to nephrotoxicity following exposure to tetrachloroethylene. These findings support a LOAEL of 200 ppm and a NOAEL of 50 ppm. Overall, multiple lines of evidence support the conclusion that tetrachloroethylene causes nephrotoxicity in the form of tubular toxicity, mediated potentially through the tetrachloroethylene GSH conjugation products TCVC and TCVCS.

4.10.2.3. Liver Toxicity

Two of four studies of occupationally exposed dry cleaners showed early indications of liver toxicity, namely sonographic changes of the liver and altered serum concentrations of one enzyme indicative of liver injury (Brodkin et al., 1995; Gennari et al., 1992). Frank liver disease was not observed among these workers nor were changes in other biomarkers indicative of liver toxicity (e.g., serum transaminases), which was not unexpected, given subjects with signs of liver disease were excluded in both studies. LOAELs in these human studies were between 12 and 16 ppm (TWA).

Liver toxicity has been reported in multiple animal species by inhalation and oral exposures to tetrachloroethylene. The effects are characterized by increased liver weight, fatty changes, necrosis, inflammatory cell infiltration, triglyceride increases, and proliferation. The mouse has been shown to be more sensitive to hepatic toxicity than the rat in multiple subchronic and chronic studies [e.g., JISA (1993); NTP (1986); Schumann et al. (1980); NCI (1977)]. After subchronic or chronic inhalation exposures in mice, liver toxicity is manifested by increased liver weight (Kjellstrand et al., 1984), liver enlargement (Odum et al., 1988; Kjellstrand et al., 1984), cytoplasmic vacuolation (fatty changes) (Odum et al., 1988; NTP, 1986; Kjellstrand et al., 1984), centrilobular hepatocellular necrosis (JISA, 1993; NTP, 1986), and inflammatory cell infiltrates, pigment in cells, oval cell hyperplasia, and regenerative foci (NTP, 1986). The LOAEL for the inhalation studies, 9 ppm, is from a 30-day continuous exposure mouse study reporting increased liver weight and morphological changes, and is supported by a finding of irreversible macromolecular binding in mouse liver following a single, 6-hour exposure at 10 ppm. The JISA (1993) chronic mouse inhalation bioassay reported liver necrotic foci at 50 ppm and higher.

With oral administration in mice, liver toxicity (increased liver weight, hepatocellular swelling, necrosis, lipid accumulation, and increased DNA synthesis) has been observed at 100 mg/kg-day (Buben and O'Flaherty, 1985; Schumann et al., 1980) and above (Ebrahim et al., 1996; Jonker et al., 1996; Berman et al., 1995; Goldsworthy and Popp, 1987). At 150 mg/kg-day administered for 30 days (Philip et al., 2007), tetrachloroethylene increased ALT levels transiently and stimulated fatty degeneration and necrosis, with ensuing regenerative repair. These findings support a LOAEL of 100 mg/kg-day and a NOAEL of 20 mg/kg-day.

4.10.2.4. Immunotoxicity and hematologic toxicity

The strongest human study examining immunologic and hematologic effects of tetrachloroethylene exposure in terms of sample size and use of an appropriately matched control group is of 40 male dry-cleaning workers (mean exposure levels: <140 ppm; mean duration: 7 years; mean blood tetrachloroethylene levels: 1,685 µg/L) by Emara et al. (2010). Statistically significant decreases in red blood cell count and hemoglobin levels and increases in total white cell counts and lymphocyte counts were observed in the exposed workers compared to age- and smoking-matched controls. Similar effects were observed in mice (Ebrahim et al., 2001). In addition, increases in several other immunological parameters, including T-lymphocyte and natural killer cell subpopulations, IgE, and interleukin-4 levels were observed in tetrachloroethylene-exposed dry-cleaning workers (Emara et al., 2010). These immunologic effects suggest an augmentation of Th2 responsiveness. However, the limited available data from studies in children (Delfino et al., 2003a; Lehmann et al., 2002; Lehmann et al., 2001) do not provide substantial evidence of an effect of tetrachloroethylene exposure during childhood on

allergic sensitization or exacerbation of asthma symptomology. 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. The available data pertaining to risk of autoimmune disease in relation to tetrachloroethylene exposure are limited for ascertainment of disease incidence and exposure-assessment difficulties in population-based studies.

The available data from experimental studies assessing immunotoxic responses in animals are very limited (Hanioka et al., 1995b; Germolec et al., 1989; Aranyi et al., 1986). 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. The data from Aranyi et al. (1986) suggest that short-term exposures may result in decreased immunological competence (immunosuppression) in CD-1 mice. The relative lack of data, taken together with the concern that other structurally related solvents have been associated with immunotoxicity, particularly relating to autoimmune disease (Cooper et al., 2009), contributes to uncertainty in the database for tetrachloroethylene. The limited laboratory animal studies of hematological toxicity demonstrated an effect of tetrachloroethylene exposure on red blood cells (decreased RBCs) (Ebrahim et al., 2001), or decreased erythrocyte colony-forming units (Seidel et al., 1992), with reversible hemolytic anemia observed in female mice exposed to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age in one series of studies (Marth et al., 1989; Marth, 1987; Marth et al., 1985a; Marth et al., 1985b). Ebrahim et al. (2001) also observed decreased hemoglobin, platelet counts, and packed cell volume, and increased WBC counts. Although limited experimental animal studies examining the immunotoxicity and hematologic toxicity of tetrachloroethylene are available in the peer-reviewed published literature, the results of these studies support the human epidemiology studies described above.

4.10.2.5. Reproductive and Developmental Toxicity

4.10.2.5.1. Reproductive toxicity

The literature contains few studies of effects on reproduction among subjects with exposure to tetrachloroethylene. One study of primarily unionized workers in the dry-cleaning and laundry industries in California observed subtle deficits in sperm quality in relation to increasing levels of three measures of exposure, including tetrachloroethylene in exhaled breath (Eskenazi et al., 1991a). However, three clinically recognized measures of sperm quality were not associated with exposure in the study population. The results of Eskenazi et al. (1991a) are compelling, but more studies are needed to understand the spectrum of effects on sperm and their

impact on fecundity. Some studies that relied on detailed work histories and monitoring data to classify exposure suggested that maternal or paternal exposure to tetrachloroethylene or work in dry cleaning reduces fertility or delays conception (Sallmen et al., 1998; Sallmen et al., 1995; Eskenazi et al., 1991b). However, the risk estimates were imprecise because the number of participants reporting exposure to tetrachloroethylene was small. As a consequence, the existing literature is limited and inconclusive concerning effects of tetrachloroethylene on reproduction and fertility.

Results of several studies of maternal occupational exposure to tetrachloroethylene suggest an increased risk of spontaneous abortion, particularly at higher levels of exposure (Doyle et al., 1997; Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989). Most of the studies evaluated exposure during the first trimester of pregnancy. Some of the studies observed an increased odds ratio ranging from 1.4 to 4.7, but risk estimates were statistically imprecise, and some studies were limited in their ability to evaluate potential confounding (Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Bosco et al., 1987). In general, the studies that used a more precise definition of exposure, or categorized exposure into levels of increasing dose or intensity, observed higher risk estimates (Doyle et al., 1997; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989). The Finnish studies controlled for reported exposure to other substances in the workplace as well as for several potential confounders. Increased risks were not found among dry cleaners in Sweden using a similar study design (Ahlborg, 1990a; Olsen et al., 1990). Although there is no evidence of an increased risk associated with paternal exposure, the studies were not of sufficient size or detail in exposure estimates to draw conclusions (Eskenazi et al., 1991a; Lindbohm et al., 1991; Taskinen et al., 1989). No associations with incidence of spontaneous abortion were observed between two populations exposed to tetrachlorethylene in drinking water (Aschengrau et al., 2008; Lagakos et al., 1986). The populations were likely exposed to lower levels compared to the occupational populations. In addition, the window of exposure used to assess risk in both studies may not have been precise enough to detect a small elevation in risk for spontaneous abortion.

The database of experimental animal studies for tetrachloroethylene includes evaluations of reproductive and fertility outcomes in rats and mice following inhalation exposures. Additionally, an in vitro assay of oocyte fertilizability is available. An assessment of fertility and reproductive function in rats exposed to tetrachloroethylene via inhalation over the course of two generations was conducted by Tinston (1994). Effects on offspring included decreased pup weights and postnatal survival in both generations, as well as behavioral alterations in the F1 pups. Decreased mean testes weight was observed in F1a males; however, no effects on male or female fertility or other evidence of alterations in reproductive function were observed. For

males, this finding is supported by the results of a study by Beliles et al. (1980), who found no sperm abnormalities in rats following up to 10 weeks of tetrachloroethylene inhalation exposures. While Beliles et al. (1980) identified an increase in abnormal sperm heads in mice after 4 weeks of exposure, no other reproductive toxicity data in mice were available to aid in the interpretation of this finding. A limitation of the Tinston (1994) study included a concern about a short peri-parturition exposure gap. Additionally, the study was conducted in accordance with standard EPA and OECD toxicological study guidelines in place at the time but did not assess endpoints that are included in the guidelines that were revised and harmonized in 1998.

4.10.2.5.2. Developmental toxicity

A few epidemiologic studies evaluated developmental toxicity endpoints such as decreased birth weight, intrauterine growth restriction (IUGR; also known as small for gestation age [SGA]), and congenital anomalies. Overall, no associations were noted in several studies that assessed maternal or paternal occupational exposure to tetrachloroethylene and increased incidence of stillbirths, congenital anomalies, or decreased birth weight (Lindbohm, 1995; Windham et al., 1991; Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989; Bosco et al., 1987). However, congenital anomalies were analyzed as a combined group, and the number of exposed cases for specific types of anomalies was not sufficient to evaluate risk with statistical precision. Some studies of tetrachloroethylene in drinking water reported that exposure during pregnancy is associated with low birth weight (Bove et al., 1995; Lagakos et al., 1986), eye/ear anomalies (Lagakos et al., 1986), and oral clefts (Aschengrau et al., 2009b; Bove et al., 1995; Lagakos et al., 1986). No associations with tetrachloroethylene exposure were reported for small for gestational age (Bove et al., 1995) or other classifications of congenital anomalies [e.g., musculoskeletal, cardiovascular (Lagakos et al., 1986)]. Although a small increase in risk of small for gestational age was reported for infants exposed prenatally to tetrachloroethylene at the Camp Lejeune military base, the finding remains inconclusive until ATSDR completes its reanalysis. Aschengrau et al. (2008) did not observe associations with birth weight or gestational age in a Cape Cod population living in communities receiving drinking water containing a wide range of tetrachloroethylene concentrations. Participants in some of the studies of drinking water contamination were exposed to multiple pollutants (Bove et al., 1995; Lagakos et al., 1986), and it was not possible to disentangle substance-specific risks. Diagnoses of attention deficit or educational histories reported by the mothers were not increased in relation to the amount of tetrachloroethylene delivered to the homes during pregnancy or childhood (Janulewicz et al., 2008). Finally, a more than threefold risk of schizophrenia was associated with dry cleaning as a surrogate for prenatal tetrachloroethylene exposure (Perrin et al., 2007). The longitudinal design and use of a national registry to identify psychiatric

diagnoses were strengths of the study, but tetrachloroethylene exposure was not directly analyzed.

The animal inhalation study database includes assessments of developmental toxicity in rats, mice, and rabbits following exposures during gestation and assessments of developmental neurotoxicity in rats following pre- and/or postnatal exposures of the offspring. Additional supportive studies include in vitro assays of embryo development and oocyte fertilizability, a developmental assay in Japanese medaka, and two oral gavage studies that assessed developmental toxicity in rats and developmental neurotoxicity in mice. The tetrachloroethylene database included assessments of the various potential manifestations of developmental toxicity, i.e., alterations in survival, growth, morphology, and functional development. Indications of effects on prenatal survival following in utero exposure included increased pre- and/or postimplantation loss in rats, mice, and rabbits (Szakmáry et al., 1997; Schwetz et al., 1975). These findings were supported by evidence of embryo mortality in a rat whole embryo culture (WEC) assay (Saillenfait et al., 1995) and decreased viability in a Japanese medaka assay (Spencer et al., 2002). Decreased prenatal growth was observed in mice (Schwetz et al., 1975) and rats (Szakmáry et al., 1997). Morphological alterations associated with prenatal exposures to tetrachloroethylene included delays in skeletal ossification in mice (Schwetz et al., 1975) and rats (Carney et al., 2006; Szakmáry et al., 1997), which were often associated with fetal weight decrements, and increased incidences of malformations in mice, rats, and rabbits (Szakmáry et al., 1997). Evidence of tetrachloroethylene exposure-related malformations was also observed in the rat WEC and medaka assays (Spencer et al., 2002; Saillenfait et al., 1995) and in a gavage prenatal developmental toxicity screening study in rats (Narotsky and Kavlock, 1995). Alterations in neurological function following pre- and/or postnatal inhalation exposures to tetrachloroethylene were observed in rats by Szakmáry et al. (1997), Nelson et al. (1979), and Tinston (1994). These findings were supported by a study that found altered spontaneous motor activity in young adult rats that had been exposed orally to tetrachloroethylene postnatally during a critical period of nervous system development (Fredriksson et al., 1993). Additionally, reductions in brain acetylcholine and dopamine were observed in rat offspring following gestational tetrachloroethylene exposures (Nelson et al., 1979). Limitations of the inhalation developmental toxicity studies include the lack of dose-response information due to the use of a single treatment level in the prenatal developmental toxicity assessment by Schwetz et al. (1975); the lack of either maternal or developmental toxicity in Hardin et al. (1981); and absence of methodological details in study reporting (Szakmáry et al., 1997).

4.10.2.5.3. Synthesis of human and animal reproductive and developmental 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. Although not a consistent finding in epidemiology studies, the finding of low birth weight in a study of contaminants in drinking water (Bove et al., 1995) is supported by reduced birth weight in five animal studies (Carney et al., 2006; Szakmáry et al., 1997; Nelson et al., 1979; Schwetz et al., 1975) 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 effects on sperm observed in humans (Eskenazi et al., 1991a) correspond to one report of abnormal sperm in mice. Overall, the developmental and reproductive toxicity database for tetrachloroethylene was judged to include a range of data from appropriate well-conducted studies in several laboratory animal species plus limited human data and was considered sufficient for hazard characterization and dose-response assessment, based upon EPA risk assessment guidelines (U.S. EPA, 2006b, 1991b). Based upon a consideration of the available database of animal developmental and reproductive toxicity studies for tetrachloroethylene, the overall inhalation NOAEL is 100 ppm, based on Tinston (1994). The overall inhalation 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.

4.10.2.6. Summary of Noncancer Toxicities and Identification of Studies for Dose-Response Analyses

Noncancer effects of tetrachloroethylene identified in exposed humans and animals include toxicity to the central nervous, renal, hepatic, immune, and hematologic systems, and on development and reproduction. Neurotoxic effects have been characterized in human occupational and residential studies, as well as in experimental animal studies, providing evidence of an association between tetrachloroethylene exposure and neurobehavioral deficits. Tetrachloroethylene exposure primarily results in visual changes, increased reaction time, and decrements in cognition in humans; in animal studies, effects on vision, visual-spatial function, and reaction time, as well as brain-weight changes were also seen. Adverse effects on the kidney in the form of tubular toxicity, potentially mediated through the tetrachloroethylene GSH conjugation products TCVC and TCVCS, have been reported in numerous well-conducted animal studies. Although epidemiological studies have not systematically investigated nephrotoxicity, an association between inhalation tetrachloroethylene exposure and chronic kidney disease, as measured by urinary excretion of renal proteins and ESRD, is supported. The developmental and reproductive toxicity database for tetrachloroethylene includes a range of data from appropriate well-conducted studies in several laboratory animal species plus limited

human data. Evidence of liver toxicity is primarily from several well-conducted rodent studies, including chronic bioassays.

Other toxicity endpoints are less well characterized. The few published reports of experimental studies examining immune or hematologic system toxicity are consistent with the limited findings in the human occupational exposure studies. The limited laboratory animal studies of hematological toxicity demonstrated an effect of tetrachloroethylene exposure on red blood cells (decreased RBCs (Ebrahim et al., 2001), or decreased erythrocyte colony-forming units (Seidel et al., 1992)), with reversible hemolytic anemia observed in female mice exposed to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age in one series of studies (Marth et al., 1989; Marth, 1987; Marth et al., 1985a; Marth et al., 1985b). Ebrahim et al. (2001) also observed decreased hemoglobin, platelet counts, and packed cell volume, and increased WBC counts. The relative lack of additional data, including confirmatory reports of immunotoxic or hematologic toxicity with low continuous exposures beginning in early lifestages, taken together with evidence of immunotoxicity from structurally related solvents (Cooper et al., 2009), contributes to uncertainty in the database for tetrachloroethylene. No epidemiological studies identified potential noncancer respiratory toxicities, and no lung effects in rodents were reported in chronic bioassays (NTP, 1986; NCI, 1977) or other published reports.

The tables above present the inhalation (refer to Table 4-49) and oral (refer to Table 4-50) findings of tetrachloroethylene toxicity, arranged by organ, which are suitable for dose-response analyses. The NOAELs and LOAELs from candidate dose-response studies are identified. In examining the studies judged to be suitable for dose-response analyses, it is evident that the neurotoxicological findings consistently occur at the lowest exposure levels. Additionally, the database for neurotoxicity comprises a number of both occupational and residential human studies as well as animal studies that are suitable for dose-response analyses. Residential inhalation exposures to tetrachloroethylene resulted in visual contrast sensitivity changes and cognitive and motor changes at exposures approximately 5- to 10-fold lower than the lowest sensitive exposure for other toxicological endpoints. Similarly, with oral doses, developmental neurotoxicity effects were observed at levels at least fivefold lower (Fredriksson et al., 1993). Therefore, the CNS effects are identified as a sensitive endpoint following either oral or inhalation exposure to tetrachloroethylene. Section 5 presents dose-response analyses of the neurotoxicity data set as a basis for derivation of inhalation and oral reference values. Quantitative dose-response analyses of the findings for other endpoints (i.e., kidney, liver, reproductive and developmental toxicity) are also presented in Section 5. In addition to providing information regarding the relative sensitivity of different organs/systems to

tetrachloroethylene, such quantitative analyses may be useful for cumulative risk assessment in which multiple chemicals have a common target organ/system other than the CNS.

4.10.3. Characterization of Cancer Hazard

Following EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, tetrachloroethylene is "likely to be carcinogenic in humans by all routes of exposure." This characterization is based on suggestive evidence of carcinogenicity in epidemiologic studies and conclusive evidence that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, increases tumor incidence (JISA, 1993; NTP, 1986; NCI, 1977). Several rodent tumor types were significantly increased with tetrachloroethylene administration in at least two studies. Mouse liver tumors (hepatocellular adenomas and carcinomas) and rat mononuclear cell leukemia were reported in both sexes in two lifetime inhalation bioassays employing different rodent strains, and mouse liver tumors were also reported in both sexes in an oral bioassay (NCI, 1977). Tumors reported in single inhalation bioassays include kidney and testicular interstitial cell tumors in male F344 rats (NTP, 1986), brain gliomas in male and female F344 rats (NTP, 1986), and hemangiomas or hemangiosarcomas in male Crj:BDF1 mice (JISA, 1993). Several metabolites of tetrachloroethylene have also been analyzed for rodent carcinogenicity. TCA and DCA produce liver tumors in mice, and DCA also induces liver tumors in rats. Other tetrachloroethylene metabolites have not been tested in a rodent bioassay.

The specific active moiety(ies) and mode(s) of action involved in the carcinogenicity of tetrachloroethylene and its metabolites are not known. For rat kidney tumors, it is generally believed that metabolites resulting from GSH conjugation of tetrachloroethylene are involved. The hypothesized modes of action for this endpoint include mutagenicity, peroxisome proliferation, α2u-globulin nephropathy, and cytotoxicity not associated with α2u-accumulation. For mouse liver tumors, it is generally believed that metabolites resulting from P450-mediated oxidation of tetrachloroethylene are involved. The mode of action (MOA) hypotheses for this endpoint concern mutagenicity, epigenetic effects (especially DNA hypomethylation), oxidative stress, and receptor activation (focusing on a hypothesized PPARα-activation MOA). However, the available evidence is insufficient to support the conclusion that either rat kidney or mouse liver tumors are mediated solely by one of these hypothesized modes of action. In addition, no data are available concerning the metabolites or the mechanisms that may contribute to the induction of other rodent tumors (including mononuclear cell leukemia, brain gliomas, or testicular interstitial cell tumors in exposed rats and hemangiosarcomas in exposed mice). Furthermore, no mechanistic hypotheses have been advanced for the human cancers suggested to be increased with tetrachloroethylene exposure in epidemiologic studies, including bladder cancer, non-Hodgkin lymphoma and multiple myeloma. Although tetrachloroethylene is largely

negative in genotoxicity assays including in the Ames mutagenicity test, tetrachloroethylene has been shown to induce modest genotoxic effects (micronuclei induction following in vitro or in vivo exposure, and DNA binding and single-strand breaks in tumor tissue) and mutagenic effects under certain metabolic activation conditions. In addition, some tetrachloroethylene metabolites have been shown to be mutagenic. Thus, the hypothesis that mutagenicity contributes to the tetrachloroethylene carcinogenesis cannot be ruled out for one or more target organs, although the specific metabolic species or mechanistic effects are not known.

4.10.4. Synthesis of Epidemiologic Studies

The available epidemiologic studies provide a pattern of evidence associating tetrachloroethylene exposure and several types of cancer, specifically bladder cancer, non-Hodgkin lymphoma, and multiple myeloma. Associations and exposure-response relationships for these cancers were reported in studies using higher quality (more precise) exposure assessment methodologies for tetrachloroethylene. Confounding by common lifestyle factors such as smoking are unlikely explanations for the observed results. For other sites, including esophageal, kidney, lung, liver, cervical, and breast cancer, more limited data supporting a suggestive effect are available.

With respect to bladder cancer, the pattern of results from this collection of studies is consistent with an elevated risk for tetrachloroethylene of a relatively modest magnitude (i.e., a 10−40% increased risk). The effect estimates from five of the six studies with relatively high quality exposure-assessment methodologies ranged from 1.44 to 4.03 [Calvert et al. (2011); Lynge et al. (2006); Blair et al. (2003); Pesch et al. (2000b); Aschengrau et al. (1993)]. An exposure-response gradient was observed in a large case-control study by Pesch et al. (2000b), using a semiquantitative cumulative exposure assessment, with an adjusted odds ratio of 0.8 (95% CI: 0.6, 1.2), 1.3 (95% CI: 0.9, 1.7), and 1.8 (95% CI: 1.2, 2.7) for medium, high, and substantial exposure, respectively, compared to low exposure. A similar exposure-response pattern was not observed in the study by Lynge et al. (1995). This study examined exposure duration, however, rather than a measure that incorporated information on exposure concentration. In addition, relative risk estimates between bladder cancer risk and ever having a job title of dry-cleaner or laundry worker in four large cohort studies ranged from 1.01 to 1.44 (Pukkala et al., 2009; Wilson et al., 2008; Ji et al., 2005a; Travier et al., 2002). As expected, the results from the smaller studies are more variable and less precise, reflecting their reduced statistical power. Confounding by smoking is an unlikely explanation for the findings, given the adjustment for smoking by Pesch et al. (2000b) and in other case-control studies.

The results from the collection of studies pertaining to non-Hodgkin lymphoma also indicate an elevated risk for tetrachloroethylene. The results from five cohort studies that used a

relatively high quality exposure-assessment methodology generally reported relative risks between 1.7 and 3.8 (Calvert et al., 2011; Seldén and Ahlborg, 2011; Radican et al., 2008; Boice et al., 1999; Anttila et al., 1995). There is also some evidence of exposure-response gradients in studies with tetrachloroethylene-specific exposure measures based on intensity, duration, or cumulative exposure (Seidler et al., 2007; Miligi et al., 2006; Boice et al., 1999). Higher non-Hodgkin lymphoma risks were observed in these studies in the highest exposure categories, with the strongest evidence from the large case-control study in Germany in which a relative risk of 3.4 (95% CI: 0.7, 17.3) was observed in the highest cumulative exposure category (trend *p*-value = 0.12) (Seidler et al., 2007). Effect estimates in studies with broader exposure assessments showed a more variable pattern (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji and Hemminki, 2006b; Blair et al., 2003; Travier et al., 2002; Cano and Pollán, 2001; Lynge and Thygesen, 1990). Confounding by life-style factors are unlikely explanations for the observed results because common behaviors, such as smoking and alcohol use, are not strong risk factors for non-Hodgkin lymphoma (Besson et al., 2006; Morton et al., 2005).

Results from the multiple myeloma studies are based on a smaller set of studies than those of non-Hodgkin lymphoma, but results are similar. The larger cohort studies that use a relatively nonspecific exposure measure (broad occupational title of launderers and dry cleaners, based on census data) do not report an increased risk of multiple myeloma, with effect estimates ranging from 0.99 to 1.07 (Pukkala et al., 2009; Ji and Hemminki, 2006b; Andersen et al., 1999). Some uncertainty in these estimates arises from these studies' broader exposure-assessment methodology. Results from the cohort and case-control studies with a higher quality exposure-assessment methodology, with an exposure measure developed specifically for tetrachloroethylene, do provide evidence of an association, however, with relative risks of 7.84 (95% CI: 1.43, 43.1) in women and 1.71 (95% CI: 0.42, 6.91) in men in the cohort of aircraft maintenance workers (Radican et al., 2008) and 1.5 (95% CI: 0.8, 2.9) in a case-control study in Washington (Gold et al., 2010b); tetrachloroethylene exposure). Gold et al. also reported increasing risks with increasing exposure duration (based on job titles) (Gold et al., 2010a) and based on a cumulative tetrachloroethylene exposure metric (Gold et al., 2010b). A smaller case-control study (n = 76 cases) with tetrachloroethylene-specific exposure measures based on intensity, duration, or cumulative exposure, Seidler et al. (2007), observed no cases among the highest exposure groups. A small cohort study by Boice et al. (1999) of aerospace workers observed one death among routinely exposed subjects and six deaths among subjects with a broader definition of routine or intermittent exposure.

Suggestive but limited evidence was also observed in the collection of epidemiologic studies pertaining to tetrachloroethylene exposure and esophageal, kidney, lung, liver, cervical, and breast cancer. One difference between these sets of data and the data for bladder cancer,

non-Hodgkin lymphoma, and multiple myeloma is a more mixed pattern of observed risk estimates and an absence of exposure-response data from the studies using a quantitative tetrachloroethylene-specific cumulative exposure measure.

For esophageal cancer, the SIR in the only large cohort study (n = 95 cases), a study using broad exposure categories, was 1.18 (95% CI: 0.96, 1.46) (Pukkala et al., 2009). The point estimates of the association in seven of eight smaller studies, four studies with specific exposure assessments, and four other studies with less precise assessments were between 1.16 and 2.44 (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Sung et al., 2007; Blair et al., 2003; Travier et al., 2002; Boice et al., 1999; Lynge and Thygesen, 1990). Two small case-control studies with relatively high quality exposure-assessment approaches, Lynge et al. (2006) and Vaughan et al. (1997) reported an odds ratio of 0.76 (95% CI: 0.34, 1.69) and of 6.4 (95% CI: 0.6, 68.9), respectively. Some uncertainties in these estimates arise from the lack of job title information for 25% of the cases and 19% of the controls, and the variability in the results from the sensitivity analysis using different assumptions regarding the correct classification of individuals in this group or the small number of exposed cases. One of the two larger studies examining exposure-response suggested a positive relationship (Calvert et al., 2011). Based on smoking rates in blue-collar workers, the twofold risk estimate reported in Calvert et al. (2011) and Blair et al. (2003) was higher than that which could reasonably be attributable to smoking.

One primary study that supports an association between tetrachloroethylene exposure and kidney cancer, the largest international case-control study (245 exposed cases from Australia, Denmark, Germany, Sweden, and the United States), reported a relative risk of 1.4 (95% CI: 1.1, 1.7) for any exposure to dry-cleaning solvents (Mandel et al., 1995). This study was able to adjust for smoking history, BMI, and other risk factors for kidney cancer. Results from the large cohort studies, using a more general exposure classification based on national census occupation data, present more variable results, with relative risks of 0.94, 1.11, and 1.15 in Pukkula et al. (2009), Travier et al. (2002), and Ji et al. (2005b), respectively. The results from the smaller studies using a relatively specific exposure-assessment approach to refine classification of potential tetrachloroethylene exposure in dry-cleaning settings are mixed, with some studies reporting little or no evidence of an association (Lynge et al., 2006; Pesch et al., 2000b; Boice et al., 1999; Dosemeci et al., 1999; Aschengrau et al., 1993), and other studies reporting elevated risks (Calvert et al., 2011; Blair et al., 2003; Anttila et al., 1995; Schlehofer et al., 1995). An increasing trend in relative risk with increasing exposure surrogate was not observed in any of the larger occupational exposure studies with three or more exposure categories (Lynge et al., 2006; Mandel et al., 1995), but some indication of higher risk with higher exposure (or duration) was observed in other studies (Blair et al., 2003).

For lung cancer risk, the results from seven large cohort studies of dry cleaners are consistent with an elevated lung cancer risk of 10−40% (Calvert et al., 2011; Seldén and Ahlborg, 2011; Pukkala et al., 2009; Ji et al., 2005b; Blair et al., 2003; Travier et al., 2002; Schlehofer et al., 1995; Lynge and Thygesen, 1990). Similar results were observed in four of the five occupational studies that were identified as having a relatively strong exposure-assessment methodology (Calvert et al., 2011; Blair et al., 2003; Boice et al., 1999; Anttila et al., 1994). However, Seldén and Ahlborg (2011) observed similar, but slightly higher, relative risks for laundry workers compared with dry-cleaning workers in their study. These studies were unable to control for potential confounding from cigarette smoking; however, and the magnitude of the association in these studies is consistent with that expected assuming the prevalence of smoking among dry-cleaners and laundry workers was slightly higher (e.g., 10% higher) than among the general population. Features of the selection of study participants and study analysis in the available case-control studies reduce the potential for confounding by smoking, however. Two case-control studies were limited to either nonsmokers or ex-smokers and both of these studies indicate an approximate twofold increased risk with a history of work in the dry-cleaning industry [OR: 1.8, 95% CI: 1.1, 3.0 in Brownson et al. (1993), and OR: 1.83, 95% CI: 0.98, 3.40 among women in Pohlabeln et al. (2000)]. The other case-control studies adjusted for smoking history, and the results for these (somewhat smaller studies) are similar to the previously cited estimates. Among the studies that evaluated exposure-response gradients, the evidence for a trend in risk estimates was mixed (Calvert et al., 2011; Blair et al., 2003; Travier et al., 2002; Boice et al., 1999; Paulu et al., 1999; Brownson et al., 1993).

For liver cancer, studies carrying greater weight in the analysis based on the large number of observed events or exposed cases, or based on a strong exposure-assessment approach show a mixed pattern of results. The one case-control study with a large number of exposed liver cancer cases and a relatively high quality exposure-assessment methodology reported an odds ratio estimate of 0.76 (95% CI: 0.38, 1.72) for liver cancer and dry cleaning (Lynge et al., 2006). A recent multiple Nordic country cohort study and two cohort studies of Swedish subjects with broad exposure-assessment approaches, and whose subjects overlapped with Lynge et al. (2006), reported SIRs of 1.02 (95% CI: 0.84, 1.24), 1.22 (95% CI: 1.03, 1.45), and 1.23 (95% CI: 1.02, 1.49) for liver and biliary tract cancer and work as a dry-cleaner or laundry worker in Travier et al. (2002), Ji and Hemminki (2005c), and Pukkala et al. (2009), respectively. Three other studies with strong exposure-assessment approaches specific to tetrachloroethylene, but whose risk estimates are based on fewer observed liver cancer cases or deaths, reported risk estimates of 1.21 to 2.05 for the association between liver cancer and tetrachloroethylene (Seldén and Ahlborg, 2011; Boice et al., 1999; Bond et al., 1990; Blair et al., 1979). However, dry cleaning workers did not have a higher liver cancer risk estimate than laundry workers or other categories

of dry cleaning workers (Seldén and Ahlborg, 2011; Lynge et al., 2006). Exposure response was not observed, and the SIR for tetrachloroethylene-exposed subjects with the longest employment duration in Seldén and Ahlborg (2011) was lower than that for subjects with shorter employment duration. Potential confounding may be an alternative explanation as no study adjusted for known and suspected risk factors for liver cancer (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Lynge et al., 2006; Ji and Hemminki, 2005c; Travier et al., 2002; Boice et al., 1999; Bond et al., 1990). Nine other cohort and case-control studies with fewer observed events and/or a broad exposure-assessment methodology carried less weight in the analysis and reported a mixed pattern of results (Calvert et al., 2011; Lindbohm et al., 2009; Sung et al., 2007; Blair et al., 2003; Lee et al., 2003; Lynge et al., 1995; Vartiainen et al., 1993; Suarez et al., 1989; Stemhagen et al., 1983). Lee et al. (2003) reported a risk estimate of 2.57 (95% CI: 1.21, 5.46) for the association between liver cancer and residence in a village with ground water contamination, but subjects were from a region with a high prevalence of HCV infection, and HCV status may confound the observed association.

For cervical cancer, the results from the two large cohort studies with a broad exposure assessment are consistent with an elevated cervical cancer risk of 20−30% (Pukkala et al., 2009; Travier et al., 2002). Results from four smaller cohort and case-control studies with a relatively high quality exposure-assessment methodology presented a pattern of more variable results, with relative risks of 0.98 (95% CI: 0.65, 1.47), 1.19 (95% CI: 0.64, 1.93), 2.10 (95% CI: 0.68, 4.90), and 3.20 (95% CI: 0.39, 11.6) in Lynge et al. (2006), Sélden and Ahlborg (2011), Calvert et al. (2011), and Anttila et al. (1995), respectively. A fourth study with higher quality exposure-assessment specific to tetrachloroethylene did not observe any cervical cancer deaths among women, but less than one death was expected (Boice et al., 1999). Calvert et al. (2011) was the only study to report an exposure response gradient with employment duration. Dry cleaning workers did not have higher cervical cancer risks compared with laundry workers or other categories of dry cleaning workers (Seldén and Ahlborg, 2011; Lynge et al., 2006). 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. Potential confounding by socioeconomic status is an alternative explanation with some support provided by Lynge et al. (2006), a case-control study with controls of similar socioeconomic status as cases and that did not observe an association between cervical cancer and dry cleaning.

The results from the large studies of breast cancer risk in women in relation to tetrachloroethylene exposure are mixed. The largest, based on 1,757 breast cancer cases in female dry-cleaners and laundry workers, reported a statistically significant deficit in the risk of breast cancer incidence compared to the populations of Nordic countries (Pukkala et al., 2009).

Findings in the other four studies were based on fewer events or exposed cases; two of four studies with a nonspecific exposure-assessment methodology provided evidence for association between breast cancer in females and tetrachloroethylene exposure (Sung et al., 2007; Chang, 2005; Aschengrau et al., 2003; Anderson et al., 1999; Lynge and Thygesen, 1990), but no association was observed in two other large cohort studies with a relatively high quality exposure-assessment methodology to tetrachloroethylene (Seldén and Ahlborg, 2011; Blair et al., 2003). Small studies also observed mixed findings (Calvert et al., 2011; Radican et al., 2008; Peplonska et al., 2007; Sung et al., 2007; Aschengrau et al., 2003; Band et al., 2000; Boice et al., 1999). Although cohort studies were unable to control for potential confounding from reproductive history or menopausal status, observations in case-control studies controlled for these potential confounders in statistical analyses and provided support of an association between female breast cancer and tetrachloroethylene compared to controls (Peplonska et al., 2007; Aschengrau et al., 2003; Band et al., 2000). Three studies examined exposure response, and two of these studies with semiquantitative or quantitative exposure-assessment approaches reported risk estimates in females monotonically increased in higher exposure groups (Aschengrau et al., 2003; Blair et al., 2003). A third study examining exposure duration observed an inverse relation (Peplonska et al., 2007). Exposure duration is more uncertain than use of a semiquantitative surrogate given increased potential for bias associated with exposure misclassification. Because of the limitation in statistical power, none of the five studies reporting on male breast cancer is adequate to examine tetrachloroethylene exposure (Seldén and Ahlborg, 2011; Pukkala et al., 2009; Chang et al., 2005; Anderson et al., 1990; Lynge and Thygesen, 1990).

4.10.5. Synthesis of Rodent Cancer Bioassay Findings

One oral gavage (NCI, 1977) and two inhalation (JISA, 1993; NTP, 1986) cancer bioassays provide evidence of tetrachloroethylene carcinogenicity in rats and mice. In male and female rats, inhalation exposure to tetrachloroethylene significantly increased the incidence of mononuclear cell leukemia (MCL) in independent bioassays of the F344/N (JISA, 1993; NTP, 1986) or F344/DuCrj (JISA, 1993) strain. Tetrachloroethylene reduced MCL latency in females in both studies. In addition, the NTP bioassay reported dose-related increases in the severity of MCL in males and females. Additional tumor findings in rats included significant increases in the NTP bioassay of two rare tumor types, kidney tumors in males, and brain gliomas in males and females. Additionally, the NTP (1986) bioassay reported increases in the rate of testicular interstitial cell tumors, a tumor type of high incidence in unexposed male F344 rats. Other evidence, including that brain gliomas occurred earlier with tetrachloroethylene exposure than in control animals, and that the related compound trichloroethylene is a kidney carcinogen in rats and humans and a testicular carcinogen in rats, support the significance of these findings. A

third rat bioassay, of oral gavage exposure in Osborne-Mendel rats, was inconclusive with respect to carcinogenicity due to a high incidence of respiratory disease in all animals and shortened survival in tetrachloroethylene-exposed animals (NCI, 1977).

In male and female mice, tetrachloroethylene exposure via inhalation (JISA, 1993; NTP, 1986) or oral gavage (NCI, 1977) significantly increased the incidence of hepatocellular adenomas and carcinomas. The NCI and NTP studies employed the B6C3F_1 strain, while the JISA study examined the Crj:BDF1 strain. The JISA study reported increases in hemangiomas or hemangiosarcomas of the liver, spleen, fat, and subcutaneous skin in exposed male Crj:BDF1 mice.

In summary, tetrachloroethylene increased the incidence of liver tumors (hepatocellular adenomas and carcinomas) in male and female mice and of MCL in both sexes of rats. These findings were reproducible in multiple lifetime bioassays employing different rodent strains and, in the case of mouse liver tumors, by inhalation and oral exposure routes. Additional tumor findings in rats included significant increases in the NTP bioassay of testicular interstitial cell tumors and kidney tumors in males, and brain gliomas in males and females. In mice, hemangiosarcomas in liver, spleen, fat, and subcutaneous skin were reported in males in the JISA study. The rat and mouse findings are summarized in Tables 4-51 and 4-52, respectively, and in the sections below.

4.10.5.1. Carcinogenicity Findings in Rats

The NCI oral gavage study in Osborne-Mendel rats was considered to be inconclusive because of the high incidence of respiratory disease, and high mortality with tetrachloroethylene exposure. Lesions indicative of pneumonia were observed in almost all rats at necropsy. A high incidence of toxic nephropathy was evident in tetrachloroethylene-exposed male and female rats. Early mortality was also observed in tetrachloroethylene-exposed animals; 50% of the high dose males and females had died by Weeks 44 and 66, respectively. Therefore, this bioassay is not considered further in the below evaluation of the carcinogenicity of tetrachloroethylene in rats.

The NTP (1986) and JISA (1993) inhalation bioassays reported increases in the incidence of mononuclear cell leukemia (MCL) in male and female F344/N or F344/DuCrj rats. Supplemental analyses by NTP indicated that tetrachloroethylene produced a dose-related increase in the severity of MCL in both males and females. Additionally, NTP found that tetrachloroethylene exposure significantly shortened the time to onset of MCL in females. Although survival was unaffected, the incidence of advanced MCL increased in female rats that died before the scheduled study termination. MCL incidences were higher in the concurrent than in the 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 rates were also

higher than the NTP program historical rate for untreated control groups (males: 583/1,977 [29%]; females: 375/2,021 [18%]).

The Japanese bioassay (JISA, 1993) also reported a significant dose-dependent increase in MCL in male and female F344/DuCrj rats exposed for 104 weeks to 50, 200, and 600 ppm tetrachloroethylene. MCL latency was decreased in female rats, with the first appearance in Week 100 in controls and Weeks 66−70 in exposed rats. As in the NTP study, there was a higher control incidence of MCL (22% in males and 20% in females) than the reported historical rate of MCL for the Japanese laboratory of 147/1,149 [13%] in males and 147/1,048 [14.0%] in females.

Additional tumor findings in rats included a significant increase in the NTP bioassay of two rare tumor types, kidney tumors in males and brain gliomas in both sexes of exposed F344/N rats. Kidney tumors rarely occur in unexposed F344/N male rats, with historical incidences reported to be 0.2% in 1968 controls. The reported incidences with 0, 200, or 400 ppm tetrachloroethylene exposure were 1/49, 3/47, and 4/50, respectively. Additional support for the significance of the kidney tumors comes from evidence that the related chemical trichloroethylene induces this tumor type in humans and in male rats (U.S. EPA, 2011b). For brain gliomas, the laboratory and overall program historical control incidences were 2/247 (0.8%) and 4/1971 (0.2%), respectively. Reported incidence with 0, 200, or 400 ppm tetrachloroethylene exposure was 2/50, 0/48, and 4/50 in males and 1/50, 0/50, and 2/50 in females, respectively. The significance of the brain tumor findings is supported by the earlier occurrence with tetrachloroethylene exposure, suggesting an effect on latency. In males, tetrachloroethylene-induced brain tumors were observed beginning at Week 88 compared with Week 99 in controls. Female brain tumors were first observed at 75 weeks in tetrachloroethylene-exposed animals compared with 104 weeks in control group females.

Table 4-51. Tumor incidence in rats exposed to tetrachloroethylene

Bioassay	Doses/exposures		Sex	Reported cumulative tumor incidence[a](%)					
	Admin.	Continuous equivalent		Hepatocellular adenomas or carcinomas	Hemangioma or hemangio-sarcomas[b]	Renal adenomas or carcinomas	Mononuclear cell leukemia[c]	Testicular interstitial cell tumors	Brain gliomas
NCI (1977)[d] Osborne-Mendel rats Gavage: 5 d/wk, 78 wk	Vehicle 500 mg/kg-day 1,000 mg/kg-day	0[e] 471 mg/kg-day 941 mg/kg-day	Male	None reported[a]	1/20 1/49 0/50	2[f]/20 (5) 1[f]/49 (2) 0/50 (0)	None reported	None reported	None reported
	Vehicle 500 mg/kg-day[f] 1,000 mg/kg-day	0[f] 474 mg/kg-day 974 mg/kg-day	Female	None reported	None reported	0/20 (0) 0/50 (0) 0/50 (2)	None reported	N/A	None reported
NTP (1986) F344/N rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 200 ppm 400 ppm	0 36 ppm 72 ppm	Male	0/50 (0) 1/50 (2) 1/50 (2)	0/50 0/50 0/50	1/49 (2) 3/49 (6) 4/50 (8)	28/50 (56) 37/50 (77) 37/50 (74)	36/50 (76) 39/49 (80) 41/50 (82)	1/50 (2) 0/50 (0) 4/50 (8)
	0 200 ppm 400 ppm	0 36 ppm 72 ppm	Female	0/50 0/50 0/50	0/50 0/50 0/50	0/47 0/44 0/46	18/50 (36) 30/50 (60) 29/50 (58)	N/A	1/50 (2) 0/50 (0) 2/50 (4)
JISA (1993) F344/DuCrj rats Inhalation: 6 h/d, 5 d/wk, 104 wk	0 50 ppm 200 ppm 600 ppm	0 9 ppm 36 ppm 108 ppm	Male	0/50 0/50 0/50	0/50 0/50 0/50 0/50	1/50 (2) 2/50 (4) 1/50 (2) 2/50 (4)	11/50 (22) 14/50 (28) 22/50 (44) 27/50 (54)	47/50 (94) 46/50 (92) 45/50 (90) 48/50 (96)	2/50 (4) 0/50 (0) 0/50 (0) 0/50 (0)
	0 50 ppm 200 ppm 600 ppm	0 9 ppm 36 ppm 108 ppm	Female	1/50 (2) 0/50 (0) 1/50 (2) 0/50 (0)	1/50 0/50 0/50 0/50	0/50 (2) 0/50 (0) 0/50 (0) 1/50 (2)	10/50 (20) 17/50 (34) 16/50 (32) 19/50 (38)	N/A	0/50 0/50 1/50 0/50

[a]None reported: Individual animal data were not available, and summary data did not include a line item for this tumor type.
[b]These tumors were reported as hemangioendotheliomas in the JISA (1993) report. The term has been updated to hemangioma (benign) or hemangiosarcoma (malignant). 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 hemangioendotheliomas from the individual animal data provided in the JISA report.
[c]Reflects the number of animals with MCL reported under "multiple organs," spleen, or liver.
[d]This study was inconclusive with respect to carcinogenicity due to a high incidence of respiratory disease in all animals and shortened survival in PCE-exposed animals.
[e]Gavage doses listed were adjusted several times during the course of the study. Male rats received the listed TWA daily doses through Week 78, and surviving animals were observed up to study termination in Week 110.
[f] "Mixed tumor, malignant" (NCI, 1977).

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Table 4-52. Tumor incidence in mice exposed to tetrachloroethylene

Bioassay	Doses/exposures		Sex	Reported cumulative tumor incidence (%)					
	Administered exposure	Continuous equivalent exposures		Hepatocellular adenomas or carcinomas	Hemangioma or hemangio-sarcoma[a]	Renal adenomas or carcinomas	Malignant lymphoma	Testicular interstitial cell tumors	Brain gliomas
NCI (1977)[b] $B6C3F_1$ mice Gavage: 5 d/wk, 78 wk	Vehicle 450 mg/kg-day 900 mg/kg-day	0 536 mg/kg-day 1,072 mg/kg-day	Male	2/20 (10) 32/48 (67) 27/45 (60)	None reported[c]	0/20 (0) 1/49 (2) 0/48 (0)	None	None reported	None reported
	Vehicle 300 mg/kg-day[d] 600 mg/kg-day	0 386 mg/kg-day 772 mg/kg-day	Female	0/20 (0) 19/48 (40) 19/48(40)	None reported	None reported	None	N/A	None reported
NTP (1986) $B6C3F_1$ mice Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Male	17/49 (35) 31/49 (70) 41/50 (82)	1/49 (2) 0/49 (0) 0/50 (0)	0/49 (0) 1/49 (2) 0/50 (0)	None	1/49 (2) 0/48 (0) 0/49 (0)	None
	0 ppm 100 ppm 200 ppm	0 18 ppm 36 ppm	Female	4/48 (8) 17/50(38) 38/50 (76)	0/48 (0) 3/50 (6) 0/50 (0)	None	None	N/A	1/48 (2) 0/49 (0) 0/50 (0)
JISA (1993) Crj:BDF1 mice Inhalation: 6 h/d, 5 d/wk, 104 wk	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Male	13/50 (28) 21/50 (43) 19/50 (40) 40/50 (82)	4/50 (4) 2/50 (2) 7/50 (13) 9/50 (18)	0/50 1/50 1/50 0/50	9/50 7/50 7/50 9/50	3[e]/50 0/50 0/50 1[e]/50	0/50 0/50 0/50 0/50
	0 ppm 10 ppm 50 ppm 250 ppm	0 1.8 ppm 9.0 ppm 45 ppm	Female	3/50 (6) 3/47 (6) 7/49 (15) 33/49 (67)	1/50 0/47 2/49 3/49	0/50 0/47 0/49 0/49	14/50 10/47 16/49 10/49	N/A	0/50 0/47 0/49 0/49

[a]Administered gavage doses listed were increased after 11 wk by 100 mg/kg-day in each low-dose group or by 200 mg/kg-day in each high-dose group. Animals received the listed TWA daily doses through Week 78, and surviving animals were observed up to study termination in Week 90.
[b]These tumors were reported as hemangioendotheliomas in the JISA (1993) report. The term has been updated to hemangioma (benign) or hemangiosarcoma (malignant). 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 hemangioendotheliomas from the individual animal data provided in the JISA report.
[c]None reported: Individual animal data were not available, and summary data did not include a line item for this tumor type.
[d]Histiocytic sarcomas, epididymides, or seminal vesicles.

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The NTP (1986) study also reported an increase in the rate of testicular interstitial cell tumors, a tumor type of high incidence in unexposed F344 rats. The reported incidences of testicular interstitial cell tumors in male rates exposed to 0, 200, or 400 ppm tetrachloroethylene were 36/50, 39/49, and 41/50, respectively. A higher incidence (47/50, or 92%) was observed in control rats in the JISA (1993) study than in the NTP (1986) study. In the JISA study, exposure to 0, 50, 200 or 600 ppm tetrachloroethylene resulted in incidences of 47/50, 46/50, 45/50, and 48/50, respectively. Support for the significance of the testicular interstitial cell tumors comes from evidence that the related chemical trichloroethylene induces this tumor type in rats. Trichloroethylene did not induce increases in testicular interstitial cell tumors in the F344 rat in a bioassay with a reported incidence of 47/48 (98%) in the vehicle control. However, increases were observed in male Marshall rats, in which the incidences were 16/46, 17/46, 21/33, and 32/39 in the untreated control, vehicle control and 500, or 1,000 mg/kg-day trichloroethylene exposure groups, respectively.

In conclusion, evidence for the carcinogenicity of tetrachloroethylene in rats was provided by increases in MCL incidence in both sexes in two inhalation bioassays. Rare kidney tumors in males and rare brain gliomas in males and females were increased in a single bioassay (NTP, 1986). Additionally, the NTP (1986) bioassay reported increases in the rate of testicular interstitial cell tumors, a tumor type of high incidence in unexposed male F344 rats. The available oral gavage cancer bioassay was inconclusive due to respiratory infection in all groups and high mortality in tetrachloroethylene-exposed animals.

4.10.5.2. Carcinogenicity Findings in Mice

In both sexes of mice, tetrachloroethylene increased the incidence of liver tumors in multiple bioassays. In male and female B6C3F$_1$ mice exposed for 2 years by oral gavage, significant increases were noted in hepatocellular carcinomas and adenomas (NCI, 1977). The reported incidence with 0, 500, and 1,000 mg/kg-day tetrachloroethylene were 2/20, 32/48, and 27/45 in males and 0/20, 19/48, and 19/49 in females, respectively. Tumor latency was significantly decreased with tetrachloroethylene exposure. A significant association between increased mortality and dose of tetrachloroethylene was seen, with liver tumors found in many of the mice that died early. In lifetime inhalation studies of B6C3F$_1$ (NTP, 1986) and Crj:BDF1 mice, tetrachloroethylene similarly increased liver tumors. Statistically significant, dose-related increases in the incidence of hepatocellular carcinoma and in combined hepatocellular adenoma and carcinoma were observed in both sexes. The reported incidence of liver carcinomas and adenomas with 0, 100, and 200 ppm tetrachloroethylene in the NTP inhalation bioassay were 17/49, 31/49, and 41/50 in males and 4/45, 17/42, and 38/48 in females, respectively. In male mice, hepatocellular carcinomas metastasized to the lungs in 2/49, 7/49, and 1/50 animals.

Metastatic hepatocellular carcinomas were found in the lungs of 0/48, 2/50, and 7/50 female mice. In the JISA study, the reported incidence of liver carcinomas and adenomas with 0, 10, 50, and 250 ppm tetrachloroethylene were 13/50, 21/50, 19/50, and 40/50 in males and 3/50, 3/47, 7/49, and 33/49 in females, respectively.

Additional evidence of carcinogenicity from the lifetime bioassays in mice included a significant increase in the incidence of hemangiosarcomas (reported as malignant hemangioendotheliomas) or hemangiomas (reported as benign hemangioendotheliomas) of the liver, spleen, fat, and subcutaneous skin in JISA study males. This tumor type was not reported in the NCI oral gavage bioassay, and no increase was reported in the NTP inhalation bioassay. Other findings in the JISA study were Harderian gland adenomas and enlargement of the nucleus in the kidney proximal tubular cells in male mice at the highest exposure.

Other supporting evidence for carcinogenicity is the known hepatocarcinogenicity of tetrachloroethylene metabolites. The major urinary metabolite of tetrachloroethylene in humans and rodents, TCA, is hepatocarcinogenic in mice. TCA significantly increased the incidence of liver tumors in male and female B6C3F$_1$ mice exposed via drinking water for 52−104 weeks (DeAngelo et al., 2008; Bull et al., 2002; Pereira, 1996; Bull et al., 1990; Herren-Freund et al., 1987). Incidence of tumors increased with increasing TCA concentrations (DeAngelo et al., 2008; Bull et al., 2002; Pereira, 1996; Bull et al., 1990). The development of tumors in animals exposed to TCA progressed rapidly, as evidenced by significant numbers of tumors in less-than-lifetime studies of 82 weeks or less. The tetrachloroethylene metabolite DCA also causes liver cancer in mice (DeAngelo et al., 1999; Daniel et al., 1992; Bull et al., 1990; Herren-Freund et al., 1987). Additionally, DCA and TCA are hepatocarcinogenic in mice when coadministered in the drinking water for 52 weeks (Bull et al., 2002). Treatment-related liver tumors were observed in male F344/N rats exposed via drinking water to DCA (DeAngelo et al., 1996) but not TCA (DeAngelo et al., 1997) for 60 or 104 weeks. However, the extent to which DCA is available to the liver following tetrachloroethylene exposure is unclear, because it is thought to be formed in the kidney following β-lyase processing of TCVC and may be largely excreted in urine without circulating systemically. The carcinogenicity of TCA and DCA has not been evaluated in female rats or in other species of experimental animals.

In conclusion, evidence for the carcinogenicity of tetrachloroethylene in mice is provided by increases in hepatocellular carcinomas and adenomas in both sexes of mice in a gavage bioassay (B6C3F$_1$ mice) and in two inhalation bioassays (one of the B6C3F$_1$ strain and the other of the Crj:BDF1 strain). In male Crj:BDF1 mice, hemangiosarcomas or hemangiomas of the liver, spleen, fat, and subcutaneous skin were increased (JISA, 1993). Supporting evidence includes the hepatocarcinogenicity of tetrachloroethylene metabolites TCA and DCA, alone and in combination.

4.10.5.3. Carcinogenic Mode of Action Hypotheses

This section summarizes the supporting evidence for the modes of action posited for the rat and mouse tumors presented in Table 4-51 and 4-52. The discussion focuses on tetrachloroethylene-specific studies, for which the database is especially limited. Evidence from studies of metabolites of tetrachloroethylene is also summarized. A tabular summary of the hypothesized MOA and key events, and the supporting evidence from studies of tetrachloroethylene and its metabolites, are provided in Table 4-56. Overall, these findings support the conclusion that the mechanisms by which tetrachloroethylene induces rodent carcinogenesis are not yet fully characterized, completely tested, or understood.

4.10.5.3.1. Hypothesized modes of action for rat tumors

4.10.5.3.1.1. Testicular interstitial cell tumors

No data are available concerning either the metabolites or the mechanisms that may contribute to the induction of testicular interstitial cell tumors occurring in exposed rats. Evidence for the related compound trichloroethylene, while suggestive of a MOA involving hormonal disruption, is inadequate to specify and test a hypothesized sequence of key events. It is concluded that the specific active moiety(ies), mechanisms, or modes of action by which tetrachloroethylene induces this type of tumor is not known.

4.10.5.3.1.2. Brain gliomas

No data are available concerning either the metabolites or the mechanisms that may contribute to the induction of rare brain gliomas occurring in exposed rats. It is concluded that the specific active moiety(ies), mechanisms, or modes of action by which tetrachloroethylene induces this type of tumor are not known.

4.10.5.3.1.3. Mononuclear cell leukemia

Regarding the metabolites that potentially contribute to MCL development, a role for GSH-derived intermediates was posited based on findings for the related compound trichloroethylene. However, TCVC, a GSH-derived metabolite of tetrachloroethylene, induced no kidney or bone marrow effects when administered to two calves as a single dose (Lock et al., 1996). Aside from this evaluation of bone marrow toxicity of TCVC in the juvenile cow, a species of unknown sensitivity to tetrachloroethylene-induced leukemia, other studies aimed at elucidating the active metabolites contributing to leukemic effects have not been reported. In particular, no such studies are available in the F344 rat, the species and strain in which leukemic effects have been consistently observed in both sexes. Additionally, no data are available concerning the contributing mechanisms. It is, thus, concluded that the specific active

moiety(ies), mechanisms, or modes of action by which tetrachloroethylene induces this type of tumor are not known.

4.10.5.3.1.4. Renal tumors

It is likely that several mechanisms contribute to tetrachloroethylene-induced kidney cancer. Mutagenicity, peroxisome proliferation, α2u-globulin nephropathy, and cytotoxicity not associated with α2u-globulin accumulation are MOAs that have been investigated. Except for α2u-globulin accumulation, which is more likely due to tetrachloroethylene itself (Lash and Parker, 2001), other mechanisms hypothesized to contribute to tetrachloroethylene-induced renal carcinogenicity are thought to be mediated by tetrachloroethylene metabolites rather than with the parent compound. Metabolites from the GSH conjugation pathway are posited to induce renal tumorigenicity, as opposed to, or to a greater extent, than the metabolites resulting from oxidative CYP processing. The glutathione conjugation of tetrachloroethylene in the kidney, discussed in Section 3, leads sequentially to TCVG and TCVC. TCVC can be further processed by β-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., 1999; Ripp et al., 1997; Werner et al., 1996). TCVG, TCVC, and NAcTCVC are mutagenic in *Salmonella* tests, as is tetrachloroethylene in the few studies of conditions that could generate GSH-derived metabolites (Dreessen et al., 2003; Vamvakas et al., 1989c; Vamvakas et al., 1989d; Vamvakas et al., 1987; Dekant et al., 1986a). Evidence of in vivo genotoxicity in the kidney is limited to reports of modest effects following i.p. exposures, including low level binding to rat kidney DNA (Mazzullo et al., 1987) and DNA single-strand breaks in mouse kidney (Walles, 1986). 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 cannot be ruled out.

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 only in male rats. In addition, tetrachloroethylene induces kidney tumors at lower doses than those required to cause α2u-globulin accumulation, raising serious doubt that α2u-globulin plays a key role—especially any major role—in rat kidney tumor formation. Rodent studies of tetrachloroethylene addressing renal α2u-globulin accumulation are summarized in Table 4-53.

Because tetrachloroethylene has been shown to induce peroxisome proliferation, an indicator of PPARα-activation, the possibility exists that certain responses resulting from activation of this receptor might be involved in cancer-causing activity leading to tetrachloroethylene-induced renal tumors. However, as summarized in Table 4-54, chemical-specific studies are limited and show only modest effects at exposures exceeding those required for renal carcinogenesis. There is no evidence causally linking PPARα-activation to kidney tumorigenesis for tetrachloroethylene or other compounds.

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 cannot be ruled out.

4.10.5.3.2. Hypothesized modes of action for mouse tumors

4.10.5.3.2.1. Hemangiosarcomas

No data are available concerning either the metabolites or the mechanisms that may contribute to the induction of hemangiosarcomas or hemangiomas observed in the liver, spleen, fat, and subcutaneous skin in male mice. It is concluded that the mechanisms or modes of action by which tetrachloroethylene induces this type of tumor are not known.

4.10.5.3.2.2. Hepatocellular tumors

As noted by NRC (2010), it is likely that key events from several pathways, comprising several simultaneous mechanisms, operate in tetrachloroethylene-induced liver cancer. MOA hypotheses for mouse liver tumors concern genotoxicity, epigenetic effects (especially DNA hypomethylation), oxidative stress, and receptor activation (i.e., a hypothesized PPARα-activation MOA). Because it has been suggested that hepatocarcinogenesis caused through a PPARα-activation MOA is not relevant to humans [e.g., Klaunig et al. (2003)], and such a conclusion would have significant implications for hazard conclusions and dose-response analyses, this hypothesized MOA is discussed in relatively more detail than other topics.

Table 4-53. Renal α2u-globulin accumulation in tetrachloroethylene-exposed rodents

Species/strain/ sex/number	Exposure level/duration	Effects	Reference
Mouse, B6C3F$_1$, both sexes (49 or 50 mice per sex per dose group)	0, 100, 200 ppm for 104 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed mice; nephrosis in exposed females, casts increased in all exposed males and in high-dose females.	NTP (1986)
Rat, F344, both sexes (50 mice per sex per dose group)	0, 200, 400 ppm for 104 wk, inhalation	Karyomegaly and cytomegaly of the proximal tubules in all exposed rats.	NTP (1986)
Rat, F344 (both sexes, 5 per group)	0 or 1,000 mg/kg-day for 10 d, corn oil gavage	Increases in α2u-hyaline droplets in exposed males but not females. Correlated to increased cell proliferation and protein droplet nephropathy.	Goldsworthy et al. (1988)
Rat, F344 (both sexes, 12 per group)	0, 500 mg/kg-day daily for 4 wk, corn oil gavage	Increases in α2u-globulin accumulation in proximal tubule cells.	Bergamaschi et al. (1992)
Rat, F344 (both sexes) and B6C3F$_1$ mice (both sexes); 10 per group for oral studies, 5 per group for inhalation studies	0, 1,000 or 1,500 mg/kg-day daily by corn oil gavage for 42 d; 0 or 1,000 ppm for 10 d	Accumulation of α2u-globulin in proximal tubules of male rats; nephrotoxicity in male rats (formation of granular tubular casts and evidence of tubular cell regeneration). Inhalation exposure demonstrated formation of hyaline droplets in kidneys of male rats.	Green et al. (1990)

Table 4-54. Renal peroxisome proliferation in tetrachloroethylene-exposed rodents

Species/strain/sex/number	Effect	Dose	Time
Rat, F344; and mouse, B6C3F$_1$; both sexes (5/group) Odum et al. (1988)	Mice of both sexes: Analysis in mice was limited to pooled tissue, but showed slight increases in β-oxidation in mouse kidney	200, and 400 ppm, inhalation	14, 21, 28 d
	Rats: Modest increases in PCO in male rat kidneys at 200 ppm for 28 d only, but elevated in female rat kidney at all doses and times.	200, and 400 ppm, inhalation	14, 21, 28 d
Rat, F344 (male only, 5/group) and mouse, B6C3F$_1$ (male only, 5/group) Goldsworthy and Popp (1987)	Mice: Increased PCO activity	1,000 mg/kg-day for 10 d, corn oil gavage	10 d
	Rats: Increased kidney weight	1,000 mg/kg-day for 10 d, corn oil gavage	10 d

The limited tetrachloroethylene-specific data for PPARα-activation support the view that this is not the primary MOA for hepatocarcinogenesis (refer to Table 4-55). Philip et al. (2007) reported significantly increased expression of CYP4A, a marker of PPARα-activation, in SW mice at only the highest dose (1,000 mg/kg-day) and at the earliest time point (7 days), in contrast to the robust dose-dependent proliferative response of a more prolonged nature (lasting for 14−30 days post exposure) observed at the same and lower (150, 500, and 1,000 mg/kg-day) levels of tetrachloroethylene. The authors suggested that these data are not supportive of a close mechanistic relationship of carcinogenicity and PPARα-activation for tetrachloroethylene-derived TCA. Limitations of this interpretation include the possible lack of sensitivity of CYP4A protein expression as a marker of peroxisome proliferation, and the unknown sensitivity of the SW mouse to tetrachloroethylene hepatocarcinogenicity. Other investigators [e.g., Schumann et al. (1980)] have reported liver toxicity and repair at 100 mg/kg-day in the $B6C3F_1$ strain, whereas repeated exposures to 1,000 mg/kg-day were reported by Philip et al. (2007) and Odum et al. (1988) to only modestly increased peroxisomal markers in SW and $B6C3F_1$ mice, respectively. Odum et al. (1988) also observed moderate increases in peroxisome proliferation in rats, a species insensitive to tetrachloroethylene hepatocarcinogenicity. In all, these findings indicate that the modest peroxisome proliferation observed in response to tetrachloroethylene may lack specificity with respect to species, tissue, and dose. Studies of the temporal sequence of events are limited. Given the limitations in the database of tetrachloroethylene-specific studies, it can be concluded that the few studies demonstrating peroxisome proliferation by tetrachloroethylene are insufficient to demonstrate a causative role of this effect in the induction of other key events posited for the PPARα-activation MOA hypothesis, and for hepatocarcinogenesis by tetrachloroethylene.

Studies of other PPARα agonists, and of transgenic models of PPARα-activation, more generally support the view that the hypothesized PPARα-activation MOA may not be a limiting factor in rodent hepatocarcinogenesis (refer to Section 4.3.5.5). PPARα-activation may play a significant role in mouse liver tumor induction by some compounds, such as Wy-14,643. However, recent studies suggest that DEHP can induce tumors in a PPARα independent manner without any loss of potency (Ito et al., 2007a), and that PPARα-activation in hepatocytes is itself insufficient to cause tumorigenesis (Yang et al., 2007). Additional analyses, presented in Section 4.3.5.3.2, demonstrate that peroxisome proliferation and associated markers are poor quantitative predictors of hepatocarcinogenesis in rats or mice. These findings raise serious concerns about human health risk assessment MOA conclusions based exclusively on evidence of PPARα-agonism and other key events in the hypothesized PPARα-activation MOA, given that other modes, mechanisms, toxicity pathways, and molecular targets may contribute to or be required for the observed adverse effects. Indeed, for tetrachloroethylene and most other PPARα

agonists, chemical-specific data to define the range of effects that may contribute to human carcinogenesis are insufficient. Similarly, the epidemiologic data are inadequate to inform conclusions of human relevance (Guyton et al., 2009).

Table 4-55. Rodent studies of induction of hepatic peroxisome proliferation or its markers by tetrachloroethylene

Species/strain/sex/number	Effect	Dose	Time
Rat, F344; and mouse, B6C3F$_1$; both sexes (5/group) Odum et al. (1988)	Mice of both sexes: increased relative liver weight, centrilobular lipid accumulation and peroxisome proliferation; increased PCO (up to 3.7-fold)	200, and 400 ppm, inhalation	14, 21, 28 d
	Male mice: mitochondrial proliferation	400 ppm, inhalation	28 d
	Rats of both sexes: increased PCO (up to 1.3-fold)	200, and 400 ppm, inhalation	14, 21, 28 d
Rat, F344 (male only, 5/group) and mouse, B6C3F$_1$ (male only, 5/group) Goldsworthy and Popp (1987)	Mice: Increased relative liver weight; 4.3-fold PCO increase	1,000 mg/kg-day for 10 d, corn oil gavage	10 d
	Rats: Increased relative liver weight; modest but not significant (1.4-fold) PCO increase	1,000 mg/kg-day for 10 d, corn oil gavage	10 d
Mouse, Swiss-Webster, male (4 mice/group) Philip et al. (2007)	Increased plasma ALT	150, 500, and 1,000 mg/kg-day, aqueous gavage	24 hours to 14 d after initial exposure
	Mild to moderate fatty degeneration and necrosis, with focal inflammatory cell infiltration	150, 500, and 1,000 mg/kg-day, aqueous gavage	24 hours to 30 d after initial exposure
	Increased mitotic figures and DNA synthesis	150, 500, and 1,000 mg/kg-day, aqueous gavage	Peaked on 7 d, sustained at 14-30 d
	CYP4A increased at 7 but not 14 d, only at 1,000 mg/kg-day	1,000 mg/kg-day, aqueous gavage	7 but not 14 d

A recent review (Rusyn et al., 2006) addressed other mechanistic effects of the PPARα agonist DEHP and proposed that tumors arise from a combination of molecular signals and pathways, rather than from a single event such as PPARα-activation. As reviewed in Section 4.3.5.1, the metabolites of tetrachloroethylene have been shown to induce a number of effects that may contribute to carcinogenicity, including mutagenicity, alterations in DNA methylation, and oxidative stress. Given the demonstrated mutagenicity of several

tetrachloroethylene metabolites, the hypothesis that mutagenicity contributes to the MOA for tetrachloroethylene hepatocarcinogenesis cannot be ruled out, although the specific metabolic species or mechanistic effects are not known. Epigenetic effects and oxidative stress, including those produced secondary to cytotoxicity, may also contribute. Currently, the available database of tetrachloroethylene-specific studies addressing these mechanisms is very limited.

4.10.5.3.3. Mode-of-action summary

Table 4-56 reviews the hypothesized modes of action for tetrachloroethylene-induced cancer in rodents, which are not intended to be interpreted as being mutually exclusive. The evidence summarized in this table supports the view that there are significant gaps in the scientific knowledge of mechanisms contributing to tetrachloroethylene-induced cancer. Multiple metabolites formed from tetrachloroethylene are toxic and carcinogenic in rodents. Given this knowledge, and the known complexity and heterogeneity in cancer development, in general, the available evidence supports a hypothesis of multiple, contributing mechanistic effects that may, in turn, be affected by multiple modifying factors.

Table 4-56. Summary of hypothesized modes of action for tetrachloroethylene-induced cancer in rodents

Tumor type, sex, strain, species	Hypothesized MOA and key events	Evidence that PCE or PCE metabolites induces key events	Necessity of key events for carcinogenesis	Sufficiency of MOA for carcinogenesis
Testicular interstitial cell tumors in male F344/N rats	None hypothesized	N/A	N/A	N/A
Brain gliomas in male and female F344/N rats	None hypothesized	N/A	N/A	N/A
Mononuclear cell leukemia in male and female F344/N and F344/DuCrj rats	None hypothesized	N/A	N/A	N/A

Table 4-56. Summary of hypothesized modes of action for tetrachloroethylene-induced cancer in rodents (continued)

Tumor type, sex, strain, species	Hypothesized MOA and key events	Evidence that PCE or PCE metabolites induces key events	Necessity of key events for carcinogenesis	Sufficiency of MOA for carcinogenesis
Kidney adenocarcinoma in male F344/N rats	Mutagenicity induced by GSH-derived metabolites advances acquisition of the multiple critical traits contributing to carcinogenesis	PCE lacks mutagenicity in *Salmonella* (Ames), other genotoxicity tests [Emmert et al. (2006); Watanabe et al. (1998) (refer to Table 4-40); DeMarini et al. (1994); Roldán-Arjona et al. (1991); Milman et al. (1988); Warner et al. (1988); NTP (1986); Connor et al. (1985); Shimada et al. (1985); Haworth et al. (1983); Hardin et al. (1981); Kringstad et al. (1981); Bartsch et al. (1979); Greim et al. (1975)] Limited studies of PCE genotoxicity in rodent kidney: low levels of DNA binding in Wistar rats with 1.4 mg/kg i.p. (Mazzullo et al., 1987); DNA single-strand breaks in NMRI mice with 660 mg/kg i.p. (Walles, 1986) Mutagenicity of TCVG, TCVC, NAcTCVC (and PCE under conditions that could generate these GSH-derived metabolites) in Ames assays (Dreessen et al., 2003; Vamvakas et al., 1989c; Vamvakas et al., 1989d; Vamvakas et al., 1987; Dekant et al., 1986a)	No PCE-specific studies[a]	No PCE-specific studies; Mutagenicity is assumed to cause cancer, as a sufficient cause

Table 4-56. Summary of hypothesized modes of action for tetrachloroethylene-induced cancer in rodents (continued)

Tumor type, sex, strain, species	Hypothesized MOA and key events	Evidence that PCE or PCE metabolites induces key events	Necessity of key events for carcinogenesis	Sufficiency of MOA for carcinogenesis
Kidney adenocarcinoma in male F344/N rats (continued)	Tubular cell necrosis and nephrotoxicity followed by hyperplasia	Nephrotoxicity of PCE reported in multiple studies in both sexes of rats and mice at carcinogenic doses [e.g., NTP (1986)]	No PCE-specific studies[a]	No PCE-specific studies
	α2u-globulin accumulation: • Excessive accumulation of hyaline droplets containing α2u-globulin in renal proximal tubules • Subsequent cytotoxicity and necrosis • Sustained regenerative tubule cell proliferation • Development of intralumenal granular casts from sloughed cellular debris associated with tubule dilatation and papillary mineralization • Foci of tubule hyperplasia in the convoluted proximal tubules • Renal tubule tumors	In F344 rats, PCE induced hyaline droplets at 500 mg/kg-day for 4 wk (Bergamaschi et al., 1992), or ≥1,000 mg/kg-day for 10 (Goldsworthy et al., 1988) or 42 d (Green et al., 1990) No evidence of mineralization in PCE bioassays (JISA, 1993; NTP, 1986) or of hyaline droplets with ≤400 ppm for 28 d (Green et al., 1990) in F344 rats	No PCE-specific studies[a]	No PCE-specific studies
	PPARα-activation: • Metabolites (e.g., TCA) activate PPARα • Alterations in cell proliferation and apoptosis • Clonal expansion of initiated cells	In F344 rat kidney, PCE increased PCO in males only at 200 ppm for 28 d (PCO increased in females at 200 and 400 ppm, at 14, 21 and 28 d) (Odum et al., 1988); in $B6C3F_1$ male mouse kidney, PCE increased PCO with 1,000 mg/kg-day p.o. for 10 d (Goldsworthy and Popp, 1987)	No PCE-specific studies No data from other chemicals on PPARα involvement in kidney tumors.	No PCE-specific studies
Hemangiosarcomas in male Crj:BDF_1 mice	None hypothesized	N/A	N/A	N/A

Table 4-56. Summary of hypothesized modes of action for tetrachloroethylene-induced cancer in rodents (continued)

Tumor type, sex, strain, species	Hypothesized MOA and key events	Evidence that PCE or PCE metabolites induces key events	Necessity of key events for carcinogenesis	Sufficiency of MOA for carcinogenesis
Liver hepatocellular carcinoma in male and female $B6C3F_1$ and Crj:BDF_1 mice	Mutagenicity induced by one or more metabolites advances acquisition of multiple critical traits contributing to carcinogenesis	PCE lacks mutagenicity in *Salmonella* (Ames), other genotoxicity tests [Emmert et al. (2006); Watanabe et al. (1998) (refer to Table 4-40); DeMarini et al. (1994); Roldán-Arjona et al. (1991); Milman et al. (1988); Warner et al. (1988); NTP (1986); Connor et al. (1985); Shimada et al. (1985); Haworth et al. (1983); Hardin et al. (1981); Kringstad et al. (1981); Bartsch et al. (1979); Greim et al. (1975)] Limited PCE genotoxicity studies in mouse liver: Positive/equivocal Comet assay in CD1 mice (Cederberg et al., 2010a), positive micronucleus assay in ddY mice post (but not pre) partial hepatectomy (Murakami and Horikawa, 1995) at 1,000 mg/kg-day; DNA binding in male Balb/c mice at 1.4 mg/kg i.p. (Mazzullo et al., 1987); DNA single-strand breaks in NMRI mice with 660 mg/kg i.p. (Walles, 1986) Certain metabolites of PCE are mutagenic in vitro and in vivo (refer to Tables 4-41 and 4-42)	No PCE-specific studies[a]	No PCE-specific studies; Mutagenicity is assumed to cause cancer, as a sufficient cause

Tumor type, sex, strain, species	Hypothesized MOA and key events	Evidence that PCE or PCE metabolites induces key events	Necessity of key events for carcinogenesis	Sufficiency of MOA for carcinogenesis
Liver hepatocellular carcinoma in male and female $B6C3F_1$ and $Crj:BDF_1$ mice (continued)	Epigenetic changes, particularly DNA methylation, induced by one or more metabolites (TCA, DCA, and other reactive species) advance acquisition of multiple critical traits contributing to carcinogenesis	No PCE-specific studies In mouse liver, TCA and DCA decrease global DNA methylation and promoter hypomethylation (e.g., of c-myc) (Ge et al., 2001; Tao et al., 1998)	No PCE-specific studies[a]	No PCE-specific studies; dys-regulation of methylation represents a common early molecular event in most tumors and is hypothesized to cause cancer
	Cytotoxicity and secondary oxidative stress: • One or more reactive intermediates induce hepatotoxicity • Oxidative stress results (from hepatocyte injury, from infiltrating inflammatory cells and/or as part of the intra- and/or intercellular repair processes) • Oxidative stress advances acquisition of multiple critical traits contributing to carcinogenesis	PCE induces hepatotoxicity characterized by increased liver weight, fatty changes, necrosis, inflammatory cell infiltration, and proliferation [e.g., NTP (1986)]	No PCE-specific studies[a]	No PCE-specific studies

Tumor type, sex, strain, species	Hypothesized MOA and key events	Evidence that PCE or PCE metabolites induces key events	Necessity of key events for carcinogenesis	Sufficiency of MOA for carcinogenesis
Liver hepatocellular carcinoma in male and female $B6C3F_1$ and $Crj:BDF_1$ mice (continued)	PPARα-activation: • TCA, after being produced in the liver, activates PPARα • Alterations in cell proliferation and apoptosis • Clonal expansion of initiated cells	In $B6C3F_1$ mouse liver, PCE increased PCO (three- to fourfold) with 200 and 400 ppm (Odum et al., 1988) or 1,000 mg/kg-day p.o. (Goldsworthy and Popp, 1987) In SW mouse liver, PCE increased CYP4A at 7 but not 14 d, at 1,000 mg/kg-day; increased mitotic figures and DNA synthesis at 7−30 d with 150, 500, and 1,000 mg/kg-day (Philip et al., 2007) TCA activates PPARα, induces peroxisome proliferation and hepatocyte proliferation in mice and rats [e.g., DeAngelo et al. (2008); Laughter et al. (2004); Stauber and Bull (1997); Pereira and Phelps (1996); Dees and Travis (1994); Sanchez and Bull (1990)]	No PCE-specific studies; liver tumor response from WY dramatically diminished in PPARα-null mice (Peters et al., 1997); liver tumor response from DEHP unchanged in PPARα-null mice (Ito et al., 2007a). No inference possible with PCE.	No PCE-specific studies; PPARα-activation in a transgenic mouse model caused all the key events in the MOA, but not carcinogenesis, suggesting that the MOA is not sufficient for carcinogenesis (Yang et al., 2007). Consistent with hypothesis that PCE liver carcinogenesis involves multiple mechanisms.

[a] Associations [e.g., per Hill (1965) considerations] noted for some chemicals between hypothesized sequence of key events and carcinogenesis.

5. DOSE-RESPONSE EVALUATION

5.1. INHALATION REFERENCE CONCENTRATION (RfC)

This section presents quantitative risk estimates for chronic noncancer inhalation tetrachloroethylene exposure. 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 RfD was developed by route-to-route extrapolation of the RfC to the oral route of exposure. The analysis is based on the noncancer hazard characterization for tetrachloroethylene presented in Section 4.10.2, which identified neurotoxicity as a sensitive endpoint following either inhalation or oral exposure to tetrachloroethylene. Neurotoxicity is thus selected as the critical effect for deriving the noncancer inhalation RfC. All neurotoxicity studies suitable for dose-response analysis are evaluated in the selection of principal studies.

5.1.1. Choice of Principal Studies and Critical Effect

5.1.1.1. Choice of Critical Effect

The database of human and animal studies on inhalation toxicity of tetrachloroethylene is adequate to support derivation of inhalation reference values. As summarized in Section 4.10, a number of targets of toxicity from chronic exposure to tetrachloroethylene have been identified in published animal and human studies. These targets include the central nervous system (CNS), kidney, liver, immune and hematologic systems, and development and reproduction. In general, neurological effects were judged to be associated with lower tetrachloroethylene concentrations compared with other noncancer endpoints of toxicity.

5.1.1.2. Overview of Candidate Principal Studies

The evidence for neurotoxicity in humans includes controlled experimental chamber (Altmann et al., 1990; Hake and Stewart, 1977) and epidemiologic (Spinatonda et al., 1997; Altmann et al., 1995; Echeverria et al., 1995; Ferroni et al., 1992; Seeber, 1989; Hake and Stewart, 1977) studies that used standardized neurobehavioral batteries or employed assessment of visual function (Storm et al., 2011 [previously reported in NYSDOH, 2010]; Schreiber et al., 2002; Gobba et al., 1998; Cavalleri et al., 1994), a neurological outcome known to be sensitive to volatile organic compounds. Of the 12 candidate studies in humans, seven epidemiological studies of tetrachloroethylene examined occupational exposure (Schreiber et al., 2002; Gobba et al., 1998; Spinatonda et al., 1997; Echeverria et al., 1995; Cavalleri et al., 1994; Ferroni et al., 1992; Seeber, 1989), three epidemiological studies examined residential exposure to

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tetrachloroethylene (Storm et al., 2011 [previously reported in NYSDOH, 2010]; Schreiber et al., 2002; Altmann et al., 1995), and 2 were acute experimental chamber studies (Altmann et al., 1990; Hake and Stewart, 1977). Together, the epidemiologic evidence supports an inference of a broad range of cognitive, motor, behavioral, and visual functional deficits following tetrachloroethylene exposure (U.S. EPA, 2004).

The research in animal models comprises acute and subchronic studies of the effects of tetrachloroethylene on functional neurological endpoints (functional observation battery, motor activity) (Oshiro et al., 2008; Kjellstrand et al., 1985), on sensory system function as assessed by evoked potential (Boyes et al., 2009; Mattsson et al., 1998) or pathological changes in the brain (Wang et al., 1993). The studies in animal models support the human studies, with notable effects on motor activity and motor function following exposure to tetrachloroethylene during either adulthood or the developmental period. Changes in evoked potentials following acute and subchronic exposures were also seen. In addition, postmortem effects in animals were observed with pathological alterations in brain DNA, RNA, or protein levels and brain-weight changes.

The studies considered for derivation of the RfC are summarized in the following sections and in Table 5-1 and Figure 5-1. Table 5-1 identifies the species, exposure duration, and ambient (experimental) concentrations. For epidemiologic studies, the reported concentrations, and the observed effect and its magnitude associated with the NOAEL or the LOAEL are provided. Additionally, human equivalent concentrations (HECs) for LOAELs or NOAELs are presented to better allow examination of effect levels across studies and species. HECs are calculated using the RfC methodology for a Category 3 gas, extrathoracic effects, and adjusted to equivalent continuous exposure (U.S. EPA, 1994).[28] The studies in Table 5-1 are listed in order of increasing HEC and displayed graphically in Figure 5-1.

5.1.1.3. Selection of Principal Studies

The candidate principal studies of CNS effects listed in Table 5-1 were evaluated according to study characteristics identified in Table 5-2. Human studies were preferred to animal studies, as were studies of chronic duration. Certain human studies are considered as more methodologically sound based on study quality attributes identified in Table 5-2 and are preferred for supporting an RfC. The sections below summarize the evaluation of these studies.

[28] $NOAEL^*_{[HEC]} = NOAEL^*_{[ADJ]}\ (ppm) \times (H_{b/g})_A/(H_{b/g})_H$, 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 hours exposed/24 hours) times week (number of days of exposure/7 days), and $(H_{b/g})_A/H_{b/g})_H$ = the ratio of the blood/gas (air) partition coefficient of the chemical for the laboratory animal species to the human value. The value of one is used for the ratio if $(H_{b/g})_A > H_{b/g})_H$.

Table 5-1. Neurotoxicological inhalation studies considered in the development of an RfC

Study	Species	Duration	NOAEL/LOAEL[a] ppm	Effect (effect magnitude) at LOAEL	Human equivalent continuous concentrations[b]		
					NOAEL/ LOAEL	ppm	mg/m^3
(Storm et al., 2011 [previously reported in NYSDOH, 2010]	Human	10 yr (mean), continuous	0.002, 0.05 (children) 0.002, 0.07 (adults)	Visual contrast sensitivity (6% ↑ in children)	NOAEL	0.002	0.01
Schreiber et al. (2002)	Human	4 yr (mean), occupational	0.3 (daycare workers, mean and median)	Visual contrast sensitivity[c]	LOAEL	0.1	0.7
Schreiber et al. (2002)	Human	5.8 yr (mean), continuous	0.1 (residents, median, and mean), maybe as high as 0.4 (mean) and 0.3 (median)	Visual contrast sensitivity[c]	LOAEL	0.4[d]	3[d]
Altmann et al. (1995)	Human	10.6 yr (median) continuous	0.7 (mean) 0.2 (median)	Cognitive function (14% ↑), reaction time (15%–20 ↑) visual memory (15% ↓)	LOAEL	0.7	5
Cavalleri et al. (1994); Gobba et al. (1998)	Human	8.8 yr (mean), occupational	6 (Cavalleri et al., 1994)	Dyschromatopsia (color vision) (6% ↑)[d]	LOAEL	2	15
Spinatonda et al. (1997)	Human	Inhalation (no duration information), occupational	8 (median)	Reaction time (15% ↑)	LOAEL	3	19

Table 5-1. Neurotoxicological inhalation studies considered in the development of an RfC (continued)

Study	Species	Duration	NOAEL/LOAEL[a] ppm	Effect (effect magnitude) at LOAEL	Human equivalent continuous concentrations[b]		
					NOAEL/ LOAEL	ppm	mg/m^3
Seeber (1989)	Human	>10 yr (mean), occupational	12, 53	Visuospatial function and information processing speed (5−30% change depending on subtest)	LOAEL	4	29
Ferroni et al. (1992)	Human	10.6 yr (mean), occupational	15	Reaction time (10% ↑), continuous performance (7−11% ↓)	LOAEL	5	36
Echeverria et al. (1995)	Human	15 yr (high-exposure group; mean), occupational	11, 23, 41	Cognitive and visuospatial measures (4−14% change depending on subtest)	LOAEL	8	56
Altmann et al. (1990)	Human	4 hr/d for 4 d	10, 50	Visual evoked potentials (2−3 ms ↑)	NOAEL	4	24
Mattsson et al. (1998)	Rat	Subchronic (13 wk) 6 hr/d, 5 d/wk	0, 50, 200, 800	Flash-evoked potential (3 ms ↑)	NOAEL	36	240
Rosengren et al. (1986)	Gerbil	Subchronic (12 wk, with 16-wk follow-up) continuous	0, 60, 300	Brain: protein, DNA concentration (10−15% change depending on brain region; there were both ↑ and ↓)	LOAEL	60	410
Kjellstrand et al. (1985)	Mouse	60 min	0, 90, 320, 400, 600, 800, 1,200, 1,800, 3,600	Increased locomotor activity (20% ↑)	LOAEL	90[e]	6,100[e]
Boyes et al. (2009)	Rat	90 min	0, 250, 500, 1,000	Impairment in steady state visual evoked potential (10% ↓)	LOAEL	250[e]	1,700[e]
		120 min	0, 1,000, 2,000, 3,000, 4,000	Impairment in steady state visual evoked potential (20% ↓)	LOAEL	1,000[e]	6,800[e]

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Table 5-1. Neurotoxicological inhalation studies considered in the development of an RfC (continued)

Study	Species	Duration	NOAEL/LOAEL[a] ppm	Effect (effect magnitude) at LOAEL	Human equivalent continuous concentrations[b]		
					NOAEL/ LOAEL	ppm	mg/m^3
Wang et al. (1993)	Rat	Subchronic (12 wk) continuous	0, 300, 600	Reduced brain weight (↓0.10 g), DNA (↓0.05−0.06 mg), protein (↓2.5−3.5 mg)	NOAEL	300[e]	2,000[e]
Oshiro et al. (2008)	Rat	60 min	0, 500, 1,000, 1,500	False alarms (10% ↑)	LOAEL	500[e]	3,400[e]
			0, 500, 1,000, 1,500	Reaction time (200 ms ↑)	NOAEL	500[e]	3,400[e]

Note: Studies from which candidate RfCs were derived shaded in blue. 1 ppm = 6.78 mg/m^3.
[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(d) × 10/20 (m^3/d, breathing rate), and experimental exposures were multiplied by hours exposed/24 (hr) × 5/7(d).
[c]Effect magnitude could not be determined from information in published paper.
[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) for four families living in apartments above active dry cleaning facilities 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) and is used as the LOAEL for this study.
[e]HECs are the human equivalent concentrations for the same duration as in the experiments, not adjusted to continuous daily exposures.

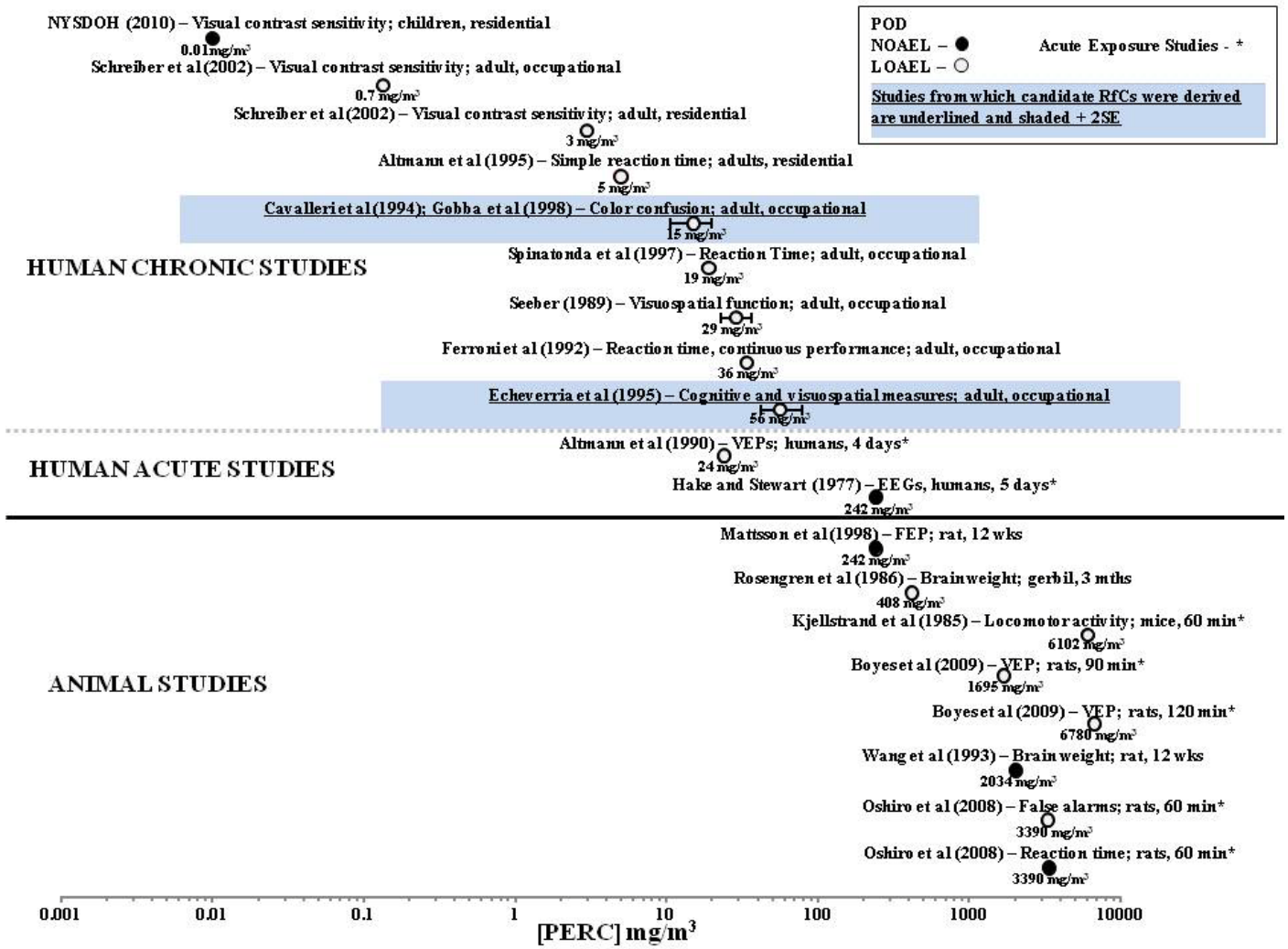

Figure 5-1. Exposure-response array for neurotoxicological inhalation studies considered for RfC development (listed in Table 5-1).

PODs (HEC for LOAELs and NOAELs) are displayed and labeled by study, effect, and duration. Studies from which candidate RfCs were derived are shaded in blue, and the POD ranges (± 2SE) are presented.

Table 5-2. Summary of rationale for identifying studies on tetrachloroethylene for RfC development

Consideration	Data characteristics	Decision context
Species studied	Animal and human neurotoxicity studies	Human data are preferred to reduce interspecies extrapolation uncertainties. Animal data are considered as supporting studies when adequate human studies are available.
Relevance of exposure paradigm scenario	Acute, subchronic, and chronic exposure durations Peak and chronic exposure intensities	Subchronic or chronic studies, if adequate, are preferred over studies of acute exposure durations. Studies of residential exposures, if available and of adequate quality, are preferred. In residential settings, exposure is more likely to be continuous, and of lower concentrations compared with the more intermittent, higher concentration exposure experienced in work settings. The potential influence of peak or intensity concentrations is more common with occupational than residential exposures.
Study quality attributes for human toxicity studies		
Study populations	Comparability of referent and exposed groups	Referent and exposed groups were evaluated and compared. In addition to age, potential confounders for neurobehavioral measures including education, lifestyle factors such as alcohol consumption, and SES are controlled for to limit selection bias and confounding. Use of a study design (e.g., matching procedures) or analysis (procedures for statistical adjustment) that adequately addresses the relevant sources of potential confounding for a given outcome adds weight to the consideration of the study as principal rather than supportive.
Measurement of exposure	Area or individual measures of exposure	Stronger studies have exposure estimates that are supported by ambient monitoring and/or biological monitoring. Measurement or assignment of exposure should not be influenced by knowledge of results of tests of neurobehavioral function. Higher quality assessment strategies in occupational studies are based on assignment of exposure potential to individual subjects considering individual job titles and tasks with consideration of changes over time. Use of higher quality assessment strategies adds weight to the consideration of the study as principal rather than supportive.

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Table 5-2. Summary of rationale for identifying studies on tetrachloroethylene for RfC development (continued)

Consideration	Data characteristics	Decision context
Measurement of effect(s)	Standardized neurological tests: validity and reliability	Neurobehavioral function (reaction time measures, cognitive function, and motor activity) assessed using a standardized test battery (e.g., Neurobehavioral Evaluation System) is preferred, because wide administration to populations in different settings has resulted in a high degree of validity within the context of potential population norms. WHO and ATSDR recommend these test methods to evaluate nervous system deficits in adults and children. Other standardized methods were used to evaluate color vision and visual contrast sensitivity. Administration or interpretation of the test should not be influenced by knowledge of exposure status. This information adds weight to the consideration of the study as principal rather than supportive. Use of standardized neurological tests and sensitive methods to detect neurological changes adds weight to the consideration of the study as principal rather than supportive.
Study quality attributes for animal toxicity studies		
Study populations	Comparability of animal models to effects observed in humans	Studies in animal models reporting effects concordant to observed solvent-associated effects in humans were considered preferable.
Measurement of effect(s)	Validity and comparability of neurological tests	Neurological tests and methods that have been validated in animal models were preferred. Endpoints in animals that were concordant or comparable with evaluated endpoints in humans were the most preferred.

5.1.1.3.1. Evaluation of epidemiologic studies of residentially exposed populations

Three epidemiological studies of residential exposures were examined as candidate principal studies for deriving a RfC (Storm et al., 2011 [previously reported in NYSDOH, 2010]; Schreiber et al., 2002; Altmann et al., 1995). As outlined in Table 5-2, residential exposures come closest to the chronic, continuous exposures addressed by reference values. The exposed populations in these studies lived in buildings colocated with dry cleaners. Additional strengths of all of these studies included high quality exposure assessment, matching of controls by age and sex, and use of standardized testing. In addition, statistical analyses adjusted for race/ethnicity, age, and other covariates such as smoking or alcohol use. On the other hand, there were differences in comparability between referent and exposed groups in each of these studies for which statistical analyses could not sufficiently adjust, limiting their use as principal

studies. Section 4 describes the studies in detail; study-specific issues relevant to principal study selection are summarized below.

The NYSDOH pilot study (Schreiber et al., 2002) reported deficits in visual contrast sensitivity (VCS) in residents exposed to tetrachloroethylene compared to controls. Schreiber et al. (2002) evaluated 17 exposed subjects, including four children (in New York City) and 17 control subjects (recruited from among NYSDOH employees living in Albany, NY) and reported reduced group-mean VCS scores in residents compared to unexposed referents at a human equivalent LOAEL ($LOAEL_{HEC}$) of 3 mg/m^3 (arithmetic mean concentration). A key limitation of this study, in addition to its small sample size and potential for selection bias owing to health department employees being referents for exposed residents, was that vision testing was not blinded to exposure classification.

NYSDOH (2010) and Storm et al. (2011) report on a larger study of 104 exposed adult and children residents of 24 buildings with colocated dry cleaners using tetrachloroethylene and 101 unexposed adults and children in 36 buildings without colocated dry cleaners. High quality exposure assessment addressed some of the concerns of selection bias in the previous study of Schreiber et al. (2002); for example, the study employed a larger number of subjects and referents from the same geographical area. Additionally, exposure and effects were assessed in family units, allowing comparison of parents and children in the same household. Storm et al. (2011) identified a human equivalent NOAEL ($NOAEL_{HEC}$) of 0.01 mg/m^3 (median concentration) in children and a $NOAEL_{HEC}$ of 0.48 mg/m^3 (median concentration) in adults. However, there are other concerns as to the comparability of referent and exposed subjects. Those living in households with higher levels of tetrachloroethylene were more likely to be of minority race and of lower income status compared to referent families. Additionally, exposed subjects were younger ($p < 0.05$) and of lower educational attainment ($p < 0.05$) than those in referent buildings. Another concern is that, although a standardized visual test (Functional Acuity Contrast Test [FACT]) was used, it was of far distance VCS only. The test was also less sensitive than that employed in other studies because the response was scored as either maximum (perfect) or less than maximum, with no gradations of reduced response. Statistical analyses appropriately examined the association between these exposure metrics and vision and adjusted for a number of relevant covariates. However, the small number of nonminority and high income subjects in the highest tetrachloroethylene exposure group, and the lower mean education level of the high exposure group, limit conclusions that observed effects were completely independent of education level, race/ethnicity, or income. This raises concerns about the comparability between exposed and referent subjects. Consequently, due to the ceiling effect of the testing method and potential confounding of education level, race/ethnicity, or income, NYSDOH (2010) and Storm et al. (2011) were not selected as principal studies.

Altmann et al. (1995) reported visuospatial and cognitive deficits (from two tests of simple reaction time, continuous performance, and visual memory) among 19 residents compared to 30 unexposed referents at a $LOAEL_{HEC}$ of 5 mg/m^3 (arithmetic mean concentration). Statistical analyses appropriately adjusted for covariates and possible confounders of age, gender, and education in logistic regression models; however, the paper lacked reporting of logistic regression coefficients and effect magnitudes, limiting a clear assessment of the effects observed. Furthermore, the referent group in Altmann et al. (1995) had a higher educational attainment than tetrachloroethylene-exposed subjects. Altmann et al. (1995) adjusted for a potential effect of education, a surrogate for socioeconomic status, and on visuospatial test performance in multiple regression models. However, the National Research Council (NRC, 2010) noted the potential for residual confounding, as education was examined as a categorical, not continuous, variable using three groups, which might affect interpretation of cognitive testing of continuous performance and visual memory. Nonetheless, effects of tetrachloroethylene exposure were observed on reaction time, an endpoint that is not influenced by education level. There was potential bias in subject selection: 19 of 95 potentially eligible subjects participated in the study, and the study did not identify reasons for excluding the remaining 76 subjects. Altmann et al. (1995) was not selected as a principal study given the limited reporting, concern about potential selection bias, and concern about residual confounding for some of the adverse outcomes observed.

In sum, none of these residential studies was selected as a principal study. These studies nonetheless provide qualitative evidence for hazard identification of neurological deficits in visual function, reaction time, and cognitive function. The database of residential studies also adds support for the choice of key endpoints in principal studies and informs uncertainty factor (UF) selection, as described in Section 5.1.3.

5.1.1.3.2. Evaluation of epidemiologic studies of occupationally exposed populations

Seven occupational studies assessed visual function or other neurobehavioral effects and were considered as candidate studies for deriving the RfC (Schreiber et al., 2002; Gobba et al., 1998; Spinatonda et al., 1997; Echeverria et al., 1995; Cavalleri et al., 1994; Ferroni et al., 1992; Seeber, 1989). The primary strength of each of these studies is their use of standardized test methodology to evaluate neurobehavioral or visual function. Additional details regarding the evaluation of occupational study characteristics that informed selection of candidate studies are provided below.

Ferroni et al. (1992) was a prevalence study of 60 female dry cleaners or other dry-cleaning workers and 30 sex-, age-, and vocabulary test score-matched controls from an industrial cleaning plant that did not use organic solvents. Compared to responses in referents,

dry cleaners had a 10% increased simple reaction time and decrements in response on two subtests of the shape comparison test, one of vigilance (7% decrease) and one of stress (11% decrease) at the LOAEL of 102 mg/m^3 [$LOAEL_{HEC}$ = 36 mg/m^3] (median concentration). Study details are sparsely reported, and results are not accurately reported in the published paper. Ferroni et al. (1992) does not clearly identify whether age-matching was for individual subjects, or for the group's average age. A crude exposure assessment was used based on ambient monitoring data assigned to the group of dry cleaners, and statistical analyses did not control adequately for confounding characteristics among participants. As compared to the other occupational studies, this study had poorer quality in terms of comparability of referent and exposed groups and measurement of exposure and analysis methods, in part because of poor reporting of study details and results and, therefore, was not selected as a principal study.

Spinatonda et al. (1997) was a prevalence study of 35 dry cleaners and 39 age- and education-matched unexposed subjects that reported a 15% increased latency to a vocal response time at a LOAEL of 54 mg/m^3 [$LOAEL_{HEC}$ = 19 mg/m^3] (median concentration). The study design is sparsely reported, and the paper lacks details of subject selection, including the population from which controls were drawn, and demographic information for evaluation of comparability of dry cleaners and controls. Exposure was assessed by a "grab sample" which is inferior to a time-weighted average estimate. The study developed an index of cumulative exposure to tetrachloroethylene for each exposed subject by multiplying the tetrachloroethylene concentration by the number of years worked. Statistical analyses comprised *t*-tests comparing average latency in dry cleaner and control groups, and regression models fit to responses of exposed subjects only, a weaker approach than fitting multiple logistic regression models to data from all subjects. Additionally, the statistical analyses did not control for alcohol consumption, which is also associated with response time, indicating a greater potential for confounding. As compared to the other occupational studies, this study had poorer quality in terms of comparability of referent and exposed groups and measurement of exposure, in part because of poor reporting of study details and results, as well as less robust statistical analyses controlling for alcohol consumption. Therefore, Spinatonda et al. (1997) was not selected as a principal study.

Schreiber et al. (2002) was a small study examining nine adult staff at a day-care facility colocated in the same building as a dry cleaner, comparing group mean visual contrast values to age- and sex-matched referents values and identifying a LOAEL of 2 mg/m^3 [$LOAEL_{HEC}$ = 0.7 mg/m^3] (arithmetic mean concentration). Referents in this study were acquaintances, local retail shop employees, staff of other local day-care centers, or NYSDOH employees. Exposed and referent subjects were similar on sex and age; however, the paper lacks any details of whether referents were of similar education or socioeconomic status. Use of

NYSDOH employees located in Albany, NY, may indicate referents and exposed subjects may be different on education and other variables. Exposure assignment to subjects was based on ambient monitoring during time of active dry cleaning; no personal monitoring was conducted. Schreiber et al. (2002) used a standardized test (FACT) for near vision; however, a shortcoming is that assessment of vision was 6 weeks after exposure ceased, when measured tetrachloroethylene concentrations were 100-fold lower than during active dry cleaning. While Schreiber et al. (2002) adopted a valid and sensitive test to measure vision, it was not selected as a principal study due to its few subjects, concern that testers were not blinded to exposure classification, concern about comparability of exposed and referent subjects, and lack of concurrent exposure and outcome assessment.

Seeber (1989) evaluated the neurobehavioral effects of tetrachloroethylene on 101 dry-cleaning workers (employed in coin-operated or while-you-wait shops) and reported effects on several measures of cognition at a LOAEL of 83 mg/m^3 [$LOAEL_{HEC}$ = 29 mg/m^3] (time-weighted average mean concentration), compared to referents from several department stores and receptionists from large hotels (refer to section 4.1.1.2 for more details). Statistically significant changes from control of 5−30% were seen at the LOAEL for the measures of perceptual speed, digit reproduction, cancellation, and digit symbol. The exposure assessment used estimates of long-term exposure from interview data, active sampling of room air, and passive sampling of personal air to assign dry cleaners to two exposed groups (mean ± SD: 83 ± 53 and 364 ± 114 mg/m^3). Strengths of the study included the relatively large sample sizes for all three groups (57, 44, and 84 subjects in the lowest, highest, and referent groups, respectively), measurement of effects using recognized methods (standardized tests of symptoms and personality; tests of sensorimotor function, including finger tapping and aiming; digit reproduction and digit symbol) and use of examiners blinded to subjects' exposure status. Stratified regression analysis was used to statistically control for the influence of potentially confounding factors—gender ratios, age, and scores on the intelligence test—on test scores. Additional adjustment for group differences in alcohol consumption were considered, and did not alter the results. A limitation of the study was that no information was provided on the methods used to identify subjects or their reasons for participating in the study, but this was offset by the ascertainment of potentially confounding factors and the use of multiple regression to adjust for these factors. Another limitation was the lack of individual data to clarify dose-response relationships, as test outcomes and exposures were reported only as summary measures (mean ± SD) for two substantially overlapping exposure groups. There is some uncertainty in the quantitative dose-response relationships for this study, given the substantial overlap in exposure estimates between the low- and high-exposure groups and the wide confidence intervals around the mean test results. For many test outcomes, one or both exposure groups was

statistically significantly different from controls, but no significant differences between the low and high exposure were reported. For some outcomes, NRC (2010) characterized the study as having discrepant results based on worse mean test scores (for neurologic signs, emotional lability, choice reaction time, cancellation d2 and digit symbol) in the low- compared with high-exposure group. This study was not among those recommended by NRC for consideration in deriving the RfC.

Cavalleri et al. (1994) and Gobba et al. (1998) are two studies of the same exposed population. Cavalleri et al. (1994) reported poorer performance (6% decrement on average) on a test of color vision among 35 dry cleaning and laundry workers compared to 35 controls matched on age, alcohol consumption, and smoking. The LOAEL for all workers in this study was 42 mg/m^3 [$LOAEL_{HEC}$ = 15 mg/m^3] (time-weighted average mean concentration). Controls were not matched on education or intelligence, but these factors have not been shown to be associated with color vision. Exposure was assessed for individual subjects from personal monitoring over the full work shift and represented an 8-hour time-weighted average. Standard testing methods, including an established protocol, were used to detect changes in color vision, which were assessed by the Lanthony D-15 Hue desaturated panel. The investigators' statistical analyses included comparison of group mean Color Confusion Indexes (CCIs) by the arithmetic mean of three exposure groupings: all workers (42 mg/m^3), dry cleaners (49 mg/m^3), and ironers (33 mg/m^3), and multiple logistic regression analyses which adjusted for effects of age, alcohol consumption, and smoking.

Gobba et al. (1998) examined color vision in 33 of these 35 dry cleaners and laundry workers after a 2-year period and reported a further decrement in color vision (9% decrement on average) among 19 subjects whose geometric mean exposure had increased from 12 mg/m^3 to 29 mg/m^3 over the 2-year period. No improvement was observed among 14 subjects whose geometric mean exposure had decreased from 20 mg/m^3 to 5 mg/m^3. The mean responses of both subgroups supported a persistence of deficits in visual function and suggested a worsening of effects when exposure increased for individuals. A strength of Gobba et al. (1998) is subjects serving as their self-controls, with scores on the test of color vision compared from the initial and follow-up studies. Given the vision deficits reported by Cavalleri et al. (1994), Gobba et al. (1998) serves to confirm and extend those findings.

Cavalleri et al. (1994) is preferred to Gobba et al. (1998) for candidate reference value derivation for several reasons. First, the earlier study more clearly associated a deficit in color vision with tetrachloroethylene exposure through comparison to a suitable and well characterized, unexposed reference group. The Gobba et al. (1998) study did not include unexposed controls and, therefore, cannot distinguish the possible impact of age on the CCI scores of subjects who were 2 years older at the second evaluation. Second, the Gobba et al.

(1998) study suggests that the earlier exposure was sufficient to cause the CCI deficit in at least those subjects (n = 14) whose exposure decreased after the earlier evaluation. While the Gobba et al. (1998) study also demonstrated further deficits in workers whose exposure increased after the first study (n = 19), it is unclear how to relate the higher measurement to the incremental deficit, given the lack of improvement in the subset with decreased exposure and the lack of information concerning the other confounding variables considered in the first evaluation—absolute age, smoking, and alcohol status. In any case, a deficit existed in this subset before the follow-up period, at a lower exposure than that of the second evaluation. Third, the exposures in Cavalleri et al. (1994) were reported as time-weighted average arithmetic means, which are expected to represent total risk better than time-weighted average geometric means [as reported in Gobba et al. (1998)] when data are grouped (Crump, 1998). The point of departure (POD) was, therefore, taken from the Cavalleri et al. (1994) study. The exposure level for the full study sample is used as the LOAEL for several reasons. Although no apparent CCI deficit was observed in ironers, their reported exposure range (0.52−11.28 ppm, or 3.5−76 mg/m^3) was completely contained within the range of exposures for dry cleaners (0.38−31.19 ppm, or 2.6−210 mg/m^3). Yet elevated CCI scores were observed at exposures lower than the mean exposure of the ironers (4.8 ppm, or 33 mg/m^3), indicating that the mean exposure of the ironers cannot be considered a NOAEL. For these reasons, Cavalleri et al. (1994) is used to derive a candidate RfC.

Echeverria et al. (1995) examined 65 dry cleaners in Detroit, MI, using a standardized neurobehavioral battery and found changes in cognitive and visuospatial function. A LOAEL of 156 mg/m^3 [$LOAEL_{HEC}$ = 56 mg/m^3] (time-weighted average mean concentration) was identified, based on comparison of the two higher exposure categories with an internal referent group comprising mainly counter clerks, who were matched to exposed dry cleaners on age and education. Changes of 4−14% from internal referent levels, depending on subtest, were observed at the LOAEL. The study had a high quality exposure-assessment approach and appropriate statistical analyses that adjusted for covariates including alcohol. A potential selection bias may have resulted from the 18% participation rate among dry-cleaning shop owners, if the low participation could be explained by the health status of employees. The study also lacked an unexposed referent group; subjects were categorized into three exposure groups. Without an unexposed control group, the exposure level for the lowest exposure group (i.e., the internal referent group) cannot be classified as a NOAEL or a LOAEL. This study was of relatively good quality in terms of the comparability of referent and exposed groups, measurement of effect, and measurement of exposure and, although there are concerns about the lack of an unexposed referent group, this study was used to derive a candidate RfC.

5.1.1.3.3. Evaluation of experimental human exposure studies

The two human controlled exposure studies (Altmann et al., 1990; Hake and Stewart, 1977) were of fewer subjects and shorter exposure durations, and effects were observed at higher exposure concentrations than chronic studies of residential and occupational exposure. While subjects in Altmann et al. (1990) could serve as their own controls, there was not an unexposed group. Therefore, neither study was selected as a principal study given the availability of suitable human data of chronic duration. These studies do provide qualitative evidence for hazard identification of neurological deficits in visual function and neurological function and add support for choice of key endpoints in principal studies.

5.1.1.3.4. Evaluation of animal neurotoxicity studies

The animal neurotoxicity studies mostly consist of acute duration studies (Boyes et al., 2009; Oshiro et al., 2008; Kjellstrand et al., 1985) and subchronic (repeated dosing) studies, which generally involve lower exposures than the acute animal studies (Mattsson et al., 1998; Wang et al., 1993; Rosengren et al., 1986). However, these studies covered shorter exposure duration periods than the available human studies and require extrapolation of animal observations to humans. They were not considered principal studies given the availability of suitable human data from chronic exposures. The findings in the animal studies contribute to the weight of evidence that tetrachloroethylene exposure results in neurological deficits and is considered supportive of the human studies in terms of hazard identification.

5.1.1.3.5. Selection of studies

To summarize, three studies (Echeverria et al., 1995; Cavalleri et al., 1994; Seeber, 1989) had more of the preferred qualities listed in Table 5-2 compared to other epidemiologic studies of occupational and residential exposure. However, NRC (2010) characterized the Seeber (1989) study as having discrepant results based on worse mean test scores (for neurologic signs, emotional lability, choice reaction time, cancellation d2 and digit symbol) in the low- compared with high-exposure group. Therefore, Seeber (1989) was not among those recommended by NRC for consideration in deriving the RfC.

NRC (2010) recommended five studies for consideration in deriving the RfC (Altmann et al., 1990; Boyes et al., 2009; Echeverria et al., 1995; Cavalleri et al., 1994; and Gobba et al., 1998). Two acute studies recommended for consideration by NRC [the human chamber study of Altmann et al. (1990) and the rodent study of Boyes et al. (2009)] were judged by EPA to be supportive, but were not considered further for deriving candidate RfCs because of the preference to use quality studies of chronic, human exposures over studies of acute exposures. In addition, two of the other studies recommended by NRC (2010), Cavalleri et al. (1994), and

Gobba et al. (1998), evaluated the same cohort, and the earlier study was preferred by EPA due to its use of a control group and the clearer identification of a POD (refer to section 5.1.1.3.2). Thus, two studies—Cavalleri et al. (1994) and Echeverria et al. (1995)—are considered principal studies by EPA for the derivation of the RfC. Endpoints selected for the candidate RfCs were reaction time measures (Echeverria et al., 1995), cognitive changes (Echeverria et al., 1995), and visual function changes (Cavalleri et al., 1994).

5.1.2. Additional Analyses: Feasibility of Dose-Response Modeling

The present analysis defines a POD using the traditional NOAEL/LOAEL approach. The NOAELs/LOAELs were adjusted to an equivalent continuous exposure (U.S. EPA, 1994), and described in Section 5.1.1) so that comparisons could be made between studies. Ambient (inhaled) concentration of tetrachloroethylene was used as the dose metric in deriving the RfC.

Because the application of dose-response modeling offers advantages over the use of NOAELs/LOAELs, the data sets from the endpoints in the two studies (refer to Table 5-3) (Echeverria et al., 1995; Cavalleri et al., 1994) were evaluated for feasibility of dose-response modeling. In both studies, it was determined that PODs could not be derived using dose-response modeling, for varying reasons as detailed below.

In evaluating the CCIs in Cavalleri et al. (1994), normative data for color confusion (Lomax et al., 2004; Iregren et al., 2002) were considered. However, the normal ranges are influenced strongly by age. Although the investigators reported statistical significance of the cleaning workers' CCIs using analyses which adjusted for age (as well as smoking and alcohol consumption), no individual ages were provided with the individual CCIs and exposure measurements, and no individual measurements for the control subjects were provided. Insufficient information was available to make use of the investigators' model, including response measures adjusted for age.

Echeverria et al. (1995) identified three exposure groups, but there was no unexposed group for comparison. Historical control data from the Echeverria group were unavailable, precluding the derivation of PODs from the logistic regression they reported. In some cases, particularly with individual exposure and response data available, a control level might be inferred by extrapolating from the observed data. However, only summary values for exposure and responses were available. Therefore, projecting a control response and biologically relevant level of change from that point was judged to be too uncertain.

Table 5-3. Application of uncertainty factors for the neurological endpoints from the studies used to derive candidate RfCs [Echeverria et al. (1995) and Cavalleri et al. (1994)]

Neurological endpoint	Human equivalent NOAEL/LOAEL (mg/m^3)	Uncertainty factors (UFs)						Candidate RfC (mg/m^3)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Cognitive Domain									
Visual reproduction, pattern memory, pattern recognition—adult, occupational	56 (LOAEL)	1,000	1	10	1	10	10	0.056	Echeverria et al. (1995)
Reaction Time Domain									
Reaction time in pattern memory—adult, occupational	56 (LOAEL)	1,000	1	10	1	10	10	0.056	Echeverria et al. (1995)
Visual Function Domain									
Color confusion—adults, occupational	15 (LOAEL)	1,000	1	10	1	10	10	0.015	Cavalleri et al. (1994)

5.1.3. Reference Concentration (RfC) Derivation, Including Application of Uncertainty Factors

The RfC is the midpoint of the range of candidate reference values from two principal studies. Candidate RfCs for tetrachloroethylene were derived by dividing the PODs of 15 mg/m^3 (Cavalleri et al., 1994) and 56 mg/m^3 (Echeverria et al., 1995) by a total UF of 1,000, comprised of 10 for interindividual variability, 10 for extrapolation from a LOAEL to a NOAEL, and 10 for database uncertainty. The application of uncertainty factors is based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002); Section 4.4.5], which address five areas of uncertainty.

- An UF of 10 was applied to account for human variability in the effects that were used for the derivation of the RfC. The principal studies are based on occupationally exposed subjects, who are generally healthier than the overall population, and, thus, provide no data to determine the relative effects of susceptible population including children, elderly, and/or people with compromised health. Additionally, no information was presented in the human studies with which to examine variation among subjects. Quantitative analyses have been carried out by Clewell et al. (2004) and Pelekis et al. (2001) evaluating pharmacokinetic variation between adults and children for tetrachloroethylene and its metabolites using physiologically based pharmacokinetic

(PBPK) models. However, validation of these results for various life-stages and further refinement of the parameters in the model have not been conducted.

- An UF of 1 was applied to account for interspecies variability in extrapolation from laboratory animals to humans because the principal studies and critical endpoints were from human studies.

- An UF of 1 was applied for the use of data from subchronic study to assess potential effects from chronic exposure because the PODs are based on studies involving chronic exposure (refer to Section 5.1.3).
- An UF of 10 was applied for the extrapolation from a LOAEL to a NOAEL because the PODs from the studies were LOAELs.
- An UF of 10 was applied to address the lack of data to adequately characterize the hazard and dose response in the human population. The following critical data gaps have been identified: uncertainties associated with database deficiencies on neurological, developmental, and immunological effects. The two studies (Echeverria et al., 1995; Cavalleri et al., 1994) used to derive the RfC evaluated neurotoxicity following occupational exposures with PODs 3- to 100-fold higher than those identified from residential studies (Storm et al., 2011 [previously reported in NYSDOH, 2010]; Schreiber et al., 2002; Altmann et al., 1995). In comparison to the occupational studies, the available residential studies were judged to be more limited for developing an RfC, based on consideration of the study design (population comparability) and/or selection of neurological methods. However, they provide human evidence of neurotoxicity following tetrachloroethylene exposure in a residential setting, with reaction time deficits, visual system dysfunction, and cognitive performance deficits.

  In addition, data characterizing dose-response relationships and chronic visuospatial functional deficits and the cognitive effects of tetrachloroethylene exposure under controlled laboratory conditions are lacking. Data from acute studies in animals (Oshiro et al., 2008; Umezu et al., 1997; Warren et al., 1996) 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., Altmann et al., 1995; Echeverria et al., 1995; Seeber, 1989). There is also a lack of cognitive testing following exposures of longer than acute duration, including during development. Visual system dysfunction and processing of visuospatial 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 effects of chronic exposure to tetrachloroethylene on 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 in humans.

Finally, additional data are needed to assess the potential hematological and immunological effects of tetrachloroethylene. In humans, Emara et al., (2010) reported changes in various standard hematological measures in subjects with mean tetrachloroethylene blood levels of 1.685 mg/L. The limited laboratory animal studies of hematological toxicity demonstrated an effect of tetrachloroethylene exposure on red blood cells (decreased RBCs (Ebrahim et al., 2001), or decreased erythrocyte colony-forming units (Seidel et al., 1992)), with reversible hemolytic anemia observed in female mice exposed to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age in one series of studies (Marth et al., 1989; Marth, 1987; Marth et al., 1985a; Marth et al., 1985b). Ebrahim et al. (2001) also observed decreased hemoglobin, platelet counts, and packed cell volume, and increased WBC counts. Although additional corroborating studies are lacking, the observation of an effect at a low exposure level raises additional concern about hematological and immunological effects. The fact that other solvents [e.g., toluene, and the structurally similar solvent trichloroethylene (Cooper et al., 2009)] have been associated with immunotoxicity contributes further concern about this gap in the database for tetrachloroethylene.

These UFs were applied to each of the following endpoints from the selected neurotoxicological studies of occupational tetrachloroethylene exposure: color vision changes (Cavalleri et al., 1994); and cognitive and reaction time changes (Echeverria et al., 1995). The UFs for each study and endpoint are presented in Table 5-3 as well as in Figure 5-2. The, candidate RfCs from these studies span a range from 0.015 to 0.056 mg/m^3. The RfC for tetrachloroethylene is **0.04 mg/m^3**, the midpoint of this range rounded to one significant figure.

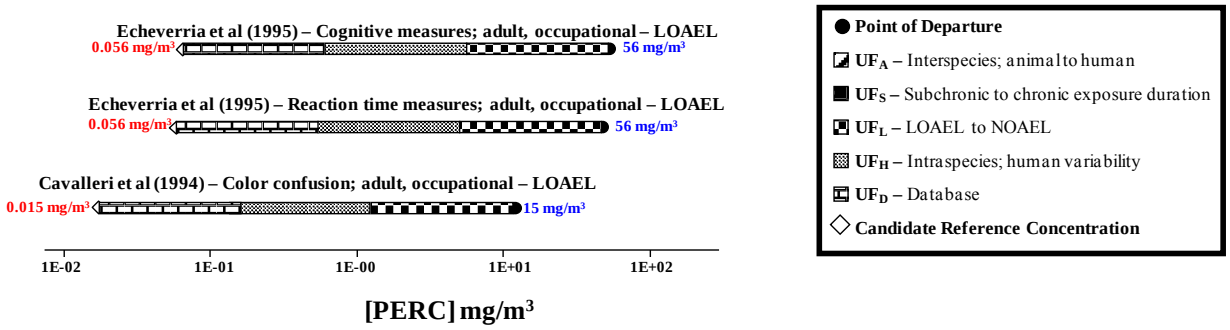

Figure 5-2. Candidate reference concentration values for inhalation exposure to tetrachloroethylene.

5.1.4. Dose-Response Analyses for Comparison of Noncancer Effects Other Than Critical Effects in Neurotoxicity

This section presents inhalation dose-response analyses for noncancer effects other than the critical effect of neurotoxicity. The purpose of these analyses is twofold: (1) to provide a quantitative characterization of the relative sensitivity of different organs/systems to tetrachloroethylene, and (2), to provide information that may be useful for cumulative risk assessment in which multiple chemicals have a common target organ/system other than the central nervous system. Therefore, for each organ/system, "sample reference concentrations" (sRfCs) are calculated based on the same methodology as is used for the critical effect of neurotoxicity. These sRfCs are based on an evaluation of studies identified in Section 4.10 as suitable for dose-response analysis.

The method of analysis is the same as that described above for neurotoxicity, using the NOAEL/LOAEL approach. Benchmark dose modeling was not performed because these sample RfCs are meant for comparison purposes only (across organs/tissues or across chemicals). HECs are derived using either (1) the RfC methodology for a Category 3 gas, extrathoracic effects, adjusted for equivalent continuous exposure; or (2) the PBPK model with an appropriate dose metric. For liver effects, the dose metric of liver oxidative metabolism was used, based on the view that oxidative metabolites are involved in tetrachloroethylene-induced liver effects. For kidney effects, while it is generally thought that GSH conjugation metabolites are involved, the large uncertainty in estimates of human GSH conjugation preclude use of that dose metric. Instead, the AUC of tetrachloroethylene in blood is used as a surrogate. For the other non-cancer effects, the AUC of tetrachloroethylene in blood was used as the preferred dose metric due to the lack of data on what the active toxic moeity(ies) may be for those effects. In addition, the PBPK model is being used to perform route-to-route extrapolation from oral to inhalation exposure, so both inhalation and oral studies are considered together here. The HEC is then treated as a POD to which the following uncertainty factors may be applied:

- An UF of 10 was applied for human variation to all PODs. The rationale is the same as described above for neurotoxicity. Furthermore, there is some indication that human variability (at least for one endpoint) may be substantially more than that implied by the default UF. Kidney toxicity is thought to be associated with metabolism of tetrachloroethylene along the glutathione (GSH) conjugation pathway. As described in Section 3.5, PBPK model predictions for GSH conjugation span a wide range that may be due to uncertainty, variability, or both. Glutathione S-transferases (GSTs) are known to be polymorphic in the human population, with some isoforms exhibiting a substantial population of null phenotypes.

- An UF of 3 was applied to the PODs from all rodent studies to account for interspecies variability in extrapolation from laboratory animals to humans. The PODs from rats and mice are expressed as HECs calculated using either the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (hereafter referred to as the RfC Methodology (U.S. EPA, 1994) or the PBPK model. Therefore, the UF of 3 was applied for animal-to-human uncertainty to the PODs from these studies of rats and mice to account for potential pharmacodynamic differences. This factor is not applied to PODs from human studies.

- An UF of 10 was applied to PODs of studies of subchronic or shorter duration to address the potential for additional or more severe toxicity from chronic or lifetime exposure.

- An UF of 10 was applied when a LOAEL is used due to a lack of a NOAEL. This factor was applied to the PODs of studies that identified a LOAEL but not a NOAEL.

- A database UF of 10 was applied to all PODs to address the lack of data to adequately characterize the hazard and dose response. The rationale is the same as described above for neurotoxicity.

5.1.4.1. Sample Reference Concentrations (RfCs) for Kidney Toxicity

As discussed in Section 4, numerous studies have reported adverse effects in the kidney from tetrachloroethylene. Five studies reporting kidney toxicity were identified in Section 4.10 as suitable for dose-response analysis. The only human study was Mutti et al. (1992), which reported statistically significant increases in retinol binding protein (RBP), $\beta_{2\mu}$-globulin, and albumin in urine among dry cleaners as compared to matched controls. In addition, for seven different urinary markers, the prevalence of individuals with abnormal values (>95th percentile of controls) was four- to fivefold greater in the exposed group. This study was in humans chronically exposed and was, thus, used to calculate an sRfC. Of the rodent studies reporting nephrotoxicity, only JISA (1993) identified a chronic NOAEL, with the other three rodent studies reporting subchronic (Jonker et al., 1996) or chronic LOAELs (NTP, 1986; NCI, 1977).

Therefore, among the rodent studies, only JISA (1993), which reported effects in both mice and rats, was used in sRfC calculations. A summary of the PODs and UFs applied is in Table 5-4. The resulting sRfCs range from 0.05−0.2 mg/m^3 based on nuclear enlargement (karyomegaly) in the proximal tubules of chronically exposed mice and rats (JISA, 1993) with a slightly lower sRfC of 0.03 mg/m^3 based on urinary markers of nephrotoxicity in occupationally exposed humans (Mutti et al., 1992).

5.1.4.2. Sample Reference Concentrations (RfCs) for Liver Toxicity

As discussed in Section 4, numerous studies have reported adverse effects in the liver from tetrachloroethylene. Six studies, none in humans, reporting liver toxicity were identified in Section 4.10 as suitable for dose-response analysis. Only JISA (1993) reported a chronic NOAEL, and so was carried forward for derivation of an sRfC. However, it is unclear whether the reported effect of angiectasis, or enlargement of the blood vessels, is related to the other liver effects of tetrachloroethylene, which generally involve hepatocytes. Therefore, two other studies were utilized, one of which reported a chronic LOAEL for liver degeneration and necrosis (NTP, 1986), and the other of which reported a NOAEL for liver weight increases after 6-week exposures (Buben and O'Flaherty, 1985). The remaining studies either only reported a LOAEL (Jonker et al., 1996; Kjellstrand et al., 1984), or reported a NOAEL for a very short duration [14 days, Berman et al. (1995)], and were, therefore, not considered further.

Therefore, JISA (1993), NTP (1986), and (Buben and O'Flaherty, 1985) were used to calculate sRfCs. In addition, PBPK modeling was used to calculate the total rate of oxidative metabolism in the liver as a dose metric for deriving the HECs.[29] Table 5-5 summarizes the PODs and UFs applied. The resulting sRfCs range from 0.09 mg/m^3 based on increased liver/body-weight ratios after 6-week exposures (Buben and O'Flaherty, 1985) to 0.7 mg/m^3 based on liver effects after chronic exposures (JISA, 1993; NTP, 1986). It should also be noted that in the chronic studies, increased liver tumors were observed at the lowest doses tested.

Therefore, under chronic exposure conditions, cancer effects are likely to be more important than noncancer effects in the liver.

[29] The MOA for tetrachloroethylene-induced liver toxicity is not clear. It appears that TCA as the sole contributory metabolite cannot explain tetrachloroethylene-induced hepatotoxicity (Clewell et al., 2005; Buben and O'Flaherty, 1985). It is not known whether reactive intermediates such as tetrachloroethylene oxide and trichloroacetyl chloride are involved in induced liver toxicity. In consideration of these uncertainties, it appears more appropriate to use total rate of oxidative metabolism as the dose metric for tetrachloroethylene-induced liver toxicity. This quantity is then scaled by body weight to the 3/4th power so as to enable extrapolation of risk across species.

Table 5-4. Sample RfCs for kidney effects

Kidney endpoint (species)	HEC in mg/m^3 (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfC (mg/m^3)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Urinary markers of nephrotoxicity (human)	34 (LOAEL)	1,000	1	10	1	10	10	0.03	Mutti et al. (1992)
Nuclear enlargement in proximal tubules (rat)	61 (NOAEL)	300	3	10	1	10	1	0.2	JISA (1993)
Nuclear enlargement in proximal tubules (mouse)	14 (NOAEL)	300	3	10	1	10	1	0.05	JISA (1993)

Table 5-5. Sample RfCs for liver effects

Liver endpoint (species)	HEC[a] in mg/m^3 (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfC (mg/m^3)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Increased angiectasis (mouse)	210 (NOAEL)	300	3	10	1	10	1	0.7	JISA (1993)
Increased liver degeneration/necrosis (mouse)	2,100 (LOAEL)	3,000	3	10	1	10	10	0.7	NTP (1986)
Increased liver/body-weight ratio (mouse)	270[b] (NOAEL)	3,000	3	10	10	10	1	0.09	Buben & O'Flaherty (1985)

[a]Calculated with PBPK model using the dose metric of liver oxidative metabolism.
[b]Route-to-route extrapolation from oral exposure.

5.1.4.3. Sample Reference Concentrations (RfCs) for Immunotoxicity and Hematologic Toxicity

As discussed in Section 4, a number of studies have reported changes in hematologic or immunologic parameters with tetrachloroethylene exposure. Two studies reporting hematologic effects were identified in Section 4.10 as suitable for dose-response analysis. The human study (Emara et al., 2010) reported changes in various standard hematological measures in subjects with mean blood levels of 1.685 mg/L. Application of the PBPK model gives an air concentration estimate during exposure of 18 ppm corresponding to this blood level, assuming constant concentration during exposure. Adjustment to equivalent continuous exposure gives an

HEC of 6.4 ppm, or 43 mg/m^3. This can be treated as a chronic LOAEL, given the 7-year mean exposure duration (≥10% of lifespan). The other study (Marth, 1987) reported reversible hemolytic anemia after drinking water exposure to 2-week-old mice for 7 weeks. Because only a LOAEL was identified, the exposures were subchronic, and the effect has not been reproduced at such low exposure in other studies, Marth (1987) was not considered further for sRfC derivation. However, it should be noted that the LOAEL identified was low—0.05 mg/kg-day—and may be a cause for additional concern about hematologic effects. Therefore, Emara et al. (2010) was used to calculate an sRfC. A summary of the POD and UFs applied is in Table 5-6. The result is an sRfC of 0.04 mg/m^3.

5.1.4.4. Sample Reference Concentrations (RfCs) for Reproductive and/or Developmental Toxicity

As discussed in Section 4, a number of studies have reported reproductive and developmental effects from tetrachloroethylene exposure. Four studies, none in humans, reporting reproductive or developmental effects, were identified in Section 4.10 as suitable for dose-response analysis. All of these studies reported NOAELs. The developmental studies were all of appropriate duration for detecting those effects. The reproductive study (Beliles et al., 1980) was short term (5 days exposure), but was the only suitable study for reproductive toxicity, and assessment was limited to males. Therefore, all four studies were used to calculate sRfCs. A summary of the PODs and UFs applied is in Table 5-7. For all these endpoints, the UF for subchronic to chronic extrapolation was not used because the studies sufficiently covered the developmental window or window of sperm development. The resulting sRfCs range from 0.4−0.7 mg/m^3 for different developmental effects (Carney et al., 2006; Tinston, 1994; Nelson et al., 1979), with an intermediate value of 0.5 mg/m^3 for reduced sperm quality (Beliles et al., 1980).

5.1.4.5. Summary of Sample Reference Concentrations (RfCs) for Noncancer Endpoints Other Than the Critical Effect

The lowest sRfCs for these noncancer endpoints are similar to the values calculated based on the critical effect of neurotoxicity (refer to Figure 5-3), therefore supporting the selection of the critical effect: 0.03 mg/m^3 from Mutti et al. (1992) and 0.04 mg/m^3 from Emara et al. (2010). The other sRfCs are less than 20-fold greater than the RfC. This suggests that multiple effects may begin to occur as exposure rises above those at which tetrachloroethylene begins to induce neurotoxicity. These results also suggest that it is important to take into account effects from tetrachloroethylene other than neurotoxicity when assessing the cumulative effects of multiple exposures.

Table 5-6. Sample RfCs for immunological and hematological effects

Immunotoxicity/hematotoxicity endpoint (species)	HEC in mg/m^3 (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfC (mg/m^3)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Reduced RBC, hemoglobin; increased WBC, lymphocytes, IgE (human)	43 (LOAEL)	1,000	1	10	1	10	10	0.04	Emara et al. (2010)

RBC = red blood cells. WBC = white blood cells.

Table 5-7. Sample RfCs for reproductive and developmental effects

Reproductive/developmental endpoint (species)	HEC in mg/m^3 (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfC (mg/m^3)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Decreased weight gain; altered behavior, brain acetylcholine (rat)	200 (NOAEL)	300	3	10	1	10	1	0.7	Nelson et al. (1979)
Reduced sperm quality (mouse)	140 (NOAEL)	300	3	10	1	10	1	0.5	Beliles et al. (1980)
Increased F2A pup deaths by Day 29; CNS depression in F1 and F2	122 (NOAEL)	300	3	10	1	10	1	0.4	Tinston et al. (1994)
Decreased fetal and placental weight; skeletal effects (rat)	110 (NOAEL)	300	3	10	1	10	1	0.4	Carney et al. (2006)

CNS = central nervous system.

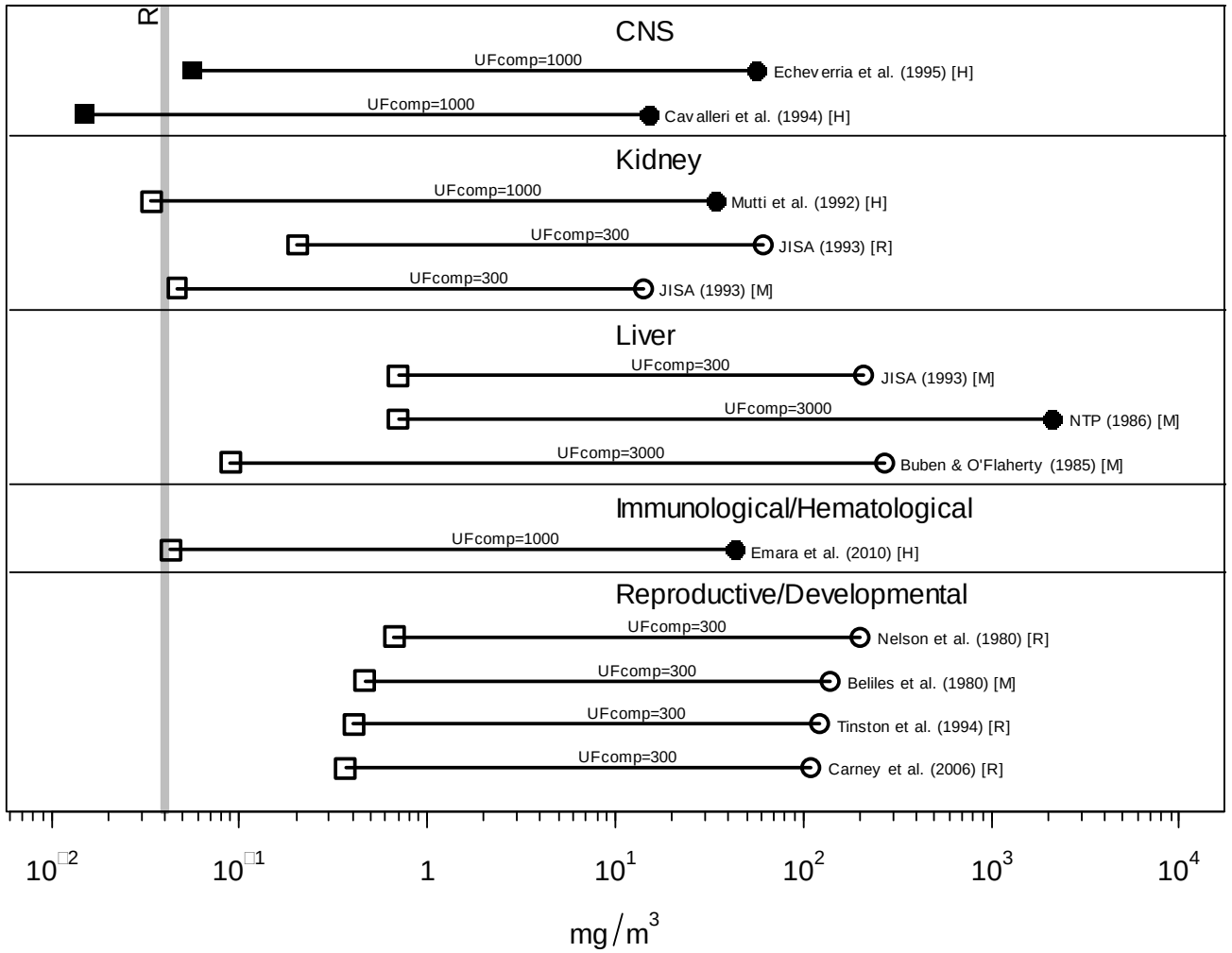

Figure 5-3. Comparison of candidate RfCs (filled squares) supporting the RfC (vertical line) and sample RfCs (open squares) for effects other the critical effect (CNS toxicity).

Filled circles = study/endpoint LOAEL in terms of human equivalent concentrations. Open circles = study/endpoint NOAEL in terms of human equivalent concentrations. Species in each study is shown in brackets after the reference (mouse: M; rat: R; human: H).

5.1.5. Previous Inhalation Assessment

There is no previous IRIS RfC for tetrachloroethylene.

5.1.6. Uncertainties in Inhalation Reference Concentration

As presented above, the uncertainty factor approach was applied to PODs consisting of LOAELs from two epidemiologic studies of neurological effects. These studies were considered to be methodologically sound based on study quality attributes, including study population selection, exposure measurement methods, and endpoint measurement methods. Other strengths are that they are human studies of chronic duration, obviating the need for extrapolation across species and exposure duration. However, there are a number of uncertainties deriving the RfC from POD, as discussed below.

First, there is uncertainty in the POD, which was based on a LOAEL. In particular, a LOAEL or a NOAEL is a reflection of the particular exposure concentrations or doses at which a study was conducted. These lack characterization of the dose-response curve and for this reason is less informative than a POD obtained from dose-response modeling. In addition, the PODs are all LOAELs because a NOAEL was not identified in the studies and benchmark dose-response modeling was not feasible. An UF of 10 is applied as an adjustment to the LOAEL, but the actual extent of adjustment necessary may be larger or smaller.

Second, there is uncertainty related to human variability. Subjects in the principal studies comprise a population of occupationally exposed adult subjects, and there is uncertainty in extrapolating doses 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 human variation. Actual human variation in tetrachloroethylene susceptibility may be larger or smaller; however, there are inadequate tetrachloroethylene-specific data to examine the potential magnitude of any over- or under-estimation.

Finally, critical data gaps have been identified that also contribute to uncertainty in the RfC. In particular, there is a need for high quality epidemiologic studies of residential exposures and chronic-duration animal studies (including in developing animals). A fuller characterization is also needed of the noncancer effects other than the critical effect of neurotoxicity, particularly immunological and hematological effects. Given these limitations, a factor of 10 was used to account for database limitations, but the actual factor necessary may be larger or smaller.

5.2. ORAL REFERENCE DOSE (RfD)

5.2.1. Choice of Principal Studies and Critical Effects

Generally, the studies of greatest duration of exposure and conducted via the oral route of exposure have the most confidence for derivation of an RfD.[30] However, the application of pharmacokinetic models for a route-to-route extrapolation of the inhalation studies expands the database of studies suitable for RfD calculation.

As discussed in Section 5.1.1, based on evidence that neurological effects were associated with lower tetrachloroethylene concentrations, neurotoxicity is selected as the critical noncancer health effect of tetrachloroethylene. The nervous system is an expected target following oral exposure, because tetrachloroethylene and metabolites produced from inhalation exposures will also reach the target tissue via oral exposure. In addition, other organ systems such as the liver and kidney are common targets associated with both inhalation and oral routes of exposure, which supports the use of route extrapolation to compare PODs for oral and inhalation exposure. In addition, differences in first-pass metabolism between oral and inhalation exposures can be adequately accounted for by the PBPK model. For these reasons, the inhalation neurotoxicity studies used in deriving the candidate RfCs are chosen for deriving candidate RfDs.

5.2.2. Additional Analyses: Route-to-Route Extrapolation Using PBPK Modeling

The present analysis defines a POD using the traditional NOAEL/LOAEL approach. As discussed in Section 5.1.2, dose-response modeling was not feasible with these studies. 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 (refer to 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, 1997b). Additionally, the metabolic pathways and kinetics of excretion with oral exposure are similar to those of inhalation exposure (ATSDR, 1997b). Furthermore, the data for oral administration indicate a pattern of effects similar to that of inhalation exposure. PBPK modeling was also used with suitable studies in animals in order to extrapolate to human equivalent doses (HEDs). It is not clear if the noncancer effects observed in humans are the result of tetrachloroethylene itself and/or one or more metabolites. However, tetrachloroethylene in the blood can safely be presumed to be a step

[30] The RfD is expressed in units of milligrams per kilogram of 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.

in the toxicity pathway. Therefore, area under the curve (AUC) of blood tetrachloroethylene concentration derived from PBPK modeling is considered the best surrogate for an internal dose. The use of blood tetrachloroethylene provides some attempt to account for breathing rates and to adjust for processes related to tetrachloroethylene toxicokinetics, and it is assumed to better reflect tetrachloroethylene toxicokinetics than use of default methodologies. Moreover, based on the results of the harmonized PBPK model (Chiu and Ginsberg, 2011), the sensitivity to the choice of dose metric for route-to-route extrapolation is low, with alternative dose metrics such as GSH metabolism, oxidative metabolism, or trichloroacetic acid (TCA) in blood giving route-to-route conversions within 1.4-fold of the conversion based on tetrachloroethylene in blood. Importantly, the PBPK model accounts for the potential first-pass effect of liver metabolism from oral exposure, which was found to be minimal.

The harmonized PBPK model of Chiu and Ginsberg (2011) was used to derive the continuous oral dose (i.e., in mg/kg-day) that would result in the same tetrachloroethylene in blood AUC as that following a continuous inhalation exposure from the two studies (Echeverria et al., 1995; Cavalleri et al., 1994. The route-to-route extrapolation starts with the estimation of the average venous blood tetrachloroethylene AUC resulting from continuous inhalation exposure at the adjusted LOAELs from the neurological endpoints in the two studies (Echeverria et al., 1995; Cavalleri et al., 1994;). The venous blood tetrachloroethylene AUC at steady state resulting from continuous exposure to these tetrachloroethylene concentrations was estimated to range from 4.5 to 17 mg-hr/L-day, according to the Chiu and Ginsberg (2011) harmonized model. While the model utilizes data from some healthy adult volunteers, it cannot be considered to address pharmacokinetic variation in the full human population. The oral exposure scenario was also modeled as continuous (i.e., a constant oral dose rate in mg/kg-day), because at these exposure levels, the AUC of tetrachloroethylene in blood is insensitive to the exposure pattern. The route-to-route extrapolation oral ingestion values at the LOAELs were 2.6 mg/kg-day for Cavalleri et al. (1994) and 9.7 mg/kg-day for Echeverria et al. (1995). The results are presented in Table 5-8.

5.2.3. Reference Dose (RfD) Derivation, Including Application of Uncertainty Factors

The RfD is the midpoint of the range of candidate reference values from two principal studies. Candidate RfDs for tetrachloroethylene were derived by dividing the route-to-route extrapolated PODs of 2.6 mg/kg-day (Cavalleri et al., 1994) and 9.7 mg/kg-day (Echeverria et al., 1995) by a total UF of 1,000, comprised of 10 for interindividual variability, 10 for extrapolation from a LOAEL to a NOAEL, and 10 for database uncertainty. The application of UFs was similar to that for the different endpoints used to derive the candidate RfCs. The application of uncertainty factors is based on EPA's *A Review of the Reference Dose and*

Reference Concentration Processes [(U.S. EPA, 2002); Section 4.4.5], which address five areas of uncertainty.

- An UF of 10 was applied for human variation in the effects that were chosen for the derivation of the RfD. As indicated in the RfC discussion (Section 5.1.3), the studies selected do not include evaluation of potential sensitive populations including children, elderly, and immune-compromised individuals.
- An UF of 1 was applied to account for interspecies variability in extrapolation from laboratory animals to humans because the studies and critical endpoints were from human studies.
- An UF of 1 was applied for the use of data from subchronic study to assess potential effects from chronic exposure because, as with the RfC derivation described in Section 5.1.3 for the human studies, the PODs are based on studies involving chronic exposure.
- An UF of 10 was applied for the extrapolation from a LOAEL to a NOAEL because the PODs from the studies were LOAELs.
- An UF of 10 was applied to address the lack of data to adequately characterize the hazard and dose response in the human population as was done for the derivation of the inhalation RfC. The rationale is the same as described above for the RfC.

UFs for the different endpoints were applied similarly to that for the RfC. The PODs from each neurological endpoint were derived from a route-to-route extrapolation using a PBPK model to obtain oral exposure equivalents. A composite UF of 1,000 was applied to the PODs for the critical endpoints. A summary for each endpoint can be found in Table 5-8 and Figure 5-4.

In summary, candidate RfDs for tetrachloroethylene were developed through route-to-route extrapolation from the PODs for the following endpoints from neurotoxicological studies of occupational tetrachloroethylene exposure: color vision changes (Cavalleri et al., 1994); and cognitive and reaction time changes (Echeverria et al., 1995). The oral exposure POD equivalent to the continuous inhalation exposure NOAELs or LOAELs was estimated via PBPK modeling. The resulting PODs were 2.6 mg/kg-day (Cavalleri et al., 1994) and 9.7 mg/kg-day (Echeverria et al., 1995). The same composite UF of 1,000 that was used for the RfC derivation was applied to each of these PODs. The candidate RfDs from these studies span a range from 2.6×10^{-3} to 9.7×10^{-3} mg/kg-day. The RfD for tetrachloroethylene is **6×10^{-3} mg/kg-day**, the midpoint of this range rounded to one significant figure. This RfD is equivalent to a drinking water concentration of 0.21 mg/L, assuming a body weight of 70 kg and a daily water consumption of 2 L.

Table 5-8. Application of uncertainty factors for neurological endpoints from the studies used to derive candidate RfDs

Neurological endpoint	Oral human equivalent dose[a], mg/kg-day (NOAEL/LOAEL)	Uncertainty factors (UFs)						Candidate RfD mg/kg-day	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Cognitive domain									
Visual reproduction, pattern memory, pattern recognition—adult, occupational	9.7 (LOAEL)	1,000	1	10	1	10	10	0.0097	Echeverria et al. (1995)
Reaction time domain									
Reaction time in pattern memory, adult, occupational	9.7 (LOAEL)	1,000	1	10	1	10	10	0.0097	Echeverria et al. (1995)
Visual function domain									
Color confusion—adults, occupational	2.6 (LOAEL)	1,000	1	10	1	10	10	0.0026	Cavalleri et al. (1994)

[a]Equivalent oral exposure from application of the PBPK model on the basis of equivalent AUC of blood tetrachloroethylene for humans.

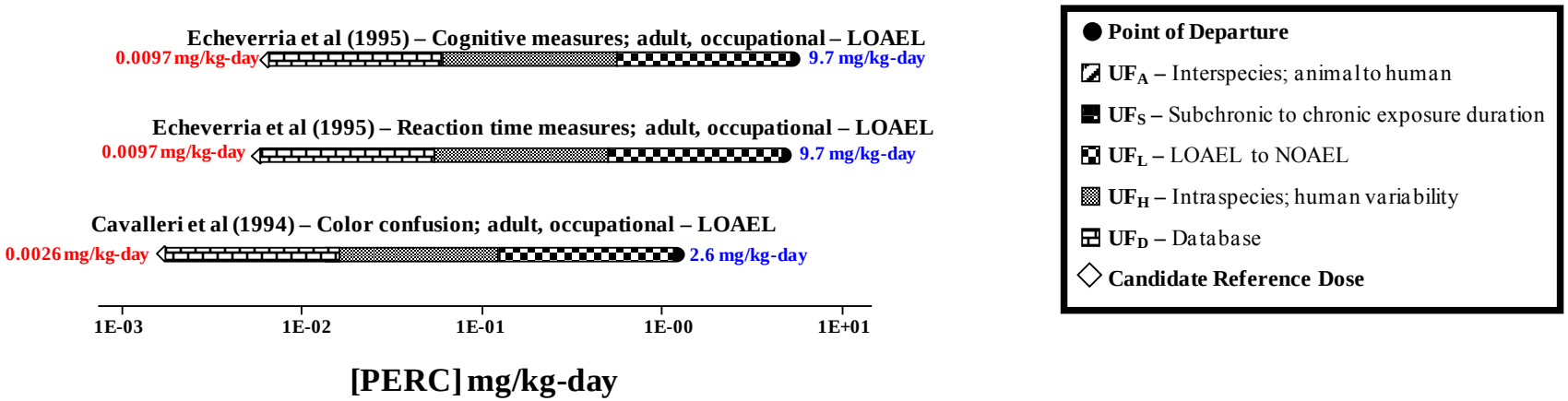

Figure 5-4. Candidate reference dose values for exposure to tetrachloroethylene.

5.2.4. Dose-response Analyses for Noncancer Effects Other Than Critical Effect of Neurotoxicity

This section presents oral dose-response analyses for noncancer effects other than the critical effect of neurotoxicity. The purpose of these analyses is twofold: (1) to provide a quantitative characterization of the relative sensitivity of different organs/systems to tetrachloroethylene, and (2), to provide information that may be useful for cumulative risk assessment in which multiple chemicals have a common target organ/system other than the central nervous system. Therefore, for each organ/system, "sample reference doses" (sRfDs) are calculated based on the same methodology as is used for the critical effect of neurotoxicity. These sRfDs are based on an evaluation of studies identified in Section 4.10 as suitable for dose-response analysis.

The method of analysis is the same as that described above for neurotoxicity, using the NOAEL/LOAEL approach. Benchmark dose modeling was not performed because these sample RfDs are meant for comparison purposes only (across organs/tissues or across chemicals). HEDs are derived using either (1) mg/kg-day dose adjusted for equivalent continuous exposure; or (2) the PBPK model with an appropriate dose metric. For liver effects, the dose metric of liver oxidative metabolism was used, based on the view that oxidative metabolites are involved in tetrachloroethylene-induced liver effects. For kidney effects, while it is generally thought that GSH conjugation metabolites are involved, the large uncertainty in estimates of human GSH conjugation preclude use of that dose metric. Instead, the AUC of tetrachloroethylene in blood is used as a surrogate. For the other non-cancer effects, the AUC of tetrachloroethylene in blood was used as the preferred dose metric due to the lack of data on what the active toxic moeity(ies) may be for those effects. In addition, the PBPK model is being used to perform route-to-route extrapolation from inhalation to oral exposure, so both inhalation and oral studies are considered together here. For each endpoint where PBPK modeling is used, the dose metric used to derive the HED is the same as that used to derive the HEC. The HED is then treated as a POD to which the following UFs may be applied:

- An UF of 10 was applied for human variation to all PODs. The rationale is the same as described above for neurotoxicity. Furthermore, there is some indication that human variability (at least for one endpoint) may be substantially more than that implied by the default UF. Kidney toxicity is thought to be associated with metabolism of tetrachloroethylene along the glutathione (GSH) conjugation pathway. As described in Section 3.5, PBPK model predictions for GSH conjugation span a wide range that may be due to uncertainty, variability, or both. Glutathione S-transferases (GSTs) are known to be polymorphic in the human population, with some isoforms exhibiting a substantial population of null phenotypes.

- An UF of 3 was applied to the PODs from all rodent studies to account for interspecies variability in extrapolation from laboratory animals to humans. The PODs from studies of rats and mice are expressed as HEDs calculated using the PBPK model. Therefore, an UF of 3 was applied for animal-to-human uncertainty to the PODs from these rodent studies to account for potential pharmacodynamic differences. This factor was not applied to PODs from human studies.

- An UF of 10 was applied to PODs of studies of subchronic or shorter duration to address the potential for additional or more severe toxicity from chronic or lifetime exposure.

- An UF of 10 is applied when a LOAEL is used due to a lack of a NOAEL. This factor was applied to the PODs of studies that identified a LOAEL but not a NOAEL.

- A database UF of 10 is applied to all PODs to address the lack of data to adequately characterize the hazard and dose response. The rationale is the same as described above for neurotoxicity.

5.2.4.1. Sample Reference Doses (RfDs) for Kidney Toxicity

As discussed in Section 4, numerous studies have reported adverse effects in the kidney from tetrachloroethylene. Five studies reporting kidney toxicity were identified in Section 4.10 as suitable for dose-response analysis. The only human study was Mutti et al. (1992), which reported statistically significant increases in RBP, β2μ-globulin, and albumin in urine among chronically exposed dry cleaners as compared to matched controls. In addition, for seven different urinary markers, the prevalence of individuals with abnormal values (>95th percentile of controls) was four- to fivefold greater in the exposed group. This study was considered adequate to derive an sRfD. Of the rodent studies reporting nephrotoxicity, only JISA (1993) identified a chronic NOAEL, with the other three rodent studies reporting subchronic (Jonker et al., 1996) or chronic LOAELs (NTP, 1986; NCI, 1977).

Therefore, among the rodent studies, only JISA (1993), which reported effects in both mice and rats, was carried forward to calculate sRfDs. Because all the studies are inhalation studies, route-to-route extrapolation was performed using the PBPK model with the AUC of tetrachloroethylene in venous blood dose metric. A summary of the extrapolated PODs and UFs applied is in Table 5-9. The resulting sRfDs range from 0.007−0.03 mg/kg-day, based on nuclear enlargement in the proximal tubules of chronically exposed mice and rats (JISA, 1993), with a slightly lower sRfD of 0.005 mg/kg-day based on urinary markers of nephrotoxicity in occupationally exposed humans (Mutti et al., 1992).

5.2.4.2. Sample Reference Doses (RfDs) for Liver Toxicity

As discussed in Section 4, numerous studies have reported adverse effects in the liver from tetrachloroethylene. Six studies reporting liver toxicity, none in humans, were identified in Section 4.10 as suitable for dose-response analysis. Only JISA (1993) reported a chronic NOAEL, and so was carried forward for derivation of an sRfD. However, it is unclear whether the reported effect of angiectasis, or enlargement of the blood vessels, is related to the other liver effects of tetrachloroethylene, which generally involve hepatocytes. Therefore, two other studies were included at this stage, one of which reported a chronic LOAEL for liver degeneration and necrosis (NTP, 1986), and the other of which reported a NOAEL for liver-weight increases after 6-week exposures (Buben and O'Flaherty, 1985). The remaining studies either only reported a LOAEL (Jonker et al., 1996; Kjellstrand et al., 1984), or reported a NOAEL for a very short duration [14 days, Berman et al. (1995)], and were, therefore, not considered further.

Therefore, JISA (1993), NTP (1986), and (Buben and O'Flaherty, 1985) were used to calculate sRfDs. In addition, PBPK modeling was applied using the liver oxidative metabolism dose metric to derive the HEDs. A summary of the PODs and UFs applied is in Table 5-10. The resulting sRfDs range from 0.01 mg/kg-day based on increased liver/body-weight ratios after 6-week exposures (Buben and O'Flaherty, 1985) to 0.08 mg/kg-day based on liver effects after chronic exposures (JISA, 1993; NTP, 1986). It should also be noted that in the chronic studies, increased liver tumors were observed at the lowest doses tested. Therefore, under chronic exposure conditions in this organ, liver cancers are likely to be more important than noncancer effects in the liver.

Table 5-9. Sample RfDs for kidney effects

Kidney endpoint (species)	HED[a] in mg/kg-day (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfD (mg/kg-day)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Urinary markers of nephrotoxicity (human)	5.4 (LOAEL)	1,000	1	10	1	10	10	0.005	Mutti et al. (1992)
Nuclear enlargement in proximal tubules (rat)	9.5 (NOAEL)	300	3	10	1	10	1	0.03	JISA (1993)
Nuclear enlargement in proximal tubules (mouse)	2.2 (NOAEL)	300	3	10	1	10	1	0.007	JISA (1993)

[a]Calculated with PBPK model using the dose metric of AUC of tetrachloroethylene in venous blood.

Table 5-10. Sample RfDs for liver effects

Liver endpoint (species)	HED[a] in mg/kg-day (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfD (mg/kg-day)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Increased angiectasis (mouse)	24.5 (NOAEL)	300	3	10	1	10	1	0.08	JISA (1993)
Increased liver degeneration/necrosis (mouse)	252 (LOAEL)	3,000	3	10	1	10	10	0.08	NTP (1986)
Increased liver/body-weight ratio (mouse)	32 (NOAEL)	3,000	3	10	10	10	1	0.01	Buben & O'Flaherty (1985)

[a]Calculated with PBPK model using the dose metric of liver oxidative metabolism.

Table 5-11. Sample RfDs for immunological and hematological effects

Immunotoxicity/ hematotoxicity endpoint (species)	HED[a] in mg/kg-day (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfD (mg/kg-day)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Reduced RBC, hemoglobin; increased WBC, lymphocytes, IgE (human)	6.8 (LOAEL)	1,000	1	10	1	10	10	0.007	Emara et al. (2010)

[a]Calculated with PBPK model using the dose metric of AUC of tetrachloroethylene in venous blood.

RBC = red blood cells; WBC = white blood cells.

Table 5-12. Sample RfDs for reproductive and developmental effects

Reproductive/developmental endpoint (species)	HED[a] in mg/kg-day (LOAEL/ NOAEL)	Uncertainty factors (UFs)						Sample RfD (mg/kg-day)	Reference
		Composite UF	UF_A	UF_H	UF_S	UF_D	UF_L		
Reduced sperm quality (mouse)	22 (NOAEL)	1000	3	10	3	10	1	0.02	Beliles et al. (1980)

[a]Calculated with PBPK model using the dose metric of AUC of tetrachloroethylene in venous blood.

5.2.4.3. Sample Reference Doses (RfDs) for Immunotoxicity and Hematologic Toxicity

As discussed in Section 4, a number of studies have reported changes in hematologic or immunologic parameters with tetrachloroethylene exposure. Two studies reporting hematologic effects were identified in Section 4.10 as suitable for dose-response analysis. The human study (Emara et al., 2010) reported changes in various standard hematological measures in subjects with mean blood levels of 1.685 mg/L. Application of the PBPK model provides an estimated HED of 6.8 mg/kg-day. This was treated as a chronic LOAEL, given the 7-year mean exposure duration (≥10% of lifespan) and is carried forward to calculate an sRfD. The other study (Marth, 1987) reported reversible hemolytic anemia in mice after 7 weeks drinking water exposure to 2-week-old mice for 7 weeks. Although Marth (1987) was not considered further, as summarized in Section 5.1.4.3, it should be noted that the LOAEL identified was low—0.05 mg/kg-day—and may be a cause for additional concern about hematologic effects.

Therefore, Emara et al. (2010) was used to calculate an sRfD. A summary of the POD and UFs applied is in Table 5-11. The result is an sRfD of 0.007 mg/kg-day.

5.2.4.4. Sample Reference Doses (RfDs) for Reproductive and Developmental Toxicity

As discussed in Section 4, a number of studies have reported reproductive and developmental effects from tetrachloroethylene exposure. Four studies, none in humans, reporting reproductive or developmental effects, were identified in Section 4.10 as suitable for dose-response analysis. All of these studies reported NOAELs. The developmental studies were all of appropriate duration for detecting those effects. The reproductive study (Beliles et al., 1980) was short term (5 days exposure) but was the only suitable study for reproductive toxicity. The PBPK model does not include gestational, fetal, or neonate compartments, so none of the inhalation studies could be converted to oral equivalents. However, the reproductive study was performed in mature male mice, for which the PBPK model could be used.

Therefore, only Beliles et al. (1980) was used to calculate an sRfD. A summary of the POD and UFs applied is in Table 5-12. The UF for subchronic-to-chronic extrapolation was not used because the study period sufficiently covered the window of sperm production. The resulting sRfD is 0.07 mg/kg-day for reduced sperm quality (Beliles et al., 1980).

5.2.4.5. Summary of Sample Reference Doses (RfDs) for Noncancer Endpoints Other Than the Critical Effect

The lowest sRfDs for these noncancer endpoints are similar to the values calculated based on the critical effect of neurotoxicity (refer to Figure 5-5): 0.005 mg/kg-day from Mutti et al. (1992), and 0.007 mg/kg-day from both JISA (1993) and Emara et al. (2010). All of the

other sRfDs are within about 10-fold of the RfD. This suggests that multiple effects may occur at about the same exposures at which tetrachloroethylene begins to induce neurotoxicity. These results also suggest that it is important to take into account effects from tetrachloroethylene other than neurotoxicity when assessing the cumulative effects of multiple exposures.

5.2.5. Previous Oral Assessment

The previous RfD of 1×10^{-2} mg/kg-day was posted on the IRIS database on March 1, 1988. It was based on a NOAEL of 14 mg/kg-day (Buben and O'Flaherty 1985), and a composite UF of 1,000 (10 for extrapolation from rats to humans, 10 for human variation, and 10 for extrapolating to chronic exposure conditions).

5.2.6. Uncertainties in Oral Reference Dose

As presented above, the uncertainty factor approach was applied to oral equivalent PODs extrapolated from inhalation LOAELs from two epidemiologic studies of neurological effects. These studies were considered to be methodologically sound based on study quality attributes, including study population selection, exposure measurement methods, and endpoint measurement methods. Other strengths are that they are human studies of chronic duration, obviating the need for extrapolation across species and exposure duration. Because of the adequacy of the PBPK model (Chiu and Ginsberg, 2011) for extrapolating from inhalation to oral exposures, there is little uncertainty in the use of inhalation studies for deriving the RfD. For instance, the sensitivity to the choice of dose metric for route-to-route extrapolation is low, with alternative dose metrics giving route-to-route conversions within 1.4-fold of the conversion selected based on tetrachloroethylene in blood. Other uncertainties in deriving the RfD are the same as those for the RfC, discussed above in Section 5.1.6.

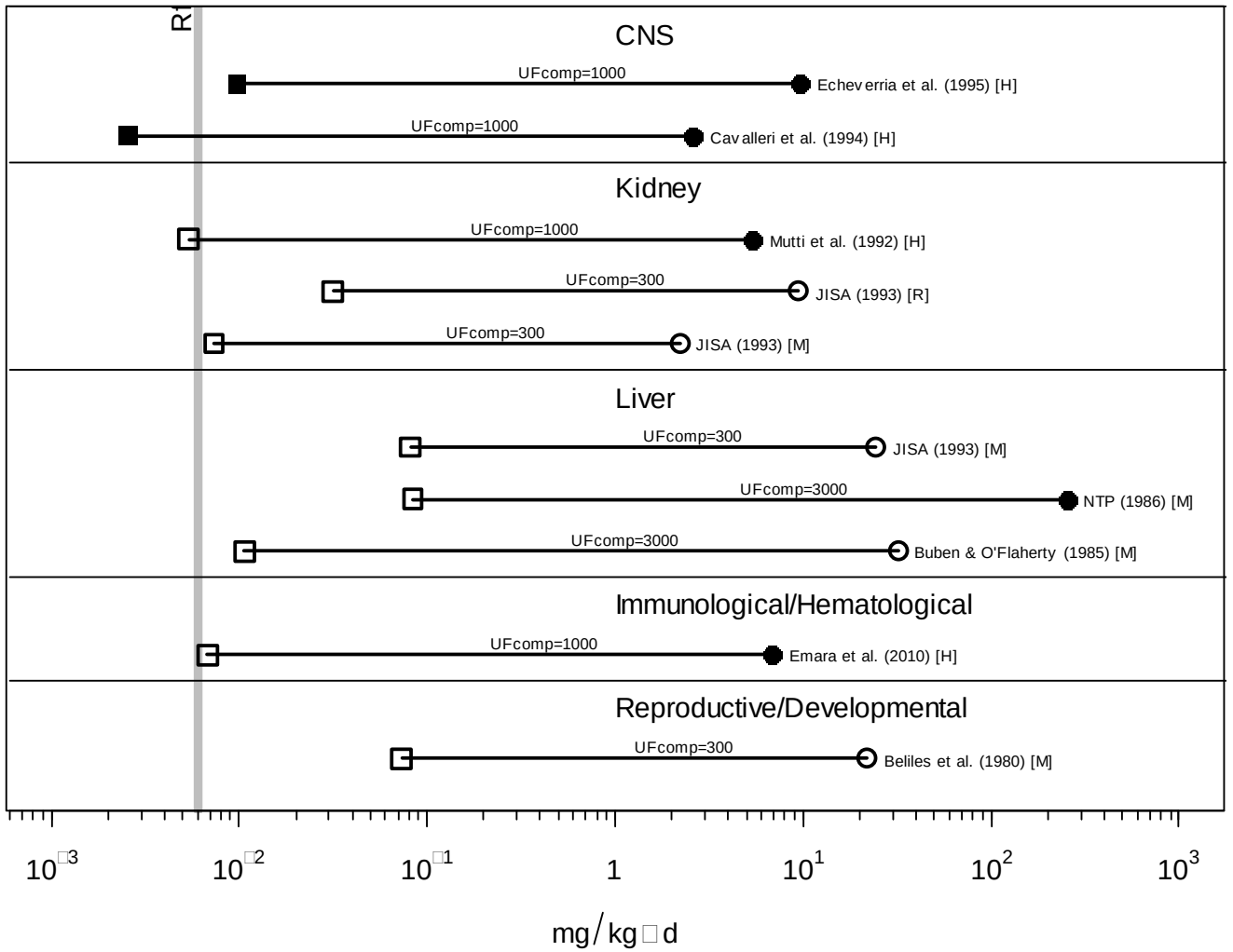

Figure 5-5. Comparison of candidate RfDs (filled squares) supporting the RfD (vertical line) and sample RfDs (open squares) for effects other the critical effect (CNS toxicity).

Filled circles = study/endpoint LOAEL in terms of human equivalent dose. Open circles = study/endpoint NOAEL in terms of human equivalent dose. Species in each study is shown in brackets after the reference (mouse: M; rat: R; human: H).

5.3. CANCER DOSE-RESPONSE ASSESSMENT

The following dose-response assessment was developed following the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). As discussed in Section 4.10.2, tetrachloroethylene is characterized as "likely to be carcinogenic in humans by all routes of exposure," based on suggestive epidemiologic evidence and conclusive evidence in mice and rats. No available human studies of cancer were found to be suitable for dose-response assessment. Therefore, the following dose-response assessment is based on data from rodent bioassays. Because the MOAs for tetrachloroethylene carcinogenicity are not known, the tumors reported in rodent bioassays are considered relevant to humans, and a low-dose linear extrapolation is used to estimate human cancer risk from rodent dose-response data.

5.3.1. Choice of Study/Data with Rationale and Justification

As discussed in Section 4, the several chronic exposure studies in rats and mice include an oral gavage study in mice and female rats by the National Cancer Institute (NCI, 1977) and two inhalation studies in mice and rats (JISA, 1993; NTP, 1986). These studies established that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, results in increased incidence of tumors. Mouse liver tumors (hepatocellular adenomas and carcinomas) and rat mononuclear cell leukemia (MCL) were reported in both sexes in two lifetime inhalation bioassays employing different rodent strains, and mouse liver tumors were also reported in both sexes in an oral bioassay (NCI, 1977). Tumors reported in a single inhalation bioassay include kidney and testicular interstitial cell tumors in male F344 rats (NTP, 1986), brain gliomas in male and female F344 rats (NTP, 1986), and hemangiomas or hemangiosarcomas in male Crj:BDF1 mice (JISA, 1993).

This analysis considers all three bioassays but focuses primarily on the JISA (1993) study results. The NCI (1977) oral gavage study in Osborne-Mendel rats was considered to be inconclusive because of the high incidence of respiratory disease, and high mortality with tetrachloroethylene exposure. Lesions indicative of pneumonia were observed in almost all rats at necropsy. A high incidence of toxic nephropathy was evident in tetrachloroethylene-exposed male and female rats. Early mortality was also observed in tetrachloroethylene-exposed animals; 50% of the high dose males and females had died by Weeks 44 and 66, respectively. Regarding the NCI (1977) gavage study in mice, several issues contribute to judging the results to be less useful for quantitative risk assessment than the inhalation studies. First, dosing lasted 78 weeks rather than 104 weeks as in the inhalation studies. Thus, 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, the dosing schedule was variable, and

doses 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, while time-weighted averages and PBPK modeling provide means for estimating the effective level of exposure, the actual correspondence of exposure with the observed effects is less clear. Further, mortality was significantly increased in both treated groups over that of controls, suggesting that the maximum tolerated dose had been exceeded. Therefore, dose-response modeling of the NCI (1977) rat and mouse bioassay data was not conducted.

The JISA (1993) bioassay was used for dose-response modeling of rodent cancer endpoints also observed with higher exposures in the earlier NTP (1986) bioassay. The lower exposure of both mice and rats in the JISA bioassay and the use of three—rather than two—exposure groups provides a stronger basis 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 and mid-dose exposure concentrations in the JISA (1993) study were 10- and twofold lower, respectively, than the lower exposure concentration (100 ppm) in the NTP (1986) inhalation study. For rats, the low-exposure concentration in the JISA (1993) study was fourfold lower than in the NTP study (200 ppm). The JISA (1993) bioassay was also used for dose-response modeling of the increased hemangiomas and hemangiosarcomas primarily in spleen, liver, skin, and adipose tissue of male mice because it was the only bioassay that reported this tumor type. Therefore, for most endpoints, including liver tumors, MCL, and hemangiosarcomas, the JISA (1993) study was used for dose-response modeling. The NTP (1986) study was utilized for modeling the increased incidence in renal cancers, brain cancers, and testicular tumors with treatment reported only in this bioassay. The sections below summarize the rodent tumor findings and additional considerations for data set selection.

5.3.2. Dose-Response Data

5.3.2.1. Liver Tumors in Mice

All three bioassays showed increases in hepatocellular tumors in male and female mice. Table 5-13 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 liver tumor incidences in the control group were fairly typical for this laboratory (refer to Table 5-14). 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.[31]

The liver tumor results of the two inhalation studies are reasonably concordant for both male and female mice when adjusted for background tumor incidence (refer to Figure 5-6). The incidence among male mice in the JISA (1993) study did not follow a clearly monotonic pattern, with a higher response in the lowest dose group than that in the next higher dose group. However, when considering the degree of expected variability given the number of animals in each dose group, this pattern appeared consistent with the overall supralinear dose-response patterns for the male and female mice in both the NTP (1986) and JISA (1993) studies.

The NCI (1977) study, in addition to the dosing and duration limitations noted above, only reported hepatocellular carcinomas but not adenomas. This was consistent with other NCI study reports of that time. Because, as stated above, hepatic adenomas and carcinomas are considered part of the same continuum of tumor development, the other two bioassays provide a more complete evaluation of hepatocarcinogenesis associated with tetrachloroethylene exposure.

[31] Combined historical incidence of adenomas or carcinomas was not available. Presumably the incidence of carcinomas slightly underestimates the overall incidence of adenomas or carcinomas.

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Table 5-13. Tumor incidence in mice exposed to tetrachloroethylene

Bioassay	Doses/exposures		Sex	Body weight[a] (kg)	Survival-adjusted tumor incidence[b] (%)	
	Administered	Continuous equivalent				
Hepatocellular adenomas or carcinomas						
NCI (1977)[c] $B6C3F_1$ mice Gavage: 5 d/wk, 78 wk	Vehicle control 450 mg/kg-day 900	0[e] mg/kg-day 332 663	Male	0.030	2/20 32/48 27/45	(10) (67) (60)
	Vehicle control 300 mg/kg-day[d] 600	0[e] mg/kg-day 239 478	Female	0.025	0/20 19/48 19/45	(0) (40) (42)
NTP (1986) $B6C3F_1$ mice Inhalation: 6 hr/d, 5 d/wk, 104 wk	0 ppm 100 200	0 ppm 18 36	Male	0.037	17/49 31/47 41/50	(35) (70) (82)
	0 ppm 100 200	0 ppm 18 36	Female	0.032	4/45 17/42 38/48	(9) (40) (79)
JISA (1993) **Crj:BDF1 mice** **inhalation:** **6 hr/d,** **5 d/wk,** **104 wk**	0 ppm 10 50 250	0 ppm 1.8 9.0 45	Male	0.048	13/46 21/49 19/48 40/49	(28) (43) (40) (82)
	0 ppm 10 50 250	0 ppm 1.8 9.0 45	Female	0.035	3/50 3/47 7/48 33/49	(6) (6) (15) (67)
Hemangiosarcomas[e], liver or spleen						
JISA (1993)	0 ppm 10 50 250	0 ppm 1.8 9.0 45	Male	0.048	4/46 2/49 7/48 11/49	(4) (2) (13) (18)

Note: Data sets carried through dose-response modeling shown in **bold**.

[a]Average body weight reached during adulthood.

[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 not to have adequate time on study to develop tumors.

[c]No adenomas were reported in this study.

[d]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 Week 90 study termination. Lifetime average daily (administered) doses (LADDs) were calculated as follows:

$$\text{LADD (mg/kg-day)} = \text{Cumulative administered dose (mg/kg)}/\text{(total days on study)}$$
$$= \{[(\text{initial dose rate} \times 11 \text{ weeks}) + (\text{later dose rate} \times 67 \text{ weeks})]/90 \text{ weeks}\} \times 5/7 \text{ (days)}$$

[e]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 Tables 11 and 12 of the JISA report summary. The incidences reported here represent a tabulation of hemangioendotheliomas in liver or spleen from the individual animal data provided in the JISA report.

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Table 5-14. 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.

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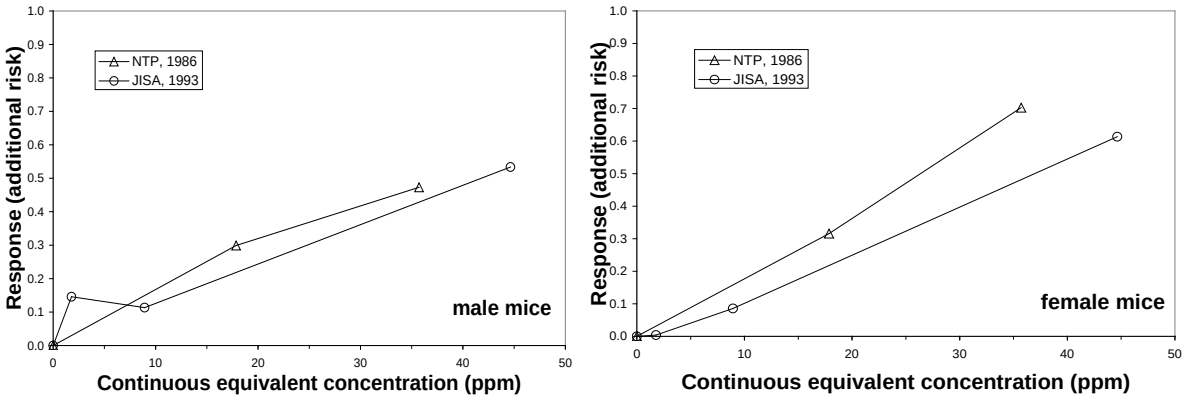

Figure 5-6. Mouse liver tumor responses (hepatocellular adenomas or carcinomas), as additional risk, for two chronic inhalation bioassays (refer to Table 5-13), plotted against continuous equivalent concentration (ppm), for male and female mice.

5.3.2.2. Other Tumor Sites in Male Mice

In addition to elevations in hepatocellular adenomas and carcinomas, the JISA (1993) study demonstrated increases in hemangiomas and hemangiosarcomas. These tumors were observed primarily in spleen and liver, with several instances also reported in subcutaneous skin and in adipose tissue, in mid- and high-dose male mice (Cochran-Armitage trend test [two-sided] = 0.008). The incidences of spleen hemangiosarcomas in control and low-dose male mice—2/46 and 2/49, respectively, each about 4%—were similar to the JBRC historical control incidence for spleen only (3.2%, range 0−8%; refer to Table 5-14). The increase in this tumor type with tetrachloroethylene exposure was not replicated in the NCI (1977) or NTP (1986) studies. In the NTP male mice, hemangiomas or hemangiosarcomas were only reported in liver; the incidences were as follows: controls, 3/49 (6%); low dose, 2/49 (4%); high dose, 2/50 (4%); within the range of NTP historical controls incidence for all sites, 2−8% (average 4.4%) (http://ntp.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/m_inhar.txt). The reasons the two bioassays differ with regard to identifying increases in hemangiomas and hemangiosarcomas have not been elucidated; differences may be due to the strain of mouse used or other factors. For this endpoint, therefore, the JISA (1993) study was selected for dose-response modeling.

5.3.2.3. Mononuclear Cell Leukemia in Rats

The NTP (1986) and JISA (1993) studies demonstrated increased MCL incidences for male and female F344/N or F344/DuCrj rats (refer to Table 5-15). Although the NCI study, in Osborne-Mendel rats, did not demonstrate any MCL increases, this study is considered inconclusive because of low survival and for other reasons noted above in Section 5.3.1.

Table 5-15. Incidence of mononuclear cell leukemia, kidney tumors, and brain gliomas in rats exposed to tetrachloroethylene by inhalation

Bioassay	Exposure concentration (ppm)		Sex	Body weight[a] (kg)	Survival-adjusted tumor incidence[b] (%)	
	Administered	Continuous equivalent				
Mononuclear cell leukemia						
NTP (1986) F344/N rats inhalation 6 hr/d, 5 d/wk, 104 wk	0 200 400	0 36 71	Male	0.44	28/50 37/48 37/50	(56) (77) (74)
	0 200 400	0 36 71	Female	0.32	18/50 30/50 29/50	(36) (58) (60)
JISA (1993) **F344/DuCrj rats** **inhalation** **6 hr/d,** **5 d/wk,** **104 wk**	0 50 200 600	0 8.9 36 110	Male	0.45	11/50 14/50 22/50 27/50	(22) (28) (44) (54)
	0 50 200 600	0 8.9 36 110	Female	0.3	10/50 17/50 16/50 19/50	(20) (34) (32) (38)
Kidney: tubular cell adenoma or adenocarcinoma						
NTP (1986)	0 200 400	0 36 71	Male	0.44	1/49 3/47 4/50	(2) (6) (8)
Brain gliomas						
NTP (1986)	0 200 400	0 36 71	Male	0.44	1/50 0/48 4/50	(2) (0) (8)
Testicular interstitial cell tumors						
NTP (1986)	0 200 400	0 36 71	Male	0.44	35/50 39/47 41/50	(70) (83) (82)

Note: Data sets carried through dose-response analysis shown in **bold**.
[a]Average body weight reached during adulthood.
[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).

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Table 5-16. 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	147/1,149 (12.8)	6.0–22.0	76/549 (13.8)	6.0–22.0
Kidney Renal cell adenoma Renal cell carcinoma	 2/1,149 (0.2) 2/1,149 (0.2)	 0–2.0 0–2.0	 1/549 (0.2) 2/549 (0.4)	 0–2.0 0–2.0
Female rats				
Mononuclear cell leukemia	147/1,048 (14.0)	2.0–26.0	68/448 (15.2)	8.0–20.0
Kidney Renal cell adenoma Renal cell carcinoma	 1/1,048 (0.1) 0/1,048 (0.0)	 0–2.0 NA	 1/448 (0.2) 0/448 (0.0)	 0–2.0 NA

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.

The responses in the NTP (1986) study were approximately twofold higher than for the corresponding groups in the JISA (1993) study in all groups, including controls. Control groups for both laboratories were consistent with their respective historical controls (refer to Table 5-16 for the JISA historical controls). Like the hepatocellular tumor results in mice (refer to Section 5.3.2.1), the MCL results from the NTP and JISA studies were plotted in terms of additional risk versus administered concentration to evaluate relative increases in tumor incidence (refer to Figure 5-7). The NTP and JISA studies are consistent for male rats at the administered concentration of 200 ppm (36 ppm continuous equivalent) in terms of the relative increases in tumors over background incidences. For female rats, the dose-response patterns are less similar. A higher overall response is observed in the NTP study. However, the JISA female rats have a steeper increase at the lowest exposure level (50 ppm administered concentration, 9 ppm continuous equivalent) than would be expected based on the NTP study, which did not include that exposure level. Both studies suggest some degree of saturation of effects in the range of exposures considered (refer to Figure 5-7).

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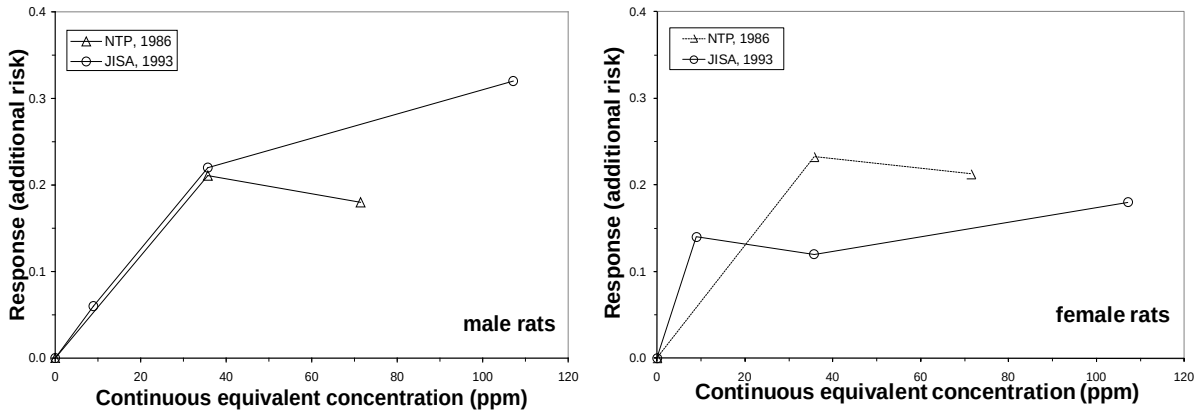

Figure 5-7. Rat mononuclear cell leukemia responses (minus control) in two chronic bioassays (refer to Table 5-15), plotted against continuous equivalent exposure (ppm) for (a) male and (b) female rats.

Overall, the NTP and JISA studies show concordant MCL responses for both male and female F344 rats. F344 rats were used in both studies, so residual differences could be attributable to the specific lines of animals used at each laboratory and to laboratory-specific procedures. As discussed in Section 5.3.1, the JISA study rather than the NTP study was selected for dose-response modeling because it provides data on tumor incidences at lower exposure, and the use of three exposures provides a stronger basis for dose-response analyses.

5.3.2.4. Other Tumor Sites in Rats

Additional tumor findings in rats included a significant increase in the NTP bioassay of two rare tumor types, kidney tumors in males, and brain gliomas in both sexes of exposed F344/N rats. The NTP (1986) bioassay also reported increases in the rate of testicular interstitial cell tumors, a tumor type of high incidence in unexposed male F344 rats. Table 5-15 summarizes the incidence data for these tumor sites.

The potential significance of the NTP brain tumor findings is supported by their relative rarity (evidenced by a low historical control incidence) and earlier occurrence with increasing tetrachloroethylene exposure, indicating an effect of exposure on latency. In males, tetrachloroethylene-induced brain tumors were observed beginning at Week 88 compared with Week 99 in controls. Female brain tumors were first observed at 75 weeks in tetrachloroethylene-exposed animals compared with 104 weeks in control group females. Additionally, the nervous system is known to be a target of tetrachloroethylene exposure in humans and animals (refer to Sections 4.1 and 5.1.1). Therefore, although the overall incidences

are low relative to other tumor sites, and the finding was not replicated in the JISA study, the rarity of rat brain tumors in control animals and the additional data suggesting biological plausibility support dose-response modeling of this tumor type.

The evidence for kidney tubule cell adenomas and adenocarcinomas differed slightly between the two bioassays (refer to Table 5-15). The JISA study showed no apparent trend among incidences compared with either concurrent or historical controls (refer to Table 5-16). In contrast, the elevation in exposed male rats in the NTP study, while not statistically significant when compared with concurrent controls, was significant when compared using a trend test with the historical control rate for the same facility ($p = 0.0002$, Cochran-Armitage, two-sided trend test). The investigators noted the relative rarity of these tumors, with incidences of 1/549 among historical controls for the study facility, and of about 0.2% in 1968 untreated controls in the NTP program overall. Further support for the significance of the kidney tumors comes from evidence that the related chemical trichloroethylene induces this tumor type in humans and in male rats (U.S. EPA, 2011b). Additional biological plausibility for this endpoint includes toxicokinetic data that nephrotoxic and mutagenic metabolites are formed in the kidney following tetrachloroethylene exposure. Therefore, although the overall incidences are low relative to other tumor sites, the rarity of rat kidney tumors in control animals and the additional data suggesting biological plausibility support dose-response modeling of this tumor type. The NTP (1986) study was better suited for modeling because it had a stronger trend, and was, therefore, selected for dose-response modeling.

The NTP (1986) study also reported an increase in the rate of testicular interstitial cell tumors, a tumor type of high incidence in unexposed F344 rats. The reported incidences of testicular interstitial cell tumors in male rates exposed to 0, 200, or 400 ppm tetrachloroethylene were 36/50, 39/49, and 41/50, respectively. A higher incidence (47/50, or 92%) was observed in control rats in the JISA (1993) study than in the NTP (1986) study. In the JISA study, exposure to 0, 50, 200, or 600 ppm tetrachloroethylene resulted in incidences of 47/50, 46/50, 45/50, and 48/50, respectively. Support for the significance of the testicular interstitial cell tumors comes from evidence that the related chemical trichloroethylene induces this tumor type in rats. Trichloroethylene did not induce increases in testicular interstitial cell tumors in the F344 rat in a bioassay with a reported incidence of 47/48 (98%) in the vehicle control. However, increases were observed in male Marshall rats, in which the incidences were 16/46, 17/46, 21/33, and 32/39 in untreated, vehicle control, 500, or 1,000 mg/kg-day trichloroethylene, respectively. Therefore, although the overall increases in incidence are low relative to other tumor sites, the additional data suggesting biological plausibility support dose-response modeling of this tumor type.

5.3.3. Dose Adjustments and Extrapolation Methods

This section provides details of the dose-response modeling carried out for developing cancer risk values. The steps include estimation of dose metrics using relevant PBPK modeling [refer to Section 3; Chiu and Ginsberg (2011)], suitable adjustment to continuous daily exposures from intermittent bioassay exposures, dose-response modeling in the range of observation, interspecies extrapolation, extrapolation to low exposures, and route-to-extrapolation. An overview of these steps is provided in Figure 5-8. The schematic also addresses route-to-route extrapolation using the Chiu and Ginsberg (2011) PBPK model because after the slope factor is expressed in terms of risk per unit of internal human dose, the PBPK model can be used to estimate the risk per unit of oral or inhalation exposure, regardless of the route of administration in the original study.

5.3.3.1. Estimation of Dose Metrics for Dose-Response Modeling

Several factors inform the criteria for selection of dose metrics in this assessment: the association of the metric with the toxic moiety relevant to the endpoint under consideration, the availability of data and models for estimating that metric, and whether the resulting estimate is sufficiently robust. When PBPK modeling is used, it is generally preferable to use a single model for estimating all the dose metrics for dose-response modeling.

5.3.3.1.1. Hepatocellular tumors

Several metabolites of tetrachloroethylene are 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. Oxidative metabolism is thought to predominate in the liver, and TCA is the major resultant urinary excretion product. As discussed in Section 3, TCA appears to be formed from spontaneous decomposition of trichloroacetyl chloride, which is known to bind to macromolecules. Dichloroacetic acid (DCA) may be formed from dechlorination of TCA, but DCA produced from this pathway is likely to be rapidly metabolized in the liver and not detected in blood or urine. DCA that has been detected in urine is thought to be the result of kidney-specific β-lyase metabolism of the results of GSH conjugation of tetrachloroethylene, and DCA produced from this pathway is presumed to not play a role in liver toxicity or cancer. The potential role of GST conjugates of tetrachloroethylene in liver carcinogenicity, although unknown, is presumed to be less important than the role of oxidative metabolites.

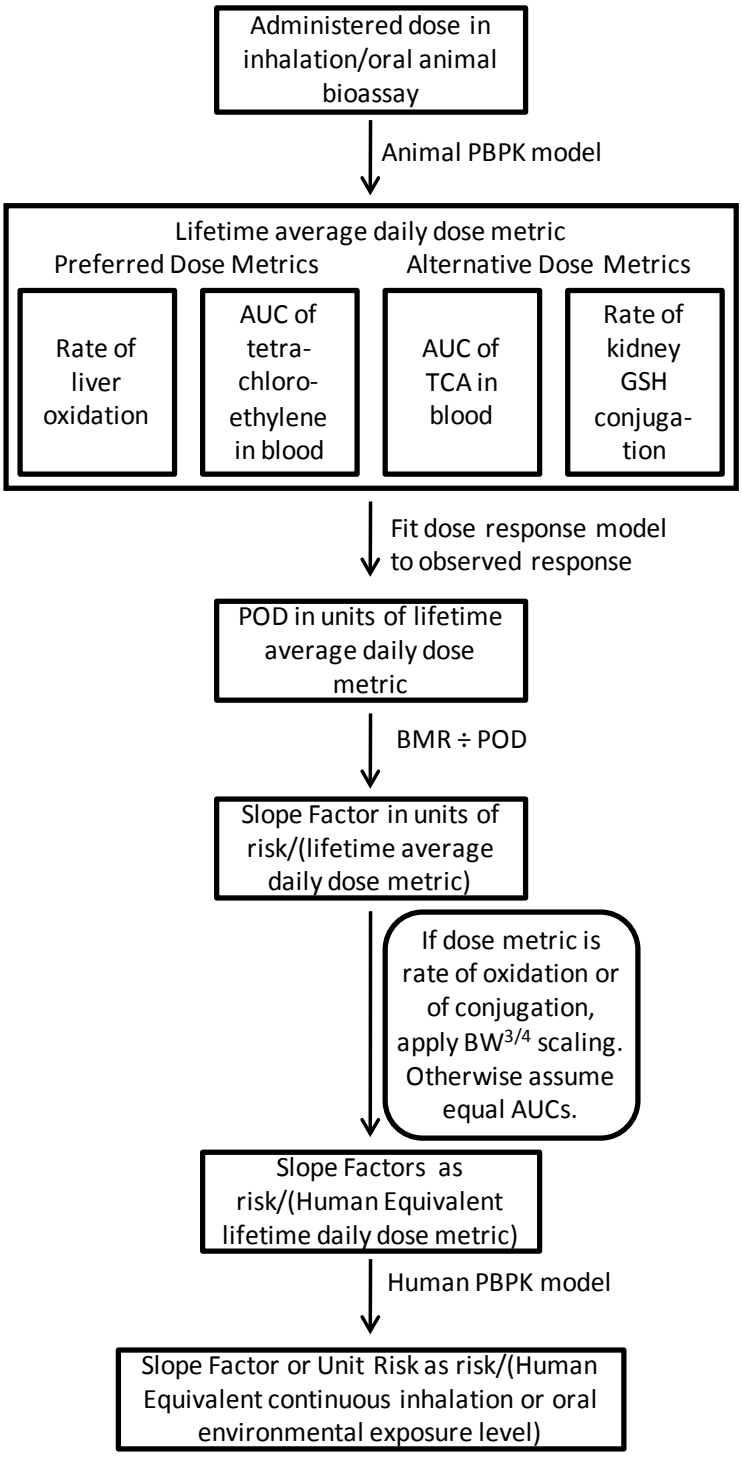

Figure 5-8. Sequence of steps for extrapolating from tetrachloroethylene bioassays in animals to human-equivalent exposures expected to be associated with comparable cancer risk (combined interspecies and route-to-route extrapolation).
Refer to Table 5-17 for units.

The focus of most hypotheses with respect to contributors to tetrachloroethylene hepatocarcinogenicity has been on TCA and, to a lesser extent, DCA. Data on the hepatocarcinogenicity of TCA and DCA in rodents, alone and in combination, are summarized in Tables 4-17, 4-18, and 4-19. TCA statistically significantly increased the incidence of liver tumors in male and female B6C3F$_1$ mice exposed via drinking water for 52−104 weeks (DeAngelo et al., 2008; Bull et al., 2002; Pereira, 1996; Bull et al., 1990; Herren-Freund et al., 1987). Incidence of tumors increased with increasing TCA concentrations (DeAngelo et al., 2008; Bull et al., 2002; Pereira, 1996; Bull et al., 1990). The development of tumors in animals exposed to TCA progressed rapidly, as evidenced by significant numbers of tumors in less-than-lifetime studies of 82 weeks or less. The tetrachloroethylene metabolite DCA also causes liver cancer in mice (DeAngelo et al., 1999; Daniel et al., 1992; Bull et al., 1990; Herren-Freund et al., 1987). Additionally, DCA and TCA are hepatocarcinogenic in mice when coadministered in the drinking water for 52 weeks (Bull et al., 2002). Treatment-related liver tumors were observed in male F344/N rats exposed via drinking water to DCA (DeAngelo et al., 1996) but not TCA (DeAngelo et al., 1997) for 60 or 104 weeks. However, the extent to which DCA is available to the liver following tetrachloroethylene exposure is unclear, because it is thought to be formed in the kidney following β-lyase processing of TCVC and may be largely excreted in urine without circulating systemically. The carcinogenicity of TCA and DCA has not been evaluated in female rats or in other species of experimental animals.

Data on tumor phenotype support the view that TCA may not be the sole tumorigenic metabolite of tetrachloroethylene but also do not provide definitive evidence testing any particular hypothesis. For instance, liver tumor genotypes (e.g., with regard to H-*ras* codon 61 mutation) and phenotypes (e.g., with regard to c-Jun staining) appear to differ among tumors induced by TCA, DCA, the combination of TCA and DCA, and the structurally related compound trichloroethylene (Bull et al., 2002). Bull et al. (2002) suggest that for trichloroethylene, the data are not consistent with the hypothesis that TCA is the sole active moiety, but a similar experiment has not been conducted for tetrachloroethylene. However, by analogy, it is possible that TCA and DCA, in combination with each other (and with other reactive intermediates produced during the oxidative metabolism of tetrachloroethylene) may contribute to the production of liver tumors. This appears to be the case for noncancer effects, as the spectrum of endpoints caused by tetrachloroethylene includes effects broader than that produced by TCA, and including fatty degeneration, focal necrosis, and regenerative repair, some of which may play a role in liver carcinogenesis (refer to Section 4.3.5).

The hepatocarcinogenic potencies of TCA and tetrachloroethylene have not been directly compared in a single rodent bioassay. Appendix C presents a comparative quantitative analysis of the carcinogenicity of TCA (including that predicted using PBPK modeling to be produced

from tetrachloroethylene) with the carcinogenicity of tetrachloroethylene. Statistically, this analysis did not reject the hypothesis of equivalent carcinogenic potencies of TCA and the internal dose of TCA resulting from tetrachloroethylene exposure. However, the analysis also concluded that a contribution of TCA of as little as 12% could be not ruled out. In addition, several factors, including the much higher control incidence of liver tumors and the relatively high body weights of the animals in the TCA bioassay, limit the direct comparability of the tetrachloroethylene and TCA bioassay data. Therefore, this analysis is only of limited utility in elucidating the contribution of TCA to tetrachloroethylene hepatocarcinogenic potency.

In consideration of these uncertainties, total rate of oxidative metabolism in the liver is the most relevant dose-metric for tetrachloroethylene-induced liver toxicity. AUC for TCA in the liver is also presented as a plausible alternative dose metric. The PBPK-derived estimates of liver total oxidative metabolism and TCA AUC corresponding to the JISA bioassay exposures for male and female mice are provided in Table 5-17.

Table 5-17. Summary of PBPK-derived dose metric estimates used for dose-response analysis of rodent tumor data

Study group	Administered concentration (ppm)	Liver total oxidative metabolism (mg/kg$^{3/4}$-day[a])		Tetrachloroethylene AUC in blood (mg-hr/L-d[b])		TCA AUC in liver (mg-hr/L-d[c])		Total GSH metabolism (mg/kg$^{3/4}$-day[d])	
		Males	Females	Males	Females	Males	Females	Males	Females
Mice, JISA (1993)	0 10 50 250	0 2.25 8.25 33.6	0 2.13 7.75 31.6	0 4.11 22.3 116	0 4.18 22.6 117	0 78.5 280 1120	0 77.0 272 1090	Not used	
Rats, JISA (1993)	0 50 200 600	Not used		0 20.0 80.9 247	0 20.1 81.4 248	Not used		Not used	
Rats, NTP (1986)	0 200 400	Not used		0 81.0 164	0 81.3 164	Not used		0 0.303 0.615	Not used

[a]Primary dose metric for mouse hepatocellular tumors.
[b]Primary dose metric for mouse hemangiomas or hemangiosarcomas, rat MCLs, rat kidney tumors, rat brain gliomas, and rat testicular tumors.
[c]Alternative dose metric for mouse hepatocellular tumors.
[d]Alternative dose metric for rat kidney tumors.

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5.3.3.1.2. Mononuclear Cell Leukemia

Mononuclear cell leukemia has been observed in rats following exposure to tetrachloroethylene. Regarding the metabolites that potentially contribute to MCL development, a role for GSH-derived intermediates was posited based on findings for the related compound trichloroethylene in bovine species. However *S*-(1,2,2,-trichlorovinyl)-*L*-cysteine (TCVC), a GSH-derived metabolite of tetrachloroethylene, induced no kidney or bone marrow effects when administered to two calves as a single dose (Lock et al., 1996). Aside from this evaluation of bone marrow toxicity of TCVC in the juvenile cow, a species of unknown sensitivity to tetrachloroethylene-induced leukemia, other studies aimed at elucidating the active metabolites contributing to leukemic effects have not been reported. In particular, no such studies are available in the F344 rat, the species and strain in which leukemic effects have been consistently observed in both sexes. It is thus concluded that the specific active moiety(ies) by which tetrachloroethylene induces this type of tumor are not known.

In summary, because considerable uncertainty surrounds the identification of the causative chemical species, AUC of the parent compound in the blood is considered a viable dose metric for MCL and has the advantage of being a more proximal dose than administered dose. The estimates of tetrachloroethylene AUC in blood corresponding to the JISA bioassay exposures for male and female rats are provided in Table 5-17.

5.3.3.1.3. Kidney tumors

Tetrachloroethylene causes tubular toxicity in mice and rats and is associated with small increases in the incidences of kidney tumors reported in multiple strains of rats (JISA, 1993; NTP, 1986). These effects, including kidney cancer, are thought to be associated with tetrachloroethylene metabolism by GSH conjugation, based on the production in the kidney of nephrotoxic and genotoxic metabolites from this pathway (Lash and Parker, 2001). As noted in Section 3, the PBPK model by Chiu and Ginsberg (2011) allows calculation of this dose metric. GSH conjugation occurs in the kidney as well as in the liver from where the metabolic products may be transported to the kidney. Therefore, the most appropriate dose metric for kidney toxicity would be the total rate of metabolism of tetrachloroethylene via the GSH conjugation pathway.

However, overall, the estimates of GSH conjugation in Chiu and Ginsberg (2011) were highly uncertain and/or variable, and to a very different extent across species (also refer to Section 3). Uncertainty in this estimate was the least, roughly twofold, in rats. In mice, the range of estimates based on the different optimization runs was about 10-fold. In the human, the range of predicted estimates spanned several orders of magnitude. In particular, two local maxima were observed for the posterior modes, each of which the fit to the data was good and

substantially similar. However, the model predictions corresponding to each estimate differed by 3,000-fold. It was not clear as to whether this 3,000-fold spread represented uncertainty or variability in the form of a bimodal distribution for human GSH conjugation or both (refer to Section 3 for a discussion of plausible reasons for a multimodal distribution).

In view of this large uncertainty/variability, and the inability to differentiate uncertainty from variability, it appears more prudent to use AUC of the parent compound in the blood as a preferred dose metric for kidney toxicity. This has the advantage of being a more proximal dose to the kidney than administered dose. Total rate of metabolism of tetrachloroethylene via the GSH conjugation pathway is also used as an alternative dose metric. PBPK-derived estimates of tetrachloroethylene AUC in blood and total GSH metabolism corresponding to the JISA (1993) male rat exposures are provided in Table 5-17.

5.3.3.1.4. Other dose metrics

No data are available concerning the metabolites that may contribute to the induction of other rodent tumor types, including hemangiosarcomas or hemangiomas in male mice, kidney tumors and testicular interstitial cell tumors in male rats, or brain gliomas in male and female rats. It is concluded that the specific active moiety(ies), mechanisms, or modes of action by which tetrachloroethylene induces these rodent tumor are not known. Accordingly, AUC of tetrachloroethylene in the blood was used for these tumors because it is more proximal to the target tissues than administered dose (refer to Table 5-17 for dose estimates used for dose-response modeling).

In addition, all tumor sites considered for modeling were also modeled using administered inhalation concentration, for comparison purposes. These concentrations (in ppm) were adjusted for continuous exposure by averaging the five 6-hour daily exposures over the full week, by multiplying by 6 hours/24 hours × 5 days/7 days (0.179) to yield equivalent continuous concentrations. Tables 5-13 and 5-15 provide these adjusted concentrations.

5.3.3.1.5. Uncertainties in PBPK modeling and dose metrics

A detailed discussion of uncertainties in the dosimetric estimates, derived using a PBPK model that considered all the available tetrachloroethylene PK data in the literature, was provided in Sections 3.5.1.2.2 and 3.5.1.2.3. A full Bayesian analysis of the uncertainty/variability was not performed. Nonetheless, the range of posterior modes provided for the various dose metrics in Section 3.5.1.2.2 gives an estimate of the range of uncertainty associated with each dose metric, which, in turn, results in a range of human unit risk estimates associated with each dose metric used for any given end point in Tables 5-18 and 5-20.

In particular, the predictions for GSH conjugation in humans were found to be highly uncertain. In the rat, the ranges of chain-specific posterior modes for GSH conjugation spanned

up to twofold, and in mice, up to 10-fold. However, in humans, the ranges spanned several orders of magnitude, reflecting the two "clusters" of posterior modes with estimates of GSH conjugation clearance differing by up to 3,000-fold. Tetrachloroethylene AUC was associated with a twofold pharmacokinetic uncertainty/variability. The range in estimates of tetrachloroethylene oxidation in humans was found to be largely dominated by a twofold interindividual variability.

In terms of the selection of dose metric, tetrachloroethylene is metabolized to several intermediates with carcinogenic potential. Although much data exist for TCA, they are inadequate to support the conclusion that TCA alone is able to explain the hepatocarcinogenicity associated with tetrachloroethylene exposure. Whether total oxidative metabolism, total GSH metabolism, or tetrachloroethylene AUC in blood—either as measures of a precursor or intermediate or as surrogates directly proportional to the toxic agent(s)—are adequate indicators of potential risk is unclear. A role for the parent compound has not been ruled out nor is it clear whether the specific active moiety(ies) are proportional to administered concentration.

5.3.3.2. Extrapolation Methods

5.3.3.2.1. 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 has been used by EPA in the majority of quantitative cancer assessments, initially because of its parallelism to the multistage carcinogenic process. A benefit of the multistage model is its flexibility in fitting a broad array of dose-response patterns, including allowing linearity at low dose. Occasionally, the multistage model does not fit the available data, in which case, alternate models should be considered. The related multistage-Weibull model has been the preferred model when individual data are available for time-to-tumor modeling, which incorporates more of the information about response than does the simpler dichotomous response model. Use of this decision scheme has contributed to greater consistency among cancer risk assessments.

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The multistage model is given by

$$P(d) = q_0 + (1 - q_0) \times [1\text{-}exp(-\sum_{i=1:n} q_i \times d^i)] \quad (5\text{-}1)$$

where:

d = exposure level (including internal dose metric) and

q_i = parameters estimated in fitting the model, $q_i \geq 0$; n is degree of the model

The multistage model in BMDS [Benchmark Dose Software, version 2.1.1 (U.S. EPA, 2009)] was used initially to fit all data sets. Using the method of maximum likelihood, all feasible orders of the multistage model up to the number of dose groups (n) less one were evaluated for fit. Model fits with goodness-of-fit p-values >0.05 are generally considered acceptable, with good visual fit and evaluation of standardized residuals for the control group and points near the benchmark dose (the dose corresponding to a predetermined increase above control levels, or BMD) also important. Among the model fits satisfying these criteria, the most parsimonious model fit was generally selected.

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.

$$P(d,t) = q_0 + (1 - q_0) \times [1\text{-}exp(-\sum_{I=1:n} q_i \times d^i) \times t^z] \quad (5\text{-}2)$$

where:

d = exposure level (or dose metric)

t = time to observation of the tumor

q_i, z = parameters estimated in fitting the model; $q_i \geq 0$, $z \geq 1$; n is degree of the model

The multistage-Weibull model is the same as the multistage model when z = 0. MSW (U.S. EPA, 2010) 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 concentration (BMCL) associated with a level of risk from the low end of the observed range, usually 10% extra risk. Extra risk has been used consistently throughout EPA risk assessments and is given by

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$$\text{Extra risk} = [P(d) - P(0)] / [1 - P(0)] \quad (5\text{-}3)$$

where:

$P(d)$ = estimated response at exposure d and

$P(0)$ = estimated response in the control group

The slope factor (risk per mg/kg-day for oral exposure, risk per dose metric unit for PBPK-modeled dose metrics) and risk per unit concentration (risk per mg/L for drinking water exposure, or per $\mu g/m^3$ for inhalation exposure) are estimated using linear extrapolation from the PODs because of the lack of information supporting another extrapolation approach (U.S. EPA, 2005a), by dividing the risk level by its associated BMCL:

$$\text{Risk/(unit of exposure)} = \text{Extra risk/BMCL.} \quad (5\text{-}4)$$

5.3.3.2.2. Uncertainties in low-dose extrapolation approach

The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. However, MOA data are lacking or limited for all candidate cancer endpoints for tetrachloroethylene (i.e., rat MCL, brain, testicular and kidney tumors, mouse hepatocellular tumors and hemangiosarcomas). When the MOA cannot be established, EPA uses a linear approach to estimate low-exposure risk as outlined in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The overall uncertainty in low-dose risk estimation could be reduced to some degree if the MOA for tetrachloroethylene were known. However, even in such a case, 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 in addition to the MOA, other factors, such as human response variability, may influence the dose-response function in humans.

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, including the multistage model used in this assessment, treats tumorigenesis as a multievent 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.

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 NRC report on

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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 NRC (2006) 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 was supported, it would need to be developed based on an examination of predicted variability within the 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 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 may eliminate population thresholds if present; and that the approach provides consistency across assessments, facilitating direct comparison of the derived risk values.

5.3.3.2.3. 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). The discussions below include a consideration of uncertainties inherent in each of these approaches.

5.3.3.2.3.1. Internal dose metrics

Because of the availability of PBPK modeling to estimate a plausible dose metric either in terms of specific metabolites or metabolic pathways or blood concentration of the parent compound in both laboratory rodents and humans, extrapolation to human equivalent environmental exposure entailed the steps as shown in Figure 5-8. First, consistent with the *Guidelines for Carcinogen Risk Assessment* (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, in order to convert the slope factor to units of risk per unit of human equivalent internal dose metric. Then the slope factor was converted to units of risk per unit of human equivalent environmental exposure by using the relationship between continuous human exposure and internal dose metric estimated via the human PBPK model. These last two considerations are further described below.[32]

[32] Typically, the POD would be expressed in terms of a human equivalent exposure. However, in this case, it is expressed in terms of the internal dose metric. This is because the relationship between exposure and internal dose may be nonlinear at the POD, even if the relationship between risk and internal dose is assumed to be linear below the POD. Therefore, the slope factor is first expressed in terms of internal dose, reflecting the assumption of low-dose linearity in internal dose. Then, provided the slope factor is applied at exposures well below the POD, where the relationship between exposure and internal dose is linear, it can be converted to a risk per unit exposure.

EPA's cross-species scaling methodology, grounded in general principles of allometric variation of biologic processes, was used for describing toxicological equivalence because of the extensive empirical evidence supporting it (U.S. EPA, 1992; Crump et al., 1989; Allen et al., 1988). Briefly, in the absence of adequate information to the contrary, the methodology determines toxicological equivalence across species through equal average lifetime concentrations, or AUCs, of the carcinogen. One typical application of this methodology is to oral exposures in mg/kg-day in the absence of pharmacokinetic or pharmacodynamic information. However, the same principles apply to the parent compound and metabolites (U.S. EPA, 1992).

For the orally administered dose, 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 $(BW_{animal}/BW_{human})^{1/4}$, based on the principle that clearance, on average, scales allometrically according to $BW^{-1/4}$ across species (U.S. EPA, 2005a). 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 enzymatically derived metabolites 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 (when clearance of a specific metabolite is specifically modeled) or mg/kg$^{3/4}$-day (when rate of metabolism is calculated) because metabolites are also subject to scale-affected clearance processes. The equivalence of considering equal AUCs of a metabolite to scaling the rate of metabolism by $BW^{3/4}$ can be easily understood if one assumes clearance rates *for the metabolite* scales allometrically adjusted by the fraction metabolized. There is a wide body of empirical evidence that metabolic rates associated with enzymatic processes scale with body weight to the ¾ power (U.S. EPA, 2005a), or $BW^{-1/4}$, if one thinks of the scaling as applied to the administered dose (U.S. EPA, 1992). Furthermore, when this scaling is applied to an internal dose expressed as a rate of production of metabolite(s), it is applicable regardless of the route of exposure. As an example, in EPA's trichloroethylene assessment, the human equivalent risk for liver and kidney effects was estimated using $BW^{3/4}$ scaling of the daily rate of the toxicologically relevant metabolic pathway (U.S. EPA, 2011b).

As discussed earlier in this subsection, rates of liver oxidative metabolism and total GSH metabolism are considered plausible dose metrics for the liver and kidney, respectively. In order

to estimate equivalent toxic effects in humans using the cross-species scaling methodology, tetrachloroethylene metabolized via either of these pathways was scaled using $BW^{3/4}$ so that the dose metric was expressed as $mg/kg^{3/4}$-day. As explained earlier, the AUC of TCA in the liver, the predominant metabolite along the oxidative pathway, is also presented as a plausible dose metric for liver cancer. No additional scaling was needed as the average concentration of TCA so determined was assumed to be equipotent when applied continuously over a lifetime in either species. Likewise, AUC of tetrachloroethylene, used as the preferred dose metric for MCL and kidney tumors, was not scaled further to extrapolate to humans.

Note that the involvement of reactive metabolites cleared nonenzymatically through which all other metabolites may follow has been hypothesized in many cases, and scaling by BW as opposed to $BW^{3/4}$ has been proposed to be more appropriate in such cases. However, scaling by BW was not considered pertinent for tetrachloroethylene because the possible reactive metabolites cleared nonenzymatically have not been identified and because the majority of the metabolites formed are thought to be sufficiently stable to be cleared enzymatically.

In the last step of the extrapolation to risk per human equivalent exposure, the slope factors in terms of internal dose metrics (associated with parent or metabolites) were converted to slope factors or unit risks in terms of human equivalent environmental inhalation and oral exposures using pharmacokinetic modeling. Refer to footnote c in Tables 5-18 and 5-21 for the inhalation and oral conversion factors. For animals, the study-specific body weights were used (refer to Tables 5-13 and 5-15), and for humans, the default of 70 kg was used.

In summary, an adjustment for cross-species scaling ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between each rodent species and humans for two dose metrics, total liver oxidative metabolism and total GSH metabolism, consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). 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, based on allometry (U.S. EPA, 1992).

5.3.3.2.3.2. Administered inhaled 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 (extrarespiratory) effects. Because the ratio of blood/air partition coefficients for the experimental animal species relative to humans is greater than or equal to one (for F344 rats, 15.1/14.7 = 1.03; for $B6C3F_1$ mice, 18.6/14.7 = 1.3), a default value of one was

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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.

In summary, for MCL, hemangiomas or hemangiosarcomas, and brain, testicular, and kidney tumors, tetrachloroethylene AUC in blood was judged to be more proximal than administered tetrachloroethylene concentration to the adverse effect, and, therefore, more relevant for estimating unit risks. Also based on allometry, average daily AUCs are expected to be equipotent across species without any additional scaling involved. The true correspondence is unknown, and risk may be higher or lower in humans than in rodents to an unknown degree.

5.3.4. Cancer Risk Values

Human cancer risk was assessed using four different sex-species animal data sets and a PBPK model for interspecies and route-to-route extrapolation. 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). For each data set, multistage modeling (refer to Section 5.3.3.2.1) using preferred and alternative (if available) PBPK model-based dose metrics was conducted, in addition to multistage modeling using administered concentration.

In addition, the NRC (2010) peer review recommended more extensive quantitative evaluation of the uncertainty due to different forms of dose-response models. Moreover, NRC (2010) agreed that for several datasets, the multistage model does not fit the data at lower doses, noting evidence of supralinearity in the underlying dose-response relationship. NRC (2010) also noted that in such cases, low-dose linear extrapolation is not conservative, and the external review draft of the Toxicological Review did not present the full ranges of variation and uncertainty in relation to model choice. Therefore, for the JISA (1993) datasets, additional analyses were conducted using administered concentration and the range of dichotomous models included in BMDS. In addition to the multistage model, these include the gamma, Weibull, log-logistic, log-probit, dichotomous Hill, probit, and logistic models. For the dichotomous Hill model, the slope was fixed at 1, making it equivalent to a Michaelis-Menten model. Statistically, a simpler model (fewer free parameters) was needed so that goodness-of-fit statistics could be derived given the number of dose groups (three exposed plus one control). Biologically, the Michaelis-Menten model is a natural choice for saturable biological processes, such as enzyme kinetics, that are not accounted for in the selected dose metrics. Hereafter, the dichotomous Hill model with slope fixed at 1 is referred to as the Michaelis-Menten model.

The results of the suite of models were evaluated for goodness-of-fit. For datasets exhibiting supralinearity, models that led to both a better fit to the supralinear shape and a stable BMDL were considered for further application using PBPK model-based dose metrics.

The sections below provide the results of the dose-response modeling using the male and female mouse and rat data from the JISA (1993) inhalation bioassay and male and female rat data from the NTP (1986) inhalation bioassay. Route-to-route extrapolation for estimating human cancer risk via oral exposure to tetrachloroethylene is then presented. Finally, quantitative and qualitative uncertainties underlying the risk estimation process are discussed.

5.3.4.1. Dose-Response Modeling Results

5.3.4.1.1. Hepatocellular tumors, male mice

In accordance with standard practice in the absence of MOA data supporting a particular dose-response model form, multistage modeling of the JISA bioassay data was carried out, using the preferred and alternative dose metrics of total liver oxidative metabolism and TCA AUC in liver. Modeling for both dose metrics generated fits for one-, two-, and three-stage models (details in Appendix D). All model fits had adequate goodness-of-fit *p*-values ($p > 0.05$), and overall adequate fit given the nonmonotonicity in the observed dose-response range (with standardized residuals within ± 2). There was no statistical improvement (by likelihood ratio test) in adding higher order terms to the first-order term, and a one-stage model was selected (refer to Figure 5-9 for the fit using total oxidative metabolism).

Extrapolation to humans using total oxidative metabolism led to a BMD_{10} of 2.9, and its lower bound benchmark dose ($BMDL_{10}$) was 1.4-fold lower at 2.1 $mg/kg^{3/4}$-day liver oxidative metabolism (refer to Figure 5-9). Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 1.8×10^{-3} per ppm.

Extrapolation to humans using TCA AUC in liver led to a human equivalent internal dose POD ($BMCL_{10}$) of 69 mg-hr/L-day TCA in blood. The corresponding central tendency estimate was approximately 1.5-fold higher, at 97 mg-hr/L-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 1.5×10^{-3} per ppm, slightly lower than the estimate using total liver oxidative metabolism.

Dose-response modeling of the male mouse liver tumor data using administered exposure fit the data points similarly to when using total oxidative metabolism or TCA AUC in liver (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 2.7 ppm tetrachloroethylene in air. The corresponding central tendency estimate

was nearly twofold higher, at 3.9 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 37×10^{-3} per ppm, more than an order of magnitude higher than using either PBPK-estimated dose metric.

The NRC (2010) peer review recommended more extensive quantitative evaluation of the uncertainty due to different forms of dose-response models. The analysis was conducted using administered concentration and the range of dichotomous models included in BMDS. Among the models fitted, five models fit worse than the multistage (gamma, Weibull, log-logistic, log-probit, and Michaelis-Menten), and two models fit better than the multistage (probit and logistic). However, the multistage model had the lowest residual for the control group, indicating that the alternative models were no better than the multistage model in addressing the supralinear shape in this data set. Nonetheless, the estimated $BMCL_{10}$s from the better-fitting models were less than twofold different than that using the multistage model.

Therefore, due to the limited sensitivity to the selection of dose-response models and the finding that none of the alternative models was clearly superior to the standard multistage model for addressing this data set's supralinearity at the lower doses, the multistage model results were carried forward to support cancer risk estimates (refer to Table 5-18). Due to the data supporting oxidative metabolism as being involved in hepatocellular tumors, the estimates carried forward were those using total oxidative metabolism as the dose metric (preferred), and those using TCA AUC in liver as the dose metric (alternative). The remaining analyses (refer to Tables 5-19 and 5-20) of administered concentration using multistage and other dose-response models are retained only to better characterize the range of results from different dose-response models.

5.3.4.1.2. Hepatocellular tumors, female mice

As was done for the male mouse hepatocellular tumors, in accordance with standard practice in the absence of MOA data supporting a particular dose-response model form, multistage modeling of the JISA bioassay data was carried out, using the preferred and alternative dose metrics of total liver oxidative metabolism and TCA AUC in liver. Modeling for both dose metrics included one-, two-, and three-stage models. Adequate fits were obtained with all three models, with adequate goodness-of-fit p-values ($p > 0.05$), and overall adequate visual fit (refer to details in Appendix D). The second-order term led to a statistically significant improvement in fit, but there was no statistical improvement with the third-order term, as it was estimated to be zero. Therefore a two-stage model was selected for both dose metrics (refer to Figure 5-10 for the fit using total oxidative metabolism).

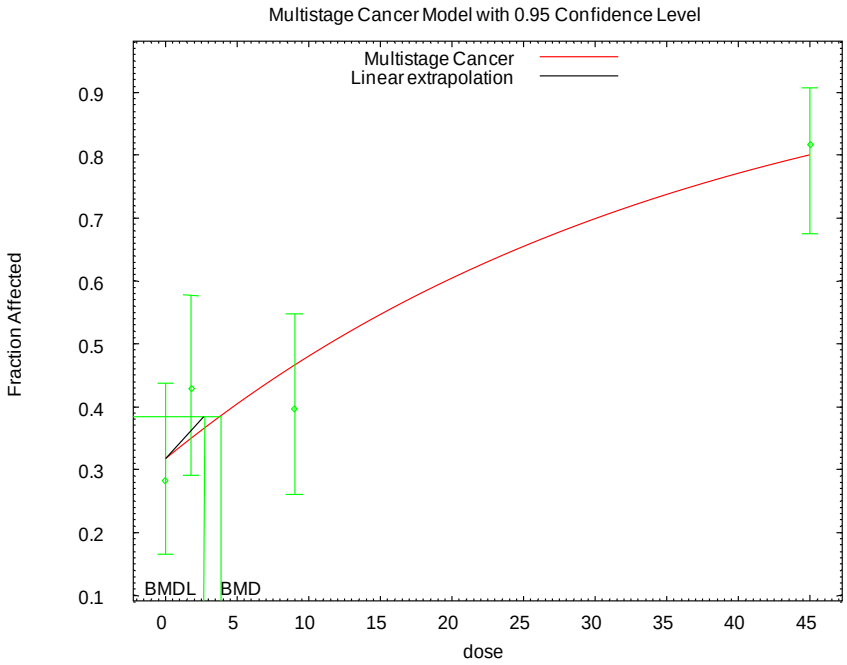

Figure 5-9. Dose-response modeling of male mouse hepatocellular tumors associated with inhalation exposure to tetrachloroethylene, in terms of liver total oxidative metabolites; response data from JISA (1993).
Details in Appendix D.

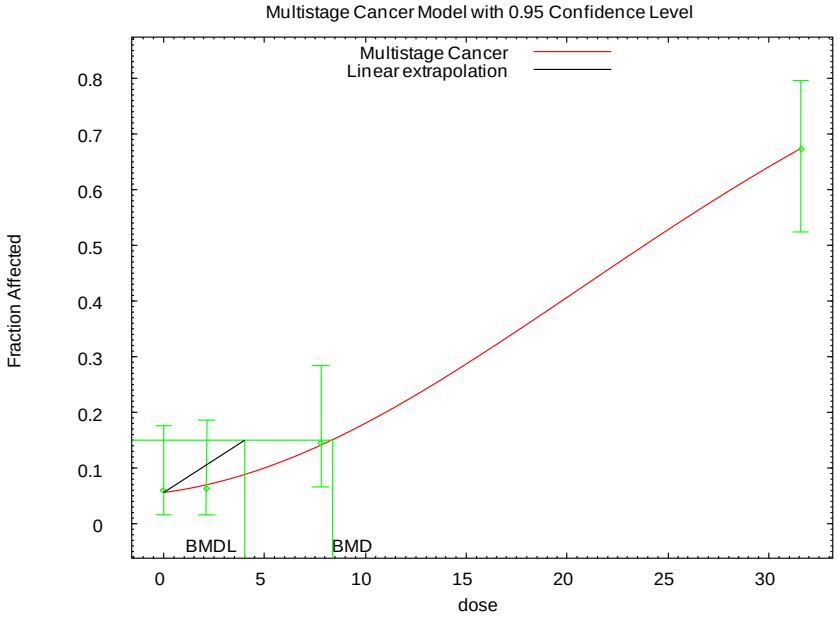

Figure 5-10. Dose-response modeling of female mouse hepatocellular tumors associated with inhalation exposure to tetrachloroethylene, in terms of liver total oxidative metabolites; response data from JISA (1993).
Details in Appendix D.

Table 5-18. Human equivalent candidate unit risks, derived using PBPK-derived dose metrics and multistage model; tumor incidence data from JISA (1993) and NTP (1986)

Study Group	Tumor type (multistage model with all dose groups unless otherwise specified)	Human Equivalents: POD[a], in internal dose units			SF×10^{-3} /internal dose unit[b]	Candidate IUR×10^{-3} /ppm (PBPK range)[c]
Primary dose metrics						
Male mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	2.9 2.1	Total liver oxidative metabolism, mg/$kg^{0.75}$-d	49	**1.8** (1.6–1.8)
	Hemangiomas, hemangiosarcomas,	BMD_{10} $BMDL_{10}$	63 34	PCE AUC in blood, mg-hr/L-d	2.9	**5.9** (5.9–6.9)
Female mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	8.4 4.0	Total liver oxidative metabolism, mg/$kg^{0.75}$-d	25	**0.90** (0.84–0.93)
Male rats JISA (1993)	MCL	BMD_{10} $BMDL_{10}$	46 30	PCE AUC in blood, mg-hr/L-d	3.4	**6.8** (6.8–8.0)
	MCL (Michaelis-Menten)	BMD_{10} $BMDL_{10}$	20 5.0	PCE AUC in blood, mg-hr/L-d	20	**40** (40–47)
Female rats JISA (1993)	MCL	BMD_{10} $BMDL_{10}$	136 61	PCE AUC in blood, mg-hr/L-d	1.6	**3.3** (3.3–3.9)
	MCL (control and low dose groups only)	BMD_{10} $BMDL_{10}$	11 5.2	PCE AUC in blood, mg-hr/L-d	19	**39** (39–45)
Female and male rats JISA (1993)	MCL (Michaelis-Menten)	BMD_{10} $BMDL_{10}$	17 3.0	PCE AUC in blood, mg-hr/L-d	33	**68** (67–71)
Male rats NTP (1986)	Kidney tumors	BMD_{10} $BMDL_{10}$	250 110	PCE AUC in blood, mg-hr/L-d	0.90	**1.8** (1.8–2.1)
	Brain gliomas	BMD_{10} $BMDL_{10}$	410 170	PCE AUC in blood, mg-hr/L-d	0.62	**1.2** (1.2–1.4)
	Testicular interstitial cell tumors	BMD_{10} $BMDL_{10}$	30 14	PCE AUC in blood, mg-hr/L-d	7.1	**14** (14–17)
	MCL	BMD_{10} $BMDL_{10}$	28 15	PCE AUC in blood, mg-hr/L-d	7.2	**15** (14–17)
	Total risk for any of above four tumor types	BMD_{10} $BMDL_{10}$	13 8.1	PCE AUC in blood, mg-hr/L-d	12	**25** (25–29)
Alternate Dose Metrics						
Male mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	97 69	TCA AUC in liver, mg-hr/L-d	1.5	**1.5** (1.4–1.5)
Female mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	292 141	TCA AUC in liver, mg-hr/L-d	0.72	**0.72** (0.68–0.74)
Male rats NTP (1986)	Kidney tumors	BMD_{05} $BMDL_{05}$	0.46 0.21	Total GSH metabolism, mg/$kg^{0.75}$-d	243	**100** (0.047–110)

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Table 5-18. Human equivalent candidate unit risks, derived using PBPK-derived dose metrics and multistage model; tumor incidence data from JISA (1993) and NTP (1986) (continued)

SF = Slope Factor; IUR = Inhalation Unit Risk; MCL= Mononuclear cell leukemias.

[a]PODs were estimated at the indicated BMRs in terms of extra risk; i.e., $BMDL_{10}$ = lower bound for the level of the internal dose metric associated with 10% extra risk. Dose metric units are in the first column and include cross-species scaling to a human equivalent internal dose metric. Refer to Appendix D for dose-response modeling details.

[b]Slope Factor = $BMR/BMDL_{BMR}$ in units of risk per dose metric unit (as given in the first column).

[c]Inhalation unit risk (IUR) is given by the product of the slope factor in units of risk per dose metric unit and an inhalation dose metric conversion factor ($DMCF_{ppm}$): $IUR = BMR/BMDL_{BMR} \times DMCF_{ppm}$, where the $DMCF_{ppm}$ is derived from the PBPK model. The $DMCF_{ppm}$ for each dose metric is shown below:

Dose metric	$DMCF_{ppm}$	
	Overall posterior mode	**Range of posterior modes**
Total liver oxidative metabolism	**0.0363**	0.0339–0.0372
Tetrachloroethylene blood AUC	**2.03**	2.01–2.36
TCA AUC in liver	**1.02**	0.956–1.04
Total GSH metabolism	**0.428**	0.00019–0.44

Values in **bold** correspond to using the overall posterior mode and are carried forward for consideration in the derivation of the IUR. The difference between the overall and alternative posterior modes is negligible (relative to other uncertainties) except for the Total GSH metabolism dose metric.

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Table 5-19. Dose-response summary and candidate unit risk estimates using continuous equivalent administered tetrachloroethylene levels as dose metric, from NTP (1986) and JISA (1993)

Study group	Tumor type (multistage model and all dose groups unless otherwise specified)	POD (ppm)		Candidate Unit risk [a,b] $\times 10^{-3}$/ppm
Male mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMC_{10} $BMCL_{10}$	3.9 2.7	37
	Hemangiomas or hemangiosarcomas	BMC_{10} $BMCL_{10}$	24 13	7.5
	Overall risk of either tumor type above[c]	BMC_{10} $BMCL_{10}$	3.3 2.4	42
Female mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMC_{10} $BMCL_{10}$	5.0 3.8	27
Male rats JISA (1993)	MCL	BMC_{10} $BMCL_{10}$	21 13	7.6
	MCL (Michaelis-Menten model)	BMC_{10} $BMCL_{10}$	8.6 2.2	45
Female rats JISA (1993)	MCL	BMC_{10} $BMCL_{10}$	60 27	3.7
	MCL (control and low-dose groups only)	BMC_{10} $BMCL_{10}$	4.9 2.3	43
Female and male rats JISA (1993)	MCL	BMC_{10} $BMCL_{10}$	32 21	4.8
	MCL (Michaelis-Menten model)	BMC_{10} $BMCL_{10}$	7.7 1.4	71
Male rats NTP (1986)	Kidney tumors	BMC_{10} $BMCL_{10}$	110 50	2.0
	Brain gliomas	BMC_{10} $BMCL_{10}$	180 73	1.4
	Testicular interstitial cell tumors	BMC_{10} $BMCL_{10}$	13 6.1	16
	Mononuclear cell leukemia	BMC_{10} $BMCL_{10}$	12 6.5	15
	Overall risk for any of above four tumor types[c]	BMC_{10} $BMCL_{10}$	5.7 3.5	29

MCL = Mononuclear cell leukemia.

[a]Using dose coefficients in terms of administered ppm of tetrachloroethylene adjusted to equivalent continuous exposure, consistent with RfC methodology (U.S. EPA, 1994). BMCs/BMCLs estimated in terms of extra risk.

[b]Unit risks, which are approximations for extrapolation to lower doses, should not be used with exposures greater than the POD from which they were derived without considering the curvature of the dose-response function (refer to Appendix D for modeling details).

[c]Overall risk estimated using maximum likelihood method. Refer to Appendix D.3.1 for details.

Data source: Refer to Tables 5-13 and 5-15 and Appendix D.

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Table 5-20. Range of outputs from fitting different BMDS models using continuous equivalent administered tetrachloroethylene levels as dose metric, from JISA (1993)[a]

Study group	Tumor type (all dose groups unless otherwise specified)	Range of PODs (ppm)		Range of $0.1/BMCL_{10} \times 10^{-3}$/ppm
Male mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMC_{10} $BMCL_{10}$	2.5 − 11 0.4 – 4.8	21 – 250
	Hemangiomas or hemangiosarcomas	BMC_{10} $BMCL_{10}$	16 − 32 4.1 − 22	4.5 – 24
	Hepatocellular adenomas or carcinomas	BMC_{10} $BMCL_{10}$	5.0 – 13 3.8 − 11	9.4 – 27
Female mice JISA (1993)	MCL	BMC_{10} $BMCL_{10}$	6.9 − 30 0.062 − 22	4.5 – 1600
Male rats JISA (1993)	MCL	BMC_{10} $BMCL_{10}$	4.9 − 88 0 − 37	2.7 – +∞
Female and male rats JISA (1993)	Mononuclear cell leukemia	BMC_{10} $BMCL_{10}$	4.5 − 42 0.001 − 31	3.3 – 71

MCL = Mononuclear cell leukemia

[a]Using dose coefficients in terms of administered ppm of tetrachloroethylene adjusted to equivalent continuous exposure, consistent with RfC methodology (U.S. EPA, 1994). BMCs/BMCLs estimated in terms of extra risk. Range from use of different dose-response models (gamma, Weibull, log-logistic, log-probit, Michaelis-Menten, probit, logistic, and multistage) using all dose groups, only including models with goodness-of-fit p-values ≥ 0.1.

Extrapolation to humans using total liver oxidative metabolism led to a human equivalent internal dose POD ($BMCL_{10}$) of 3.9 mg/kg$^{3/4}$-day liver oxidative metabolism. The corresponding central tendency estimate was 2.2-fold higher, at 8.4 mg/kg$^{3/4}$-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures led to a human equivalent unit risk of 0.92×10^{-3} per ppm.

Extrapolation to humans using TCA AUC in liver led to a human equivalent POD ($BMCL_{10}$) of 139 mg-hr/L-day TCA in blood. The corresponding central tendency estimate was approximately 2.1-fold higher, at 292 mg-hr/L-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 0.73×10^{-3} per ppm, slightly lower than the estimate using total liver oxidative metabolism.

Dose-response modeling using administered exposure fit the data points similarly to when using total oxidative metabolism, or TCA AUC, in liver (details in Appendix D). The

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result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 3.8 ppm tetrachloroethylene in air. The corresponding central tendency estimate was approximately twofold higher, at 5.0 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 27×10^{-3} per ppm, more than an order of magnitude higher than using either PBPK-estimated dose metric.

The NRC (2010) peer review recommended more extensive quantitative evaluation of the uncertainty due to different forms of dose-response models. The analysis was conducted using administered concentration using the range of dichotomous models included in BMDS. All the models (gamma, Weibull, log-logistic, Michaelis-Menten, log-probit, probit, and logistic) fit similar to or better than the multistage. The estimated $BMCL_{10}$s from the better-fitting models were less than threefold different than that using the standard multistage model.

Therefore, due to the limited sensitivity to the selection of dose-response models, the multistage model results were carried forward to support cancer risk estimates (refer to Table 5-18). Due to the data supporting oxidative metabolism in hepatocellular tumors, the estimates carried forward were those using total oxidative metabolism as the dose metric (preferred), and those using TCA AUC in liver as the dose metric (alternative). The remaining analyses (refer to Tables 5-19 and 5-20) using administered concentration using multistage and other dose-response models are retained only to better characterize the range of results from different dose-response models.

5.3.4.1.3. Hemangiosarcomas, male mice

Hemangiosarcomas were also observed in the JISA male mice: in liver, spleen, fat, and subcutaneous skin. Because these tumors differ etiologically from the hepatocellular adenomas and carcinomas, they were modeled separately. In accordance with standard practice in the absence of MOA data supporting a particular dose-response model form, multistage modeling of the JISA bioassay data was carried out, using the preferred dose metric of tetrachloroethylene AUC in blood, including fits for one-, two-, and three-stage models (details in Appendix D). A one-stage model was found to be sufficient, with an adequate goodness-of-fit *p*-value ($p = 0.38$), and overall adequate visual fit (refer to Figure 5-11). There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero.

Extrapolation to humans led to an internal dose POD ($BMCL_{10}$) of 34 mg-hr/L-day tetrachloroethylene in blood (refer to Table 5-18). The corresponding central tendency estimate was nearly twofold higher, at 63 mg-hr/L-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures led to a human equivalent unit risk of 5.9×10^{-3} per ppm.

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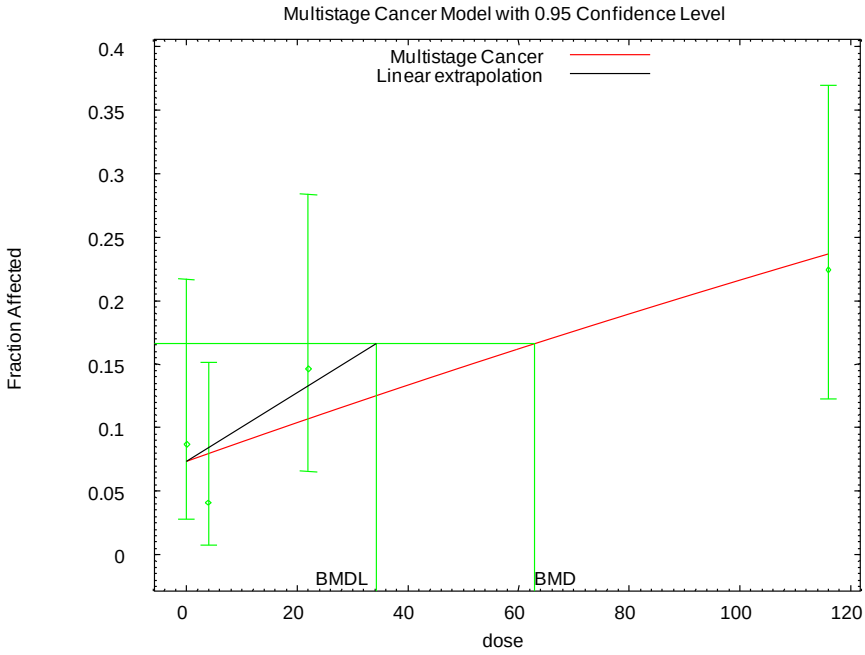

Figure 5-11. Dose-response modeling of male mouse hemangiomas or hemangiosarcomas associated with inhalation exposure to tetrachloroethylene, in terms of tetrachloroethylene AUC in blood; response data from JISA (1993).
Details in Appendix D.

Dose-response modeling using administered exposure fit the data points similarly to when using tetrachloroethylene AUC in blood (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 13 ppm tetrachloroethylene in air (refer to Table 5-19). The corresponding central tendency estimate was approximately twofold higher, at 24 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 7.5×10^{-3} per ppm, slightly higher than using tetrachloroethylene AUC in blood.

These results raise some concern that total cancer risk based on the male mice data may be underestimated by considering only one site. Methods for estimating overall risk from sites with very different dose metrics are not currently available. However, when an analysis using administered concentration as the dose metric for both sites was carried out, using a method based on maximum likelihood estimation,[33] the overall risk was estimated to be only slightly

[33] An approach suggested in the EPA *Guidelines for Carcinogen Risk Assessment* (2005a) to characterize total risk from multiple tumor sites would be to estimate cancer risk from tumor-bearing animals. EPA traditionally used this approach until *Science and Judgment in Risk Assessment* (NRC, 1994) made a case that this approach would tend to underestimate composite risk when tumor types occur in a statistically independent manner— that is, that the occurrence of a hemangiosarcoma, say, would not be dependent on whether there was a hepatocellular tumor. This assumption cannot currently be verified and if not correct could lead to an overestimate of risk from combining

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higher than that using hepatocellular tumors alone (refer to Table 5-19). The analysis yielded an overall risk value of 0.042 per ppm, compared with the unit risk of 0.037 based on hepatocellular tumors alone. On the other hand, using administered concentration for the hepatocellular tumors may substantially overestimate human equivalent risk as compared to that estimated by using total liver metabolism, under the assumption that oxidative metabolism is likely an important component of this process. Refer to Appendix D.1.1.3 for a summary of the calculations.

The NRC (2010) peer review recommended more extensive quantitative evaluation of the uncertainty due to different forms of dose-response models. The analysis was conducted using administered concentration using the range of dichotomous models included in BMDS. All of the models had similar or worse fits than the multistage (gamma, Weibull, log-logistic, log-probit, Michaelis-Menten, probit, and logistic). The estimated $BMCL_{10}$s ranged from 3.2-fold less to 1.7-fold more than that using the multistage model.

Therefore, due to the limited sensitivity to the selection of dose-response models, the multistage model result was carried forward to support cancer risk estimates (refer to Table 5-18). Due to the lack of data on the active moiety for this endpoints, the result carried forward used AUC of tetrachloroethylene in blood as the preferred dose metric. The remaining analyses (refer to Table 5-19) using administered concentration using multistage and other dose-response models are retained only to better characterize the range of results from different dose-response models.

5.3.4.1.4. Mononuclear cell leukemia (MCL), male rat

In accordance with standard practice in the absence of MOA data supporting a particular dose-response model form, multistage modeling of the JISA bioassay data was carried out considering fits for one-, two-, and three-stage models (details in Appendix D). Using the preferred dose metric of tetrachloroethylene AUC in blood, a one-stage model had a goodness-of-fit p-value = 0.52, generally considered adequate, and the standardized residuals were within the recommended limit of ±2 units (refer to Figure 5-12a). There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero. Extrapolation to humans led to an internal dose POD ($BMCL_{10}$) of 30 mg-hr/L-day tetrachloroethylene in blood (refer to Table 5-18). The corresponding central tendency estimate was less than twofold higher, at 46 mg-hr/L-day. Linear extrapolation from the POD to low

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across tumor sites. However, NRC (1994) argued that a general assumption of statistical independence of tumor-type occurrences within animals was not likely to introduce substantial error in assessing carcinogenic potency from rodent bioassay data.

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exposures, followed by conversion to human exposures, led to a human equivalent unit risk of 6.8×10^{-3} per ppm.

Dose-response modeling using administered exposure fit the data points similarly to that using tetrachloroethylene AUC in blood (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 13 ppm tetrachloroethylene in air (refer to Table 5-19). The corresponding central tendency estimate was approximately twofold higher, at 21 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 7.6×10^{-3} per ppm, very similar to that using tetrachloroethylene AUC in blood.

To address NRC (2010) peer review comments, additional dose-response models were evaluated for this data set in order to obtain a better model fit, particularly at lower doses where the data set exhibited some supralinearity (refer to Appendix D, Table D-11). The analysis was conducted using administered concentration using the range of dichotomous models included in BMDS. Among the models fitted, five models fit better than the multistage (gamma, Weibull, log-logistic, log-probit, and Michaelis-Menten), with two models leading to worse fits than the multistage (probit and logistic). Visually, the Michaelis-Menten model better captured the supralinear dose-response shape of the data. Because of the better dose-response fit, the Michaelis-Menten model was preferred over the standard multistage model for this data set using administered concentration. The human equivalent POD ($BMCL_{10}$) was 2.2 ppm tetrachloroethylene in air (refer to Table 5-19), with the corresponding central tendency estimate 3.8-fold higher at 8.6 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 45×10^{-3} per ppm, sixfold higher than using the multistage model.

Based on this analysis, the Michaelis-Menten model was also fitted using the preferred dose metric of tetrachloroethylene AUC in blood (the analysis was not conducted using other dose-response models because of the near proportionality between this dose metric and administered tetrachloroethylene). Extrapolation to humans led to an internal dose POD ($BMCL_{10}$) of 5 mg-hr/L-day tetrachloroethylene in blood (refer to Table 5-18). The corresponding central tendency estimate was about 4-fold higher, at 20 mg-hr/L-day. Linear extrapolation from the POD to low exposures, followed by conversion to human exposures, led to a human equivalent unit risk of 40×10^{-3} per ppm.

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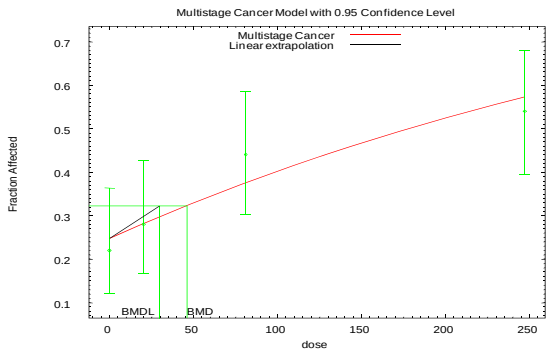
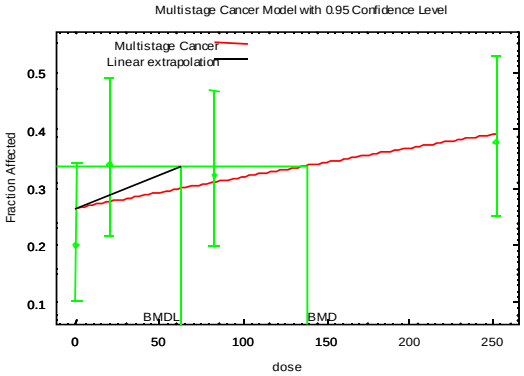
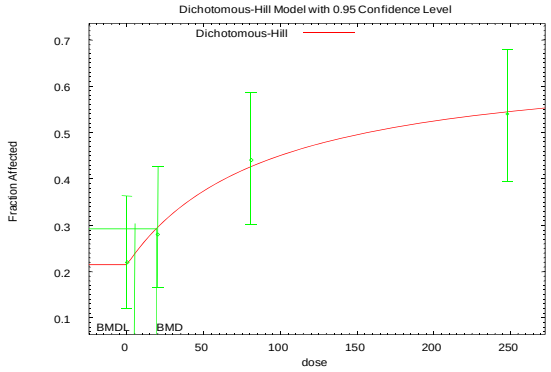


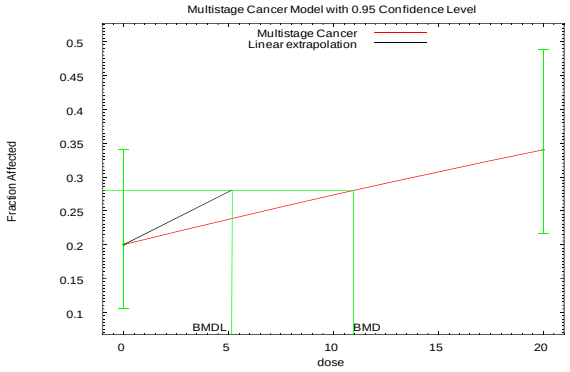
a. One-degree multistage model fit to male rat MCL data, all dose groups.



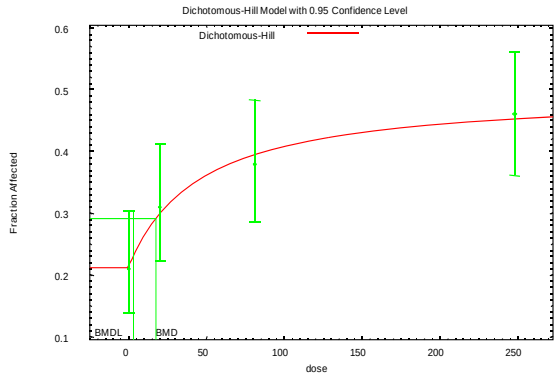
b. One-degree multistage model fit to female rat MCL data, all dose groups.



c. Michaelis-Menten model fit to male rat MCL data, all dose groups.



d. Multistage model fit to female rat MCL data, control and lowest dose group only.



e. Michaelis-Menten model fit to female and male rat MCL data, all dose groups.

Figure 5-12. Dose-response modeling of female and male rat MCLs associated with inhalation exposure to tetrachloroethylene, in terms of tetrachloroethylene AUC in blood; response data from JISA (1993).
Details in Appendix D.

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Therefore, two approaches were carried forward to support cancer risk estimates: the standard approach using the multistage model and a better-fitting approach using the Michaelis-Menten model, both on the basis of AUC of tetrachloroethylene in blood. The remaining analyses using administered concentration using these and (less preferred) alternative approaches are retained only to better characterize the range of results from different dose-response models.

5.3.4.1.5. Mononuclear cell leukemia (MCL), female rat

In accordance with standard practice in the absence of MOA data supporting a particular dose-response model form, multistage modeling of the JISA bioassay data was carried out considering fits for one-, two-, and three-stage models (details in Appendix D). Using the preferred dose metric of tetrachloroethylene AUC in blood, a one-stage model had a goodness-of-fit *p*-value ($p = 0.34$) generally considered adequate, and the standardized residuals were within the recommended limit of two units (refer to Figure 5-12b). There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero. Extrapolation to humans led to an internal dose POD ($BMCL_{10}$) of 61 mg-hr/L-day tetrachloroethylene in blood. The corresponding central tendency estimate was about twofold higher, at 136 mg-hr/L-day. Linear extrapolation from the POD to low exposures, followed by conversion to human exposures, led to a human equivalent unit risk of 3.4×10^{-3} per ppm.

Dose-response modeling using administered exposure fit the data points similarly to that using tetrachloroethylene AUC in blood (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 27 ppm tetrachloroethylene in air. The corresponding central tendency estimate was approximately twofold higher, at 60 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 3.7×10^{-3} per ppm, essentially the same as using tetrachloroethylene AUC in blood.

To address NRC (2010) peer review comments, additional options were evaluated for this data set in order to obtain a better model fit, particularly for lower doses at which the data set exhibited some supralinearity (refer to Appendix D, Table D-6). These analyses were conducted using administered concentration, due to its close proportionality with AUC of tetrachloroethylene in blood. This case was the most extreme among the supralinear datasets, with the multistage model estimate of the control incidence markedly above the data, and the estimate of the lowest dose group markedly below the data. Briefly, use of a wider range of dose-response models [as suggested by NRC (2010)] for the full data set was considered first. When those attempts proved unsuccessful, incorporation of historical controls and exclusion of higher exposure groups were also considered. These approaches are described in more detail below.

First, the range of dichotomous models included in BMDS was considered. Among the models fitted, four models fit better than the multistage (gamma, Weibull, log-logistic, and Michaelis-Menten), two models fit similarly to the multistage (probit and logistic), and one model fit worse than the multistage (log-probit). However, for the better-fitting models, the predicted response rate became virtually infinite in slope, approaching zero dose. Thus, no $BMCL_{10}$ could be estimated (refer to Appendix D), indicating that the statistical uncertainty is too great to support the BMC estimates. While data are lacking to inform the dose-response relationship below 50 ppm in female rats, these fits are consistent with the possibility that a response plateau extends below the lowest observed response. Therefore, none of these options were successful in both improving upon the multistage model fit and estimating a BMCL.

The next strategy for obtaining an adequate fit to the female rat MCL data involved focusing model fitting on the low-exposure range. First, the sensitivity of the fit to the use of historical controls was examined in an attempt to constrain the estimated control response at a level representative of previously observed values. Thus, the concurrent control was replaced with the overall historical control incidence for inhalation studies in this laboratory (66/448 among control female rats in inhalation studies; refer to Table 5-16), and all models above were fitted. None of these fits was both adequate and an improvement on the fits obtained with concurrent controls (results not shown).

Next, exposure groups were excluded from analysis, starting with the highest exposure group (600 ppm). All models used above were considered, as was the use of either the concurrent or historical controls. All model fits were essentially the same as when using the full data set (refer to Appendix D). Consequently, the next highest exposure group's data (200 ppm) were also excluded. Only the multistage model was fit to the two remaining data points (control and 50 ppm) because the other models use more parameters and need more data points. The $BMCL_{10}$ was 2.3 ppm, and the BMC_{10} was about twofold higher at 4.9 ppm (refer to Figure 5-12d; details in Appendix D). Linear extrapolation from the POD to low exposures, followed by conversion to human exposures, led to a human equivalent unit risk of 43×10^{-3} per ppm. In sum, dose-response modeling of the full female rat MCL data set was only superior to the multistage model for models that could not provide a lower bound estimate for a POD. The only method that both led to a better fit to the control data and provided a lower bound BMC estimate for a POD was use of just the concurrent control and lowest female rat exposure group. This analysis is, therefore, consistent with the suggestion by the NRC (2010) that use of the multistage model for the full datasets is not likely to provide a conservative upper bound estimate of risk for this data set, and may, therefore, underestimate risk.

Based on this analysis, the multistage model was also fitted to only the concurrent control and lowest exposure group using the preferred dose metric of tetrachloroethylene AUC in blood

(the analysis was not conducted using other models because of the near proportionality between this dose metric and administered tetrachloroethylene). Extrapolation to humans led to an internal dose POD ($BMCL_{10}$) of 5.2-mg-hr/L-day tetrachloroethylene in blood. The corresponding central tendency estimate was about twofold higher, at 11 mg-hr/L-day. Linear extrapolation from the POD to low exposures, followed by conversion to human exposures led to a human equivalent unit risk of 39×10^{-3} per ppm, essentially the same as the result using administered concentration.

Therefore, two approaches were carried forward to support cancer risk estimates: the standard approach using the multistage model and the full data set, and the only available better-fitting approach using the multistage model, and only the control and lowest dose group data, both on the basis of AUC of tetrachloroethylene in blood. However, neither method fully captures the potential extent of supralinearity into the region below the lowest dose. The remaining analyses using administered concentration using these and (less preferred) alternative approaches are retained only to better characterize the range of results from different dose-response models.

5.3.4.1.6. Mononuclear cell leukemia (MCL), combined female and male rat

The MCL data for male rats and especially female rats were challenging to fit because of the apparent supralinearity at lower doses. It was hypothesized that the male and female MCL responses reflect the same underlying dose response to tetrachloroethylene. The presence of a supralinear shape to the dose response for both male and female rats, in both the NTP (1986) and JISA (1993) bioassays (refer to Figure 5-7), and the similar background MCL rates between sexes in the JISA rats, are consistent with this hypothesis. Combining the datasets would increase statistical power and, thus, perhaps better stabilize the BMDL estimates while being able to fit the supralinear shape.

Two analyses were conducted to evaluate the consistency of the two JISA datasets. A test described by Stiteler et al. (1993) evaluates whether two datasets are consistent with an underlying dose-response model. In this case, the Michaelis-Menten model was used, given its relative success at fitting both datasets in the low-dose region. The test involves comparing the maximum log-likelihoods for the separate and combined datasets. The resulting *p*-value was 0.54, indicating insufficient reason to conclude that the datasets differ from one underlying model. The other analysis used a logistic regression to test whether the datasets differed significantly between males and females. The advantage of this approach is that it does not require assuming a specific functional form to represent the dose-response relationship. This

analysis yielded a *p*-value of 0.197, indicating no significant relationship of sex in the pattern of responses. Refer to Appendix D for more details of both analyses.

The analysis began with fitting all dichotomous models to the combined male and female MCL data on the basis of administered concentration. As compared to the sex-specific analyses, only the Michaelis-Menten model provided an overall improved fit to all dose groups relative to the multistage model (refer to Figure 5-12d). The resulting BMC_{10} was 7.7 ppm, and the $BMCL_{10}$ was about sixfold lower at 1.4 ppm (refer to Table 5-19). Thus, combining the male and female rat MCL data generated a result with slightly greater statistical uncertainty (shown in the wider confidence interval) than POD estimates for the sex-specific results. Linear extrapolation from this POD to low exposures led to a human equivalent unit risk of 71×10^{-3} per ppm.

Based on this analysis, the Michaelis-Menten model was also fitted using the preferred dose metric of tetrachloroethylene AUC in blood (the analysis was not conducted using other models because of the near proportionality between this dose metric and administered tetrachloroethylene). The result was a human equivalent POD ($BMCL_{10}$) of 3.0 mg-hr/L-day. The corresponding central tendency estimate was approximately sixfold higher, at 17 mg-hr/L-day. Linear extrapolation from this POD led to a human equivalent unit risk of 68×10^{-3} per ppm, essentially the same as the estimates using administered tetrachloroethylene.

Therefore, the approach carried forward to support cancer risk estimates was the Michaelis-Menten model on the basis of AUC of tetrachloroethylene in blood. The remaining analyses using administered concentration are retained only to better characterize the range of results from different dose-response models.

5.3.4.1.7. Other tumors in male rats

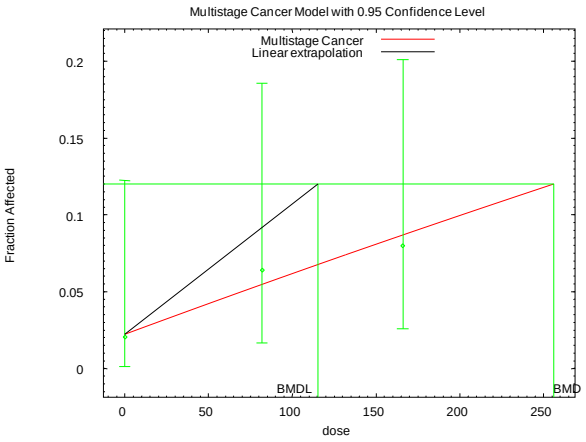
As discussed in Section 5.3.1, tumors occurred at multiple sites in male rats exposed to tetrachloroethylene in the NTP (1986) bioassay. While the design of NTP study is less suitable than the JISA study for developing risk estimates, due to the higher exposures and the fewer dose groups, dose-response modeling of these data was conducted to address variability in responses across animal strains and bioassays. Estimates were developed for the risk of each tumor type individually, as well as for the risk of any combination of tumor types. Because these analyses are considered less preferred alternatives to those based on the JISA study, additional analyses with respect to dose-response model selection were not conducted for these data.

5.3.4.1.7.1. Kidney tumors, male rat

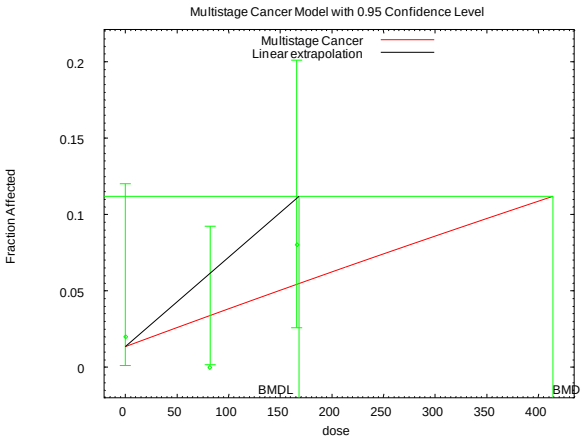
As discussed in Section 5.3.3.3 regarding selection of dose metrics, metabolism of tetrachloroethylene via the GSH conjugation pathway was calculated as a dose metric relevant for effects in the kidney. Multistage modeling of the NTP bioassay was carried out in units of tetrachloroethylene conjugated with GSH per kg body weight to the ¾ power per day considering fits for one- and two-stage models. A one-stage model was found to be sufficient, with an adequate goodness-of-fit *p*-value ($p = 0.75$) and overall adequate visual fit (refer to Figure 5-13, details not provided). There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero. Extrapolation to humans led to an internal POD ($BMDL_{10}$) of 0.21 $mg/kg^{0.75}$-day in blood (refer to Table 5-18). The corresponding central tendency estimate was about twofold higher, at 0.46 $mg/kg^{0.75}$-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 100×10^{-3} per ppm. However, using the range of posterior modes for the PBPK model predictions led to human equivalent risks of 0.047×10^{-3} to 110×10^{-3}, a range of more than 2000-fold. In view of this large range (much larger than the range for any of the other endpoints), and the inability to discern from the toxicokinetic data whether this spread represented uncertainty or variability or both [refer to Section 3; Chiu and Ginsberg (2011)], AUC of the parent compound in the blood was preferred as the dose metric for kidney toxicity, while carrying forward the results of using the GSH conjugation dose metric for comparison.

Thus, multistage modeling of the kidney tumor data was also carried out in units of tetrachloroethylene AUC in blood, considering fits for one-, two-, and three-stage models. A one-stage model had an adequate goodness-of-fit *p*-value ($p = 0.74$) and overall adequate visual fit. There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero. Extrapolation to humans led to an internal POD ($BMDL_{10}$) of 110 mg-hr/L-day tetrachloroethylene in blood (refer to Table 5-18). The corresponding central tendency estimate was about twofold higher, at 250 mg-hr/L-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 1.8×10^{-3} per ppm.

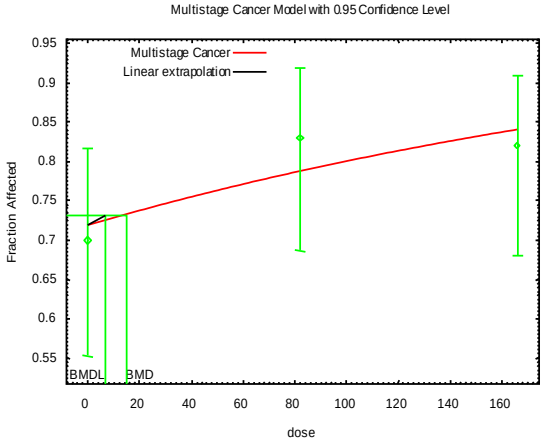
Dose-response modeling using administered exposure fit the data points similarly to when tetrachloroethylene AUC in blood was used (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 50 ppm tetrachloroethylene in air (refer to Table 5-19). The corresponding central tendency estimate was approximately twofold higher, at 110 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 2.1×10^{-3} per ppm, essentially the same as the estimate using tetrachloroethylene AUC in blood.



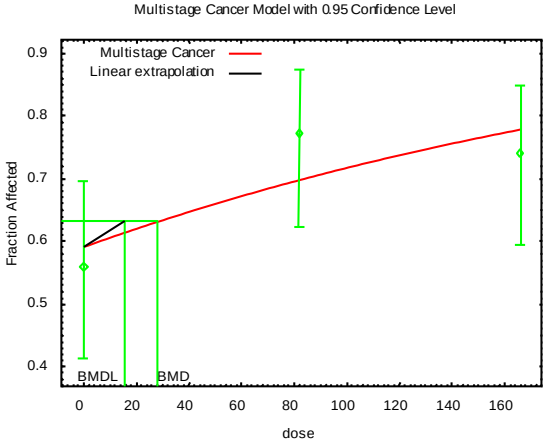
a. One-stage model fit to kidney tumors.



b. One-stage model fit to brain gliomas.



c. One-stage model fit to testicular interstitial cell tumors.



d. One-stage model fit to MCLs.

Figure 5-13. Dose-response modeling of male rat tumors—kidney, brain gliomas, interstitial cell tumors, MCLs—associated with inhalation exposure to tetrachloroethylene, in terms of tetrachloroethylene AUC in blood; response data from NTP (1986).
Details in Appendix D.

Two multistage model results were carried forward to support cancer risk estimates (refer to Table 5-18): that using AUC of tetrachloroethylene in blood as the dose metric (preferred), and those using GSH conjugation metabolism as the dose metric (alternative). For the alternative dose metric, it is also noted that the range of PBPK model-based estimates is carried forward to characterize the impact of uncertainty in GSH conjugation metabolism in humans.

5.3.4.1.7.2. Brain tumors, male rat

Multistage modeling of the NTP bioassay data for brain gliomas in male rats was carried out in units of tetrachloroethylene AUC in blood, considering fits for one- and two-stage models. A one-stage model was found to be sufficient, with an adequate goodness-of-fit *p*-value (p = 0.11) and overall adequate visual fit (refer to Figure 5-13). There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero.

Extrapolation to humans led to an internal POD ($BMDL_{10}$) of 170 mg-hr/L-day tetrachloroethylene AUC in blood (refer to Table 5-18). The corresponding central tendency estimate was less than twofold higher, at 410 mg-hr/L-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 1.3×10^{-3} per ppm.

Dose-response modeling using administered exposure fit the data points similarly to when tetrachloroethylene AUC in blood was used (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 73 ppm tetrachloroethylene in air (refer to Table 5-19). The corresponding central tendency estimate was about twofold higher, at 180 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 1.4×10^{-3} per ppm, essentially the same as the estimate using tetrachloroethylene AUC in blood.

The multistage modeling result using tetrachloroethylene AUC in blood was carried forward to support cancer risk estimates (refer to Table 5-18).

5.3.4.1.7.3. Testicular tumors, male rat

Multistage modeling of the NTP bioassay data for testicular tumors was carried out in units of tetrachloroethylene AUC in blood, considering fits for one- and two-stage models. A one-stage model had an adequate goodness-of-fit *p*-value (p = 0.40) and overall adequate visual fit (refer to Figure 13c). There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero.

Extrapolation to humans led to an internal POD ($BMDL_{10}$) of 14 mg-hr/L-day tetrachloroethylene in blood (refer to Table 5-18). The corresponding central tendency estimate was about twofold higher, at 30 mg-hr/L-day. Linear extrapolation from the POD to low internal

dose, followed by conversion to human exposures, led to a human equivalent unit risk of 14×10^{-3} per ppm.

Dose-response modeling using administered concentration fit the data points similarly to when tetrachloroethylene AUC in blood was used (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 6.1 ppm tetrachloroethylene in air (refer to Table 5-19). The corresponding central tendency estimate was approximately twofold higher, at 13 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 16×10^{-3} per ppm, the same as the higher estimate using tetrachloroethylene AUC in blood.

The multistage modeling result using tetrachloroethylene AUC in blood was carried forward to support cancer risk estimates (refer to Table 5-18).

5.3.4.1.7.4. Mononuclear cell leukemia, male rat

Multistage modeling of the NTP bioassay data for male rat MCL was carried out in units of tetrachloroethylene AUC in blood, considering fits for one- and two-stage models. A one-stage model had an adequate goodness-of-fit *p*-value ($p = 0.18$) and overall adequate visual fit (refer to Figure 5-13d). There was no statistical improvement in fitting higher order models, as all the higher order parameters were estimated to be zero. Extrapolation to humans led to an internal POD ($BMDL_{10}$) of 15 mg-hr/L-day tetrachloroethylene in blood, and a corresponding central tendency estimate about twofold higher, at 28 mg-hr/L-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 15×10^{-3} per ppm.

Dose-response modeling using administered exposure fit the data points similarly to when tetrachloroethylene AUC in blood was used (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 6.5 ppm tetrachloroethylene in air (refer to Table 5-19). The corresponding central tendency estimate was approximately twofold higher, at 12 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 15×10^{-3} per ppm, essentially the same as the estimate using tetrachloroethylene AUC in blood.

The multistage modeling result using tetrachloroethylene AUC in blood was carried forward to support cancer risk estimates (refer to Table 5-18).

5.3.4.1.7.5. Total risk estimate for NTP (1986) male rats

The increased incidences of kidney, brain, and testicular interstitial cell tumors observed in the NTP (1986) male rats led to unit risks that ranged from about 1×10^{-3} to 15×10^{-3} per ppm, all lower than the unit risk based on male rats in the JISA (1993) study using the Michaelis-

Menten model. In order to compare the results of both studies more equitably, the overall impact of these multiple tumor types, or the risk of developing any combination of the four tumor types, was estimated. First, the tumor types were judged likely to occur independently of each other or not only in the presence of one of the other tumor types. The individual risk estimates developed above were combined for an overall estimate of risk of any combination of these four tumor types, using the approach based on maximum likelihood estimation described in Section 5.3.4.1.3.

In terms of tetrachloroethylene AUC, the POD ($BMDL_{10}$) was 8.1-mg-hr/L-day tetrachloroethylene in blood (refer to Table 5-18). The corresponding central tendency estimate was almost twofold higher, at 13 mg-hr/L-day. Linear extrapolation from the POD to low internal dose, followed by conversion to human exposures, led to a human equivalent unit risk of 25×10^{-3} per ppm.

Using administered exposure, the estimated overall risk was similar to when tetrachloroethylene AUC in blood was used (details in Appendix D). The result was directly interpretable as a human equivalent POD ($BMCL_{10}$), at 3.5 ppm tetrachloroethylene in air (refer to Table 5-19). The corresponding central tendency estimate was approximately twofold higher, at 6.1 ppm. Linear extrapolation from this POD led to a human equivalent unit risk of 29×10^{-3} per ppm, essentially the same as the higher estimate using tetrachloroethylene AUC in blood.

The combined overall risk using tetrachloroethylene AUC in blood as the dose metric for each tumor type was carried forward to support cancer risk estimates (refer to Table 5-18). Overall, the combined unit risk estimate was less than twofold higher than the highest individual unit risk. While this bioassay is less ideal for low-dose extrapolation than the JISA bioassay, it is still notable that the combined risk estimate supports the JISA study results, less than threefold lower than the highest JISA study estimate of $\sim 70 \times 10^{-3}$ per ppm.

5.3.4.1.8. Summary and discussion of site-specific dose-response modeling

The standard approach of applying the multistage model to the candidate data sets, using PBPK model-based dose metrics, yielded results that were considered adequate according to several criteria, including goodness-of-fit p-values > 0.05 and standardized residuals within ±2. However, the NRC (2010) peer review report recommended a more extensive quantitative evaluation of uncertainty due to different forms of dose-response models. In particular, NRC (2010) agreed that for several datasets, the multistage model does not fit the data at lower doses, owing to the supralinear shape in the data. Furthermore, they noted that lack of significance in goodness-of-fit tests can result from a small number of animals in each dose group, and use of

such tests to justify a selection of a dose-response model can be misleading. Therefore, for the datasets from JISA (1993), additional analyses were performed to examine whether alternative dose-response models better accounted for datasets that exhibited supralinearity, and to more generally characterize the range that would result from applying different dose-response models. The discussion here focuses on the JISA (1993) data, because these were selected as the primary source of dose-response data.

For mouse hepatocellular tumors and hemangiomas and hemangiosarcomas, the alternative analyses did not lead to better fits and did not suggest a wide range of possible results from alternative dose-response models. Therefore, for those datasets, the results from the standard multistage approach were carried forward for consideration (in some cases including an alternative dose metric in addition to the preferred one).

For male and female rat MCLs, some of the analyses yielded model results that substantially improved fit to the datasets' supralinearity. For male rat MCLs, the preferred result carried forward used the Michaelis-Menten model, with the standard multistage approach also carried forward as an alternative for comparison. However, application of the range of alternative dose-response models led to a wide (>300-fold) range of BMCL estimates, indicating that the data have difficulty supporting a robust statistical lower bound on the BMC.

For female rat MCLs, the only approach that was successful in both addressing the supralinearity and estimating a BMCL was multistage modeling of only the control and low-dose group. Moreover, for this data set, the standard multistage approach using the entire data set had the most pronounced inaccuracy with respect to the supralinearity in the data. These two results–the multistage model using the full data set and using only the control and low-dose group–were carried forward because they were the best available for this data set. The fit to the full data set likely substantially overestimates the BMD, due to the markedly high estimate for the control incidence and the markedly low estimate for the low-dose incidence. However, while the fit to only the control and low-dose groups leads to a good fit to those data, it cannot quantitatively address the possibility that the supralinearity extends below the lowest dose group. Finally, application of the range of alternative dose-response models led to an unbounded range of BMCL estimates, with some models unable to estimate a statistical lower bound on the BMC.

Because of these difficulties in fitting the individual rat MCL datasets, a subsequent analysis was performed using the combined male and female datasets. There are no biological data suggesting that the male and female rats would not reflect the same underlying toxicological dose response, and statistical tests indicated that these data could be combined. In fitting the range of available dichotomous models, it was found that the Michaelis-Menten model led to the best fit and was able to account for the supralinearity in the full data set. Moreover, the range of alternative dose-response models leads to stable estimates for the lower bound on the BMD.

Therefore, the analysis using the Michaelis-Menten model on the full, combined male and female rat MCL data set was carried forward for consideration.

Figure 5-14 shows the relative magnitudes of the unit risks associated with each tumor site. Also shown are the unit risks estimated using alternate dose metrics, including administered concentration, the range of estimates based on alternative PBPK model parameters, and the range of estimates based on the range of dose-response models available in BMDS. Finally, this figure also includes estimates based on dose-response modeling of the NTP (1986) bioassay. In terms of preferred dose metrics (refer to Section 5.3.3.2.3.1), the unit risks, rounded to one significant figure, ranged from 0.9 to 70 × 10^{-3} per ppm, about an 80-fold range.

5.3.4.2. Inhalation Unit Risk

Human inhalation cancer risk has been assessed using several different gender-species animal tumor data sets and a newly developed "harmonized" PBPK model. These results, and their uncertainties, have been discussed above and are summarized in Figure 5-14.

The majority of the NRC peer review panel recommended that the mouse hepatocellular tumors be used for cancer risk estimation. Therefore, the inhalation unit risk is **2 × 10^{-3} per ppm** or **3 × 10^{-7} per µg/m^3** (rounding to one significant digit), based on the male mouse hepatocellular tumor data from the JISA (1993) bioassay. The inhalation unit risk should not be used with exposures exceeding 60 ppm, or 400 mg/m^3 (the equivalent ambient exposures corresponding to the POD for male mouse hepatocellular tumors), because above this exposure level, the dose-response relationship is not linear, and the unit risk would tend to overestimate risk. The slope of the linear extrapolation from the human equivalent central estimate BMC_{10} is 1.9 × 10^{-7} per µg/m^3 [0.1/(5.4 × 10^5 µg/m^3)]. Some members of the NRC peer review panel recommended that the MCL data be used for cancer risk estimation. The inhalation unit risk would be 7 × 10^{-2} per ppm, or 1 × 10^{-5} per µg/m^3 (rounding to one significant digit) if it were based on the male and female rat MCL data from the JISA (1993) bioassay.

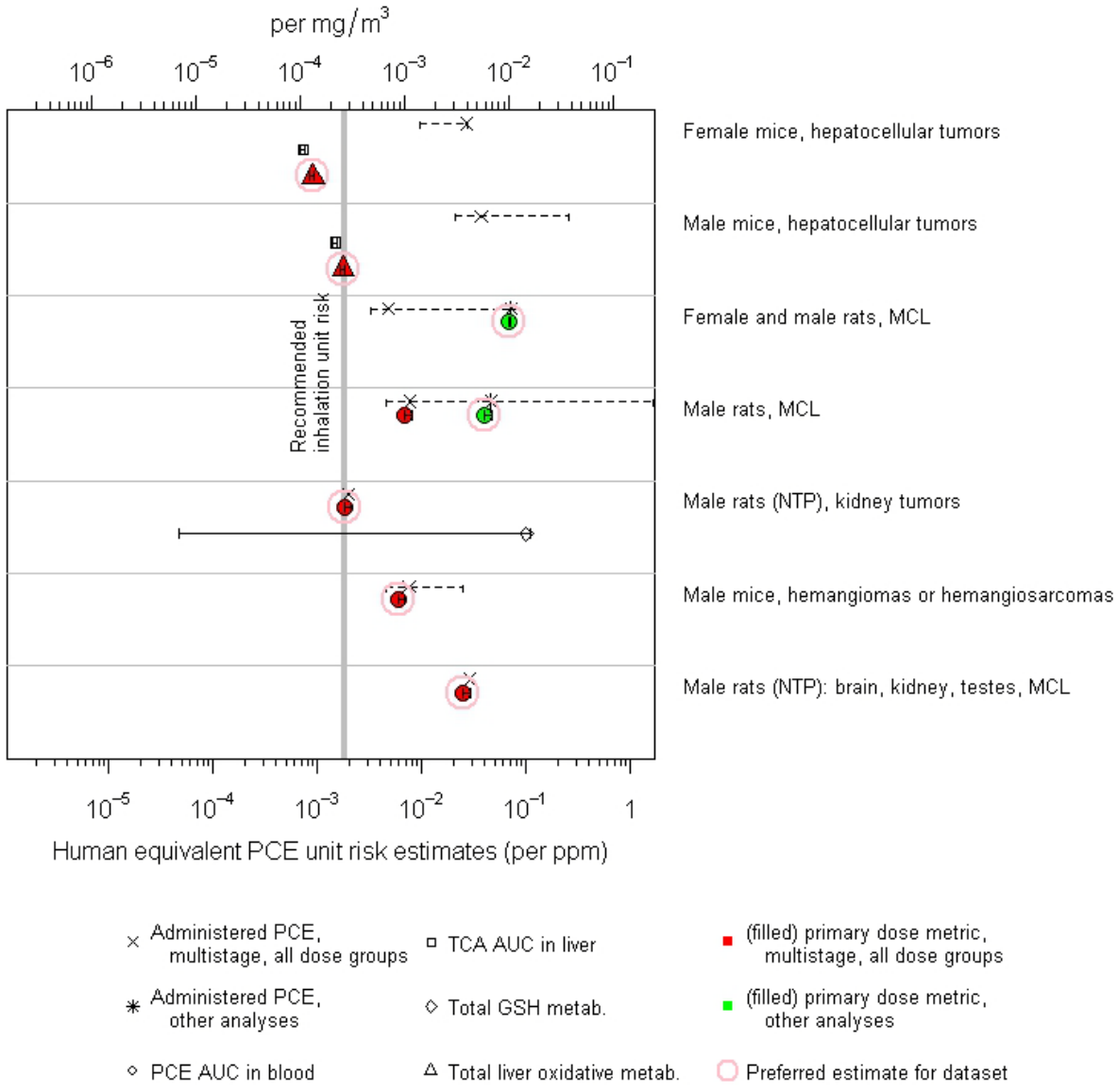


Figure 5-14. Comparison of inhalation unit risks for tetrachloroethylene derived from rodent bioassays using PBPK-based dose metrics and administered concentration.

Symbols represent results using the posterior mode PBPK model results, with filled symbols representing the preferred dose metrics (refer to Tables 5-18 and 5-19). Red-filled symbols use the multistage model with all dose groups; green-filled symbols use a different dose-response approach in response to NRC (2010) comments. Solid error bars show the range of estimates using the range of posterior modes for the human PBPK model-based conversion to a human equivalent unit risk (refer to Table 5-18). Dashed error bars show the range of unit risk estimates (based on administered concentration) using alternative dose-response models with goodness-of-fit p-values ≥ 0.10 (refer to Table 5-20). The preferred estimate for each dataset is circled in pink.

5.3.4.3. Oral Slope Factor

The oral slope factor was developed from inhalation data because the only available oral bioassay had several limitations for extrapolating to lifetime risk in humans (also refer to Section 5.3.1). First, the oral study (NCI, 1977) was conducted by gavage at relatively high doses. Human exposures are less likely to occur in boluses, and high doses are associated 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, making the oral study less useful for estimating lifetime risk. Route-to-route extrapolation from the inhalation PODs developed from the JISA study (refer to Table 5-18) was carried out using the human pharmacokinetic models described in Section 3.5. The total tumor risks from multiple sites (brain, kidney, testes, and MCL in rats and hepatocellular tumors and hemangiomas or hemangiosarcomas in mice) were estimated using the same methods as were used for the inhalation unit risk estimates, with results of 20×10^{-3} per mg/kg-day for rats in NTP (1986) and 18×10^{-3} per mg/kg-day for mice in JISA (1993). Table 5-21 and Figure 5-15 summarize all of the resulting candidate oral slope factors.

The majority of the NRC peer review panel recommended that the mouse hepatocellular tumors be used for cancer risk estimation. Therefore, the oral slope factor is **2×10^{-3} per mg/kg-day** (rounding to one significant digit) based on the male mouse hepatocellular tumor data from the JISA (1993) bioassay. The oral slope factor should not be used with exposures exceeding 50 mg/kg-day (the equivalent ambient exposure corresponding to the POD for male mouse hepatocellular tumors), because above this exposure level, the dose-response relationship is not linear, and the slope factor would tend to overestimate risk. The slope of the linear extrapolation from the human equivalent central estimate BMD_{10} is 1.5×10^{-3} per mg/kg-day [0.1/(67 mg/kg-day)]. Some members of the NRC peer review panel recommended that the MCL data be used for cancer risk estimation. The oral slope factor would be 6×10^{-2} per mg/kg-day (rounding to one significant digit) if it were based on the male and female rat MCL data from the JISA (1993) bioassay.

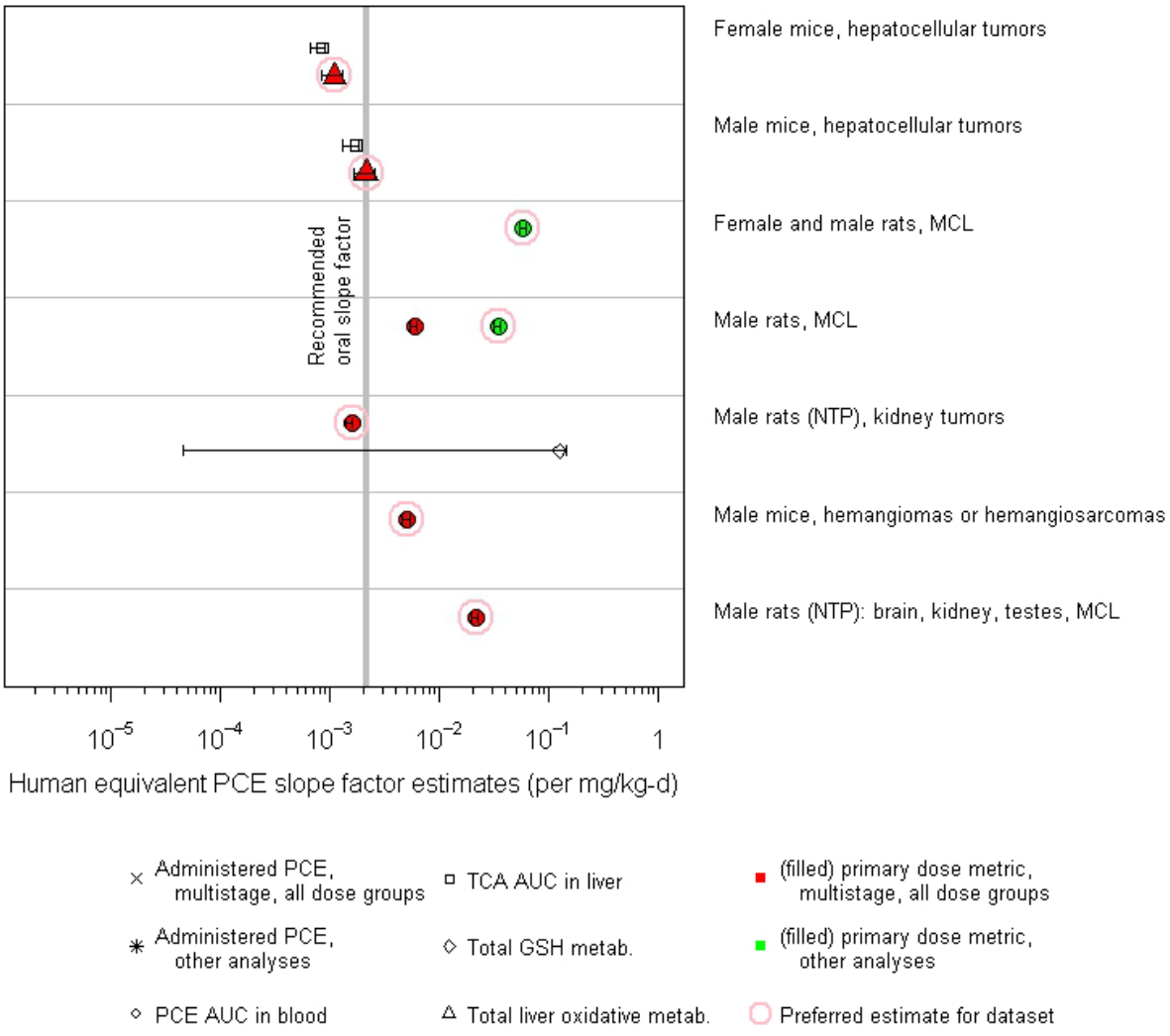


Figure 5-15. Comparison of oral slope factors for tetrachloroethylene, derived from rodent bioassays using PBPK-based dose metrics and route-to-route extrapolation.

Symbols represent results using the posterior mode PBPK model results, with filled symbols representing the preferred dose metrics (refer to Table 5-21). Red-filled symbols use the multistage model with all dose groups; green-filled symbols use a different dose-response approach in response to NRC (2010) comments. Solid error bars show the range of estimates using the range of posterior modes for the human PBPK model-based conversion to a human equivalent unit risk (refer to Table 5-21). The preferred estimate for each dataset is circled in pink.

Table 5-21. Human equivalent candidate oral slope factors, derived using primary dose metrics and multistage model; tumor incidence data from JISA (1993) and NTP (1986)

Study Group	Tumor type (multistage model with all dose groups unless otherwise specified)	Human Equivalents				
		POD[a], in internal dose units			SF×10^{-3} /internal dose unit[b]	Candidate OSF×10^{-3}/ mg/kg-day (PBPK range)[c]
Primary dose metrics						
Male mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	2.9 2.1	Total liver oxidative metabolism, mg/$kg^{0.75}$-d	49	**2.1** (1.6–2.6)
	Hemangiomas, hemangiosarcomas,	BMD_{10} $BMDL_{10}$	63 34	PCE AUC in blood, mg-hr/L-d	2.9	**5.1** (4.6–5.3)
Female mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	8.4 4.0	Total liver oxidative metabolism, mg/$kg^{0.75}$-d	25	**1.1** (0.84–1.3)
Male rats JISA (1993)	MCL	BMD_{10} $BMDL_{10}$	46 30	PCE AUC in blood, mg-hr/L-d	3.4	**5.9** (5.3–6.1)
	MCL (Michaelis-Menten)	BMD_{10} $BMDL_{10}$	20 5.0	PCE AUC in blood, mg-hr/L-d	20	**35** (31–36)
Female rats JISA (1993)	MCL	BMD_{10} $BMDL_{10}$	136 61	PCE AUC in blood, mg-hr/L-d	1.6	**2.8** (2.5–2.9)
	MCL (control and low dose groups only)	BMD_{10} $BMDL_{10}$	11 5.2	PCE AUC in blood, mg-hr/L-d	19	**33** (30–35)
Female and male rats JISA (1993)	MCL (Michaelis-Menten)	BMD_{10} $BMDL_{10}$	17 3.0	PCE AUC in blood, mg-hr/L-d	33	**58** (53–61)
Male rats NTP (1986)	Kidney tumors	BMD_{10} $BMDL_{10}$	246 110	PCE AUC in blood, mg-hr/L-d	0.90	**1.6** (1.4–1.6)
	Brain gliomas	BMD_{10} $BMDL_{10}$	400 192	PCE AUC in blood, mg-hr/L-d	0.62	**1.1** (1.0–1.1)
	Testicular interstitial cell tumors	BMD_{10} $BMDL_{10}$	31 14	PCE AUC in blood, mg-hr/L-d	7.1	**12** (11–13)
	MCL	BMD_{10} $BMDL_{10}$	28 15	PCE AUC in blood, mg-hr/L-d	6.6	**12** (10–12)
	Total risk for any of above four tumor types	BMD_{10} $BMDL_{10}$	14 8.2	PCE AUC in blood, mg-hr/L-d	12	**21** (19–22)
Alternate Dose Metrics						
Male mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	97 69	TCA AUC in liver, mg-hr/L-d	1.5	**1.7** (1.3–1.8)
Female mice JISA (1993)	Hepatocellular adenomas or carcinomas	BMD_{10} $BMDL_{10}$	292 141	TCA AUC in liver, mg-hr/L-d	0.72	**0.85** (0.65–0.89)
Male rats NTP (1986)	Kidney tumors	BMD_{05} $BMDL_{05}$	0.46 0.21	Total GSH metabolism, mg/kg0.75-d	243	**120** (0.045–140)

Table 5-21. Human equivalent candidate oral slope factors, derived using primary dose metrics and multistage model; tumor incidence data from JISA (1993) and NTP (1986) (continued)

SF = slope factor; OSF = oral slope factor.

[a]PODs were estimated at the indicated BMRs in terms of extra risk; i.e., $BMDL_{10}$ is the lower bound for the internal dose metric on the level associated with 10% extra risk. Dose units are in the first column, which include cross-species scaling to a human equivalent internal dose metric. Refer to Appendix D for dose-response modeling details.

[b]Slope Factor = $BMR/BMDL_{BMR}$ in units of risk per dose metric unit.

[c]The oral slope factor is given by the product of the slope factor in units of risk per dose metric unit and an oral dose-metric conversion factor ($DMCF_{mg/kg\text{-}day}$): Oral Slope Factor = $BMR/BMDL_{BMR} \times DMCF_{mg/kg\text{-}day}$, where the $DMCF_{mg/kg\text{-}day}$ is derived from the PBPK model. The $DMCF_{mg/kg\text{-}day}$ for each dose metric is a constant factor shown:

Dose metric	$DMCF_{mg/kg\text{-}day}$	
	Overall posterior mode	**Range of posterior modes**
Total liver oxidative metabolism	**0.0438**	0.0334–0.0459
Tetrachloroethylene blood AUC	**1.74**	1.58–1.82
TCA AUC in liver	**1.18**	0.903–1.24
Total GSH metabolism	**0.512**	0.00019–0.543

Values in **bold** correspond to using the overall posterior mode and are carried forward for consideration in the derivation of the cancer slope factor. The difference between the overall and alternative posterior modes is negligible (relative to other uncertainties) except for the Total GSH metabolism dose metric.

5.3.4.4. Uncertainties in Human Population Variability and Quantitative Adjustment for Sensitive Populations (Age-Dependent Adjustment Factors)

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. The extent of interindividual variability in tetrachloroethylene metabolism has not been characterized. As noted above, several enzymes of the oxidative and GSH metabolism, notably Cytochrome 2E1 (CYP2E1), CYP3A4, GSTZ, GSTA, GSTM, and GSTT, show genetic polymorphisms with the potential for variation in production of specific metabolites. Inducers of CYP450 enzymes such as toluene, phenobarbital, and pregnenolone-16α-carbonitrile have been shown to increase tetrachloroethylene metabolism, 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 Section 3.

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 heterogeneous 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. These considerations strengthen the scientific support for the choice of a linear nonthreshold extrapolation approach. However, because chemical-specific life-stage susceptibility data are not available, and the MOA for tetrachloroethylene has not been established, the application of age-derived adjustment factors for early life exposures, as discussed in *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), is not recommended.

5.3.4.5. Concordance of Animal and Human Risk Estimates

Sufficient human health outcome data with quality exposure characterizations linked to individual study subjects or epidemiologic studies with characterization of exposure-response using a quantitative surrogate of tetrachloroethylene exposure are not available to derive cancer risk values. An analysis of epidemiologic studies provides some limited perspectives on the human cancer risk values estimated from animal bioassays (van Wijngaarden and Hertz-

Picciotto, 2004). The analysis assigned an exposure surrogate of average tetrachloroethylene concentration to all exposed subjects based on information in the published literature (van Wijngaarden and Hertz-Picciotto, 2004). EPA prefers that the exposure-assessment approach of epidemiologic studies used for estimating lifetime cancer risk represent not only the relevant conditions and exposures (e.g., through a job-exposure matrix or exposure model), but also subject-specific quantitative estimates of exposure. The epidemiologic study (Vaughan et al., 1997) in the analysis did not meet these criteria; the study did not assign a unique exposure estimate to individual subjects, nor did it examine exposure response using a quantitative exposure surrogate. Although not sufficient to serve as a primary basis for dose-response assessment, this study does provide information without extrapolation from animals to humans.

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 the 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 unit risk based upon Vaughan et al. (1997) is $0.01/1.3 \times 10^3$ µg/m^3 = 8×10^{-6} per µg/m^3 (0.05 per ppm). A cancer risk estimate from human data using the ED_{01} as the POD is $0.01/4.8 \times 10^3$ µg/m^3 = 2×10^{-6} per µg/m^3 (0.01 per ppm). These estimates overlap with the cancer risk estimates from combined male and female rat MCL tumors in JISA (1993), and from the combined male rat brain, kidney, testes, and MCL tumors in NTP (1986).

These estimates are based on extrapolated exposure estimates, assume that laryngeal cancer is the only carcinogenic hazard in humans, and may be subject to other sources of bias. Thus, they should only be viewed as order-of-magnitude estimates.

5.3.5. Summary of Uncertainties in Cancer Risk Values

A number of uncertainties underlie the cancer unit risk for tetrachloroethylene, including the choice of study, PBPK modeling and dose metrics, cross-species scaling, low-dose extrapolation, model uncertainty, statistical uncertainty in the POD, the species/gender/tumor type combination selected, and sensitive subpopulations (refer to Table 5-22). Some suggest risks could be higher than was estimated (e.g., selection of MCL rather than mouse liver tumors, sensitive subpopulations), while others would decrease risk estimates (e.g., use of central tendency instead of lower 95% confidence bound on the POD), or have an impact of an uncertain direction. Several uncertainties are quantitatively characterized for the significantly increased rodent tumors. These include the statistical uncertainty in the POD, the range of uncertainty in PBPK modeling and dose metrics, dose-response model uncertainty, and the species/gender/tumor type combination selected. The latter three of these could either increase or decrease risk estimates. Due to limitations in the data, particularly regarding the mode of action and relative human sensitivity and variability, the quantitative impact of other uncertainties, which may have equal or greater impact, has not been explored.

Table 5-22. Summary of uncertainties in tetrachloroethylene cancer unit risk estimate

Consideration/ approach (Section)	Impact on unit risk	Decision	Justification
Bioassay (5.4.1)	↑ unit risk up to twofold if NTP study used	JISA study	JISA study used the lowest experimental exposures (reduces extrapolation uncertainty) and used three treated groups.
PBPK modeling and dose metrics (5.4.3.1.5)	Alternatives could ↑ or ↓ unit risk by an unknown extent	Relied on total liver oxidative metabolism and tetrachloroethylene AUC, in addition to administered concentration	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not clearly identified.
Cross-species scaling (5.4.3.2.2.3)	Alternatives could ↓ or ↑ unit risk (e.g., 3.5-fold ↓ [scaling by BW] or ↑ twofold [scaling by $BW^{2/3}$])	(default approach) for total oxidative or GSH metabolism; direct animal-to-human correspondence when the dose metric was an AUC	There are no data to support alternatives. Use of $BW^{3/4}$ for metabolism rates and no scaling for dose metrics expressed as AUCs are consistent treatments of the available dose metrics. While the true human correspondence is unknown, this overall approach is expected neither to over- or underestimate human equivalent risks.
Low-dose extrapolation procedure (5.4.3.2.3)	Departure from EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) POD paradigm, if justified, could ↓ or ↑ unit risk 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 nonlinearity (mutagenicity is plausible contributor and cannot be ruled out); linear approach in absence of clear support for an alternative is generally supported by scientific deliberations supporting EPA's *Guidelines for Carcinogen Risk Assessment*.
Model uncertainty	Alternatives could ↓ or ↑ unit risk	Multistage model for all tumor sites except Michaelis-Menten model for MCLs from JISA (1993) male rats, and male and female rats combined	No biologically based models available; no a priori basis for selecting a model other than multistage. Selected options tended to be intermediate among the available alternatives. Refer to Appendix D.
Statistical uncertainty at POD (5.4.4.1.7)	↓ unit risk 1.4-fold if BMC_{10} used rather than $BMCL_{10}$	BMCL (default approach for calculating plausible upper bound)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on concentration.

Table 5-22. Summary of uncertainties in tetrachloroethylene cancer unit risk estimate (continued)

Consideration/ approach (section)	Impact on unit risk	Decision	Justification
Species/gender/ tumor type combination (5.4.4.2, Figure 5-14)	Human risk could ↓ or ↑ more than an order of magnitude, depending on selected tumor type and relative species sensitivity	Male mouse hepatocellular tumors.	Recommended by majority of the NRC (2010) peer review panel.
Human population variability sensitive subpopulations (5.4.4.5)	Low-dose risk ↑ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity in metabolism or response, including whether children are more sensitive. Mutagenic MOA, which cannot be ruled out, would indicate increased early-life susceptibility.

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6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

This section summarizes the human hazard potential for tetrachloroethylene. For extensive discussions and references, refer to Section 2 for Exposure, Section 3 for toxicokinetics and physiologically based pharmacokinetic (PBPK) modeling, and Sections 4.1−4.8 for the epidemiologic and experimental studies of noncancer toxicity and carcinogenicity. Section 4.9 summarizes information on susceptibility, and Section 4.10 provides a more detailed summary of noncancer toxicity and carcinogenicity.

6.1.1. Exposure (refer to Section 2)

Tetrachloroethylene is a volatile compound with relatively low water solubility. It is widely used for dry cleaning of fabrics, for metal degreasing, and in manufacturing some consumer products and other chemicals. Tetrachloroethylene has been detected in drinking, ground, and surface water as well as in air, soil, food, and breast milk. The primary exposure routes of concern are vapor inhalation and ingestion of contaminated water. Inhalation exposure is the predominant route of exposure compared with ingestion, including from breast milk.

The highest environmental releases are to the air. Ambient tetrachloroethylene concentrations vary from source to source and with proximity to the source. Outdoors, the high volatility leads to increased ambient air concentrations near points of use (ATSDR, 1997a; U.S. EPA, 1996b). The U.S. Environmental Protection Agency (EPA) has carried out modeling to characterize the geographic distribution of tetrachloroethylene for its National-Scale Air Toxics Assessment database (U.S. EPA, 1996b). Median census tract-based tetrachloroethylene concentrations across the United States were estimated at about 0.3 $\mu g/m^3$ for urban areas and 0.1 $\mu g/m^3$ for rural areas (75% upper percentiles of 0.4 and 0.2 $\mu g/m^3$, respectively). Air exposure may also occur from vapor intrusion, or during showering or bathing as dissolved tetrachloroethylene in the warm tap water is volatilized.

Near points of use, such as dry cleaners or industrial facilities, indoor exposure to tetrachloroethylene is more significant than outdoor exposure (U.S. EPA, 2001a). Adgate et al. (2004a) measured tetrachloroethylene in outside and indoor air at school, indoor air at home, and using personal samplers on children, and demonstrated that levels are lower in homes with greater ventilation (Adgate et al., 2004a) and in homes in nonurban settings (Adgate et al., 2004b; Adgate et al., 2004a). Mean indoor air concentrations in apartments above dry-cleaning shops of 4.9 mg/m^3 have been reported [Altmann (1995); also refer to Garetano and Gochfeld

(2000); McDermott (2005); Schreiber (1993); Schreiber (2002)]. Measurements have also been made in a daycare center adjacent to a dry cleaner (NYSDOH, 2005a, b, c), and in a classroom exposed to tetrachloroethylene from an air "emission from a small chemical factory" (Monster and Smolders, 1984). Mean concentrations inside dry-cleaning facilities were reported to be 454—1,390 mg/m^3 in the United States and 164 mg/m^3 in Nordic countries during the 1960s and 1970s. Overall levels declined from 95−210 mg/m^3 in the 1980s to 20−70 mg/m^3 over the next decades in these countries (Lynge et al., 2011; Gold et al., 2008; Lynge et al., 2006).

The off-gassing of garments that have recently been dry-cleaned may be of concern [Tichenor (1990); also refer to Thomas et al. (1991)]. Relatively high tetrachloroethylene air concentrations have been measured in closets and automobiles containing freshly dry-cleaned clothing. Using dry-cleaned clothes as a source, tetrachloroethylene levels inside a stationary vehicle after 30 minutes reached 0.230 mg/m^3 (Park et al., 1998). 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 a 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).

Exposure to related compounds—including metabolites and other parent compounds that produce similar metabolites—can alter or enhance tetrachloroethylene metabolism and toxicity by generating higher internal metabolite concentrations than would result from tetrachloroethylene exposure by itself.

6.1.2. Toxicokinetics and Physiologically Based Pharmacokinetic (PBPK) Modeling (refer to Section 3)

Tetrachloroethylene is a lipophilic compound that readily crosses biological membranes. 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 (Poet et al., 2002; Nakai et al., 1999; Stewart and Dodd, 1964). Additionally, tetrachloroethylene can be transferred transplacentally and through breast milk ingestion. Refer to Section 3.1 for additional discussion of tetrachloroethylene absorption.

Once absorbed, tetrachloroethylene is distributed by first-order diffusion processes. Animal studies provide clear evidence that tetrachloroethylene distributes widely to all tissues of the body, readily crossing the blood:brain barrier and the placenta (Dallas et al., 1994b; Ghantous et al., 1986; Schumann et al., 1980; Savolainen et al., 1977b). The 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). Refer to Section 3.2 for additional discussion of tetrachloroethylene distribution.

The metabolism of tetrachloroethylene is an important determinant of its toxicity. Metabolites are generally thought to be responsible for toxicity—especially to the liver and kidney. 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 further biotransformation and processing, either through the cysteine conjugate β-lyase pathway or by other enzymes including FMO3 and CYP3A (Lash and Parker, 2001; Lash et al., 1998; Völkel et al., 1998; Birner et al., 1996; Dekant et al., 1989; Anders et al., 1988; Dekant et al., 1987; Costa and Ivanetich, 1980; Filser and Bolt, 1979; Pegg et al., 1979; Daniel, 1963). The conjugative pathway is toxicologically significant because it yields relatively potent toxic metabolites (Lash and Parker, 2001; Werner et al., 1996; Dekant et al., 1989; Vamvakas et al., 1989c; Vamvakas et al., 1989d; Anders et al., 1988; Vamvakas et al., 1987; Dekant et al., 1986b; Dekant et al., 1986d). Studies in both animals and humans indicate that overall metabolism of tetrachloroethylene is relatively limited, particularly at higher exposures [reviewed in Lash and Parker (2001)]. Although thought to be qualitatively similar, there are clear differences among species in the quantitative aspects of tetrachloroethylene metabolism (Lash and Parker, 2001; Völkel et al., 1998; Schumann et al., 1980; Ikeda and Ohtsuji, 1972). Refer to Section 3.3 for additional discussion of tetrachloroethylene metabolism.

Tetrachloroethylene is excreted from the body by pulmonary excretion of the parent compound and urinary excretion of metabolism products, with a small amount of pulmonary excretion of metabolism products. Tetrachloroethylene that is not metabolized is exhaled unchanged, and this process is the primary pathway of tetrachloroethylene excretion in humans for all routes of administration (Opdam and Smolders, 1986; Koppel et al., 1985; Monster, 1979; Stewart et al., 1977; Guberan and Fernandez, 1974; Stewart et al., 1974; Stewart and Dodd, 1964). Pulmonary excretion of (unchanged) parent compound is also important in animals (Bogen et al., 1992; Frantz and Watanabe, 1983; Schumann et al., 1980; Pegg et al., 1979; Yllner, 1961). A small amount of tetrachloroethylene has been shown to be excreted through the skin (Bolanowska and Golacka, 1972); however, it represents an insignificant percent of total tetrachloroethylene disposition. Refer to Section 3.4 for additional discussion of tetrachloroethylene excretion.

As part of this assessment, a PBPK model-based analysis of the toxicokinetics of tetrachloroethylene and its metabolites was developed in mice, rats, and humans [also reported in Chiu and Ginsberg (2011)]. This model was developed to address many of the limitations of the existing models for tetrachloroethylene. Among the most important improvements are (1) the

utilization of all the available toxicokinetic data for tetrachloroethylene and its metabolites in mice, rats, and humans; (2) the incorporation of available information on the internal toxicokinetics of TCA derived from the most current PBPK modeling of trichloroethylene and TCA; and (3) the separate estimation of oxidative and conjugation metabolism pathways. This "harmonized" PBPK model used a limited Bayesian analysis implemented using Markov chain Monte Carlo approach for parameter calibration. As expected, the major route of elimination of absorbed tetrachloroethylene is predicted to be exhalation as parent compound, with metabolism accounting for less than 20% of intake except in the case of mice exposed orally, in which metabolism is predicted to be slightly over 50% at lower exposures. In all three species, the concentration in blood, the extent of oxidation, and the amount of TCA production is well estimated, with residual uncertainties of ~twofold. However, the resulting range of estimates for the amount of GSH conjugation is quite wide in humans (~3,000-fold) and mice (~60-fold). While even high-end estimates of GSH conjugation in mice are lower than estimates of oxidation, in humans the estimated rates range from much lower to much higher than rates for tetrachloroethylene oxidation. It is unclear to what extent this range reflects uncertainty, variability, or a combination. Importantly, by separating total tetrachloroethylene metabolism into separate oxidative and conjugative pathway, this analysis reconciles the disparity between those previously published PBPK models that predicted either low or high metabolism in humans. In essence, both conclusions are consistent with the data if augmented with some additional qualifications: in humans, oxidative metabolism is low, while GSH conjugation metabolism may be high or low, with uncertainty and/or interindividual variability spanning three orders of magnitude. More direct data on the internal kinetics of tetrachloroethylene and GSH conjugation, such as trichlorovinyl glutathione or trichlorovinyl cysteine levels in blood and/or tissues, would be needed to better characterize the uncertainty and variability in GSH conjugation in humans. Because of the substantial refinements from previous PBPK models, this assessment utilizes the Chiu and Ginsberg (2011) model to calculate relevant dose-metrics that were then used in dose-response modeling. Refer to Section 3.5 for additional discussion of and details about PBPK modeling of tetrachloroethylene and metabolites.

6.1.3. Noncancer Toxicity (refer to Section 4.10.1)

Noncancer effects of tetrachloroethylene identified in exposed humans and animals include toxicity to the central nervous system, kidney, liver, immune and hematologic system, and on development and reproduction. Neurotoxic effects have been characterized in human controlled exposure, occupational and residential studies, as well as in experimental animal studies, providing evidence of an association between tetrachloroethylene exposure and neurological deficits. Tetrachloroethylene exposure primarily results in visual changes,

increased reaction time, and cognitive decrements in humans; in animal studies, effects on vision, visual-spatial function, and reaction time, as well as brain weight changes were also seen. Adverse effects on the kidney in the form of tubular toxicity, potentially mediated through tetrachloroethylene GSH conjugation, have been reported in numerous well-conducted animal studies. Although human studies have not systematically investigated nephrotoxicity, an association between tetrachloroethylene exposure via inhalation and chronic kidney disease, as measured by urinary excretion of renal proteins and end-stage renal disease, is supported. The developmental and reproductive toxicity database for tetrachloroethylene includes a range of data from appropriate well-conducted studies in several laboratory animal species plus limited human data. Evidence of liver toxicity is primarily from several well-conducted rodent studies, including chronic bioassays.

Other toxicity endpoints are less well characterized. The few published reports of experimental studies examining immune or hematologic system toxicity are consistent with the limited findings in the human occupational studies. These include evidence of an effect of tetrachloroethylene exposure on red blood cells [decreased RBCs (Ebrahim et al., 2001), or decreased erythrocyte colony-forming units (Seidel et al., 1992)], with reversible hemolytic anemia observed in female mice exposed to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age in one series of studies (Marth et al., 1989; Marth, 1987; Marth et al., 1985a; Marth et al., 1985b). Ebrahim et al. (2001) also observed decreased hemoglobin, platelet counts, and packed cell volume, and increased WBC counts. The relative lack of additional data, including confirmatory reports of immunotoxic or hematologic toxicity with low continuous exposures beginning in early lifestages, taken together with evidence of immunotoxicity from structurally related solvents (Cooper et al., 2009), contributes to uncertainty in the database for tetrachloroethylene. No human studies identified adverse effects on the respiratory tract, and no lung toxicities in rodents were reported in chronic bioassays (NTP, 1986; NCI, 1977) or other published reports.

6.1.3.1. Neurological Effects (refer to Section 4.1)

Human and animal studies provide complementary evidence regarding the association of neurobehavioral deficits and tetrachloroethylene exposure. Tetrachloroethylene exposure in humans has primarily been shown to affect visual function (including color vision) and visuospatial memory and other aspects of cognition. Brain-weight changes have been measured in animal studies. A more in-depth discussion of the human neurotoxicological studies can be found in Section 4.1.1.3, and the animal inhalation and oral or i.p. exposure studies are discussed in Sections 4.1.2.1 and 4.1.2.2, respectively.

Visual contrast sensitivity deficits as well as color discrimination deficits are commonly present prior to detectable pathology in the retina or optic nerve head. These deficits are, thus, among the earliest signs of disease and potentially more sensitive measures than evoked potentials from visual stimuli (Regan, 1989). Several independent lines of evidence can be found in the occupational and residential exposure studies to support an inference of visual deficits following chronic tetrachloroethylene exposure. The studies that observed effects on color vision using the Lanthony D-15 color vision test include cross-sectional and longitudinal designs in dry cleaning (Gobba et al., 1998; Cavalleri et al., 1994) and residential (Schreiber et al., 2002) settings. Decrements in color confusion were reported among all workers exposed to a mean TWA of 6 ppm for an average of 8.8 years (Cavalleri et al., 1994). A significant dose-response relationship between CCI value and tetrachloroethylene concentration ($p < 0.01$) was also observed in Cavalleri et al. (1994). As noted previously, the color vision testing in this study was blinded to exposure level of the study participants, and the study participants were well matched in terms of age, smoking, and alcohol use. A follow-up of these workers 2 years later (Gobba et al., 1998) showed greater loss in color discrimination in those who were subsequently exposed to a higher concentration [increase in geometric mean from 1.7 to 4.3 ppm], with no change in those exposed to lower concentrations [decrease in geometric mean from 2.9 to 0.7 ppm]). Although Gobba et al. (1998) demonstrated persistent color confusion effects in this follow up evaluation, the study exposures are not clearly characterized over the course of the 2-year duration. Nakatsuka et al. (1992) did not observe an association with color vision among dry cleaners in China ($n = 64$, geometric mean: TWA 11 and 15 ppm in females and males, respectively), but the relative insensitivity of the specific type of color vision test used in this study (Lanthony, 1978) is a likely explanation for these results. Effects on color vision were also observed among 14 dry cleaners in the small study in Malaysia by Sharanjeet-Kaur et al. (2004), but this study provides little weight to the strength of the evidence because of the lack of exposure information (other than job title), and differences between dry cleaners and controls regarding test conditions and smoking habits. Two other small studies also reported lower scores on the Lanthony D-15 color vision test in much lower exposure settings, but the differences were not statistically significant. A study of residents living above dry cleaners (mean tetrachloroethylene exposure during active dry cleaning = 0.4 ppm), reported mean CCI scores of 1.33 and 1.20 for 17 exposed and 17 controls, respectively ($p = 0.26$). A study of workers in a daycare center located in a building with a dry-cleaning business (mean tetrachloroethylene exposure: 0.32 ppm) reported mean CCI scores of 1.22 and 1.18 in the exposed daycare workers and controls, respectively ($p = 0.39$) (Schreiber et al., 2002). Overall, the evidence reveals a high degree of consistency in this aspect of visually mediated function.

Visual contrast sensitivity changes were reported in two NYSDOH residential studies. In a small pilot study (4 children and 13 adults), mean scores for visual contrast sensitivity (using a near vision visual contrast sensitivity test) across spatial frequencies were statistically significantly lower in exposed residents than in controls, indicating poorer visual function in the exposed groups (Schreiber et al., 2002). Controls were age- and sex-matched to the exposed group, and both groups were English speaking and of predominately Caucasian ethnicity; however, they were drawn from different geographic areas. In addition, two of the four exposed children had diagnoses of learning disabilities or developmental delays, which could affect performance on this type of test. In the larger study (NYSDOH, 2010, 2005a, b), the test (Functional Acuity Contrast Test, FACT) assessed far vision visual contrast sensitivity, and the test had a low rate of detecting visual contrast changes. For contrast vision, a number of analyses in NYSDOH (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a) suggest a vulnerability among children. However, exposure to >0.015 ppm (>100 $\mu g/m^3$) tetrachloroethylene was highly correlated with race and children's age. Additionally, the sample sizes in the highest exposure group, especially in higher income, nonminority groups, makes it difficult to fully examine possible effects of income, race, and age on vision. Therefore, while both studies report visual contrast sensitivity changes, with exposed children being more sensitive, there are concerns with the methodological and analytic approaches in these studies.

Acute human exposure studies reported increased latencies of up to 3.0 ms in visual evoked potentials (Altmann et al., 1990) and changes in EEGs (magnitude of effect was not specified) (Hake and Stewart, 1977; Stewart et al., 1970) at higher exposures ranging from 340 to 680 mg/m^3.

In rats, acute inhalation exposure to tetrachloroethylene results in significant changes to the flash-evoked potential at 800 ppm (Mattsson et al., 1998) and a decrease in F2 amplitudes of the steady state visual evoked potential at 250 ppm (Boyes et al., 2009). In a subchronic exposure study (13 weeks, up to 800 ppm tetrachloroethylene), changes in flash-evoked potential responses were not observed at tetrachloroethylene exposures up to 200 ppm. In the 800 ppm group, there was a significant increase in the amplitude and a significant increase in latency (~3.0 ms) of the mid-flash-evoked potential waveform (N3), but histopathological lesions were not observed in the examination of the visual system brain structures [e.g., visual cortex; optic nerve; Mattsson et al. (1998)].

Effects on visuospatial memory in humans were also reported in each of the studies that examined this measure (Altmann et al., 1995; Echeverria et al., 1995; Echeverria et al., 1994; Seeber, 1989). These effects (increased response times or cognition errors) were observed in occupational and residential studies, and the occupational studies were quite large, involving 101, 65, and 173 dry-cleaning workers in Seeber (1989), Echeverria et al. (1995), and Echeverria

et al. (1994), respectively. Several different types of tests were used including digit reproduction (Seeber, 1989), switching, pattern memory, and pattern recognition (Echeverria et al., 1995; Echeverria et al., 1994), and the Benton test (Altmann et al., 1995). Exposure ranges for the increased reaction time observations (LOAELs) ranged from 4.99 to 102 mg/m^3 (Altmann et al., 1995; Echeverria et al., 1995; Ferroni et al., 1992). The changes in the cognitive tasks were observed at exposures (LOAELs) ranging from 53.9 to 364.22 mg/m^3 (Spinatonda et al., 1997; Echeverria et al., 1995; Seeber, 1989). All of these studies except Altmann et al. (1995) indicate that the neurobehavioral assessment was blinded to knowledge of the exposure level of the subject, and all of the studies adjusted for potentially confounding factors. It should be noted, however, that residual confounding from education-level differences between exposed and referent subjects may still be present in Altmann et al. (1995).

Increased reaction time, increased number of false alarms, and decreased trial completions in a signal detection task (measures of decreased attention) were reported in an acute (60 minutes) exposure (6,782 mg/m^3 or higher) study in rats (Oshiro et al., 2008). Additionally, 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 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.

An occupational exposure study (n=60) (Ferroni et al., 1992) and a residential exposure study (n = 14) (Altmann et al., 1995), with mean exposure levels of 15 and 0.7 ppm, respectively, reported significant increases in simple reaction time of 24 ms (11% increase) (Ferroni et al., 1992) and 40 and 51.1 ms (15 and 20% increases`, respectively`, for two separate measurements) (Altmann et al., 1995) for the exposed subjects. A third study, Lauwerys et al. (1983), reported better performance on simple reaction time in 21 exposed workers (mean TWA: 21 ppm) compared with controls when measured before a work shift but not when measured after work.

The changes in brain weight, DNA/RNA, and neurotransmitter levels that were observed in the animal studies are highly supportive of the neurobehavioral changes observed with tetrachloroethylene exposure. Changes in brain DNA, RNA, or protein levels and lipid composition were altered following inhalation, with changes observed in cerebellum, hippocampus, and frontal cortex (Wang et al., 1993; Rosengren et al., 1986; Savolainen et al., 1977a; Savolainen et al., 1977b). The replication of these changes in biochemical parameters and effects in brain weight in both rats and gerbils is pathognomonic. Changes in neurotransmitters systems (Briving et al., 1986; Honma et al., 1980a; Honma et al., 1980b) and

circadian rhythm (Motohashi et al., 1993) in animal studies are consistent with neuroendocrine alterations observed in humans (Ferroni et al., 1992).

In conclusion, the weight of evidence across the available studies of humans and animals exposed to tetrachloroethylene indicates that chronic exposure to tetrachloroethylene can result in decrements in color vision, visuospatial memory, and possibly other aspects of cognition and neuropsychological function, including reaction time.

6.1.3.2. Summary of Other Noncancer Adverse Effects (refer to Sections 4.2, 4.3, 4.6, and 4.7)

In addition to evidence of toxicity to the central nervous system, tetrachloroethylene has been shown to adversely affect the kidney, liver, immune and hematologic systems, as well as development and reproduction. The human and animal evidence for these effects is summarized in the paragraphs below.

6.1.3.2.1. Kidney Toxicity (refer to Section 4.2)

The human evidence for kidney effects is limited. Most available reports do not include information on the standard battery of tests for kidney function and only one study (Calvert et al., 2011) reported on end-stage renal disease (ESRD). However, an association between tetrachloroethylene exposure via inhalation and chronic kidney disease is supported by evidence of urinary excretion of renal proteins (Verplanke et al., 1999; Mutti et al., 1992) and higher ESRD, particularly hypertensive ESRD, with higher exposures (Calvert et al., 2011). Mutti et al. (1992) reported statistically significant increases in retinol binding protein, β2μ-globulin, and albumin in urine among dry cleaners as compared with matched controls. In addition, for seven different urinary markers, the prevalence of individuals with abnormal values (>95th percentile of controls) was four- to fivefold greater in the exposed group. Adverse effects on the kidney have been observed in studies of animals exposed to high concentrations of tetrachloroethylene by inhalation (JISA, 1993; NTP, 1986), oral gavage (Ebrahim et al., 2001; Ebrahim et al., 1996; Jonker et al., 1996; Green et al., 1990; Goldsworthy et al., 1988; NCI, 1977) and by intraperitoneal injection of tetrachloroethylene metabolites (Elfarra and Krause, 2007). The nephrotoxic effects include increased kidney-to-body weight ratios, hyaline droplet formation, glomerular "nephrosis," karyomegaly (enlarged nuclei), cast formation, and other lesions or indicators of renal toxicity. Overall, multiple lines of evidence support the conclusion that tetrachloroethylene causes nephrotoxicity in the form of tubular toxicity, mediated potentially through GSH conjugation products. Limitations to the database include the lack of human studies investigating drinking water or other oral tetrachloroethylene exposures on kidney toxicity.

6.1.3.2.2. Liver Toxicity (refer to Section 4.3)

Two of four studies of occupationally exposed dry cleaners showed early indications of liver toxicity, namely sonographic changes of the liver and altered serum concentrations of one liver enzyme indicative of liver injury (Brodkin et al., 1995; Gennari et al., 1992). Frank liver disease was not observed among these workers, nor were changes in other biomarkers indicative of liver toxicity (e.g., serum transaminases), not unexpected given that subjects with signs of liver disease were excluded in both studies. Liver toxicity was reported in multiple animal species exposed to tetrachloroethylene via inhalation and oral routes of exposure. The effects were characterized by increased liver weight, fatty changes, necrosis, inflammatory cell infiltration, triglyceride increases and proliferation (Philip et al., 2007; Ebrahim et al., 1996; Jonker et al., 1996; Berman et al., 1995; JISA, 1993; Odum et al., 1988; Goldsworthy and Popp, 1987; NTP, 1986; Buben and O'Flaherty, 1985; Kjellstrand et al., 1984; Schumann et al., 1980).

6.1.3.2.3. Immunologic and Hematopoietic Toxicity (refer to Section 4.6)

The strongest human study examining immunologic and hematologic effects of tetrachloroethylene exposure in terms of sample size and use of an appropriately matched control group is the study of 40 male dry-cleaning workers (mean exposure levels <140 ppm; mean duration 7 years; mean blood tetrachloroethylene levels 1,685 µg/L) by Emara et al. (2010). Statistically significant decreases in red blood cell count and hemoglobin levels and increases in total white cell counts and lymphocyte counts were observed in the exposed workers compared to age- and smoking-matched controls. Similar effects were observed in mice (Ebrahim et al., 2001). In addition, increases in several other immunological parameters, including T lymphocyte and natural killer cell subpopulations, IgE, and interleukin-4 levels were observed in tetrachloroethylene-exposed dry-cleaning workers (Emara et al., 2010). These immunologic effects suggest an augmentation of Th2 responsiveness. The available data from experimental studies assessing immunotoxic responses in animals are limited (Hanioka et al., 1995b; Germolec et al., 1989; Aranyi et al., 1986), with one study (Aranyi et al., 1986) suggesting that short-term exposures may result in decreased immunological competence (immunosuppression) in CD-1 mice. The limited laboratory animal studies of hematological toxicity demonstrated an effect on red blood cells [decreased RBC (Ebrahim et al., 2001), or decreased erythrocyte colony forming units (Seidel et al., 1992)], with reversible hemolytic anemia observed in female mice exposed to low drinking water levels (0.05 mg/kg-day) of tetrachloroethylene beginning at 2 weeks of age in one series of studies (Marth et al., 1989; Marth, 1987; Marth et al., 1985a; Marth et al., 1985b). Ebrahim et al. (2001) also observed decreased hemoglobin, platelet counts and packed cell volume, and increased WBC counts. The results of these studies, while limited, support the human epidemiology studies. 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. The relative lack of additional data, including confirmatory reports of immunotoxic or hematologic toxicity with low continuous exposures beginning in early lifestages, taken together with evidence of immunotoxicity from structurally related solvents (Cooper et al., 2009), contributes to uncertainty in the database for tetrachloroethylene.

6.1.3.2.4. Reproductive Toxicity (refer to Section 4.7)

The epidemiologic database is inconclusive concerning potential effects of tetrachloroethylene exposure on spermatogenesis, menstruation, fertility or delayed conception (Sallmen et al., 1998; Sallmen et al., 1995; Eskenazi et al., 1991a; Eskenazi et al., 1991b; Zielhuis et al., 1989; Rachootin and Olsen, 1983). One study of primarily unionized workers in the dry-cleaning and laundry industries in California observed subtle deficits in sperm quality in relation to increasing levels of three measures of exposure, including tetrachloroethylene in exhaled breath (Eskenazi et al., 1991a). This observation is supported by one report of abnormal sperm in mice (Beliles et al., 1980). Several studies of maternal occupational exposure to tetrachloroethylene suggest an increased risk of spontaneous abortion, particularly at higher levels (Doyle et al., 1997; Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989), but other studies did not report an association with maternal (Ahlborg, 1990a; Olsen et al., 1990) or paternal (Eskenazi et al., 1991a; Lindbohm et al., 1991; Taskinen et al., 1989) exposure. Some studies observed an increased odds ratio ranging from 1.4 to 4.7, but risk estimates were statistically imprecise and some studies were limited in their ability to evaluate potential confounding (Windham et al., 1991; Lindbohm et al., 1990; Olsen et al., 1990; Bosco et al., 1987). In general, the studies that used a more precise definition of exposure, or categorized exposure into levels of increasing dose or intensity, observed higher risk estimates (Doyle et al., 1997; Lindbohm et al., 1990; Olsen et al., 1990; Kyyronen et al., 1989). No associations with incidence of spontaneous abortion were observed among two populations exposed to tetrachloroethylene in drinking water, although the window of exposure used to assess risk in both studies may not have had been precise enough to detect a small elevation in risk (Aschengrau et al., 2009a; Aschengrau et al., 2008; Lagakos et al., 1986). 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 (Carney et al., 2006; Szakmáry et al., 1997; Nelson et al., 1979; Schwetz et al., 1975) [and in the F1 generation but not the F2 generation of Tinston (1994)].

6.1.3.2.5. Developmental Toxicity (refer to Section 4.7)

Stillbirths, congenital anomalies, or decreased birth weight were not associated with maternal or paternal occupational exposure to tetrachloroethylene in several epidemiologic studies (Lindbohm, 1995; Windham et al., 1991; Olsen et al., 1990; Kyyronen et al., 1989; Taskinen et al., 1989; Bosco et al., 1987). However, the studies analyzed congenital anomalies in a combined category, and the number of exposed cases for specific types of anomalies was not sufficient to evaluate risk with statistical precision. Some studies of tetrachloroethylene in drinking water reported that exposure during pregnancy is associated with low birth weight (Bove et al., 1995; Lagakos et al., 1986), eye/ear anomalies (Lagakos et al., 1986), and oral clefts (Aschengrau et al., 2009b; Bove et al., 1995; Lagakos et al., 1986). No associations with prenatal tetrachloroethylene exposure in drinking water were reported for small for gestational age (Aschengrau et al., 2008; Bove et al., 1995), other classifications of congenital anomalies [e.g., musculoskeletal, cardiovascular (Lagakos et al., 1986)], or deficits in attention or educational performance (Janulewicz et al., 2008). Although a small increase in risk of small for gestational age was reported for infants exposed prenatally to tetrachloroethylene at the Camp Lejeune military base (Sonnenfeld et al., 2001), the finding remains inconclusive until ATSDR completes its reanalysis. Participants in some of the studies of drinking water contamination were exposed to multiple pollutants (Bove et al., 1995; Lagakos et al., 1986), and it was not possible to disentangle substance-specific risks. In animals, the developmental toxicity database provides evidence of decreased prenatal survival, decreased fetal growth, delays in skeletal ossification, and increased incidences of malformations following in utero exposure in rats, mice, and/or rabbits (Carney et al., 2006; Szakmáry et al., 1997; Narotsky and Kavlock, 1995; Schwetz et al., 1975). The decreased survival and malformation findings in laboratory mammals were supported by data from whole embryo culture (Saillenfait et al., 1995) and Japanese medaka assays (Spencer et al., 2002; Saillenfait et al., 1995). Alterations in neurological function following pre- and/or postnatal inhalation exposures to tetrachloroethylene were observed in rats by Szakmáry et al. (1997), Nelson et al. (1979), Fredriksson et al. (1993), and Tinston (1994). These findings were supported by a study that found reductions in brain acetylcholine and dopamine in rat offspring following gestational tetrachloroethylene exposures (Nelson et al., 1979). Limitations of the inhalation developmental toxicity studies include the lack of dose-response information due to the use of a single treatment level in the prenatal developmental toxicity assessment by Schwetz et al. (1975); the lack of either maternal or developmental toxicity in Hardin et al. (1981); and absence of methodological details in study reporting (Szakmáry et al., 1997).

6.1.4. Carcinogenicity (refer to Section 4.10.2)

Following EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, tetrachloroethylene is "likely to be carcinogenic in humans by all routes of exposure." This characterization is based on suggestive evidence of carcinogenicity in epidemiologic studies and conclusive evidence that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, increases tumor incidence (JISA, 1993; NTP, 1986; NCI, 1977). Tetrachloroethylene increased the incidence of liver tumors (hepatocellular adenomas and carcinomas) in male and female mice and of mononuclear cell leukemia (MCL) in both sexes of rats. These findings were reproducible in multiple lifetime bioassays employing different rodent strains and, in the case of mouse liver tumors, by inhalation and oral exposure routes. Additional tumor findings in rats included significant increases in the NTP bioassay of testicular interstitial cell tumors and kidney tumors in males, and brain gliomas in males and females. In mice, hemangiosarcomas in liver, spleen, fat, and subcutaneous skin were reported in males in the JISA study. The available epidemiologic studies provide a pattern of evidence associating tetrachloroethylene exposure and several types of cancer, specifically bladder cancer, non-Hodgkin lymphoma, and multiple myeloma. Associations and exposure-response relationships for these cancers were reported in studies using higher quality (more precise) exposure-assessment methodologies for tetrachloroethylene. Confounding by common lifestyle factors such as smoking are unlikely explanations for the observed results. For other sites, including esophageal, kidney, lung, liver, cervical, and breast cancer, more limited data are available.

The specific active moiety(ies) and mode(s) of action involved in the carcinogenicity of tetrachloroethylene and its metabolites are not fully characterized. For rat kidney tumors, it is generally believed that metabolites resulting from GSH conjugation of tetrachloroethylene are involved. The hypothesized modes of action for this endpoint include mutagenicity, peroxisome proliferation, α2u-globulin nephropathy, and cytotoxicity not associated with α2u-globulin accumulation. For mouse liver tumors, it is generally believed that metabolites resulting from P450-mediated oxidation of tetrachloroethylene are involved. The mode of action (MOA) hypotheses for this endpoint concern mutagenicity, epigenetic effects (especially DNA hypomethylation), oxidative stress, and receptor activation (focusing on a hypothesized PPARα activation MOA). However, the available evidence is insufficient to support the conclusion that either rat kidney or mouse liver tumors are mediated solely by one of these hypothesized modes of action. In addition, no data are available concerning the metabolites or the mechanisms that may contribute to the induction of other rodent tumors (including mononuclear cell leukemia, brain gliomas, or testicular interstitial cell tumors in exposed rats and hemangiosarcomas in exposed mice). Furthermore, no mechanistic hypotheses have been advanced for the human

cancers suggested to be increased with tetrachloroethylene exposure in epidemiologic studies, including bladder cancer, non-Hodgkin lymphoma and multiple myeloma. Although tetrachloroethylene is largely negative in genotoxicity assays—including in the Ames mutagenicity test—tetrachloroethylene has been shown to induce modest genotoxic effects (e.g., micronuclei induction following in vitro or in vivo exposure, and DNA binding and single strand breaks in tumor tissue) and mutagenic effects under certain metabolic activation conditions. In addition, some tetrachloroethylene metabolites have been shown to be mutagenic. Thus, the hypothesis that mutagenicity contributes to the tetrachloroethylene carcinogenesis cannot be ruled out for one or more target organs, although the specific metabolic species or mechanistic effects are not known.

6.1.5. Susceptibility (refer to Section 4.9)

There is some evidence that certain populations might be more susceptible to exposure to tetrachloroethylene. Attributes that may increase susceptibility to tetrachloroethylene include age, gender, race/ethnicity, genetics, preexisting disease, lifestyle factors, nutritional status, socioeconomic status, and multiple exposures and cumulative risk. Although there is more information on early life exposure to tetrachloroethylene than on other potentially susceptible populations, there remain a number of uncertainties regarding childhood susceptibility. Although inhalation of tetrachloroethylene is believed to be of most concern, pathways of exposure for children are not well characterized. Tetrachloroethylene has been shown to pass through the placenta in rodent studies (Szakmáry et al., 1997; Ghantous et al., 1986), but the extent to which this occurs in humans is not known. For some infants the primary route of exposure may be through breast milk ingestion (refer to Sections 2.2.4 and 3.2), while for other infants the dose received through ingestion of breast milk will become insignificant when compared with inhalation exposure (Schreiber, 1997). The amount of tetrachloroethylene ingested from food is not well described; and it is not known to what extent tetrachloroethylene is absorbed by a child and to which organs tetrachloroethylene and its metabolites may be distributed. The neurological effects of tetrachloroethylene may constitute the most sensitive endpoints of concern for noncancer effects, and limited data show that early life-stages may be more susceptible to visual deficits than are adults (Storm et al., 2011 [previously reported in NYSDOH, 2010]; NYSDOH, 2005a; Schreiber et al., 2002), yet developmental neurotoxic effects, particularly in the developing fetus, need further evaluation using age-appropriate testing for assessment. There are a number of adverse health effects observed uniquely in early lifestages, with no comparable observations in adults to determine relative sensitivity (e.g., birth outcomes, autism, or allergy); conversely, there are some adverse outcomes that have been observed only in adults.

There is suggestive evidence that there may be greater susceptibility among the elderly, but the available data are much more limited with related uncertainties. Improved PBPK modeling that contains physiologic parameter information for infants and children (including, for example, the effects of maternal inhalation exposure and the resulting concentration in breast milk) and for older adults, and validation of these models, will aid in determining differences in life stage toxicokinetics of tetrachloroethylene. The differences reported in the literature may reflect a true difference in susceptibility by life stage, an incomplete assessment of these outcomes in all life-stages, or latent outcomes associated with earlier exposure. 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.

For other susceptibility factors, the data are more limited and based mainly on nonchemical specific data that provides information on variation in physiology, exposure, and toxicokinetics. Until quantitative conclusions can be made for each susceptibility factor, it will be difficult to consider the impacts of changes in multiple susceptibility factors. In addition, further evaluation of the effects of aggregate exposure to tetrachloroethylene from multiple routes and pathways is needed. Similarly, the effects due to coexposures to other compounds with similar or different MOAs need to be evaluated.

6.2. DOSE-RESPONSE ASSESSMENT

This section summarizes the major conclusions of the dose-response analysis for tetrachloroethylene noncancer effects and carcinogenicity, with more detailed discussions in Section 5.

6.2.1. Noncancer Effects (refer to Section 5.1)

The database of human and animal studies on inhalation toxicity of tetrachloroethylene is adequate to support derivation of inhalation and oral reference values. A number of targets of toxicity from chronic exposure to tetrachloroethylene have been identified in published animal and human studies. These targets include the central nervous system, kidney, liver, immune and hematologic system, and development and reproduction. In general, neurological effects were judged to be associated with lower tetrachloroethylene exposures.

6.2.1.1. Selection of Principal Studies and Critical Effect (refer to Section 5.1.1)

The evidence for human neurotoxicity includes 12 well-conducted epidemiological studies of tetrachloroethylene exposure. Of these, seven examined occupational exposure (i.e., Schreiber et al., 2002; Gobba et al., 1998; Spinatonda et al., 1997; Echeverria et al., 1995; Cavalleri et al., 1994; Ferroni et al., 1992; Seeber, 1989), several examined residential exposure

(i.e., Storm et al., 2011 [previously reported in NYSDOH, 2010]; Schreiber et al., 2002; Altmann et al., 1995) and two were acute-duration experimental chamber studies (i.e., Altmann et al., 1990; Hake and Stewart, 1977). The animal database comprises acute-duration and subchronic-duration studies of the effects of tetrachloroethylene on functional neurological endpoints (functional observation battery, motor activity) (i.e., Oshiro et al., 2008; Kjellstrand et al., 1985), on sensory system function as assessed by evoked potential (i.e., Boyes et al., 2009; Mattsson et al., 1998), or pathological changes in the brain (i.e., Wang et al., 1993).

Principal study selection from these candidate studies of central nervous system effects involved evaluation of study characteristics as identified in Table 5-2. To summarize, human studies are preferred to animal studies, as are studies of chronic duration and in residential settings, if available and of adequate quality. Study quality attributes evaluated include the comparability of study populations and the quality of exposure information (in human studies), and effect measurements. Three human studies—Seeber (1989), Cavalleri et al. (1994), and Echeverria et al. (1995)—were considered to be more methodologically sound based on study quality attributes, including study population selection, exposure measurement methods, and endpoint measurement methods listed in Table 5-2. However, the NRC (2010) characterized the Seeber (1989) study as having discrepant results based on worse mean test scores (for neurologic signs, emotional lability, choice reaction time, cancellation d2 and digit symbol) in the low- compared with high-exposure group. Therefore, Seeber (1989) was not among those recommended by NRC for consideration in deriving the RfC.

NRC (2010) recommended five studies for consideration in deriving the RfC (Altmann et al., 1990; Boyes et al., 2009; (Echeverria et al., 1995); Cavalleri et al., 1994; and Gobba et al., 1998). Two acute studies recommended for consideration by NRC [the human chamber study of Altmann et al. (1990) and the rodent study of Boyes et al. (2009)] were judged by EPA to be supportive, but were not considered further for deriving candidate RfCs because EPA gives preference to quality studies of chronic, human exposures over studies of acute exposures. In addition, two of the other studies recommended by NRC (2010), Cavalleri et al. (1994), and Gobba et al. (1998), evaluated the same cohort, and the earlier study was preferred by EPA due to its use of a control group and the clearer identification of a POD (refer to section 5.1.1.3.2). Thus, two studies—Cavalleri et al. (1994) and Echeverria et al. (1995)—are considered principal studies by EPA for the RfC. Endpoints selected for the candidate RfCs were reaction time measures (Echeverria et al., 1995), cognitive changes (Echeverria et al., 1995), and visual function changes (Cavalleri et al., 1994).

6.2.1.2. Uncertainties and Application of Uncertainty Factors (UFs) (refer to Sections 5.1.3, 5.2.3)

For the studies from which candidate reference values were derived, it was determined that PODs could not be derived using dose-response modeling, due to no control group (Echeverria et al., 1995) or lack of an important covariate (age) (Cavalleri et al., 1994). Each of the candidate studies provided lowest-observed-adverse-effect levels (LOAELs) that were selected as PODs. The adjusted LOAELs are as follows: 56 mg/m^3 [for either visual reproduction, pattern memory, and pattern recognition, or reaction time in pattern memory in Echeverria et al. (1995)] and 15 mg/m^3 [for color confusion in Cavalleri et al. (1994)]. The application of uncertainty factors is based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002); Section 4.4.5], which address five areas of uncertainty. No adjustment of the PODs was needed for animal-to-human extrapolation uncertainty. Additionally, no adjustment was needed for subchronic-to-chronic uncertainty because these studies involved chronic exposures. An overall uncertainty factor of 1,000 was applied to each selected POD, comprised of the following uncertainty factors (UFs): An UF of 10 was applied to account for human variability in the effects that were used for the derivation of the RfC. An UF of 10 was applied for the extrapolation from a LOAEL to a NOAEL because the PODs from the studies were LOAELs. An UF of 10 was applied to address the lack of data to adequately characterize the hazard and dose response in the human population. The following critical data gaps have been identified: uncertainties associated with database deficiencies on neurological, developmental, and immunological effects.

6.2.1.3. Reference Concentration (refer to Section 5.1.3)

An uncertainty factor of 1,000 was applied to the PODs for the following endpoints from the two principal neurotoxicological studies: color vision changes (Cavalleri et al., 1994); and cognitive and reaction time changes (Echeverria et al., 1995). The candidate RfCs derived from these endpoints span a range from 0.015 to 0.056 mg/m^3. The RfC for tetrachloroethylene is **0.04 mg/m^3**, the midpoint of this range rounded to one significant figure.

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The overall confidence in the RfC is medium. Although the confidence in the evidence of neurotoxicological hazard is high, the estimates from studies for which candidate RfCs were calculated are of medium confidence. These studies were considered to be methodologically sound based on study quality attributes, including study population selection, exposure measurement methods, and endpoint measurement methods. Other strengths are that

they are human studies of chronic duration, obviating the need for extrapolation across species and exposure duration. However, high confidence was not attained for the studies for which candidate RfCs were calculated because they identified a LOAEL rather than a NOAEL, and dose-response modeling could not be used for POD derivation due to lack of sufficient data [e.g., no control group (Echeverria et al., 1995) or lack of an important covariate (age) (Cavalleri et al., 1994)]. Additionally, the studies for which candidate RfCs were calculated are of occupationally exposed subjects; no data concerning potential susceptibility or variability among subjects were available.

Medium confidence in the database is based on a number of limitations of both the human and animal literature. Regarding neurotoxicity, there is a need for high quality epidemiologic studies of residential exposures and chronic-duration animal studies (including in developing animals). A fuller characterization is also needed of the noncancer effects other than the critical effect of neurotoxicity, particularly immunological and hematological effects.

6.2.1.4. Reference Dose (refer to Section 5.2)

Candidate RfDs for tetrachloroethylene were developed through route-to-route extrapolation from the inhalation PODs from two neurotoxicological studies of occupational tetrachloroethylene exposure (i.e., Echeverria et al., 1995; Cavalleri et al., 1994). First, the harmonized PBPK model of Chiu and Ginsberg (2011) was used to predict the tetrachloroethylene in blood area under the curve (AUC) at the inhalation PODs from the two principal studies. Then, using the same PBPK model, the oral equivalent POD for each study was derived as the oral dose that would result in the same tetrachloroethylene in blood AUC. Although it is not clear if the noncancer effects observed in humans are the result of tetrachloroethylene itself and/or one or more metabolites, it is reasonable to assume that the appearance of tetrachloroethylene in the blood is a step in the toxicity pathway. Moreover, the sensitivity to the choice of dose metric for route-to-route extrapolation is low, with alternative dose metrics giving route-to-route conversions within 1.4-fold of the conversion based on tetrachloroethylene in blood. The resulting PODs were 2.6 mg/kg-day (Cavalleri et al., 1994) and 9.7 mg/kg-day (Echeverria et al., 1995), respectively, for each of the critical endpoints. The composite UF of 1,000 that was used for the RfC derivation as described above was applied to each of these PODs. Candidate RfDs span a range from 2.6×10^{-3} to 9.7×10^{-3} mg/kg-day. The RfD for tetrachloroethylene is **6×10^{-3} mg/kg-day**, the midpoint of this range rounded to one significant figure. This RfD is equivalent to a drinking water concentration of 0.21 mg/L, assuming a body weight of 70 kg and a daily water consumption of 2 L.

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods*

for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994). The overall confidence in the RfD is medium. Although the confidence in the evidence of neurotoxicological hazard is high, the estimates from studies for which candidate RfDs were calculated are of medium confidence. These studies were considered to be methodologically sound based on study quality attributes, including study population selection, exposure measurement methods, and endpoint measurement methods. Other strengths are that they are human studies of chronic duration, obviating the need for extrapolation across species and exposure duration. However, high confidence was not attained for the studies for which candidate RfDs were calculated because they identified a LOAEL rather than a NOAEL, and dose-response modeling could not be used for POD derivation due to lack of sufficient data [e.g., no control group (Echeverria et al., 1995) or lack of an important covariate (age) (Cavalleri et al., 1994)]. Additionally, the studies for which candidate RfDs were calculated are of occupationally exposed subjects; no data concerning potential susceptibility or variability among subjects were available. Because of the adequacy of the PBPK model (Chiu and Ginsberg, 2011) for extrapolating from inhalation to oral exposures, the use of inhalation studies for deriving the RfD did not decrease confidence.

Medium confidence in the database is based on a number of limitations of both the human and animal literature. Regarding neurotoxicity, there is a need for high quality epidemiologic studies of residential exposures and chronic-duration animal studies (including in developing animals). A fuller characterization is also needed of the noncancer effects other than the critical effect of neurotoxicity, particularly immunological and hematological effects.

6.2.1.5. Dose-Response Analyses for Noncancer Effects Other Than Critical Effect of Neurotoxicity (refer to Sections 5.1.4 and 5.2.4)

Inhalation and oral dose-response analyses for noncancer effects other than the critical effect of neurotoxicity were also conducted. The purpose of these analyses is twofold: (1) to provide a quantitative characterization of the relative sensitivity of different organs/systems to tetrachloroethylene, and (2), to provide information that may be useful for cumulative risk assessment in which multiple chemicals have a common target organ/system other than the central nervous system. The method of analysis is analogous to that described above for neurotoxicity, using the NOAEL/LOAEL approach and the application of uncertainty factors to studies of kidney, liver, immunologic and hematologic, and reproductive and developmental toxicity. Specifically, human equivalent concentrations [HECs] and human equivalent doses [HEDs] are derived using either (1) for inhalation exposure, the RfC methodology for a category 3 gas with extrarespiratory effects, adjusted for equivalent continuous exposure; (2) for oral exposure, mg/kg-day dose adjusted for equivalent continuous exposure; or (3) for either

route of exposure, the PBPK model with an appropriate dose metric. The HECs and HEDs are then treated as PODs to which uncertainty factors are applied.

The sample values for renal and hematologic toxicity overlap with the range of values based on the critical effect of neurotoxicity. Specifically, for renal effects, the resulting values range from 0.03−0.2 mg/m^3 for inhalation and 0.005−0.03 mg/kg-day for oral exposure, based on effects in chronically exposed mice and rats (JISA, 1993) and occupationally exposed humans (Mutti et al., 1992). For hematologic toxicity, the resulting values were 0.04 mg/m^3 for inhalation and 0.007 mg/kg-day for oral exposure, based on changes in hematological measures in occupationally exposed humans (Emara et al., 2010). These overlap with the ranges of 0.02−0.06 mg/m^3 for inhalation and 0.003−0.01 mg/kg-day for oral exposure based on the critical effect of neurotoxicity, and thereby provide additional support for the RfC and RfD. The sample values from the other endpoints are up to 20-fold greater than the RfC, and up to 10-fold greater than the RfD. This suggests that multiple effects may occur at about the same exposure levels at which tetrachloroethylene begins to induce neurotoxicity. These results suggest that it is important to take into account effects from tetrachloroethylene other than neurotoxicity, particularly when assessing the cumulative effects of multiple exposures.

6.2.2. Cancer (refer to Section 5.2)

As summarized above, following EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, tetrachloroethylene is characterized as *"Likely to be carcinogenic to humans"* by all routes of exposure based on suggestive epidemiologic evidence of carcinogenicity and conclusive evidence of carcinogenicity in mice and rats. No available epidemiologic studies of cancer were found to be suitable for dose-response modeling. Therefore, cancer risk estimation is based on data from rodent bioassays. Because the mode(s) of action for tetrachloroethylene carcinogenicity has not been adequately established, the tumors reported in rodent bioassays are considered relevant to humans and a low-dose linear extrapolation is used to estimate human cancer risk from rodent dose-response data, in accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Chronic studies in rats and mice include an oral gavage study in mice and rats by NCI (1977) and two inhalation studies in mice and rats (JISA, 1993; NTP, 1986). The NCI (1977) rat and mouse oral gavage study had a number of limitations that made it less suitable for dose-response modeling as compared to the other studies, including significantly higher early noncancer morbidity and mortality in treated groups, a variable dosing schedule, and the study duration was substantially less than the other available bioassays. With respect to the other two bioassays, the JISA (1993) bioassay included lower exposures of both mice and rats than the NTP (1986) study, and it included three exposure groups as compared to two exposure groups in

the NTP (1986) study. Therefore, JISA (1993) provides a stronger basis for deriving dose-response relationships for risk assessment purposes, insofar as all other aspects of these studies can be considered comparable. Thus, for endpoints which were reported to be tetrachloroethylene-related in multiple studies—i.e., liver tumors and MCLs—the JISA (1993) study was used for dose-response modeling. The JISA (1993) bioassay was also used for dose-response modeling of the increased hemangiomas and hemangiosarcomas in male mice because it was the only bioassay that reported this tumor type. The NTP (1986) study was utilized for modeling the increased incidence of renal cancers, brain cancers, and testicular tumors in male rats, which were reported only in this bioassay. In male mice and male rats, multiple treatment-related tumors were reported in the same study [(JISA, 1993) and (NTP, 1986), respectively]; thus, dose-response analyses of the combined risk of multiple tumors for those experiments were also conducted.

The harmonized PBPK model of Chiu and Ginsberg (2011) was used to perform the interspecies extrapolation from rodents to humans, and for route-to-route extrapolation of the inhalation bioassay results to oral exposures. The choice of the preferred dose-metric to use for each endpoint was based on the strength of its association with the toxic moiety relevant to the endpoint and an evaluation of uncertainties in the calculation of that dose-metric. For cancer, total rate of oxidative metabolism in the liver was considered the most relevant dose metric for tetrachloroethylene-induced liver tumors, and AUC of the parent compound in the blood was considered the preferred dose metric for all other sites, including MCL. Alternative dose-metrics were also used for the purposes of comparison. These include the AUC of TCA in the liver for mouse liver tumors and the rate of GSH conjugation for rat kidney tumors.

6.2.2.1. Inhalation Unit Risk

Several animal tumor data sets were analyzed for estimating cancer risk values. The majority of the NRC peer review panel recommended that the mouse hepatocellular tumors be used for cancer risk estimation. Therefore, the inhalation unit risk is **2×10^{-3} per ppm** or **3×10^{-7} per µg/m^3**, based on the male mouse hepatocellular tumor data from the JISA (1993) bioassay. The unit risk should not be used with exposures exceeding 60 ppm, or 400 mg/m^3 (the equivalent ambient exposures corresponding to the POD for male mouse hepatocellular tumors), because above this exposure level, the dose-response relationship is not linear, and the unit risk would tend to overestimate risk. Some members of the NRC peer review panel recommended that the MCL data be used for cancer risk estimation. The inhalation unit risk would be 7×10^{-2} per ppm, or 1×10^{-5} per µg/m^3 if it were based on the male and female rat MCL data from the JISA (1993) bioassay.

6.2.2.2. Oral Slope Factor

The oral slope factor was developed from inhalation data because the only available oral bioassay had several limitations for extrapolating to lifetime risk in humans (see also Section 5.3.1). Route-to-route extrapolation from the inhalation PODs developed from the JISA study (see Table 5-18) was carried out using the harmonized PBPK model (Section 3.5). The oral slope factor is **2×10^{-3} per mg/kg-day** based on the male mouse hepatocellular tumor data from the JISA (1993) bioassay. The unit risk should not be used with exposures exceeding 50 mg/kg-day (the equivalent ambient exposure corresponding to the POD for male mouse hepatocellular tumors), because above this exposure level, the dose-response relationship is not linear, and the slope factor would tend to overestimate risk. The oral slope factor would be 6×10^{-2} per mg/kg-day if it were based on the male and female rat MCL data from the JISA (1993) bioassay.

6.2.2.3. Uncertainties in Cancer Dose-Response Assessment (refer to Section 5.3.5)

A number of uncertainties underlie the cancer unit risk for tetrachloroethylene, including the choice of study, PBPK modeling and dose metrics, cross-species scaling, low-dose extrapolation, model uncertainty, statistical uncertainty in the POD, the species/gender/tumor type combination selected, and sensitive subpopulations. Some suggest risks could be higher than was estimated (e.g., selection of MCL rather than mouse liver tumors, sensitive subpopulations), while others would decrease risk estimates (e.g., use of central tendency instead of lower 95% confidence bound on the POD), or have an impact of an uncertain direction. Several uncertainties are quantitatively characterized for the significantly increased rodent tumors. These include the statistical uncertainty in the POD, the range of uncertainty in PBPK modeling and dose metrics, dose-response model uncertainty, and the species/gender/tumor type combination selected. The latter three of these could either increase or decrease risk estimates. Due to limitations in the data, particularly regarding the mode of action and relative human sensitivity and variability, the quantitative impact of other uncertainties, which may have equal or greater impact, has not been explored.

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APPENDIX A. EPA RESPONSE TO MAJOR EXTERNAL PEER-REVIEW AND PUBLIC COMMENTS

The 2008 external review draft (ERD) of EPA's *Toxicological Review of Tetrachloroethylene (Perchloroethylene)* underwent a formal external peer review in accordance with U.S. Environmental Protection Agency (EPA) guidance on peer review (U.S. EPA, 2006c). The external peer review was performed by the National Research Council (NRC). NRC was tasked with evaluating the adequacy of the EPA assessment, the data and methods used for deriving the noncancer values for inhalation and oral exposures and the oral and inhalation cancer unit risks posed by tetrachloroethylene; whether the key studies underlying the draft IRIS assessment are of requisite quality, reliability, and relevance to support the derivation of the reference values and cancer risks; and whether the uncertainties in EPA's risk assessment were adequately described and, where possible, quantified. The major peer review comments below (Sections A.1 – A.3) include all comments from the Summary section (with introductory or background text omitted), and several additional comments that potentially have a major impact on the revision of the ERD, and are quoted verbatim from NRC (2010). Page numbers for each quotation are also noted.

In addition, Sections A.4 and A.5 contain a summary of public comments and EPA's responses. In revising the ERD *Toxicological Review of Tetrachloroethylene*, EPA utilized a harmonized PBPK model that was developed in response to NRC (2010) recommendations. EPA conducted a focused peer review on the application of the harmonized model to support its use in the final document. The focused peer review report is publicly available (www.epa.gov/iris). A summary of the focused peer review comments and EPA's responses can be found in Section A.6.

A.1. Major NRC Introductory Comments and EPA Response

NRC Comment: [pp 3-4] The committee appreciates the extensive work that EPA has invested in the development of its draft assessment of tetrachloroethylene. However, the committee has identified concerns about some of the approaches that EPA used to evaluate the data on tetrachloroethylene and subjects about which inadequate information or rationales are used to support its risk assessment—factors that call into question the soundness and reliability of EPA's proposed reference values and cancer risk estimates for tetrachloroethylene. One of the overarching weaknesses of the draft assessment was a lack of critical analysis of the data on which EPA relied in evaluating methodologic strengths and weaknesses. That lack was particularly evident in the assessment of the epidemiologic data: study selection and conclusions

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appeared to be based heavily on results that showed positive associations, and other data and the strengths and weaknesses of the selected studies were not adequately taken into consideration. The committee observed similar problems in its review of EPA's evaluation of the genotoxicity evidence, in which preference appeared to be given to studies that reported positive results. Specifically, EPA did not analyze studies critically with respect to their methodologic strengths and weaknesses, nor did it organize its discussion clearly to provide an integrated consideration of the weight of evidence on the genotoxicity of tetrachloroethylene. Other mode of action evaluations were also hampered in this way.

EPA Response: EPA agrees that a balanced critical analysis of the data is necessary, and has significantly revised its assessment to make its evaluation of study methodological strengths and weaknesses more organized and transparent, and to ensure impartial consideration of all pertinent studies. Specific changes with respect to evaluation of epidemiologic data (A.3.1), genotoxicity (A.3.6), and modes of action (A.3.4) are described in the more detailed responses below.

A.2. Noncancer Assessment

A.2.1. Major NRC Comments on "Critical Noncancer End Point and Studies" and EPA Responses

NRC Comment: [pp 4-5] The committee found that EPA adequately supported its selection of neurotoxicity as the critical effect on which to base the RfC and RfD. The draft IRIS document illustrates that neurotoxic effects are the most sensitive effects of tetrachloroethylene and that reference values based on neurotoxic effects would be protective against other noncancer effects that occur at higher concentrations.

EPA Response: EPA accepts these NRC recommendations, and continues to rely on neurotoxicity as the critical effect (Section 5.1.1.1).

NRC Comment: [p 5] EPA provides descriptions of the relevant neurotoxicity studies, but its evaluation of the epidemiologic literature could be improved by providing a critical evaluation of the validity of study designs and evaluation of the methods used for data collection and analysis, which the committee judges to be most important in selecting key studies.

EPA Response: EPA accepts these NRC recommendations. The rationale for selecting principal studies of neurotoxicity has been more fully and transparently articulated (see Section 5.1.1). Study strengths and weaknesses are judged according to the recommended criteria (e.g., study populations, exposure durations, quality of neurotoxicological tests and

exposure measurements) (see Table 5.1). EPA has also strengthened the presentation of human and animal studies and reorganized them by the toxicological endpoint, particularly (1) neurobehavior, (2) neurophysiology, (3) brain pathology, and (4) developmental neurotoxicity. As also suggested by the NRC, the developmental neurotoxicity studies are grouped together in one section, and a more robust discussion of these studies is provided. EPA focuses on the neurotoxic effects (including developmental neurotoxicity) observed in studies of tetrachloroethylene, and does not comprehensively review the neurotoxicity of structurally related solvents. The MOA discussion for neurotoxic effects (see Section 4.1.3) addresses mechanistic commonalities with other volatile organic solvents and alcohols but likewise focuses on tetrachloroethylene. Hypothesized mechanisms for the different neurological domains affected by tetrachloroethylene are addressed, as are potential molecular targets. EPA addresses recent animal studies identified by NRC (Boyes et al., 2009; Oshiro et al., 2008) and also includes three new epidemiological studies published or available since the release of the 2008 ERD of EPA's *Toxicological Review of Tetrachloroethylene (Perchloroethylene)*, including the final peer-reviewed report of New York State Department of Health study [NYSDOH (2010); published by Storm et al. (2011)] that was presented to NRC during the committee deliberations.

NRC Comment: [p 5] EPA chose the study by Altmann et al. (1995) as the critical one for determining the RfC and RfD because it involved an environmental exposure and used a standardized computer-assisted testing battery. Those are reasonable bases for the choice, but they do not outweigh methodologic deficiencies that seriously compromised the results of the study. Most important, the referent group was not appropriate. The group had more education than the exposed group and appeared to have pre-existing differences in cognitive abilities, which could account for its better test results. Evidence of residual confounding by education can be seen in the variability in reported results. For example, there was no association between tetrachloroethylene and visual evoked potentials; this is important because changes in the visual system and abnormalities in visual evoked potentials have been associated with tetrachloroethylene and other related solvents, and they are essentially unrelated to education. Other limitations of the study included the lack of a rationale for initial selection of study subjects, inadequacy of exposure characterization, and lack of a dose-response relationship. Finally, even though the test battery was performed properly, some of the tests have not been well validated with regard to what they reveal about brain damage.

Thus, the committee disagrees with EPA's selection of the Altmann et al. (1995) study as the basis of its risk calculations.

EPA Response: EPA acccepts these NRC recommendations. In particular, based on application of the criteria described above for conducting its critical review (see Table 5.2), EPA acknowledges the limitations of Altmann et al. (1995) identified by NRC, and as indicated in Section 5.1.1, relies on other studies as the basis for determining the RfC and RfD (see next response).

NRC Comment: [p 5] In reviewing the database, the committee gave greater weight to studies that had the strongest methods; it neither chose nor excluded studies on the basis of their results. The set of studies that the committee judged to be more appropriate for supporting the RfC and RfD include those of Altmann et al. (1990), Cavalleri et al. (1994), Gobba et al. (1998), Echeverria et al. (1995), and Boyes et al. (2009).

EPA Response: EPA modified its approach based on these recommendations. EPA's revised assessment relies on two of the chronic neurotoxicity studies recommended by the NRC, Echeverria et al. (1995) and Cavalleri et al. (1994). Acute studies recommended for consideration by NRC [the chamber study of Altmann et al. (1990) and the rodent study of Boyes et al. (2009)] are supportive, but were not selected for POD derivation because EPA gives preference to quality studies of chronic human exposures for reference value derivation. Gobba et al. (1998) evaluated the same cohort as Cavalleri et al. (1994), and the earlier study was preferred due to its use of a control group and the clearer identification of a POD (refer to section 5.1.1.3.2).

A.2.2. Major NRC Comments on "Derivation of Reference Values" and EPA Responses

NRC Comment: [p 5] EPA derived sample inhalation reference values by using results from several supporting neurotoxicity studies for comparison with its principal study by Altmann et al. (1995). The committee found that some uncertainty factors (UFs) were applied inconsistently; specifically, the application of the uncertainty factor to account for subchronic exposures in epidemiologic studies should be justified better. In some cases, EPA did not use such a factor; in other cases, it applied a value of 10 with weak justification.

EPA Response: EPA accepts these NRC recommendations, and has provided more thorough justification for the selection of all UFs (Sections 5.1.3 and 5.2.3). With respect to the UF to account for subchronic exposures, because each of the selected studies was of chronic exposure, EPA did not apply this UF to any of the PODs. Comments regarding other UFs are discussed in the response to comments that follow.

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NRC Comment: [Chapter 10, p 90] A factor of 10 was used consistently by EPA when a lowest observed-adverse-effect level (LOAEL) from a study was used instead of a no-observed-adverse-effect level (NOAEL). That is consistent with EPA policy. A benchmark dose (BMD) can be treated as a NOAEL, but no studies of neurotoxicity that could support a BMD calculation had been published when the draft was written. More recent studies of neurotoxicity would support such a calculation (Benignus et al., 2009; Boyes et al., 2009; Oshiro et al., 2008).

EPA Response: EPA accepts these NRC comments, and has retained the value of 10 for the UF_L (LOAEL-to-NOAEL extrapolation) (Sections 5.1.3 and 5.2.3). Regarding studies of neurotoxicity that would support dose-response modeling, NRC noted the lack of suitable studies at the time of the 2008 external review draft, and recommended consideration of more recent studies of neurotoxicity by Oshiro et al. (2008), Benignus et al. (2009) and Boyes et al. (2009). The studies of Oshiro et al. (2008) and Boyes et al. (2009) are in rats and covered shorter, acute exposure duration periods than the available human studies and require extrapolation of animal observations to humans. The Benignus et al. (2009) analysis of tetrachloroethylene included three acute exposure studies, the two rat studies above and the acute-exposure study in humans of Altmann et al. (1992). While subjects in Altmann et al. (1992) could serve as their own controls, there was not an unexposed group. Further, these three studies were not considered principal studies given the availability of suitable human data from chronic exposures.

NRC Comment: [Chapter 10, pp 90-91] The uncertainty factor for extrapolating animal data to humans is considered to have toxicokinetic and toxicodynamic aspects. EPA judged that an uncertainty factor of 3 was adequate to address these uncertainties. EPA applied that approach consistently, but the rationale for doing so was not adequately described. Specifically, the draft cites an EPA (1994) document, but it would have enhanced transparency if it summarized briefly why an uncertainty factor of 3, rather than the default factor of 10, was used.

EPA Response: EPA accepts this NRC recommendation. With respect to the UF for interspecies extrapolation from animals (UF_A), because each of the selected studies was in humans, EPA did not apply this UF to any of the PODs. For the "sample" RfCs and RfDs, where some animal studies were used, it is explained that the PODs are expressed as human equivalent concentrations, so the UF of 3 is applied to account for potential pharmacodynamic differences (Sections 5.1.3 and 5.2.3).

NRC Comment: [Chapter 10, p 91] The application of a default factor of 10 to account for interindividual variation is justified because of the paucity of data on sensitive populations,

including developing and aging organisms. Its use is appropriate and in accordance with EPA guidance.

EPA Response: EPA accepts these NRC comments, and has retained the value of 10 for the UF_H (human variability) (Sections 5.1.3 and 5.2.3).

NRC Comment: [Chapter 10, p 92] In the derivation of RfCs on the basis of neurotoxicity, EPA used a factor of 3 for database deficiencies because of the inadequacy of the experimental literature designed to characterize hazard and dose-response. Key deficiencies identified were inadequate data to address childhood or other life-stage susceptibility, a paucity of animal studies (especially studies of developing animals and of chronic, low-level exposures) designed to investigate neurotoxicity or to define and characterize dose-response relationships, and inadequate database on cognitive testing. It was unclear whether a factor of 3 was adequate to address these uncertainties because there was some overlap with the factor of 10 applied for human variation, which also addressed developmental concerns.

The committee recommends that EPA revisit and defend more clearly its decision to apply a factor of 3 for database deficiencies in light of new data and the committee's findings in Chapter 3. New studies include, for example, recent papers from researchers in EPA's National Health and Environmental Effects Research Laboratory provide excellent data from well-designed studies using controlled, acute exposures that link deficits in visual function and signal detection with atmospheric tetrachlorethylene concentrations and instantaneous concentrations in the brain. This includes papers by Oshiro et al. (2008) and Boyes et al. (2009) investigating function and by Shafer et al. (2005) on mechanisms, which is described in the IRIS document but not fully integrated. These studies link neural or behavioral effects to actual brain concentrations of tetrachloroethylene or to their estimated concentration using PBPK modeling. Thus, the animal literature on controlled acute exposure is now stronger. Notable gaps in the animal literature still include the paucity of studies of developmental or chronic exposures. Another consideration is that the committee found the human study of exposed children (Schreiber et al., 2002) to be methodologically flawed. The committee judges these to be serious gaps in the database, which suggests that a factor of 3 may be inadequate to account for database deficiencies.

EPA Response: EPA accepts these NRC recommendations. Based on concerns raised by the NRC, EPA re-examined the adequacy of the database and increased the UF_D from 3 to 10 (Sections 5.1.3 and 5.2.3). EPA's application of a UF_D of 10 to address the lack of data to adequately characterize the hazard and dose response in the human population is consistent with EPA's *A Review of the Reference Dose and Reference Concentration* (U.S. EPA, 2002). EPA provides scientific justification for choosing this UF_D in Section 5 where the reference

concentration (Section 5.1.3) and dose (Section 5.2.3) are derived. EPA's justification is based on a number of data gaps identified from both the human and animal literature. Regarding neurotoxicity, animal studies of chronic exposures (including in developing animals) examining sensitive neurotoxic endpoints are lacking. Moreover, the most sensitive neurotoxic endpoint associated with tetrachloroethylene exposure in humans—decrement in visual contrast sensitivity—was identified in residential studies that were judged to be limited for developing an RfC (Storm et al., 2011 [previously reported in NYSDOH, 2010]; Schreiber et al., 2002; Altmann et al., 1995). This specific endpoint was not evaluated in any of the occupational studies used for developing the RfC. Regarding sensitive endpoints other than neurotoxicity, the available human and animal studies of immunologic and hematologic toxicity [e.g., Emara et al. (2010); Marth (1987)] are limited.

NRC Comment: [p 8] The committee derived candidate values by using the same studies as EPA and additional studies. The committee found that the reference values from the strongest studies were in the range of 6-50 ppb (or 0.04-0.34 mg/m^3). That range is higher than the RfC of 0.016 mg/m^3 derived by EPA and is further supported when considered in the context of the full database (see further discussion below).

EPA Response: EPA revisited the above calculation based on NRC's annotation that their exercise was illustrative, and that some candidate values were subject to change based on implementation of their advice regarding the UFs. As discussed above, due to concerns raised by the NRC, EPA increased the UF_D from 3 to 10. With this change, the NRC-suggested range would be lowered to 0.01−0.10 mg/m^3, which fully encompasses EPA's revised range of candidate RfCs (0.02 to 0.06 mg/m^3) and EPA's selected RfC (0.04 mg/m^3) (see Section 5.1.3).

NRC Comment: [p 8] EPA extrapolated the results of inhalation studies to derive the oral RfD for tetrachloroethylene. Physiologically based pharmacokinetic (PBPK) modeling was used to support the route-to-route extrapolation. The rationale behind that approach is sound and adequately explained by EPA, and the choice of dose metric (blood area-under-the-curve) was appropriate and adequately supported by the available evidence. However, the three models used by EPA were formulated and validated with data from inhalation exposures—none was validated against blood concentrations that result from oral exposure. EPA empirically assumed a value for the rate of oral absorption of tetrachloroethylene; this assumption is inferior to direct estimation. Other PBPK models that use direct estimation are available, and their use may help to reduce the uncertainty in the assumed values; or additional PBPK models could be developed (see recommendation below for a harmonized PBPK model).

EPA Response: EPA accepts these NRC recommendations. EPA followed the NRC recommendations and developed a new harmonized PBPK model that incorporated available oral data from which the oral absorption rate could be estimated (Section 3.5) (Chiu and Ginsberg, 2011), which was used in the route-to-route extrapolations for the RfD calculations (Section 5.2.2). The response to recommendations with respect to PBPK modeling is discussed in more detail below (Section A.3.7).

A.2.3. Major NRC Comments on "Graphical Presentation" and EPA Responses

NRC Comment: [p 8] EPA provides graphical comparisons of reference values, values that could be derived from supporting studies. Reference values derived from neurotoxicity data are presented, as are values based on other noncancer effects to illustrate dose dependence of multiple forms of observed toxicity. Overall, the committee supports the approach of presenting the evidence in this visual format. However, the committee recommends some revisions to improve illustration of the uncertainties being represented and to expand the presentation to include the larger body of literature on a particular end point to show how the RfC compares with sample reference values derived from studies that are methodologically sound but not judged to be critical for the RfC. Consistency between the RfC and such studies would provide additional support.

Figure S-1 provides an example illustration developed by the committee. It shows that the majority of sample values is centrally clustered, but there is a wide spread at the lower and higher ends. The overall range of the 19 sample reference values is 0.03-333 ppb (0.0002-2.6 mg/m^3), but the range is reduced to about 6-50 ppb (0.04-0.34 mg/m^3) when consideration is restricted to the five strongest studies. The RfC of 0.016 mg/m^3 calculated by EPA on the basis of the Altmann et al. (1995) study falls below the range. The figure shows that sample reference values that could be derived from the full database of neurotoxicity studies provide some support for the range.

EPA Response: EPA accepts these NRC recommendations. In particular, EPA agrees that the graphical presentation of studies and resulting risk values is useful. EPA graphically portrayed the PODs for all the tetrachloroethylene neurotoxicity studies considered for dose-response analysis, with the studies used to derive candidate reference values highlighted (see Section 5.1, Figure 5-1). Separately, for the studies used to derive candidate reference values, EPA graphically presented the PODs and uncertainty factors used in the derivation of the candidate noncancer RfCs (see Section 5.1, Figure 5-2). Additionally, in agreement with NRC, EPA continues to provide "sample" reference values based on reproductive and developmental, kidney, liver, immunological, and hematological noncancer endpoints.

Sample PODs and composite UFs for noncancer effects other than the critical effect of neurotoxicity are also graphically displayed (see Section 5.1, Figure 5-3), in accordance with the NRC recommendations.

A.2.4. Major NRC Comments on "Reproductive and Developmental Effects" and EPA Responses

NRC Comment: [Chapter 4, p 48] EPA's identification of the key animal and epidemiologic reproductive and developmental studies of tetrachloroethylene appears to be complete, but the committee recommends some reorganization and reconsideration of data to provide a more transparent and balanced characterization of the data.

EPA Response: EPA accepts this NRC recommendation, and has made revisions throughout Section 4.7. Consistent with NRC advice, the presentation of developmental and reproductive toxicity studies was reordered, and developmental studies were separated from reproduction studies, to emphasize the differences in exposure paradigm and types of endpoints assessed. Study strengths and deficiencies are presented in the individual study descriptions. Evidence from supportive in vitro and in vivo studies and the consistency of outcomes across species and protocols are described. Findings of parental (including maternal) toxicity and the treatment levels at which those effects were observed are also described for each study.

NRC Comment: [Chapter 4, p 48] The committee agrees with the selection of the Tinston (1994) two-generation reproductive-toxicity study and the Carney et al. (2006) developmental-toxicity study as supportive of a point of departure and an RfV [reference value]. EPA's derivation of a comparative RfV based on the developmental toxicity of tetrachloroethylene is an important contribution to the tetrachloroethylene database.

EPA Response: EPA accepts this recommendation, and has included these studies in developing comparative "sample" RfCs and RfDs (Sections 5.1.4 and 5.2.4).

NRC Comment: [Chapter 4, pp 48-49] However, the committee recommends that EPA revise the chapter to address the specific deficiencies discussed above regarding information presented on the animal reproductive and developmental studies. In particular, the revision should include: (1) a critical analysis of the described studies, including an assessment of the relationship of maternal toxicity to developmental toxicity and the strengths, limitations, and consistency of the various study results; (2) characterization of maternal toxicity (e.g., mild or severe) associated with the studies listed in Table 4-10 and use of consistent nomenclature (ppm or mg/m^3) for listing tetrachloroethylene concentrations; (3) the scientific basis for selecting the Tinston (1994)

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and Carney et al. (2006) studies as supportive of an RfV; (4) the scientific rationale for selecting the Tinston (1994) study instead of the Carney et al. (2006) study for derivation of the comparative RfV; (5) information on the mode of action for tetrachloroethylene-induced developmental toxicity which addresses the apparent contradictions raised in the committee's review that TCA may be the causative agent; and (6) characterization of the evidence for tetrachloroethylene-induced reproductive and developmental toxicity in animals based on EPA risk assessment guidelines. Stating explicitly whether the animal evidence is sufficient or insufficient for these important end points will help risk managers and others to more readily identify and protect against potential adverse health effects. It will also help to identify data gaps in the tetrachloroethylene database.

EPA Response: EPA has made revisions based on the NRC recommendations throughout Section 4.7. In particular, EPA accepts Recommendation (1), and has revised the individual study descriptions to include the critical analysis elements noted. Regarding (2), EPA clarified that there is no evidence in the tetrachloroethylene mammalian developmental or reproductive toxicity study database of severe maternal toxicity that compromised or confounded the evaluation of offspring toxicity, noting the difficulty in determining the relationship between maternal and developmental toxicity in a developmental or reproductive toxicity study. EPA accepts Recommendations (3) and (4) and has revised the basis and rationale for selecting studies in Section 5.1. EPA accepts Recommendation (5) and has expanded the discussion of the MOA hypotheses for developmental outcomes to address the potential involvement of the metabolite TCA. EPA accepts Recommendation (6), and in accordance with EPA risk assessment guidelines for reproductive and developmental toxicity, explicitly states that the database of animal and human studies was sufficient for the evaluation of developmental and reproductive toxicity.

NRC Comment: [Chapter 4, p 49] In addition to revising the chapter, the committee also recommends that EPA consider conducting a bench-mark dose analysis and deriving an RfV based on the Carney et al. (2006) study in addition to, or instead of, the Tinston (1994) study. This will address the potential confounding effects of maternal toxicity at the 1,000 ppm exposure level observed in the Tinston (1994) study.

EPA Response: Endpoints from both Tinston (1994) and Carney et al. (2006)—as well as from Beliles et al. (1980) and Nelson et al. (1979)—were carried forward for potential RfC development (see Table 4-49). Sample RfCs were derived for reproductive and developmental effects (see Table 5-7) and were one order of magnitude greater than the candidate RfCs derived for neurological effects (see Table 5-3). The possible—but

uncharacterized—influence of maternal toxicity on offspring outcomes at the highest dose tested (1,000 ppm) in the Tinston (1994) study would have no impact on the final noncancer reference value derivation.

A.3. Cancer Assessment

A.3.1. Major NRC Comments on "Epidemiologic Evidence Pertaining to Cancer" and EPA Responses

NRC Comment: [Chapter 9, p 85] One of the biggest difficulties in assessing the cogency of the EPA's assessment related to cancer is how the data are organized in the tables and some parts of the text. It would be much easier to evaluate the overall picture of results regarding tetrachloroethylene and a particular cancer if the tables were organized by cancer type as opposed to the current format, which organizes them by study design. The current format requires the reader to jump between sections for cohort mortality, incidence, and case-control studies. Studies are sometimes further categorized as to the type of worker included (for example, dry-cleaner vs degreaser); this makes it extremely difficult to evaluate the overall consistency or lack of consistency in results related to specific cancers.

EPA Response: EPA accepts these NRC recommendations. EPA has significantly reorganized the data presentation by type of cancer as follows: kidney and bladder toxicity and cancer (see Section 4.2); liver toxicity and cancer (see Section 4.3); esophageal cancer (see Section 4.4); lung and respiratory cancer (see Section 4.5); immunotoxicity, hematologic toxicity, and cancers of the immune system (see Section 4.6); developmental and reproductive toxicity, and reproductive cancers (see Section 4.7). Epidemiologic observations on tetrachloroethylene and breast cancer are included in Section 4.7.

NRC Comment: [Chapter 9, p 85] Errors in reporting results also occur occasionally. For example, the draft reports (on page 4-150, lines 1-3), in relation to Hodgkin disease, "a statistically significantly elevated risk for male [sic] with a job title of dry cleaner or laundry worker (Costantini et al., 2001)." The result from Costantini et al. (2001) for that group in relation to Hodgkin disease was an OR of 2.5 (95% CI, 0.3-24.6), which is not significant and was based on a single case.

EPA Response: EPA accepts this recommendation and has corrected reporting errors.

NRC Comment: [Chapter 9, p 85] The overall impression is that data are presented to support a positive association between tetrachloroethylene and cancer and that studies that found no such

association are criticized or minimized. EPA should provide a clearer discussion of criteria used to identify studies of merit and a more balanced critique to strengthen the draft IRIS assessment.

EPA Response: In agreement with NRC, EPA has also now included an updated and more balanced evaluation of the epidemiologic literature on tetrachloroethylene and cancer (throughout Section 4, as described above). The revised discussion of epidemiologic observations achieves a more balanced review by clarifying how EPA considered study methodological strengths and weaknesses, including evaluation of the exposure-assessment approach, study size, number of observed cancer events, choice of referent population, presence or absence of an exposure-response relationship, and the potential for alternative explanations such as chance, bias, or confounding. A synthesis of the epidemiologic cancer data is provided considering evidence across cancer sites. Recent literature added to the epidemiologic evaluation comprised 27 epidemiologic studies on occupational tetrachloroethylene exposure and cancer, and one meta-analysis of bladder cancer and dry cleaning. These studies were published since 2004, the date of the comprehensive literature review in support of the 2008 ERD of EPA's *Toxicological Review of Tetrachloroethylene (Perchloroethylene)*. As a supplement to the tabular summaries of epidemiologic observations organized by cancer site in Section 4, Appendix B characterizes the design and methods more fully.

A.3.2. Major NRC Comments on "Cancer Characterization" and EPA Responses

NRC Comment: [p 8] EPA classified tetrachloroethylene as "likely to be carcinogenic to humans." The committee reviewed the classification guidance in EPA's 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005) and the bioassay data available on tetrachloroethylene and concluded that EPA adequately documented that its classification has been based on the results of bioassays that found increased incidences of hepatocellular tumors, mononuclear-cell leukemia (MCL), renal tumors, and hemangiosarcomas in laboratory animals and to a lesser extent on epidemiologic evidence. EPA's decision to characterize tetrachloroethylene as likely to be a human carcinogen as opposed to "carcinogenic to humans" appropriately reflects the possibility that there are deficiencies or potential inaccuracies in interpretation of the data. Some of the possible deficiencies and inaccuracies are discussed below for each of the datasets.

EPA Response: EPA accepts these NRC recommendations, and continues to characterize tetrachloroethylene as "likely to be carcinogenic to humans" (Section 4.10.3). EPA agrees with NRC that the epidemiologic literature on cancers provides limited evidence that tetrachloroethylene is carcinogenic in humans.

NRC comments and responses regarding individual animal bioassay datasets are discussed below.

NRC Comment: [pp 8-9] *Mononuclear-Cell Leukemia*

An increased incidence of MCL in F344 rats has been reported in two bioassays. The biologic significance of the increases was debated by the committee because increases were observed in only one strain of rat, which is known to have a high background incidence of MCL, and because MCL's relevance to humans and the mode of action of tetrachloroethylene causing it are not understood. In considering the high background of MCL, the committee found a published assessment by Thomas et al. (2007) that applied statistical approaches (life-table analyses) to bioassays of the National Toxicology Program (NTP) to interpret dose-response relationships. Tetrachloroethylene was one of five chemicals of 500 tested by NTP that showed statistically significant increases in MCL in both male and female rats despite the high background rates. The publication advocated that such statistical evidence be supported with a weight-of-evidence analysis of biologic data before conclusions were drawn.

The committee found some support from epidemiologic studies that suggested an association between tetrachloroethylene and lymphoma, but the data were relatively weak and inconsistent. A difficulty in interpreting the findings is a difference of opinion about the human relevance of MCL. Some committee members judged that similarities between a form of human leukemia (natural killer-cell large granular lymphocyte leukemia) and rat MCL and results of mechanistic studies that the committee recommended be added to EPA's assessment were adequate to establish human relevance; others believed that more research was needed to establish the relevance. The committee agreed that there was little information on a mode of action of tetrachloroethylene in increasing MCL and that it therefore was not possible to determine whether exposure to tetrachloroethylene results in initiation of new tumors or enhances the expansion or promotion of existing tumors.

EPA Response: EPA added the studies that NRC judged to provide indirect evidence that tetrachloroethylene induces effects associated with MCL and with known leukemogens (see Section 4.6). Particularly, EPA now includes studies of tetrachloroethylene exposure by Marth et al. (1989; 1985) and Marth (1987) demonstrating hemolysis and a study by Seidel et al. (1992) showing effects on bone marrow function. Additionally, EPA summarizes the findings of Thomas et al. (2007) and presents and discusses the data relevant to interpreting tetrachloroethylene effects according to the approach proposed by those authors, as recommended by NRC (see Section 4.6). This includes a summary and statistical analyses of the MCL findings for tetrachloroethylene in the NTP and JISA bioassays (including of the

JISA MCL data which appropriately considered time of death), as well as a presentation of the historical control incidences of MCL for these laboratories.

NRC Comment: [p 9] *Hepatic Cancer*

Statistically significant increases in hepatic tumors were observed in male and female mice after oral or inhalation exposure. As in the case of MCL, the biologic significance of the increases was debated by the committee because B6C3F1 mice have a high background incidence of hepatic cancer. However, the findings were reproduced in several studies conducted in different laboratories and showed a dose-response relationship. There is also fairly substantial information for characterizing potential modes of action of hepatic-tumor formation relative to the data available on MCL and renal cancer. Although the committee recommended that EPA revise its presentation of the mode-of-action evidence on tetrachloroethylene-related hepatic cancer to clarify its position, most of the members agreed with EPA that the mode of action is complex and remains to be established. The latter members also agreed that there was insufficient evidence to rule out human relevance. One member objected to those conclusions and to the committee's support of using hepatic cancer to quantify risk. He argued that in the absence of evidence of other contributing modes of action, the evidence is sufficient to conclude that the mode of action in mice is predominantly through activation of the peroxisome proliferator-activated receptor-alpha, a mode of action that he considered to be of little relevance to humans. His arguments are presented in a dissenting statement in Appendix B of the report.

EPA Response: EPA agrees with the majority of the NRC panel that the mode of action for hepatic tumors observed in male and female mice is complex and remains to be established, and that, therefore, there was insufficient evidence to rule out human relevance (Sections 4.3.5 and 4.10.5.3). EPA revised the presentation of hepatic mode of action evidence in accord with the NRC panel suggestions to clarify its position.

NRC Comment: [p 9] *Renal Cancer*

Tetrachloroethylene caused a low rate of induction of renal tumors in rats. Although the increases were not statistically significant when compared with concurrent controls, EPA has used historical controls to calculate the chances of two of these rare carcinomas to occur by chance to be less than 0.001. Furthermore, a dose-response trend was shown against the low background and the tumors in the treated rats were malignant whereas the tumors in the controls were not. EPA provided a strong evaluation of the potential modes of action for tetrachloroethylene-induced kidney cancer. The committee agrees with EPA that the mode of action of tetrachloroethylene tumorigenesis is not understood but that a mutagenic mode of action cannot be ruled out. Thus, renal tumors observed in tetrachloroethylene-treated rats were

considered relevant to humans although additional characterization of quantitative relevance is desirable.

EPA Response: EPA agrees with the NRC panel that renal tumors observed in tetrachloroethylene-treated rats are considered relevant to humans. EPA has performed additional characterization of quantitative relevance through development of a harmonized PBPK model for tetrachloroethylene that includes the glutathione conjugation pathway (Section 3.5, and discussed below, Section A.3.7).

A.3.3. Major NRC Comments on "Selection of Tumor Type for Quantitative Assessment" and EPA Responses

NRC Comment: [p 10] The committee was unable to reach consensus on the selection of the critical cancer end point. The majority of the members judged that the uncertainties associated with MCL (particularly the high background incidence, uncertainty about the dose-response relationship, and poor understanding of mode of action) were too great to support using MCL data rather than data on hepatic or renal cancer for determining quantitative estimates of risk. Those members judged that the use of the MCL data could be justified only if it is EPA's policy to choose the most conservative unit risk when considering options but that such justification should be distinguished as a policy decision, not a scientific one. They believed that a more scientifically defensible approach would be to use the data set that has the least uncertainty rather than the dataset that yields the highest estimate of risk. In their judgment, the hepatic-cancer data would have the least uncertainty, followed by the data on renal cancer and MCL.

Other members judged that the MCL data should be used for cancer-risk estimation. Their opinions were based on the observation that reproducible, statistically significant increases in MCL in male and female rats above the background incidence of MCL were found and that MCL was the cancer end point with the highest magnitude of response. They believed that use of the most sensitive response to quantify cancer risk decreases the uncertainty associated with potential differences in metabolism and susceptibility to tetrachloroethylene among exposed populations. They concluded that additional statistical analyses of the dose-response data and the addition of supporting mechanistic information identified by the committee would strengthen the existing support of the use of MCL in the draft assessment.

EPA Response: In accordance with the majority of the NRC peer review panel, the oral slope factor and inhalation unit risk are now based on the male mouse hepatocellular tumor data from the JISA (1993) bioassay as shown in Sections 5.3.4.2 and 5.3.4.3. EPA also presents what the cancer risk estimates would be if they were based on the male and female rat MCL data from the JISA (1993) bioassay.

A.3.4. Major NRC Comments on "Mode-of-Action Considerations" and EPA Responses

NRC Comment: [pp 10-11] The modes of action by which tetrachloroethylene produces increases in MCL, hepatic cancer, and renal cancer were an important consideration in EPA's and the committee's evaluations of the evidence. The analytic framework described in EPA's cancer guidelines for considering hypothesized modes of action was best applied in the draft IRIS assessment's consideration of renal cancer. The evaluation focused on synthesizing the evidence to support the idea that multiple modes of action may play a role.

EPA Response: EPA accepts these NRC recommendations. In addition, EPA has included text and tabular summaries of the relevant data for the MOA hypotheses to better and more clearly support the conclusions.

NRC Comment: [p 11] However, for hepatic cancer, the committee found that the assessment lacked the organization to present and provide appropriate context for the evidence clearly. It therefore recommended that EPA revise its mode-of-action assessment for hepatic cancer to support better the conclusions that were drawn. Specifically, the committee suggested that the mode-of-action analyses would be improved by outlining the proposed sequence of hypothesized tetrachloroethylene-associated key events (possibly with a diagram). Transparency would be improved by presenting the details of experimental results in tabular form to allow the reader to understand more easily the relative potency of tetrachloroethylene, or its metabolites, in inducing both key events and tumors. In this context, species and strain differences could also be considered more easily. The goals of the presentation should be to lay out the timeline of key events explicitly in the context of dose, to evaluate concordance between early and late events, and to consider the relative contribution of chemical-specific data compared with information on categories of chemicals.

EPA Response: EPA accepts these NRC recommendations. EPA agrees with and has followed NRC's recommendations in revising the discussion of supporting evidence for the various hypothesized MOAs. In particular, the presentation of the pertinent MOA data has been reorganized, and additional analyses suggested by NRC have been included (Sections 4.3.5, 4.10.5.3, and Appendix C). For each MOA hypothesis, EPA identifies the proposed sequence of hypothesized key events. The final assessment provides tabular summaries of the relevant results for tetrachloroethylene to facilitate understanding of the relative potency of tetrachlroethylene and its metabolites in inducing both hypothesized key events and tumors. Information on species, strain, sex, dose and temporality of effect are presented so that concordance between early and late events, and differences across experimental

paradigms, can be recognized. In addition, data specific to tetrachloroethylene and on other categories of chemicals are clearly delineated, to aid consideration of their relative contribution in supporting the conclusions.

NRC Comment: [p 11] This approach should be applied to each hypothesized mode of action. Even if the data are ultimately judged to be insufficient to support a hypothesis, the exercise can be used to identify critical data gaps and to inform the direction of future research.

EPA Response: EPA accepts these NRC recommendations. In particular, EPA has undertaken significant revisions to more clearly specify the hypothesized MOAs for each tumor endpoint, and to present and analyze the evidence available to support conclusions about these hypothesized MOAs (Sections 4.2.4, 4.3.5, and 4.10.5.3). This includes presentation of experimental details in tabular form.

A.3.5. Major NRC Comments on "Low-Dose Extrapolation" and EPA Responses

NRC Comment: [p 11] EPA's dose-response analyses of the various cancer datasets involved using several models to extrapolate to doses below the experimental range. EPA considered six data sets: hepatocellular adenoma or carcinoma in male and female mice, hemangiosarcoma in male mice, MCL in male and female rats, and renal tumors in male rats. EPA used the multistage model for each dataset because mode-of-action information was lacking or uncertain and the model was able to fit a broad array of dose-response patterns. However, because the studies used small numbers of dose groups and because the benchmark-dose software automatically fixed some parameters to zero to obtain convergence in model-fitting, the fitted models were nearly linear in the low-dose range. The imposed linearity explains the similarity among the slopes of the models and among the unit risks derived from the models.

EPA Response: EPA would like to clarify that the earlier modeling did not impose linearity on the subject data sets. EPA's software uses maximum likelihood estimation, a standard method; the software merely allowed for the possibility of linearity in the chosen model and did not select or fix parameters at zero. Although multistage model parameters are restricted to be nonnegative, this only imposes monotonicity, not linearity. Also, the multistage model can take on more curvilinear forms, even with first-order models. The methods used have been clarified further in the assessment (Section 5.3.3.2.1).

NRC Comment: [p 11] In the case of hepatocellular adenoma and carcinoma in male mice and MCL in female rats, EPA considered the fitted models acceptable solely on the grounds that statistical tests for goodness of fit had nonsignificant results ($p > 0.10$). The committee considers

this to be a weak rationale in that the statistical significance of goodness-of-fit tests may not detect a poor fit when the number of animals per dose group is small.

EPA Response: EPA agrees that factors in addition to statistical significance should be taken into account in assessing goodness-of-fit, and that adequate power of statistical testing is an important consideration when interpreting lack or presence of statistical significance. For instance, EPA prefers that the absolute values of the standardized residuals for the reported fits be within the limit of ±2 units, though EPA acknowledges that this consideration was not adequately documented in the external review draft. In addition, the visual fits for both data sets were not satisfying, as pointed out by the committee. Moreover, in both cases, using the multistage model, the *modeled* benchmark concentration for an extra risk of 10% (both the maximum likelihood estimate and the 95% lower bound) was *higher* than the concentrations at which 10% or more extra risk response was *observed*. EPA has revised its discussions of the dose-response modeling to more completely document these considerations when judging goodness of fit. Additional dose-response analyses to improve model fits to these data are discussed in the response to the next comment.

NRC Comment: [pp 11-12] The questionable fitting of the multistage model to some candidate datasets and insufficient consideration of alternative models contribute to underestimation of the overall uncertainties.

EPA Response: EPA agrees that the dose-response relationships for the highlighted data sets merited reanalysis, particularly in cases where the multistage model did not fit the data at lower doses. EPA also agrees that in such cases, uncertainty may be underestimated if there is insufficient consideration of alternative models (see response below under "Uncertainty Analysis"). The additional analyses, discussed further below, resulted in better characterization of these data sets.

NRC Comment: [p 12] EPA adopted linear low-dose extrapolation, the default option, with several justifications. First, nonlinear, mechanistic models are unavailable for dose-response modeling because mode-of-action information on tetrachloroethylene is insufficient and support for dynamic models is unavailable. Second, because mathematical models are subject to uncertainties for low-dose extrapolation beyond the experimental dose range, linear extrapolation is more conservative than all sublinear (curvilinear) models. When individual thresholds in the human population are plausible, wide variation in threshold values typically implies a curvilinear shape of the dose-response relationship. Thus, linear extrapolation protects susceptible subpopulations. Third, a few of the candidate data, especially the male-rat MCL data, exhibit a linear dose-response relationship. Whereas those arguments are consistent with EPA's

Guidelines for Carcinogen Risk Assessment, there is evidence in the candidate datasets that the underlying dose-response relationship can be supralinear (for example, in MCL in female rats). When that is the case, low-dose linear extrapolation is not conservative. EPA does not present the full ranges of variation and uncertainty in relation to model choice, in large part because it applied only linear or nearly linear dose-response models to all candidate datasets.

EPA Response: EPA agrees that there is evidence in the candidate datasets that the underlying dose-response relationship can be supralinear, and that in this case low-dose linear extrapolation is not conservative. Additional analyses were performed to address the concerns with respect to dose-response fitting of supralinear data sets. Several options to find better fitting models were considered, including: other model forms; substitution of historical controls for concurrent controls; exclusion of exposure groups from the analysis, starting with the highest exposure group; and consideration of dose-response analysis of combined males and females. These analyses (provided in detail in Section 5.3.4.1) resulted in use of a one-degree multistage model for the male hepatocellular tumors and in use of combined male and female rat MCL data and an alternate model (Michaelis-Menten) for adequate characterizations of the low-dose data. Responses to comments with respect to the range of uncertainty and variability are addressed below under "Uncertainty Analysis."

A.3.6. Major NRC Comments on "Age-Adjustment Factor" and EPA Responses

NRC Comment: [p 12] EPA did not apply an age-adjustment factor to its cancer risk assessment, because there is little evidence that tetrachloroethylene or its oxidative metabolites directly damage DNA, because information about genotoxicity of glutathione (GSH) metabolites in cell assays other than Salmonella or in vitro experiments is lacking, and because the mode of action of tetrachloroethylene has not been established. In addition, there are no data on differential sensitivity to tetrachloroethylene carcinogenicity among life stages. The committee agrees that those are adequate reasons for not using an age-adjustment factor but suggests that the rationale can be strengthened if EPA follows the committee's suggestions for improving its analysis of the genotoxicity data and mode-of-action evidence.

EPA Response: EPA accepts these NRC recommendations. To better support its conclusions, EPA has substantially revised the genotoxicity section (see Section 4.8) in accord with NRC recommendations. Text and tabular study summaries of the available genotoxicity studies of tetrachloroethylene and its metabolites are presented, organized by test article (chemical entity) and further structured according to the assessed endpoint. Missing and more recently peer-reviewed and published studies as identified by the NRC

committee are included. Additionally, the data for each metabolite are summarized, and an overall synthesis section is included.

A.3.7. Major NRC Comments on "Physiologically Based Pharmacokinetic Models" and EPA Responses

NRC Comment: [pp 12-13] Tetrachloroethylene can be viewed as being metabolized by three pathways. The predominant pathway is the cytochrome P-450 (CYP) pathway that produces metabolites that have been associated with hepatic cancer. Two other pathways involve the GSH conjugation pathway that produces metabolites that are further metabolized by the β-lyase pathway or the β-lyase-independent pathway, each of which produce metabolites that have been associated with renal cancer. To take those metabolic factors into account, EPA used three PBPK models to estimate human equivalent doses from animal studies and to perform route-to-route extrapolations. Each of the models used total metabolism of tetrachloroethylene as the dose metric. In some instances, EPA used a single model; in others, it used all three. The justification for using single or multiple models is not always clear. The committee observed that the models could yield different results because they were calibrated with different datasets, so comparisons among them were not straightforward. For consistency and to allow for better comparisons among end points, the committee recommends that EPA use a single PBPK model for its assessment. Ideally, the model would be a "harmonized" version of the three models used by EPA or of other relevant models (that is, a single model that integrates multiple exposure routes and tissue compartments).

The committee notes that the use of total metabolism as the dose metric for carcinogenicity reflects primarily the CYP metabolic pathway because of large differences in the flux of the metabolism between it and the GSH pathway. Using that dose metric does not reflect the contribution of the GSH conjugation pathway, which has been implicated in the development of renal cancer. EPA did not pursue the addition of the GSH pathway to any of the PBPK models, arguing that data on GSH-dependent metabolism are from in vitro studies or constitute measurements of urinary excretion products and do not represent toxic species in vivo. The committee agrees that the available data on the GSH pathway are more limited than the available data on the CYP pathway but notes that in vitro and urinary metabolite data were used in the development of the CYP-based PBPK models chosen by EPA. Thus, better justification is necessary to rule out modeling the GSH pathway.

The committee recommends that EPA explore the possibility of adding the GSH pathway to a harmonized PBPK model. If such modeling is deter-mined to be infeasible, total metabolism can be used as a reasonably conservative dose metric. The modeling exercise would be useful in identifying data gaps that prevent successful modeling, which can be used to guide

research that will allow more comprehensive PBPK models to be developed in support of the next IRIS reassessment of tetrachloroethylene.

EPA Response: EPA accepts these NRC recommendations, agrees that a "harmonized" PBPK model that includes data regarding the GSH pathway would be beneficial, and has developed such a model that integrates multiple exposure routes and tissue compartments (Section 3.5). Additionally, EPA followed the NRC advice of separating metabolism into three pathways (oxidation, GSH-conjugation with further β-lyase metabolism, and GSH-conjugation with further β-lyase-independent metabolism). The PBPK modeling analysis showed that the GSH conjugation pathway in humans remains highly uncertain and/or variable, and that additional data are needed to better quantify that pathway in humans (see Section 3.5). Therefore, the assessment does not rely on quantitative estimates of GSH pathway metabolism provided by the new PBPK model. Instead, the quantitative risk estimates presented in the revised assessment rely on estimates of blood tetrachloroethylene, oxidation of tetrachloroethylene, and route-to-route extrapolation from this new model. These dose metric estimates from the new model are robust and consistent with prior models and, thus, insensitive to model choice.

A.3.8. Major NRC Comments on "Uncertainty Analysis" and EPA Responses

NRC Comment: [p 13] EPA has clearly identified key sources of uncertainty as part of its process of assessing the cancer risk posed by exposure to tetrachloroethylene, including human population variation, low-dose extrapolation, dose metrics, extrapolation from animals to humans, and the use of PBPK models for route-to-route extrapolation. The effect of uncertainties on risk estimates is assessed qualitatively in most parts of the IRIS draft except in dealing with such issues as the choice of dose-response models, the use of PBPK models, and, to a small degree, variation between studies. That approach reflects the current state of practice of uncertainty analysis.

EPA Response: EPA agrees with the NRC comments that its approach to uncertainty analysis reflects the current state of practice, and that emerging new methods for quantification of overarching uncertainty, of variability, and of their cumulative effects could be considered when tetrachloroethylene is re-evaluated. In addition, as recommended by the NRC committee, EPA has retained tabular presentation highlighting EPA's choices and their effects on the determination of the upper bound of the risk estimate (section 5.3.5).

NRC Comment: [p 14] In a few respects, the committee disagrees with EPA's presentation on uncertainties. For example, EPA notes narrow variation between cancer risks derived from four

dose-response models. However, in its comparison, EPA used only data on male rats, and all four models were linear or nearly linear at lower doses. Failure to consider a wider array of feasible dose-response models, including multistage models of various orders, could lead to inadequate quantification of uncertainty associated with the choice of dose-response model.

The committee supports EPA's quantitative assessments of uncertainty with regard to choice of dose-response models, the use of PBPK models, and variation between studies. In particular, the committee found EPA's consideration of uncertainty due to different forms of dose-response models to be valuable, and it recommends that such quantitative evaluations be extended to all candidate datasets so that a fuller array of uncertainties can be assessed.

EPA Response: EPA accepts these NRC recommendations and has extended the quantitative evaluation of different models to all candidate data sets to a fuller array of uncertainties at the point of departure (i.e., 10% extra risk level). EPA has conducted dose-response modeling on the basis of administered concentration for each of the JISA candidate data sets using the range of dichotomous dose-response models included in BMDS (Appendix D). The results of the suite of models were evaluated for goodness-of-fit. For datasets exhibiting supralinearity, models that led to both a better fit to the supralinear shape and a stable BMDL were considered for further application using PBPK model-based dose metrics. The results of this analysis showed that for datasets exhibiting supralinearity, the BMD estimated using the multistage model may lead to an underestimation of risk, consistent with the NRC comments. Moreover, in such cases, it can be challenging to obtain both a better fit than the multistage model and a stable lower bound estimate for the BMD.

A.4. Response to Public Comments – Noncancer Assessment

A.4.1. Critical Noncancer End Point and Studies

Public Comments: Several public commenters recommended specifying the criteria used to select studies of the best quality, to better support weight of evidence conclusions and principal study selection. Several commenters critiqued Altmann et al. (1995) based on factors such as small sample sizes, uncontrolled confounding, selection bias, the transient and subtle nature of the effects, the relevance of exposure scenario and the statistical analysis. Another public commenter submitted, and recommended consideration of (for RfC derivation and in choice of UF), the final peer-reviewed report of New York State Department of Health study [NYSDOH (2010); published by Storm et al. (2011)]. Other studies of neurological effects in residential populations were identified for use either in supporting an RfC based on Altmann et al. (1995) or in conduct of a meta-analysis together with Altmann et al. (1995). One commenter noted a lack of concordance from high to low exposures in human studies, and from human to animal studies.

EPA Response: As discussed above in Section A.2.1, EPA followed NRC recommendations in more transparently presenting the rationale for evaluating and selecting principal studies of neurotoxicity. The EPA re-evaluation included the final peer-reviewed report of New York State Department of Health study [NYSDOH (2010); published by Storm et al. (2011)] that was provided to EPA in public comments and presented to NRC during the committee deliberations.

Public Comments: A study by the Halogenated Solvents Industry Alliance that had been under final review was cited as providing a lack of evidence of immune suppression with 28-day inhalation of up to 1,000 ppm. One commenter noted that misclassification of effect and recall bias limited conclusions from human studies of autoimmunity.

EPA Response: The study cited on immune suppression is still not available in final, peer-reviewed form, and so was not addressed in the Toxicological Review. Limitations regarding ascertainment of disease incidence and exposure assessment in population-based studies are addressed in the discussion of autoimmune disease data in the Toxicological Review (see Section 4.6.1.1.2).

A.4.2. Derivation of Reference Values

Public Comments: One commenter disagreed with selection of 3 for the UF_D (database). Another commenter noted that additional accounting of sensitivity and susceptibility of children is needed in RfC derivation. One commenter remarked that median, rather than mean [adopted by EPA], tetrachloroethylene concentration is more scientifically defensible as a POD. This commenter also questioned the assumption of continuous exposure in the critical study supporting the RfC and recommended EPA develop a time-weighted average exposure estimate using an estimate of 75% time in residence from Schreiber et al. (2002), the population/percentile estimates from EPA's Exposure Factor Handbook, or using a biologically-motivated mathematical or PBPK-based approach.

EPA Response: Based on public comments and concerns raised by the NRC, EPA re-examined the adequacy of the database and increased the UF_D from 3 to 10 (See Sections A.2.2, 5.1.2, and 5.1.3). EPA generally uses mean exposure, based on the argument of Crump (1998) that arithmetic means are expected to represent total risk better than

geometric means (see Section 5.1.1.3.2). The revised assessment uses mean exposures from occupational studies, which EPA time-weighted to represent continuous exposures.

Public Comments: An objection was raised to citing Fredriksson et al. (1993) as a supporting study, because no difference in responses was noted between doses which differed by 60-fold.

EPA Response: EPA agrees with concerns that the Fredriksson et al. (1993) is limited to support dose-response analyses, and no longer includes it among the tetrachloroethylene neurotoxicity studies considered for dose-response analysis.

A.4.3. Graphical Presentation

Public Comments: Several commenters endorsed graphical presentation of studies to illustrate the support of reference values by multiple studies, and one commented that a distributional quantitative uncertainty analysis should have been undertaken.

EPA Response: EPA has followed the NRC recommendations and agrees that the graphical presentation of studies and resulting risk values is useful (see Section A.2.3). As noted by the NRC, EPA's approach with respect to uncertainty analysis represents the current state of practice. In agreement with the NRC advice, EPA will consider expanding beyond the current state of practice for uncertainty analysis – such as use of distributional quantitative uncertainty analysis for non-cancer effects – in future re-evaluations of tetrachloroethylene.

A.4.4. Reproductive and Developmental Effects

Public Comments: Several commenters raised concern that the epidemiologic studies for developmental and reproductive endpoints were not objectively reviewed. One commenter raised concern about the studies by Szakmary et al. (1997) and Fredricksson et al. (1993), noting that in the latter study effects were only reported at doses causing maternal toxicity. They also raised concern about the presentation of a potential MOA for developmental toxicity, especially regarding the potential role of the tetrachloroethylene metabolite TCA. Other concerns raised by the commenter related to POD selection and lack of transparency in LOAEL and NOAEL selection.

EPA Response: As presented in Section A.2.4, study strengths and deficiencies, and evidence from supportive in vitro and in vivo studies and the consistency of outcomes across species and protocols, are addressed. Findings of parental (including maternal)

toxicity and the treatment levels at which those effects were observed are also described for each study. The developmental neurotoxicity evaluation by Fredricksson et al. (1993) is presented in the neurotoxicity section of the assessment, with a brief summary included in the developmental toxicity section. EPA has also expanded the discussion of the MOA hypotheses for developmental outcomes to address the potential involvement of the metabolite TCA. Selection of PODs for endpoints selected for potential RfC development (see Table 4-49) has also been more transparently described.

A.5. Response to Public Comments – Cancer Assessment

A.5.1. Epidemiologic Evidence Pertaining to Cancer

Public Comments: Several commenters were critical of the presentation and interpretation of epidemiologic studies and the weight-of-evidence provided by these studies. A more clear, comprehensive and balanced review was recommended. Particular comments concerned community drinking-water studies [Aschengrau et al. (1993), as well as Lynge et al. (2006), Ruder et al. (2001), and Ma et al. (2009)].

EPA Response: As discussed in Section A.3.1, EPA has followed the NRC recommendations and significantly reorganized the data presentation by type of cancer, included updated and more comprehensive evaluation of the epidemiologic literature, and clarified presentation of data from these studies, including those identified by public commenters.

A.5.2. Cancer Characterization

Public Comments: Several commenters raised issues about the cancer characterization. Some agreed with the characterization of tetrachloroethylene as "*likely to be carcinogenic to humans*"; others disagreed based on the lack of human relevance of animal tumors, and inconclusive epidemiologic evidence.

EPA Response: As discussed in Section A.3.1, EPA has followed the NRC recommendations and continues to characterize tetrachloroethylene as "*likely to be carcinogenic to humans.*" EPA's characterization was based on the results of bioassays that found increased incidences of tumors and to a lesser extent on epidemiologic evidence.

A.5.3. Hepatic and Renal Toxicity and Cancer

Public Comments: Several commenters recommended improved transparency and clarity in the presentation of hepatic and renal toxicity and carcinogenicity (including hepatocellular tumors and hemangiosarcomas), and carcinogenic MOA information. With respect to hepatic endpoints, one commenter recommended transparency with respect to presentation and analyses of the hemangiosarcoma data of JISA (1993). Another was critical of the Kjellstrand et al. (1984) study. Regarding kidney, commenters stated that the rodent and human data were not comprehensively or critically evaluated. One commenter noted that hepatocellular and renal tumors were specific to the rodent species and strains studied.

> **EPA Response:** EPA has revised its presentation of the renal and hepatic toxicity, carcinogenicity (including hepatocellular tumors and hemangiosarcomas), and carcinogenic MOA information, following NRC recommendations. For liver toxicity, lesser emphasis has been given to the hepatotoxicity findings in the shorter-term study of Kjellstrand et al. (1984), which has also been more completely and accurately described.

A.5.4. Selection of Tumor Type for Quantitative Assessment

Public Comments: Several commenters were critical of calculating cancer potency based on MCL, highlighting issues of susceptibility and that this endpoint is a poor model for human responses. Others raised issues with the use of any of the rodent tissue endpoints based on their apparent specificity with respect to species/sex combinations.

> **EPA Response:** As discussed in Section A.3.3, the majority of the NRC peer review panel recommended that the mouse hepatocellular tumors be used for cancer risk estimation. Therefore, the oral slope factor and inhalation unit risk are now based on the male mouse hepatocellular tumor data from the JISA (1993) bioassay.

A.5.5. Mode-of-Action Considerations

Public Comments: Several commenters raised issues about the MOA presentation and conclusions, recommending improved transparency and clarity. With respect to genotoxicity, several recommended a more comprehensive review of the available studies for tetrachloroethylene and its metabolites, including tabular summaries of the available data, a discussion of study strengths and weaknesses, and a summary discussion of the evidence. Several criticized the clarity of the MOA presentation for hepatocellular tumors. Some commenters agreed, while others disagreed, that PPARα is not the MOA. Some recommended

more explicitly addressing tetrachloroethylene-specific studies, the role of metabolic activation, and alternative MOAs (cytotoxicity and hyperplasia). For renal cancers induced by tetrachloroethylene, further consideration and discussion of the PPARα activation and sustained cytotoxicity MOAs was recommended.

> **EPA Response:** As discussed in A.3.3, EPA has undertaken significant revisions to more clearly specify the hypothesized MOAs for each tumor endpoint, and to present and analyze the evidence available to support conclusions about these hypothesized MOAs. In particular, the genotoxicity section (see Section 4.8) was substantially revised to include text and tabular study summaries of the available genotoxicity studies of tetrachloroethylene and its metabolites, and an overall synthesis section. For mouse liver tumors, EPA has significantly revised the presentation of the PPARα activation MOA and added discussion of epigenetic changes and cytotoxicity and secondary oxidative stress. EPA presents quantitative analyses of TCA, DCA, other known peroxisome proliferators, PPARα endpoints (including PPARα transactivation) and hepatic cancer. Additional emphasis has been given to the deficiencies in the knowledge-base regarding the MOA for tetrachloroethylene. Similarly, with respect to rat kidney tumors, EPA has included text and tabular summaries of the relevant data for the MOA hypotheses (including PPARα activation and sustained cytotoxicity) to better and more clearly support the conclusions.

A.5.6. Low-Dose Extrapolation

Public Comments: Several commenters supported a (threshold) non-linear dose-response analysis (or approach) based on hypothesized MOAs (particularly, PPARα activation for liver and kidney). Several were critical of the range of potencies; some noted that other factors in addition to PBPK models should be considered, while others recommended selection of a point estimate within the proposed range.

> **EPA Response:** As described in Section A.3.5, EPA has retained linear low-dose extrapolation from the POD below the observed range, based on NRC advice. The NRC noted that this is in accordance with the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*, and supported EPA's use of linear low-dose extrapolation. In addition, as recommended by the NRC, EPA has evaluated multiple options for improving the model fit in the observed range for data sets showing supra-linear dose-response shapes. As discussed in Section A.3.5, the additional analyses resulted in better characterization of the available data sets for cancer risk estimation.

A.5.7. Physiologically Based Pharmacokinetic Models

Public Comments: Several commenters addressed the dose metrics and PBPK models used in risk estimation. Regarding the dose metric, some commenters endorsed total metabolism; another commenter remarked that dose metrics for cancer risk estimates do not address the distribution or elimination of metabolites likely involved with carcinogenic process. One commenter disagreed that $BW^{3/4}$-scaling is appropriate for interspecies extrapolation because 1) EPA has not established that a metabolite causes cancer; and 2) the unknown metabolite could be a highly reactive intermediate. Several commenters were critical of the PBPK models used, with some suggesting inclusion of the Clewell et al. (2005) model, one recommending inclusion of Covington et al. (2007), and another recommended utilizing only Clewell et al. (2005) and Gearhart et al. (1993). One commenter suggested using the upper 95th confidence limit of the fraction metabolized in the Chiu and Bois (2006) analysis (i.e., 61% at a modeled exposure concentration of 0.001 ppm). Limitations of the available PBPK models for predicting GSH conjugation pathway metabolism were noted. Commenters recommended clarity in the presentation of, and disagreed with some conclusions regarding, the oral and dermal metabolism of tetrachloroethylene, the rates of metabolism through the GSH pathway conjugation, the metabolism of TCA to DCA, the bioavailability of TCA, and the presentation of and selection among the available PBPK models.

EPA Response: As discussed in Section A.3.7, EPA developed a "harmonized" PBPK model (Chiu and Ginsberg, 2011), including implementation of the NRC advice to separate metabolism into three pathways (oxidation, GSH-conjugation with further β-lyase metabolism, and GSH-conjugation with further β-lyase-independent metabolism). The harmonized PBPK modeling analysis showed that the GSH conjugation pathway in humans remains highly uncertain and/or variable (yielding an approximately 3000-fold range in human estimates), and that additional data are needed to better quantify that pathway in humans (see Section 3.5). Therefore, the assessment does not rely on quantitative estimates of GSH pathway metabolism provided by the new PBPK model. Instead, the quantitative risk estimates presented in the revised assessment rely on estimates of blood tetrachloroethylene, oxidation of tetrachloroethylene, and route-to-route extrapolation information derived from this harmonized model. These dose metric estimates from the harmonized model are robust and consistent with prior models and, thus, insensitive to model choice. EPA also revised its presentation of metabolism (see Section 3) and dose metric selection (see Section 5), as well as presentation of an

empirical analysis of the contribution of TCA to tetrachloroethylene-induced hepatic tumorigenesis (see Appendix C).

Given the current understanding of tetrachloroethylene metabolism and cancer mode(s) of action, EPA maintains that $BW^{3/4}$-scaling of metabolites (oxidative metabolites for hepatocellular tumors, GSH conjugates for kidney tumors) for extrapolation to human cancer risk is supported by the principles previously outlined by U.S. EPA (1992). In this revised assessment, risks extrapolated using tetrachloroethylene AUC in blood as the dose metric were not scaled by $BW^{3/4}$, also consistent with U.S. EPA (1992).

A.5.8. Uncertainty Analysis

Public Comments: Some public commenters recommended that a more clear and concise summary of the limitations and uncertainties in the data and analyses would be more informative than the uncertainty analysis presented by EPA. Others recommended expansion of EPA's uncertainty analysis to include additional quantitative analyses.

EPA Response: EPA has followed the NRC recommendations and retained tabular presentation highlighting EPA's choices and their effects on the determination of the upper bound of the risk estimate. As discussed in Section A.3.8, EPA has followed the NRC recommendations and extended the quantitative evaluation of different models to all candidate data sets so as to more fully array the uncertainties at the point of departure.

A.6. Focused External Peer Review of the Application of Physiologically-Based Pharmacokinetic (PBPK) modeling in the Toxicological Review for Tetrachloroethylene

The IRIS Toxicological Review of Tetrachloroethylene utilizes a harmonized PBPK model that EPA developed in response to NRC (2010) recommendations. In particular, the NRC recommended that EPA:

1. Pursue development of a single harmonized PBPK model for its assessment (as was done for trichloroethylene), which synthesizes important aspects of the previously published models. This includes the use of multiple exposure routes and inclusion of all relevant tissue compartments, and utilization of data from all relevant species and routes of exposures.
2. Explore the possibility of adding the GSH pathway (in addition to the P-450 metabolic pathway) to a harmonized PBPK model. The initial goal should be to predict the fraction

of an absorbed tetrachloroethylene dose that enters the GSH pathway and the fraction that enters the P-450 pathway. That would permit the development of more discrete dose metrics and should lead to a more rational and defensible selection of dose metrics for the various cancer end points.

3. If such modeling is determined to be infeasible, then identify data gaps that prevent successful modeling. These can be used to guide future research.

EPA accepted the NRC recommendations and developed a harmonized PBPK model for mice, rats, and humans. In particular:

1. The model synthesizes the important aspects of the various previous models, and utilizes a wider range of *in vitro* and *in vivo* data than any previous individual PBPK model of tetrachloroethylene.
2. The model separately predicts the fractions of absorbed tetrachloroethylene dose that enter either the P-450 or the GSH pathways, in addition to blood levels of the parent compound tetrachloroethylene.
3. The analysis showed that modeling the GSH pathway is feasible, but that predictions for this pathway in humans remain highly uncertain and/or variable. Therefore, better quantification of this pathway, whether at the individual or the population level, would require more direct in vivo data.

This harmonized PBPK model was peer-reviewed and published by *Toxicology and Applied Pharmacology* (Chiu and Ginsberg, 2011).

EPA conducted a focused peer review on the application of the published harmonized model to support its use in the IRIS Toxicological Review of Tetrachloroethylene. Peer reviewers were asked to consider whether the harmonized PBPK model 1) is clearly and transparently described, and adequately responsive to the NRC recommendations; and 2) used appropriately in the dose-response assessment.

The following peer reviewers were independently chosen by an EPA peer review contractor according to scientific credentials and particular areas of expertise:

1. Janusz Z. Byczkowski, Ph.D., DABT - Independent Consultant, Fairborn, OH
2. Claude Emond, Ph.D. - University of Montreal, Quebec, Canada

3. Moiz Mumtaz, Ph.D. - Computational Toxicology and Methods Development Laboratory, Division of Toxicology and Environmental Medicine, Agency for Toxic Substances and Disease Registry, Atlanta, GA

The charge questions, peer reviewer comments, and EPA responses are summarized below.

A.6.1. Charge Question #1, Peer Reviewer Comments, and EPA Responses

Charge Question:

1. NRC (2010) recommended that EPA develop a harmonized PBPK model for tetrachloroethylene and metabolites for use in its risk assessment, and provided advice to EPA on how such a model should be developed. **Overall, is the harmonized PBPK model developed by EPA clearly and transparently described and adequately responsive to the NRC recommendations?**

Peer Review Comments: All three peer reviewers commented that the PBPK model developed by EPA appears to be adequately responsive to the NRC recommendations. Specifically, one reviewer found the model to be "adequately responsive to the NRC recommendations from [a] technical standpoint"; a second reviewer stated that EPA "appeared to respond clearly and transparently to the National Research Council's (NRC's) recommendation"; and a third reviewer stated that EPA "has used credible science and current information to adequately respond to the recommendations of the NRC."

EPA Response: No response needed.

Peer Review Comments: Two reviewers recommended including more detail on the PBPK model structure and changing the conceptual representation of the model. Specifically, one peer reviewer commented that the model "is not clearly and transparently described," and recommended additional documentation in the Toxicological Review of the model structure and mechanics. A second reviewer found the model to be well presented, but having room for improvement. In particular, this reviewer found the conceptual representation of the model (Figure 3-5) did not provide a full understanding of the model's structure, and thought Tables 3-2 to 3-5 could benefit from presentation of more details. The third reviewer only provided editorial comments on this point.

EPA Response: In response to the reviewers' recommendations, EPA has substantially revised Figure 3-5 and its caption in order to more completely document the model structure and associated parameters. EPA has also added text to the table notes of Tables

3-2 to 3-5 to provide more details as to how the calculations were made. The Toxicological Review contains approximately 17 pages of text and tables describing the mechanics, parameterization, and predictions of the PBPK model. Further details of the mechanics and structure of the model are contained in Chiu and Ginsberg (2011) (particularly Appendix A of the article). To further address the peer reviewers' comments, EPA has made the model code available for download via the internet. The supporting publications and detailed code are both publicly available through EPA's HERO database, and hyperlinks to these materials are contained in the Toxicological Review.

A.6.2. Charge Question #2, Peer Reviewer Comments, and EPA Responses

Charge Question:

2. EPA's dose-response assessment includes development of a chronic inhalation reference concentration (RfC) and oral reference dose (RfD) for non-cancer effects, and an inhalation unit risk and oral slope factor for carcinogenic effects. The assessment uses the following dose metric predictions from the harmonized PBPK model to conduct inter-species and/or route-to-route extrapolation for use in the dose-response assessment:

    a. For the critical non-cancer effect of neurotoxicity, in accordance with NRC recommendations, the area-under-the-curve (AUC) of tetrachloroethylene in blood is used as the preferred dose metric, and represents a surrogate for the AUC of tetrachloroethylene in the brain.

    b. For non-cancer hepatotoxicity and hepatocarcinogenesis, liver oxidative metabolism was used as the preferred dose metric, due to weight of evidence that oxidative metabolism plays a role in these endpoints for tetrachloroethylene. Results for the AUC of trichloroacetic acid (TCA) in the liver were presented as an alternative dose metric for comparison purposes.

    c. For non-cancer nephrotoxicity and nephrocarcinogenesis, from a toxicological perspective, glutathione (GSH) conjugation metabolism would have been the preferred dose metric due to the weight of evidence that conjugative metabolites play a role in these endpoints for tetrachloroethylene. However, due to the wide range of PBPK model predictions for GSH conjugation in humans, the surrogate dose metric of AUC of tetrachloroethylene in blood was preferred. Results for

GSH conjugation were presented as an alternative dose metric for comparison purposes.

d. For all other non-cancer endpoints (reproductive, developmental, immunological, hematological toxicity) and cancer endpoints (hemangiosarcomas, mononuclear cell leukemias, brain gliomas, and testicular interstitial cell tumors), the AUC of tetrachloroethylene in blood was used as the preferred dose metric, due to the lack of available data on what the active carcinogenic moiety(ies) may be for these endpoints.

Is the harmonized PBPK model used appropriately for making predictions for these dose metrics, and are the results appropriately characterized?

Peer Review Comments: The peer reviewers supported the technical soundness of the application of the PBPK model and the numerical results. In particular, one reviewer found that the technical soundness of the PBPK methodology and numerical results seems adequate. A second reviewer found that PBPK modeling improves the quality of risk assessment predictions, and that the current model will reduce the uncertainties as compared to using previous PBPK models or the default approach. A third reviewer found that using the model will result in an acceptable degree of the uncertainty in the assessment.

EPA Response: No response needed.

Peer Review Comments: One peer reviewer requested additional explanation of how the AUC was calculated, and how and when the uncertainty factors are applied. This reviewer also recommended following U.S. EPA (2006a) recommendations as to description and documentation of the PBPK model and its use. Another peer reviewer requested information about the dose metric selection for non-cancer effects.

EPA Response: EPA has added documentation as to how the AUC was calculated to Tables 3-2 to 3-5. Documentation as to how and when the uncertainty factors are applied in cases of PBPK modeling use is already contained in Sections 5.1.4, 5.2.2, and 5.2.4; additional documentation has not been provided. With respect to the comment to follow U.S. EPA (2006b) recommendations as to description and documentation of the PBPK model and its use, EPA has, as stated above, added links to the full text of the Chiu and Ginsberg (2011) article, the supplementary materials, and the model code and simulation files, rather than reproducing the information in the Toxicological Review or its appendices. Specifically, the documentation recommended by U.S. EPA (2006b) is as

follows, with the location of such documentation for the tetrachloroethylene PBPK model in square brackets:

- Graphical representation of the model [revised Figure 3-5]
- Well-annotated and complete documentation of the model code [HERO link to model code and simulation files: (U.S. EPA, 2011)]
- All data (fully referenced) that were used to calibrate and/or test the model [HERO link to model code and simulation files: (U.S. EPA, 2011)]
- A description of the calibration and testing procedures [HERO link to full text and supplementary material: (Chiu and Ginsberg, 2011)]
- Full reference of sources for all parameter values (or the optimization methods, results, and data used in optimizing parameters) [HERO link to full text: (Chiu and Ginsberg, 2011)]
- Sensitivity analysis or other rationale that guided the choice of which parameters were optimized [HERO link to full text and supplementary material: (Chiu and Ginsberg, 2011)]
- Simulation run conditions [added as notes to Tables 3-2 to 3-5, as well as contained in HERO link to model code and simulation files: (U.S. EPA, 2011)]
- Any additional analyses that help characterize or support the quality of the model [HERO link to full text and supplementary material: (Chiu and Ginsberg, 2011)]
- All supporting documentation that would be needed by an experienced modeler to run the model and accurately reproduce any simulations used (or submitted for use) in deriving reference values [HERO link to model code and simulation files: (U.S. EPA, 2011)]

Finally, with respect to the requested information about the dose metric selection for non-cancer effects, the following information has been added to Sections 5.1.4 and 5.2.4: "For liver effects, the dose metric of liver oxidative metabolism was used, based on the view that oxidative metabolites are involved in tetrachloroethylene-induced liver effects. For kidney effects, while it is generally thought that GSH conjugation metabolites are involved, the large uncertainty in estimates of human GSH conjugation preclude use of that dose metric. Instead, the AUC of tetrachloroethylene in blood is used as a surrogate. For the other non-cancer effects,

the AUC of tetrachloroethylene in blood was used as the preferred dose metric due to the lack of data on what the active toxic moeity(ies) may be for those effects."

Peer Review Comments: One peer reviewer raised a question as to whether using tetrachloroethylene in blood as a surrogate for tetrachloroethylene in brain implied that the concentrations in brain and blood are similar, that tetrachloroethylene crosses the blood-brain barrier, and that there is no metabolism in the brain. This reviewer questioned the appropriateness of these assumptions, and that they could result in "an unrealistically conservative RfC."

EPA Response: EPA notes that the inhalation RfC did not utilize PBPK modeling, so is not impacted by the choice of dose metric. Tetrachloroethylene in blood is used as a surrogate for tetrachloroethylene in brain only for route-to-route extrapolation from the inhalation RfC to the oral RfD for neurotoxicity. For this application, the only assumption is that the blood and brain levels are proportional, independent of route of exposure, not that they are similar. Therefore, no assumptions are made as to the degree to which tetrachloroethylene crosses the blood-brain barrier and the degree of metabolism in the brain. Thus, any uncertainties regarding the blood-brain barrier and metabolism in the brain do not impact the RfD derived from route-to-route extrapolation. As noted in Section 3.2, tetrachloroethylene readily crosses the blood-brain barrier, with measured brain concentrations 4-5 times higher than blood concentrations.

A.6.3. Editorial comments

Peer Review Comment: One reviewer provided editorial comments.

EPA Response: EPA has addressed the editorial comments identified by the reviewer.

A.7. References

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APPENDIX B. STUDY DESIGN CHARACTERISTICS OF TETRACHLOROETHYLENE EXPOSURE AND CANCER EPIDEMIOLOGICAL STUDIES

B.1. Cohort Studies

Tetrachloroethylene cohort studies have been organized by occupational sector with summary of study design, exposure-assessment approach and statistical methodology. Table B-1 provides summaries of the study characteristics of each paper or group of papers.

B.1.1. Dry Cleaner and Laundry Worker Studies

B.1.1.1. Andersen et al. (1999)

Andersen, A.; Barlow, L.; Engeland, A.; Kjaerheim, K.; Lynge, E.; Pukkala, E. (1999). Work-related cancer in the Nordic countries. Scand J Work Environ Health, 25, 1-116. http://www.ncbi.nlm.nih.gov/pubmed/10507118

Summary: This cohort study examined work-related cancer in Denmark, Finland, Norway, and Sweden. The Danish, Finnish, and Norwegian cohorts were identified through the 1970 Censuses, and the Swedish cohort was ascertained through the 1960 Census. Each of the country-specific cohorts consisted of those individuals who were between 25 and 64 years of age and still alive on January 1, 1971. Overall, the four cohorts included 10,101,711 people, of which 2,346,134 were Danish, 2,115,691 were Finnish, 1,792,817 were Norwegian, and 3,847,069 were Swedish. Follow-up began in 1971 and ended with death, emigration, or end of follow-up, whichever came first. The follow-up protocol for each country was as follows: Denmark: 1971−1987 and linked for identifying deaths and/or emigration with the Central Population Register; Finland: 1971−1990 and linked with Statistics Finland; Norway: 1971−1991 and linked with the Central Population Register; and Sweden: 1971−1989 and linked with the cause-of-death register. Incident cancer cases were obtained through the national cancer registries in each country. Of the more than one million cases, 228,456 were from Denmark, 197,305 were from Finland, 207,068 were from Norway, and 397,433 were from Sweden.

The censuses contained information on demographics as well as occupations and industries that was obtained through descriptions provided by the heads of households for all economically active members. These descriptions were then coded according to the Nordic Occupational Classification in Finland, Norway, and Sweden. Denmark coded their inhabitants' occupations according to their own standards. The researchers then recoded all jobs based on a set of 54 common occupational groups based on Nordic Occupational Classification standards

and included 1 group for those who were economically inactive at the time of the census. This scheme was used to evaluate occupation as a proxy for exposure. Group number 51, Code 95 consisted of launderers and dry cleaners and included 29,333 (0.3%) cohort members. There were 9,873 (0.4%) within the Danish cohort, 4,949 (0.2%) within the Finnish cohort, 4,061 (0.2%) within the Norwegian cohort, and 10,450 (0.3%) within the Swedish cohort. This occupational group contributed a total of 519,844 person-years, which were distributed as follows: 159,156 in Denmark, 94,302 in Finland, 78,086 in Norway, and 187,580 in Sweden. Overall, there were 3,254 incident cancer cases among the laundering and dry-cleaning worker population. Of these, 964 occurred in Denmark, 429 in Finland, 545 in Norway, and 1,316 in Sweden.

Age-standardized incidence ratios and their corresponding 95% confidence intervals (CIs) were calculated for launderers and dry cleaners for all cancer sites, and for cancers of the pancreas, lung and bronchus, cervix, kidney, nervous system, and lymphopoietic tissues (non-Hodgkin lymphoma and multiple myeloma), stratified by country. Expected numbers of cases were determined using the cancer incidence rates for each country's study population. A Poisson distribution was assumed for all CIs whose standardized incidence ratios were calculated with 100 cancer cases or fewer. Strengths of the study include the compulsory nature of the 1970 Census in all four countries, the 95−99% accuracy in cancer incidence data depending on the country, and the linkage of census, mortality, and emigration, and cancer incidence data based on personal identifiers. Limitations of the study include the lack of lifetime occupational histories and the inability to differentiate between launderers and dry cleaners in the analyses.

B.1.1.2. Blair et al. (2003)

Blair, A.; Petralia, S. A.; Stewart, P. A. (2003). Extended mortality follow-up of a cohort of dry cleaners. Ann Epidemiol, 13, 50-56. http://dx.doi.org/10.1016/S1047-2797(02)00250-8

Summary: This study extended the follow-up of an earlier cohort (Blair et al., 1990) of dry-cleaning workers for the purpose of providing more information on mortality and cancer risk among those occupationally exposed to dry-cleaning solvents. The cohort was identified through the dues records from the Local No. 161 (St. Louis) of the Laundry, Dry Cleaning, and Dye House Workers' International Union. This particular union was composed entirely of dry cleaners. The cohort consisted of male and female members who entered the union between 1945 and 1978, worked for at least 1 year, and had demographic information (race, sex, date of birth, date of entry) available. Of the 11,062 union members identified, 5,369 met inclusion criteria. Blair et al. (1990) followed-up subjects through January 1979, and Blair et al. (2003) began in January 1979 and ended in December 1993, an addition of 14 years. Person-years were

calculated starting at entry to the union or in 1948, whichever came later, and ended with death or December 1993, whichever came first. Deaths were identified through the National Death Index and all were coded according to the International Classification of Diseases (8th revision) standards.

Dues records were used to obtain demographic and employment information. When demographics were not accessible through this mechanism, attempts were made to identify this information through driver's license records, social security files, health care finance administration records, and credit bureaus (Blair et al., 1990). Tasks within the dry cleaning occupation were used to assess exposure. Exposure indices were determined for four different categories of jobs within the dry cleaning occupation: (1) cleaners who run the machines and handle the clothes were deemed to have the highest exposure and assigned a time-weighted index of 40 (based on an 8-hour day); (2) pressers, sewers, and counter workers who worked where the dry cleaning occurred and were deemed to receive the bulk of their exposures through the air and were assigned a time-weighted average exposure index of 7; (3) counter workers who were employed at pick-up stations were determined to have minimal exposure and assigned an index of 0; (4) maintenance workers who had high, short-term exposures were assessed to have a time-weighted average exposure of 7. Although the authors did not report the numbers of exposed within each category, there were 220 deaths from cancer among those with little/no exposure (index of 0) and 316 deaths from cancer among those with medium/high exposure (index of 7 or 40). Standardized mortality ratios (SMRs) and 95% CIs were estimated for all causes of death, stratified by the initial follow-up period, the extended follow-up period, and the full follow-up period. Expected SMRs were determined using U.S. population 5-year age and mortality statistics. SMRs were also presented by exposure score—little or no exposure and medium/high exposure—and by date of union membership, before 1960 or after 1960, a time corresponding to widespread use of tetrachloroethylene for cleaning clothes. At the end of the follow-up period in 1993, 43.8% of the cohort members were identified as deceased. The authors did not report any strengths of their methodology; limitations include the lack of information on potential confounders, the lack of detail on job history within the industry, the study's inability to determine what proportion of their cohort were exposed to tetrachloroethylene, the inability to attribute risk to occupational versus lifestyle factors, and potential misclassification due to the use of death certificates in determining the cause of death, and the notably limited ability to examine liver cancer due to disease misclassification biases.

B.1.1.3. Cano and Pollán (2001)

Cano, M. I. and Pollán, M. (2001). Non-Hodgkin's lymphomas and occupation in Sweden. Int Arch Occup Environ Health, 74, 443-449. http://dx.doi.org/10.1007/s004200100248

Summary: This study used a historical cohort design to follow 2,881,315 Swedish men and women from 1971 to 1989 to determine whether workers associated with certain occupations had a higher risk of non-Hodgkin lymphoma. The researchers conducted the follow-up by linking the Swedish cancer environment register, which provided information on cancer cases, as well as demographic variables from the 1960 and 1970 Censuses, with a population register, which housed information on occupation and residence in 1970 and occupation in 1960. Incidence rate numerators were calculated using the Swedish cancer environment register, while rate denominators were calculated using the population register. Person-years were counted starting in 1971 until either that individual's date of death or 1989. A total of 278 occupations in men and 263 occupations in women were counted.

A total of 7,610 non-Hodgkin lymphomas were reported in the study cohort, with 5,391 cases in men and 2,219 in women. Among male cases, 11 fell within the launderers and dry cleaners occupational category (Code 943), and 22 were considered textile workers (Code 701). There were no women classified as launderers/dry-cleaners or textile workers in this study. The analysis consisted of the calculation of age-standardized incidence rates, standardized incidence ratios, as well as relative risks, and their associated 95% CIs. Age-standardized incidence rates were developed for each occupation for the entire time period and used the standard European population as a reference. Standardized incidence ratios were calculated for the 10 main occupational sectors, as well as each occupation, stratified by 5-year age groups and 5-year calendar-year period. Log-linear Poisson models were used to compare occupations against the overall cohort, adjusted for geographical area. Relative risks for each occupation and of the 10 main occupational sectors were also calculated and adjusted for age, period, and geographical category and using the other occupations in the general cohort as a reference. A strength of this study is its inclusion of 1960 Census data leading to an improved definition of exposure; a limitation was its lack of control for other potential confounders beyond demographic information.

B.1.1.4. Chow et al. (1995)

Chow, W. H.; McLaughlin, J. K.; Malker, H. S.; Linet, M. S.; Weiner, J. A.; Stone, B. J. (1995). Esophageal cancer and occupation in a cohort of Swedish men. Am J Ind Med, 27, 749-757. http://dx.doi.org/10.1002/ajim.4700270509

Summary: This study aimed to generate and refine hypotheses regarding occupational risks for esophageal cancer by examining the esophageal cancer incidence by occupation and industry in Sweden. The cohort was identified from the Swedish Cancer Environment Registry, which linked employment and cancer information for all individuals registered in the 1960 Census and the National Swedish Cancer Registry. The linkage was performed using personal identifiers. The authors do not report the final cohort size. The follow-up period was from 1961 to 1979. There were three cases of laundry workers, though exposure prevalence could not be estimated because the authors did not report the total laundry worker population. Standardized incidence ratios were calculated for the entire time frame, with expected numbers of cases based on the 5-year birth cohort- and sex-specific rates for esophageal cancer in the general Swedish population during that same time period. Only those occupations that had at least 500 individuals were examined. Statistical significance was evaluated assuming a Poisson distribution. A strength of this study is its extended follow-up period, and a limitation was the small number of exposed laundry worker cases.

B.1.1.5. Ji et al. (2005a, b), Ji and Hemminki (2005a, b, c), Ji and Hemminki (2006)

Ji, J.; Granström, C.; Hemminki, K. (2005a). Occupation and bladder cancer: A cohort study in Sweden. Br J Cancer, 92, 1276-1278. http://dx.doi.org/10.1038/sj.bjc.6602473

Ji, J.; Granström, C.; Hemminki, K. (2005b). Occupational risk factors for kidney cancer: A cohort study in Sweden. World Journal of Urology, 23, 271-278. http://dx.doi.org/10.1007/s00345-005-0007-5

Ji, J. and Hemminki, K. (2005a). Occupation and upper aerodigestive tract cancers: A follow-up study in Sweden. J Occup Environ Med, 47, 785-795. http://dx.doi.org/10.1097/01.jom.0000165798.28569.b5

Ji, J. and Hemminki, K. (2005b). Occurrences of leukemia subtypes by socioeconomic and occupational groups in Sweden. J Occup Environ Med, 47, 1131-1140. http://dx.doi.org/10.1097/01.jom.0000174302.63621.e8

Ji, J. and Hemminki, K. (2005c). Variation in the risk for liver and gallbladder cancers in socioeconomic and occupational groups in Sweden with etiological implications. Int Arch Occup Environ Health, 78, 641-649. http://dx.doi.org/10.1007/s00420-005-0015-1

Ji, J. and Hemminki, K. (2006). Socioeconomic/occupational risk factors for lymphoproliferative diseases in Sweden. Ann Epidemiol, 16, 370-376. http://dx.doi.org/10.1016/j.annepidem.2005.09.002

Summary: These six studies used a cohort obtained through the Swedish Family-Cancer Database to examine the potential relationship between occupation and various cancers. The database linked national censuses (1960, 1970, 1980, and 1990), mortality data, cancer incidence data, and an administrative family register. The database was updated at two different time periods (2002 and 2004) with information from the Swedish Cancer Registry. The update in 2002 covered the period 1961−2000, and the update in 2004 covered the period 1958−2002. The cohort consisted of 1,644,958 men who were employed at the time of the 1960 Census and 1,154,091 women who were employed at the time of the 1970 Census. Follow-up began at immigration or at one of the following dates: January 1961 for those in the 1960 Census, January 1970 for those in the 1970 Census or for those who reported the same occupation in both the 1960 and 1970 Censuses, or January 1980 for those who reported the same occupation in all three censuses. Follow-up ended with cancer diagnosis, death, emigration, or on December 2000, whichever came first. Occupation was assessed as a proxy for exposure, with relevant census information (employment status, job title, work industry) coded according to Nordic Occupational Classifications. These codes corresponded to 53 occupational groups, which included launderers and dry cleaners. Overall, there were 9,255 (0.6%) male and 14,974 (1.3%) female launderers and dry cleaners. Standardized incidence ratios were calculated for each occupation in each census subcohort (1960, 1960−1970, and 1960−1970−1980). Expected numbers of site-specific cancer were estimated from 5-year-age, 10-year-period, and 6 group socioeconomic status-specific standard incidence rates. Corresponding 95% CIs were estimated assuming a Poisson distribution. Strengths of these studies include their population-based design, extended follow-up period, and utilization of three different censuses. Limitations to the studies include the inability to directly control for smoking as a potential confounder, the exception was bladder cancer (Ji et al., 2005a), the low power resulting from a small proportion of exposed cohort members that reported the same occupation in more than one census, and the high proportion of women without occupational data in the 1960 Census, which made comparisons with more than one census difficult. Also, Ji et al. (2005b) and Ji and Hemminki (2006) limited their study to those over the age of 30 years, which may have biased the external validity of the study because the findings could not be generalized to the ≤30 population.

Ji et al. (2005a) examined the relationship between occupation and first primary bladder cancers using the information from the 2002 database update. Overall, 24,041 men and 3,405 women developed bladder cancer, which included 157 male launderers and dry cleaners from the 1960 Census. There were 67 cases among male launderers and dry cleaners in both the

1960 and 1970 Census, and 19 cases among male launderers and dry cleaners in the 1960, 1970, and 1980 Censuses. The results for female launderers or dry cleaners were not reported. Two different standardized incidence ratios were calculated for occupations stratified by gender. The first estimate was adjusted for age and period, and its corresponding 95% CI was adjusted for age, period, and socioeconomic status. In order to account for the effect of smoking on bladder cancer, all standardized incidence ratios were divided by 35%, which was based on a difference in bladder and lung cancer risks for smoking 20 cigarettes per day developed by the International Agency for Research on Cancer (IARC, 2004b). This estimated a second, smoking-corrected standardized incidence ratio as well as a smoking-corrected 95% CI. All estimates were stratified by gender.

Ji et al. (2005b) examined the relationship between occupation and first primary kidney cancer, including parenchymal cancer, pelvic cancer, and unspecified cancer. This study utilized the data from the 2002 update. There were 61 cases (51 renal parenchyma, 7 renal pelvis, and 3 unspecified) among male launderers from the 1960 Census and 92 cases (79 renal parenchyma, 6 renal pelvis, and 7 unspecified) among female launderers from the 1970 census. There were 26 cases (21 parenchyma, 3 renal pelvis, 2 unspecified) that occurred among women who reported being launderers in both the 1960 and 1970 Censuses and 3 cases (1 parenchyma, 1 renal pelvis, 1 unspecified) among those who reported being launderers in the 1960, 1970, and 1980 Censuses. All standardized incidence ratio estimates were adjusted for age, time period, and socioeconomic status and stratified by gender.

Ji and Hemminki (2005a) assessed risk factors for first primary upper aerodigestive tract (lip, tongue, month, pharynx, and larynx) cancers using the 2002 database update. There were 83 cases (9 lip, 9 tongue, 13 mouth, 24 pharynx, 28 larynx) that occurred among male launderers in the 1960 Census, 32 cases (2 lip, 3 tongue, 6 mouth, 10 pharynx, 11 larynx) among male launderers who were in both the 1960 and 1970 Censuses, and 13 cases (0 lip, 2 tongue, 2 mouth, 6 pharynx, 3 larynx) among male launderers who were in the 1960, 1970, and 1980 Censuses. Among the female launderer population in the 1970 Census, there were 30 upper aerodigestive cancers (10 lip, 2 tongue, 7 mouth, 6 pharynx, 5 larynx). The results for female launderers in multiple censuses were not reported. All standardized incidence ratio estimates were adjusted for age, period, and socioeconomic status and stratified by gender.

Ji and Hemminki (2005b) examined socioeconomic and occupational risks on leukemia by histologic type after the database's 2004 update. This study was limited to cohort members aged 31 or older and diagnosed with primary leukemia, including chronic lymphatic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and polychythemia vera (PV). There were 47 cases of leukemia, of which 19 were CLL, 7 AML, and 5 CML, among male launderers and dry cleaners from the 1960 cohort and 80 cases of leukemia, of

which, 32 were CLL, 20 AML, and 2 CML, among female launders and dry cleaners from the 1970 cohort. The results for men and women employed as launderers and dry cleaners for more than one census were not reported. Standardized incidence ratios were calculated for six socio-economic groups reported in the 1960 Census, adjusted for age and period, as well as for each occupational group, adjusted for age, period, and socioeconomic status. All estimates were stratified by gender.

Ji and Hemminki (2005c) examined the relationship between occupation, socioeconomic status, and liver and gallbladder cancers using information from the 2002 database update. Overall, 7,620 men and 4,041 women developed liver and gall bladder cancer (4,211 men and 1,126 women with primary liver cancer), which included 53 male launderers and dry cleaners from the 1960 Census and 86 female launderers and dry cleaners from the 1970 Census (25 men and 25 women with primary liver cancer). All standardized incidence ratio estimates were adjusted for age and socioeconomic status and stratified by gender.

Ji and Hemminki (2006) looked at the socioeconomic and occupational risks for lymphoproliferative diseases, including non-Hodgkin lymphoma, chronic lymphatic leukemia, and multiple myeloma, after the database's 2004 update. This study was limited to cohort members aged 31 and older who were diagnosed with primary lymphoproliferative diseases, including non-Hodgkin lymphoma (NHL), CLL, and multiple myeloma (MM). There were 59 cases of NHL, 19 cases of CLL, and 29 cases of MM among male launderers and dry cleaners from the 1960 Census. Among female launderers and dry cleaners, there were 67 cases of NHL, 18 cases of CLL, and 36 cases of MM in the 1960 Census, 64 cases of NHL, 32 cases of CLL, and 31 cases of MM among those in the 1970 Census, and 12 cases of NHL, 8 cases of CLL, and 9 cases of MM among those from both the 1960 and 1970 Censuses. Standardized incidence ratios were calculated for six socio-economic groups reported in the 1960 Census, adjusted for age and period, as well as for each occupational group, adjusted for age, period, and socioeconomic status. All estimates were stratified by gender.

B.1.1.6. Lindbohm et al. (2009)

Lindbohm, M. L.; Sallmén, M.; Kyyrönen, P.; Kauppinen, T.; Pukkala, E. (2009). Risk of liver cancer and exposure to organic solvents and gasoline vapors among Finnish workers. Int J Cancer, 124, 2954-2959. http://dx.doi.org/10.1002/ijc.24309

Summary: This cohort study of economically active Finns born between 1906 and 1945 examined the relationship between job title reported on the 1970 Census and primary liver cancer incidence between 1971 and 1995. The cohort consisted of 1.2 million economically active men and women born between 1906 and 1945 who participated in the Finnish Population Census of 1970. There were 2,474 liver cancers diagnosed between 1971 and 1995 of which 9 occurred in

launderers (2 male, 7 female). Exposure was defined as longest held occupation reported on the 1970 Census and assigned to subjects using industry code (850 for launderers) or as cumulative exposure to "organic solvents" using the Finnish job exposure matrix (FINJEM) for every 5-year birth cohort and 5-year calendar period. The exposure for each birth cohort was assumed to start in the year when the average age of the birth cohort was 20 or at 65 years of age, whichever came first, because occupational histories were not available. The annual average exposure was the product of the proportion of exposed and the mean level of exposure in that occupation. A lag period was incorporated in the cumulative estimate by omitting exposure from the 10 last years. For launderers, averages of 10 ppm in 1945−1959 and 5.3 ppm in 1960−1984 were assumed. In Finland, for the later time period, this would likely be for tetrachloroethylene because this was the predominate solvent used in dry cleaning, accounting for roughly 85% of all solvents at that time (Kauppinen et al., 2009; Lynge et al., 2006; Johansen et al., 2005).

Standardized incidence ratios and 95% CIs were calculated by gender using a Poisson regression, with expected number of cancer cases estimated using site-specific cancer incidence rates of the larger Finnish population. Statistical analyses controlled for alcohol consumption, smoking, and socioeconomic status. A strength of the study includes the use of census information and the ability of statistical analysis to account for potential confounding from smoking, alcohol consumption, and socioeconomic status. The few liver cancer deaths and low-exposure prevalence and the classification of exposure based solely on census-reported information rather than a full lifetime of employment are limitations.

B.1.1.7. Lynge and Thygesen (1990), Lynge et al. (1995)

Lynge, E; Thygesen, L. (1990) Primary liver cancer among women in laundry and dry-cleaning work in Denmark. Scand J Work Environ Health 16(2):108−112.

Lynge, E; Carstensen, B; Anderson, O. (1995) Primary liver cancer and renal cell carcinoma in laundry and dry-cleaning workers in Denmark. Scand J Work Environ Health 21(4):293−295.

Summary: These studies used a retrospective cohort design to examine the relationship between work in dry-cleaning shops where tetrachloroethylene was the main solvent used and cancer in Denmark. The cohort consisted of 10,600 Danish men and women aged 20 to 64 years who were registered in the 1970 Census as "laundries, cleaning and dyeing." This encompassed industry Code 860 (laundries, cleaning, and dyeing) and occupational Codes 411 (laundry worker, ironer) and 380 (factory hand), as well as those who reported themselves as self-employed or family workers. There were 2,434 (23%) self-employed dry cleaners or launderers, 830 (7.8%) family workers, 6,837 (64.5%) laundry workers or ironers, and 499 (4.7%) factory hands. Overall, there were 2,886 laundry and dry-cleaning shops in Denmark in 1970, of which

695 were known dry-cleaning and dyeing shops where dry cleaning was the predominant activity.

Lynge et al. (1990) studied the cancer incidence within the cohort during a 10-year follow-up after the 1970 Census. The census data were linked to the Danish Cancer Registry for the period 1970 to 1980, and 24 cancer sites were examined. There were a total of 510 observed cancer cases. Standardized incidence ratios and their corresponding 95% CIs were calculated assuming a Poisson distribution if the observed number of cases was $\leq$30 and a normal distribution if the number was >30. Expected numbers were estimated by multiplying the person-years at risk within each 5-year age group with the site-specific incidence rates that were estimated for the full 1970 Census cohort. The authors do not report any strengths of their methodology; limitations include the study's inability to separate laundries from dry-cleaning shops and the lack of a sufficient period for cancer latency.

Lynge and Thygesen (1995) used a nested case-control study to differentiate the laundry workers from the dry cleaners in their examination of liver and renal cell carcinoma within this cohort. The cohort was followed from 1970 through 1987 for death, emigration, and incident cancer. During this period, there were a total of 17 liver cancer cases and 16 renal cell carcinoma cases. Controls were randomly selected from within the cohort and matched to cases on gender, 5-year age group, and occupation. In order to be included in the study, controls were required to be alive and living in Denmark at the time of the case's diagnosis; no other exclusion criteria were adopted. There were five controls matched to each case, and the final sample consisted of 33 cases (17 liver and 16 renal cell carcinoma) and 165 controls (85 liver and 80 renal cell carcinoma). Occupation was assessed as a proxy for exposure. The identification numbers of each of the 198 participants were unencrypted to obtain personal addresses, which were then used to retrieve the original census forms. These forms contained descriptions of the occupations and workplaces and allowed the researchers to recode each individual as either a launderer or a dry cleaner. The authors do not state if this assessment occurred blindly. Overall, none of the liver cancer cases, 20 (24%) liver cancer controls, 3 (18%) renal cell carcinoma cases, and 20 (29%) renal cell carcinoma controls worked as dry cleaners in 1970. Conditional logistic regression was used to calculate relative risks and their corresponding 95% CIs. A strength of this study is its ability to examine dry cleaners separately from laundry workers. Limitations include the low-exposure prevalence, the lack of adjustment for alcohol and smoking as possible confounders, the classification of exposure based solely on a census form rather than a full lifetime of employment, and the use of controls with diseases potentially associated with dry-cleaning exposure.

B.1.1.8. Pukkala et al. (2009)

Pukkala, E.; Martinsen, J.; Lynge, E.; Gunnarsdottir, H.; Sparén, P.; Tryggvadottir, L., . . . Kjaerheim, K. (2009). Occupation and cancer - follow-up of 15 million people in five Nordic countries. Acta Oncol, 48, 646-790. http://dx.doi.org/10.1080/02841860902913546

Summary: This cohort study, the Nordic Occupational Cancer Study, of 15 million subjects, aged 30−64 years in the 1960, 1970, 1980/1981, and/or 1990 Censuses in Denmark Finland, Iceland, Norway, and Sweden assessed cancer incidence through 2005 using national cancer registries. Occupational title as recorded on census records was used as a surrogate for exposure, and the investigators examined 54 broad occupational categories identified from Nordisk Yrke Klassifisering (NYK) and the International Standard Classification of Occupations (ISCO). In total, 43,496 dry-cleaners and laundry workers (n = 8,744 men, n = 34,752 women), defined by NYK and ISCO Codes 95 (http://astra.cancer.fi/NOCCA/). Both tetrachloroethylene and trichloroethylene were potential exposures to dry cleaners and launderers. Tetrachloroethylene used in Finland was less than in the other Nordic countries in 1975−1994 (Kauppinen et al., 2009). A future effort of this project is an examination of cancer incidence and 20 agents, including tetrachloroethylene and trichloroethylene (Kauppinen et al., 2009).

Follow-up began on January 1 of the year after the first available census, and person-years were counted until the date of emigration, death, or to December 31 of the following years: 2003 (subjects from Denmark and Norway), 2004 (subjects from Iceland), 2005 (subjects from Finland and Sweden). The study examined 49 cancer sites and 27 diagnostic subgroups during the 13−45 year follow-up period. Standardized incidence ratios and their corresponding 95% CIs were calculated for each site-specific cancer and occupational title with expected number of site-specific cancers calculated from separate countrywide incidence rates. Statistical analyses did not include examination of duration of employment, in this case, appearing as a dry-cleaner or laundry worker on more than one census.

This is a large study with follow-up to account for a cancer latent period of ≥15 years, and a strength is linkage with national population registries and cancer registries. The large number of dry-cleaners and laundry workers is an advantage; however, occupational title as dry-cleaner and laundry worker is broad, with subjects having differing potential to exposure intensities and to multiple solvents. Despite the large number of subjects with occupational title of dry-cleaner and laundry worker, statistical power may be compromised from the low-level detail of the exposure-assessment approach for these reasons.

B.1.1.9. Ruder et al. (2001, 1994), Calvert et al. (2011)

Ruder, A. M.; Ward, E. M.; Brown, D. P. (1994). Cancer mortality in female and male dry-cleaning workers. J Occup Med, 36, 867-874. http://www.ncbi.nlm.nih.gov/pubmed/7807267

Ruder, A. M.; Ward, E. M.; Brown, D. P. (2001). Mortality in dry-cleaning workers: An update. Am J Ind Med, 39, 121-132. http://dx.doi.org/10.1002/1097-0274(200102)39:2<121::AID-AJIM1000>3.0.CO;2-H

Calvert, G. M.; Ruder, A. M.; Petersen, M. R. (2011). Mortality and end-stage renal disease incidence among dry cleaning workers. Occup Environ Med. http://dx.doi.org/10.1136/oem.2010.060665

Summary: This retrospective cohort study examined the relationship between occupational exposures and mortality in a cohort of dry cleaners and updates earlier studies (Calvert et al., 2011; Ruder et al., 2001, 1994). An examination of end-stage renal disease incidence (ESRD) was presented in (Calvert et al., 2011) using the Renal Management Information System (REMIS) maintained by the U.S. Centers for Medicare and Medicaid Services. The cohort was obtained from union dry-cleaning records in California, Illinois, Michigan, and New York and included anyone employed for at least 1 year prior to 1960 in a dry-cleaning shop that used tetrachloroethylene. Attempts were made to verify the solvent exposure records with visits to the shops themselves. Follow-up of vital status was to 1990 (Ruder et al., 1994), 1996 (Ruder et al., 2001), and 2004 (Calvert et al., 2011). Of the cohort of 1,704 workers in the current follow-up analysis, 618 (36%) worked only in shops that used tetrachloroethylene as the primary solvent cleaner, and 1,086 (64%) worked at shops where the primary cleaner (tetrachloroethylene or Stoddard solvent) could not be verified or where other solvents were known or suspected to be used instead of tetrachloroethylene. Calvert et al. (2011) found four subjects in Ruder et al. (2001) had missing birthdates, and these subjects were not included in their latest cohort follow-up. Calvert et al. (2011), additionally, was less successful than Ruder et al. (2001) at obtaining causes of deaths; 8% of deaths were not obtained in the latest follow-up compared to 3% in Ruder et al. (2001). As of 2004, 322 deaths had occurred.

Tetrachloroethylene exposure was estimated by duration of employment in the dry-cleaning shops (1 to 5 years or more than 5 years) and by latency periods (time since first employment was less than 20 years or 20 or more years). Person-years were calculated from either January 1, 1940, or after 1 year of employment in a unionized tetrachloroethylene shop, whichever came later, through their death, the date they were lost to follow-up, or the end of 2004, whichever came earlier. SMRs and their corresponding 95% CIs were calculated for each cause of death in the full cohort, for selected causes of death by duration of employment and time since first employment, and for selected causes of death by the tetrachloroethylene-only subcohort (618 workers) and the mixed cohort (1,704 workers) separately. The expected

number of deaths was estimated using national rates. SMRs and their 95% CIs for each of the four regions were also estimated using both county and national rates, though these data were not shown. The National Death Index was used to obtain information on deaths that occurred in the cohort.

Subjects employed since 1977, the date REMIS was first available, were followed for ESRD incidence to 2004. A total of 1,296 subjects—494 in the tetrachloroethylene cohort—were followed with 30 incident cases of ESRD identified. Standardized incidence ratios and their corresponding 95% CIs were calculated for each ESRD type by the tetrachloroethylene-only subcohort and the mixed cohort separately. The expected number of deaths was estimated using all incident cases of ESRD available in REMIS as the numerators and U.S. Census data as the denominators.

A strength of this study is its estimation of tetrachloroethylene exposure based on duration and intensity. A limitation of the study is likely exposure-measurement error introduced through inability to update work histories after 1982, potentially underestimating duration, and lack of information on exposure intensity. Both aspects would tend to result in nondifferential bias that would dampen risk estimates. Additionally, a full latent period has not passed for the cohort, only 2% of the cohort had died at the end of follow-up in 2004, and only one-half of the cohort had a latent period of ≥20 years. This is also valid for analysis of ESRD incidence as the latent period is less than that for mortality. Another limitation is the lack of individual subject information on smoking and alcohol consumption as potential confounders, although the authors noted that the estimates for certain cancers were higher than what they would be if smoking was the only significant factor, and potential for multiple solvents exposures with subjects whose first employment date was before 1960.

B.1.1.10. Sélden and Ahlborg (2011)

Seldén, A. I. and Ahlborg, G. (2011). Cancer morbidity in Swedish dry-cleaners and laundry workers: Historically prospective cohort study. Int Arch Occup Environ Health, 84, 435-443. http://dx.doi.org/10.1007/s00420-010-0582-7

Summary: This study examined cancer incidence in a cohort of 9,440 Swedish dry-cleaning workers launderers, dry cleaners, and pressers identified by employers as working in laundries or dry-cleaning shops during 1973 and 1983 for a study of pregnancy outcomes (Ahlborg, 1990a). In mid-1980, a questionnaire was mailed to all washing establishments recorded in the Swedish Postal Address Registry. Of the 1,254 employers that received the questionnaire, 475 (37.9%) of the employers responded to the questionnaire and identified 10,389 employees. Data from 14 companies were lost from the original study, leaving workers from 461 companies for the cancer incidence study. The size of companies participating in the

study varied from small family businesses to large establishments. In addition to seeking information on employee identities, the questionnaire sought details of production volumes, washing techniques, and details of any chemicals used; no tetrachloroethylene exposure information was provided on individual employees. Study authors verified subjects fulfilled inclusion criteria of Ahlborg (1990a) and this study, excluding subjects who did not fulfill criteria.

Exposure assignment to three categories was carried out using company-provided information on type of business: tetrachloroethylene or PER subgroup, laundry subgroup, or other. The PER subgroup was composed of employees of dry cleaner and laundries with a proportion of dry-cleaning with tetrachloroethylene only; the laundry subgroup included employees in laundry establishments, while the "other" subgroup contained employees of businesses using a combination of chemicals for dry cleaning in addition to tetrachloroethylene (chlorofluorocarbons, white spirit, naphtha, or trichloroethylene). Tetrachloroethylene had been used in Sweden almost exclusively for dry-cleaning since the 1950s. Historical industrial monitoring data indicated exposure levels on the order of 100−200 mg/m^3 in the 1970s, with tetrachloroethylene concentrations decreasing by 50% from 1980 to 1985, and an 8-hour TWA rarely exceeding 50 ppm (Seldén and Ahlborg, 2011; Johansen et al., 2005; Ahlborg, 1990b).

Of the 10,389 subjects reported by the companies and who were employed at least 1 month, 677 were excluded for either not fulfilling the original inclusion criteria or other reasons and 272 were lost in the identification process. Overall, 9,440 subjects (2,810 men and 6,630 women) were followed for cancer incidence from January 1, 1985 to until 85 years of age, death, emigration or to December 31, 2006, whichever can first. A total of 1,106 incident cancers were identified from the Swedish Cancer Registry, 723 of which occurred in subjects categorized in the PER exposure subgroup. Site-specific standardized incidence ratios and their 95% CIs were estimated using expected numbers of cancers estimated from cancer incidence rates of the Swedish population. Additionally, SIR and 95% CIs are reported separately for each exposure category, as well as by employment duration for subjects in the PER and laundry categories.

This study differs from other included in this summary of Swedish or all-Nordic dry cleaners and launderers in that it is based on employer-reported instead of census-reported information. A strength of the study is the over 20-year follow-up. Also, the authors provide some information to evaluate potential confounding from smoking and alcohol. Ahlborg (1990a, b) collected smoking information on some of the women who also participated in the pregnancy outcome study and reported a prevalence of daily smoking before conception of 66−70%, higher than reported for women attending Swedish prenatal care centers in the early 1980s and for national data (Seldén and Ahlborg, 2011; Ahlborg and Bodin, 1991). The higher prevalence of

smoking among women may potentially confound observations for smoking-related site-specific cancers such as lung and bladder. With respect to alcohol consumption, a previous survey of women in the pregnancy outcome study found a higher prevalence of "high" consumption compared to women attending prenatal care centers (Seldén and Ahlborg, 2011; Ahlborg and Bodin, 1991; Ahlborg, 1990a). Selden and Ahlborg (2011) do not identify the average age of the cohort. As some subjects in the cohort were included in a study of pregnancy outcomes, this is not an "old" cohort, and expected cancer rates would be lower than for a cohort composed of more aged subjects. Limitations identified by Selden and Ahlborg (2011) included lack of quantitative exposure data, lack of a full occupational history, and low tetrachloroethylene exposures. The expected lower background cancer rates and low tetrachloroethylene exposures would lower the study's statistical power.

B.1.1.11. Travier et al. (2002)

Travier, N.; Gridley, G.; De Roos, A. J.; Plato, N.; Moradi, T.; Boffetta, P. (2002). Cancer incidence of dry cleaning, laundry and ironing workers in Sweden. Scand J Work Environ Health, 28, 341-348. http://www.ncbi.nlm.nih.gov/pubmed/12432988

Summary: This cohort study examined cancer incidence in Swedish launderers, dry cleaners, and pressers using a linked register that included the 1960 and 1970 Censuses, the Swedish national cancer registry, and the national register of causes of death. Person years were counted starting January 1971 until cancer diagnosis, death, or loss to follow-up December 1989, whichever came first. All individuals with second primary neoplasms were excluded. The authors did not report the total number of individuals included in the cohort. Launderers and dry cleaners comprised Nordic Classification of Occupation Code 943, and pressers were Code 944; laundry, ironing, and dyeing comprised Swedish Industrial Code 880 in 1960 and Code 9520 in 1970.

Exposure was classified into five categories: Group 1, launderer, dry cleaner, or presser occupation employed in the laundry, ironing, or dyeing industries in either the 1960 or 1970 Censuses (543,036 person-years); Group 2, launderer, dry cleaner, or presser occupation employed in the laundry, ironing, or dyeing industries in both the 1960 and 1970 Censuses (46,934 person-years); Group 3, launderer, dry cleaner, or presser occupation employed in other industries at the time of both censuses (18,960 person-years); and, Group 4, other occupational titles employed in laundry, ironing, or dyeing industry (13,395 person-years); and, Group 5, not employed in relevant industries or occupations during both censuses (69,540,184 person-years).

Multivariable Poisson regressions were used to calculate the relative risks and 95% CIs of cancer for each category of exposure, adjusted for age, calendar period, geographic region, urban setting, and gender. These analyses were also stratified by gender and adjusted for age,

calendar period, geographic region, and urban setting. Travier et al. (2002) further assessed temporal changes in solvent use, portraying relative risks by age in 1960 and noted subjects under 40 years of age in 1960 presumably used mainly tetrachloroethylene and carbon tetrachloride. A strength of this study is its detailed analysis of observed associations. Limitations of this study include its low power and the use of self-reported occupational and industrial codes to classify exposures.

B.1.1.12. Wilson et al. (2008)

Wilson, R.; Donahue, M.; Gridley, G.; Adami, J.; El Ghormli, L.; Dosemeci, M. (2008). Shared occupational risks for transitional cell cancer of the bladder and renal pelvis among men and women in Sweden. Am J Ind Med, 51, 83-99. http://dx.doi.org/10.1002/ajim.20522

Summary: This study used a retrospective cohort design to examine if incident bladder and renal pelvic cancers share similar occupational risk factors. It tested the hypothesis that bladder and renal pelvis cancers were similarly elevated in each occupation and industry category. The cohort consisted of 4,197,684 Swedish men and women employed during either the 1960 or the 1970 Census and still living at the start of 1971. Person-years were counted starting January 1, 1971, and ending with a cancer diagnosis, emigration, death, or December 31, 1989, whichever came first. Cancer information was obtained from the Swedish Cancer-Environment Registry for the study period 1971 to 1989. Overall, there were a total of 70,083,912 person-years of follow up, with a mean time of 16.7 years. Within the cohort, there were 1,374 incident renal pelvis cancers and 21,591 incident bladder cancers.

Occupation as noted on the 1960 and 1970 Censuses was assessed as a proxy for chemical exposures, including tetrachloroethylene. Job titles reported in the censuses were coded according to the National Swedish Classification of Occupations and Industries standards, for which laundry and dry-cleaning workers were occupation Code 943, and the laundry, ironing, and dyeing was industry Code 880 in 1960 and Code 9250 in 1970; 25,249 men and women (0.6% of the cohort) were employed in this industry, for which there 110 observed bladder cancer cases (55 female and 55 male) and 11 observed renal pelvic cancer cases (8 female and 3 male). A job exposure matrix was also used to assess exposure to indoor work and low physical activity, among others.

Standardized incidence ratios and their associated 95% CIs were calculated for each occupation and industry using expected site-specific cancer incidence of rates of the total employed Swedish population. Strengths include the large sample size, use of well-validated registries, adequate follow-up, and high case ascertainment. Limitations to the study include its lack of adjustment for confounders including smoking, possible misclassification of exposure

based on job title in 1 or 2 census years, and the lack of occupational history for each participant.

B.1.2. Other Occupational Cohorts

B.1.2.1. Anttila et al. (1995)

Anttila, A.; Pukkala, E.; Sallmen, M.; Hernberg, S.; Hemminki, K. (1995). Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. J Occup Environ Med, 37, 797-806. http://www.ncbi.nlm.nih.gov/pubmed/7552463

Summary: This cohort study assessed the incidence of cancer among employees who were biologically monitored by the Finnish Institute of Occupational Health (FIOH) between 1965 and 1983 in comparison to the total Finnish population. The cohort consisted of workers who had their blood and urine assessed for trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane due to their employment in occupations that exposed them to hazardous substances. Tetrachloroethylene was monitored in the blood of workers between 1974 and 1983, and median blood tetrachloroethylene concentrations were 0.7 μmol/L in males and 0.4 μmol/L in females. There were, on average, 3.2 blood tetrachloroethylene measurements per individual. In addition to the measurement information, the FIOH database provided data on demographics, date and time of sampling, workplace, solvent code, result, specific activity, and the laboratory in which the sample was analyzed. Approximately 600 codes of workplaces or sampling laboratories were included in the database.

Follow-up was conducted automatically with the Finnish Cancer Registry and began in January 1967 or on the date of first measurement of the solvent, whichever was later, and ended at emigration, death, or December 1992, whichever was first. Death and emigration were ascertained through the Population Register Center; mortality was also followed-up using cause-of-death data from the Central Statistical Office of Finland for the period 1956 to 1991. Of the 11,534 biological measurements taken between 1965 and 1983, 10,743 (93.1%) were linked to personal identifiers, which corresponded to a total of 3,976 workers. After excluding those who could not be completely identified or were not alive at the start of follow-up, the final sample consisted of 3,974 individuals who contributed a total of 71,800 person-years. Follow-up time averaged 18 years, with 27,547 person-years within the period 10−19 years after entry into the cohort and 5,877 person-years within the period ≥20 years. There were 849 (21.4%) workers monitored for exposure to tetrachloroethylene, and they contributed a total of 11,958 person-years.

The study examined 29 cancer sites during the 26-year follow-up period, which were selected on the basis of their known or suspected association with the solvents. Of these, 8 sites

(pancreas, lung/bronchus, cervix uteri, kidney, nervous system, non-Hodgkin lymphoma, multiple myeloma, and all cancer sites) were specifically evaluated with respect to tetrachloroethylene and contributed 31 observed cancer cases during the follow-up period of 1974 to 1992. Standardized incidence ratios and their corresponding 95% CIs were calculated for all halogenated hydrocarbons, trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane separately, with expected numbers of site-specific cancers calculated from incidence rates of the Finnish population. Significance was evaluated with the Mantel-Haenszel χ^2 test under the assumption that the observed cases followed a Poisson distribution. Exposure duration was not examined for tetrachloroethylene subjects, given the few site-specific cancer cases. Strengths of the study include its use of the same source for the calculation of observed and expected cases, as well as its linkage with Finnish registries, which provided complete ascertainment of death, emigration, and cancer incidence. Limitations include the study's low power for its analysis of tetrachloroethylene, incomplete registration in earlier years, low tetrachloroethylene concentrations [for comparison, Ferroni et al. (1992) reported median blood tetrachloroethylene and atmospheric monitoring of dry cleaners of 874 μmol/L and 15 ppm, respectively], the study's inability to infer lifetime exposure based on blood tetrachloroethylene, and the potential for multiple solvents exposures.

B.1.2.2. Boice et al. (1999)

Boice, J.; Marano, D.; Fryzek, J.; Sadler, C.; McLaughlin, J. (1999). Mortality among aircraft manufacturing workers. Occup Environ Med, 56, 581-597. http://dx.doi.org/10.1136/oem.56.9.581

Summary: This cohort mortality study conducted follow-up to evaluate cancer and other diseases among aircraft workers. The cohort was identified through work history cards, personnel files, and retirement records and consisted of individuals employed at Lockheed Martin aircraft manufacturing factories for at least 1 year from January 1960 onwards. Those with missing work history information or incorrect dates were excluded. The follow-up period began January 1, 1960 or after 1 year of employment and ended with death, age 95 years, or December 1996, whichever came first. The vital status of each cohort member at the end of the follow-up period was obtained through a variety of methods, which included California death tapes, the National Death Index, Pension Benefit Information Files, Social Security Death Index, Health Care Financing Administration files, California Department of Motor Vehicles records, employment work history cards, pension and retirement records, and obituaries from 1960 to 1996. Vital status could not be ascertained for 11,533 (15%) of cohort members and were assumed to be alive. This assumption was examined using a random sample of 700 subjects and demonstrated that approximately 95% of this sample was alive, and if representative of all

subjects with missing vital status information, overall, lost to follow-up was estimated to be 0.7% of the cohort. The published paper lacks information to evaluate whether the random sample was representative of all subjects lacking vital status. Of the 113,204 aircraft workers, 77,965 (68.9%) were included in the study and contributed a total of 1,889,795 person-years of follow-up. The average follow-up per cohort member was more than 20 years.

Exposure was assessed through walk-through surveys of the closed factories or similar factories, interviews with long-term employees, and industrial hygiene files or other historical documents. From job code, job title, and job change information, the researchers were able to group occupations with similar work activities, identifying job titles that may have indicated chemical exposure. All administrative and technical jobs were classified as having "no significant chemical exposure" and removed from the analysis. All factory-related jobs were categorized based on a number of chemical exposures and a job exposure matrix, to assign tetrachloroethylene exposure defined as routine (part of daily activities), intermittent (not routine or on a daily basis), or minimal to no exposure. Limited data on tetrachloroethylene levels were available, with few measurements before 1970 although tetrachloroethylene was used in vapor degreasing starting in 1966 after trichloroethylene (TCE) was discontinued until the early 1990s (Marano et al., 2000). Air sampling revealed that long-term air exposures to tetrachloroethylene measures from 1987−1988 were 3 ppm (median) and 9.5 ppm (mean) [range: 0.06−27 ppm], and short-term air exposures measured from 1978−1988 were 56 ppm (mean) and 17 ppm (median) [range: 1.7−150 ppm]. Similarly, many factory workers were exposed to multiple substances. For example, 4,421 (59%) subjects were also exposed to chromate, 2,262 (42%) were also exposed to TCE, 5,830 (18%) were also exposed to mixed solvents, and 298 (24%) were also exposed to asbestos. Among the factory worker subcohort, 2,631 (5.8%) employees were assessed as having been exposed to routine levels, and another 3,199 (7.1%) subjects were exposed to intermittent levels of tetrachloroethylene. The workers that were routinely exposed contributed a total of 51,214 person-years at risk and had 476 observed deaths (all causes).

SMRs and their corresponding 95% CIs were calculated for routine exposed subjects assuming the observed number of deaths followed a Poisson distribution. Expected numbers of deaths among the Caucasian population were based on race, age, calendar year, and sex-specific rates among the general population of California, while expected numbers of deaths among the non-Caucasian population were based on the general population rates of the United States. Poisson regression was used to estimate relative risks and their corresponding 95% CIs for a combined grouping of routine and intermittent exposed subjects for duration of exposure, adjusted for date of birth, date first employed, date of finishing employment, race, and sex. Tests of linear trend were also performed to examine the potential effect of exposure duration for subjects with routine or intermittent tetrachloroethylene exposure potential. In all analyses, the

referent population consisted of all factory workers with incidental or no exposure. The strengths of this study included its large size, although only 4% of the cohort was identified with routine tetrachloroethylene exposure potential, extended (>37 years) follow-up period, exposure assessment using a job exposure matrix, and use of an internal referent group in analyses examining exposure duration. Limitations include the study's lack of adjustment for smoking, lack of control for the healthy worker effect, and its finding that the assumption of living vital status for approximately 7% of the cohort was incorrect. This bias would lead to an inflation of the expected number of deaths due to the fact that they were assumed alive at the end of the study period. Additionally, the inclusion of subjects with intermittent exposures who likely have low-exposure potential may reduce the study's detection sensitivity. This may also introduce differential bias in duration exposure-response analyses if intermittently exposed subjects had longer employment duration than routinely exposed subjects. Marano et al. (2000) noted the number of subjects with intermittent tetrachloroethylene exposure potential was 1.5 times larger than the number of subjects assigned routine tetrachloroethylene exposure potential.

B.1.2.3. Bond et al. (1990; 1987)

Bond, G.; McLaren, E.; Cartmill, J.; Wymer, K.; Sobel, W.; Lipps, T.; Cook, R. (1987). Cause-specific mortality among male chemical workers. Am J Ind Med, 12, 353-383. http://www.ncbi.nlm.nih.gov/pubmed/3674026

Bond, G.; McLaren, E.; Sabel, F.; Bodner, K.; Lipps, T.; Cook, R. (1990). Liver and biliary tract cancer among chemical workers. Am J Ind Med, 18, 19-24. http://www.ncbi.nlm.nih.gov/pubmed/2378367

Summary: This nested case-control study, conducted as a follow-up to a cohort mortality study (Bond et al., 1987), investigated liver and biliary cancer deaths of male employees working at Dow Chemical's Midland/Bay City production, research, and headquarters units. The initial cohort was identified through work history records and consisted of men and women employed for 3 or more days between 1940 and 1982. Overall, the cohort consisted of 48,521 men and women, of whom 96% were Caucasian, 77.7% were male, and 56.9% were paid by the hour (Bond et al., 1987). Cases were identified through a review of death certificates and consisted of all male, hourly employees who died between 1940 and 1982. Of the 6,259 cohort members identified, 44 (0.7%) (11 primary liver cancer, 14 gallbladder/bile duct cancer, and 19 unspecified liver cancer) were considered eligible for and included in this study. The source of death certificates was not identified by the authors, and it is not known whether they were obtained through pension records or the National Death Index. Controls were randomly chosen from among the cohort of male workers. Of the 21,437 hourly, male subjects, a random sample

of 1,888 (8.8%) was selected as controls. Bond et al. (1990) do not identify if controls were matched to cases on age, time period of first employment or end employment, or vital status.

Dow's work history records were used to determine the employee's work area (administration, manufacturing, unknown manufacturing) as well as their possible exposure to tetrachloroethylene and 10 other chemical agents. Overall, 6 (13.6%) cases and 213 (11.3%) controls were potentially exposed to tetrachloroethylene during their time at Dow. The Mantel-Haenszel method was used to estimate risk ratios for work areas and chemical exposures separately, adjusted for birth year; Miettinen's method was used to calculate corresponding 95% CIs. Additional adjustment for period of hire produced similar results; as a result, only those analyses controlling for birth year were presented. Individual analyses were conducted for primary liver cancer and gall bladder/bile duct cancer separately. This study is of low prevalence of tetrachloroethylene exposure, unable to determine whether the cancer was primary or secondary in almost half of the cases, lacked information and statistical adjustment for alcoholism as a potential confounder, is based on pension records or deaths known to the employer, and deaths among nonpensioned employees are not used to identify deaths, used a living control population, and may have misclassified exposure and disease given the use of information on death certificates. Additionally, the study lacks description of source and process for assigning tetrachloroethylene exposure potential. The authors do not report any strengths of their methodology.

B.1.2.4. Chang et al. (2005; 2003), Sung et al. (2008; 2007)

Chang, Y.; Tai, C.; Yang, S.; Chen, C.; Shih, T.; Lin, R.; Liou, S. (2003). A cohort mortality study of workers exposed to chlorinated organic solvents in Taiwan. Ann Epidemiol, 13, 652-660. http://dx.doi.org/10.1016/S1047-2797(03)00038-3

Chang, Y.; Tai, C.; Yang, S.; Lin, R.; Sung, F.; Shih, T.; Liou, S. (2005). Cancer incidence among workers potentially exposed to chlorinated solvents in an electronics factory. J Occup Health, 47, 171-180. http://www.ncbi.nlm.nih.gov/pubmed/15824483

Sung, T.; Chen, P.; Jyuhn-Hsiarn Lee, L.; Lin, Y.; Hsieh, G.; Wang, J. (2007). Increased standardized incidence ratio of breast cancer in female electronics workers. BMC Public Health, 7, 102. http://dx.doi.org/10.1186/1471-2458-7-102

Sung, T.; Wang, J.; Chen, P. (2008). Increased risk of cancer in the offspring of female electronics workers. Reprod Toxicol, 25, 115-119. http://dx.doi.org/10.1016/j.reprotox.2007.08.004

Summary: After tetrachloroethylene and other substances were detected in the soil and groundwater surrounding a closed Taiwanese electronics factory (Bechtel Environmental Inc., 1990 and Target Environmental Services Inc., 1995), a series of retrospective cohort studies were

conducted to examine the potential effect of employment in the factory. Strengths of all of these studies are their large size and linkage with national data sets to assure that all cases had been retrieved. Limitations include the use of employment in the factory as a proxy for exposure, and subjects will have varying exposure potential to tetrachloroethylene.

Chang et al. (2005; 2003) identified the cohort through the Bureau of Labor and Insurance's records for the years 1973 to 1997. To ensure completeness of the cohort, the researchers also determined which employees had been hospitalized through labor-insurance hospitalization data and obtained a list of those associated with the United Labor Association. The cohort consisted of 86, 868 individuals (16,133 men and 70,735 women) who contributed a total of 1,380,354 person-years. The average follow-up time was 14.3 years for men and 16.3 years for women; the average age of cohort members was 39.3 years. The cohort included both white- and blue-collar workers.

Chang et al. (2003) linked the cohort with the National Mortality Database for their 13-year follow-up from 1985 to 1997. Person-years were counted starting when an individual entered the cohort or on January 1, 1985, whichever came later, and ended with either that person's death or December 31, 1997. The cohort experienced 1,357 deaths, 316 (24%) of which were due to cancer. All cause mortality rate was 1.56%, and all cancer mortality rate was 0.36%. The analysis consisted of the calculation of SMRs. The number of deaths was stratified by their underlying cause and compared with the expected numbers using the general Taiwanese population as a reference. In order to better understand any dose-response relationships, the cohort was stratified twice. First, it was stratified based on duration of employment: $\leq$1 year, >1 year but $\leq$5 years, and >5 years. Then it was stratified based on the calendar year: 1985−1990 and 1991−1997. Duration of employment consisted of the period of employment between the start and end of labor insurance coverage. Assumptions were made regarding the duration of employment for those individuals with missing data. Limitations include reliance on mortality rates from registration data sources, too brief of a follow-up time to allow for a sufficient cancer latent period, data on employment were incomplete, and the cohort was very young despite the mortality endpoint.

Chang et al. (2005) examined the cancer incidence from 1968 to 1992 by linking the cohort with the National Cancer Registry, National Mortality Registry. Follow-up time was calculated between the latter of employment start date or January 1, 1979, until the first of cancer diagnosis, death, or December 31, 1997, with assumptions made regarding duration for those with missing start or end dates. Overall, 998 individuals developed cancer. Standardized incidence ratios were calculated comparing this exposed cohort to incidence rates in the general population of Taiwan by age, calendar year, and sex. Latency periods of <3 months, 6 months, and 1, 5, and 10 years were used. Trends were examined by duration of employment (<1 year, 1

through ≤5 years, 5 through ≤10 years, and 10+ years) and period of employment (1979−1984, 1985−1990, and 1991−1997). Limitations include the study's reliance on registration data, which meant that exposure could not be quantified, and the results were not adjusted for potential confounders, such as smoking, alcohol consumption, reproductive history, or diet. The lack of company personnel records prevented verification of the completeness of cohort identification, as well as the need by the author's to make assumptions regarding length of employment. The study was unable to assess potential individual exposures, and the cohort included white-collar employees with limited potential exposure to organic solvents, which may reduce the study's detection ability. Finally, the young average age of cohort subjects and short duration of follow-up may reduce the study's sensitivity given low background cancer rates and inadequate latent period.

Sung et al. (2007) tested the hypothesis of increased breast cancer among female workers in the factory. Using a retrospective design, the cohort was identified through employment records from the Bureau of Labor Insurance and consisted of women employed between 1973 and 1992 who worked for at least 1 day and whose cancer diagnosis occurred after employment began. Of the 64,000 women employed during this time, 63,982 (99.97%) were eligible for and included in the study, contributing a total of 1,403,824 person-years. Vital statistics information was obtained from the Ministry of the Interior; cancer diagnoses were retrieved from the Taiwan National Cancer Registry for the period 1979 to 2001 and linked with the cohort through employee identification numbers. There were 29 cancer sites (oral, salivary, nasopharynx, esophagus, stomach, small intestine, colon/rectum, liver/bile ducts, gall bladder, pancreas, peritoneum, trachea/bronchus/lung, other respiratory, breast, cervix uteri, other uterus, ovary/fallopian tube/broad ligament, other genital, kidney/urinary organs, bladder, skin, brain, other nervous system, thyroid, bone, connective tissue, other/unspecified sites, leukemia, and all sites) examined, and depending on the type of cancer, the latency periods were 5 years (thyroid and leukemia), 15 years (breast and cervix uteri), or 10 years (all other cancer sites). Employment in the factory was assessed as a proxy for exposure; duration of employment (1 month; 1, 5, 10, 15, 20 years) was calculated based on the date that labor insurance started and the date that employment ended. In the event of missing employment information, two assumptions were made: (1) if the date of labor insurance was missing, this was assessed as the earliest possible age (14 years); (2) if the date that employment ended was missing, this was assessed as the date the factory closed in 1992. Periods of exposure were classified according to government regulations that were issued in 1974, 1976, and 1978, as well as documents that discussed factory violations with regard to proper ventilation. Pre-1974 was considered to be the time of highest exposure, and there were 8,461 (13.2%) women who began working during this time. Standardized incidence ratios and their corresponding 95% CIs were calculated assuming a

Poisson distribution for each cancer site separately. Additional standardized incidence ratios and 95% CIs were calculated for breast, cervical, colorectal, and thyroid cancer stratified by calendar year (pre- or post-1974) as well as duration of employment. *t*-Tests were used to compare women with breast cancer who were employed either before or after 1974 on age at diagnosis, age at first employment, and length of employment. Limitations included the lack of detailed exposure information for both the factory and individuals within the cohort, the lack of control for possible confounders, and the lack of detailed information related to the early 1970s.

Sung et al. (2008) investigated any possible link between maternal employment and childhood cancer among first live born children. The factory employment records for all women employed between 1973 and 1992 were obtained from the Bureau of Labor Insurance and linked to the Taiwan Birth Registration database for the period 1978 to 2001. Children were required to be first born singletons. Of the 103,506 children born to 47,348 women between 1978 and 2001, 40,647 children were eligible for and included in the study, contributing a total of 639,051 person-years. Demographics were obtained through the National Birth Registry and included information on birth date, sex, single/multiple pregnancy, gestational age, and birth weight, as well as parents' birth dates, education, marital status, and maternal parity. The children's identification numbers were linked with the National Cancer Registry for the period 1979 to 2001 to ascertain how many were diagnosed with cancer. Employment at the factory during the periconceptional time period was assessed as a proxy for exposure to tetrachloroethylene and the other substances previously found in the soil and groundwater around the factory (Bechtel Environmental Inc., 1990 and Target Environmental Services Inc., 1995). Periconceptional exposure was defined as having been employed at the factory during 3 months prepregnancy and 3 months after conception. Conception was calculated by subtracting the length of gestation and an additional 14 days from the date of birth. Overall, there were 8,506 (20.9%) exposed children who contributed a total of 155,121 person-years. There were 11 cases of cancer (1 liver, 2 bone, 1 skin, 1 testis, 6 leukemia) in the exposed group and 36 cases (3 buccal cavity/pharynx, 1 liver, 1 bone, 3 connective/soft tissue, 1 skin, 2 breast, 2 ovary, 1 testis, 5 brain/other nervous system, 4 multiple myeloma, 9 leukemia, and 4 others) in the nonexposed group. Poisson regression was used to calculate rate ratios and their corresponding 95% CIs, adjusted for maternal age and education level, sex, and year of birth. Strengths of this study include its use of an internal, nonexposed comparison group, which reduced the potential for confounding and selection bias. Also, the researchers compared their list of cancer cases to the death registry to verify that all cases had been retrieved. Limitations include the study's inability to link exposures to individuals, and the study's inability to separate the effects of different chemicals.

B.1.2.5. Spirtas et al. (1991), Blair et al. (1998), Radican et al. (2008)

Spirtas, R.; Stewart, P. A.; Lee, J. S.; Marano, D. E.; Forbes, C. D.; Grauman, D. J., . . . Cohen, J. L. (1991). Retrospective cohort mortality study of workers at an aircraft maintenance facility: I. Epidemiological results. Br J Ind Med, 48, 515-530. http://dx.doi.org/10.1136/oem.48.8.515

Blair, A.; Hartge, P.; Stewart, P. A.; McAdams, M.; Lubin, J. (1998). Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: Extended follow-up. Occup Environ Med, 55, 161-171. http://dx.doi.org/10.1136/oem.55.3.161

Radican, L.; Blair, A.; Stewart, P.; Wartenberg, D. (2008). Mortality of aircraft maintenance workers exposed to trichloroethylene and other hydrocarbons and chemicals: Extended follow-up. J Occup Environ Med, 50, 1306-1319. http://dx.doi.org/10.1097/JOM.0b013e3181845f7f

Summary: A retrospective cohort mortality study of workers at Hill Air Force Base in Utah was conducted with four aims: (1) to determine whether working at the aircraft maintenance facility was associated with an increased risk of death; (2) to evaluate, in detail, mortality risks associated with exposure to trichloroethylene; (3) to determine whether any raised risks for specific causes of death were associated with specific chemical exposures; and (4) to generate hypotheses for future research by evaluating the relation between various diseases and specific chemicals. Individual earnings records from the National Personnel Records Center were used to identify the cohort, which consisted of male and female civilian employees who had worked at the base for at least 1 year between 1952 and 1956. Of the 14,457 eligible workers, 14,425 (99.8%) had official personnel folders that were able to be retrieved. These files contained demographic information, as well as complete occupational histories, and were used to create a "job dictionary" with 43,000 job titles. Industrial hygienists assessed exposure through walkthrough surveys of the base, interviews with employees, industrial hygiene files, job descriptions, and other historical documents including worker compensation files, telephone books of the facility, organization charts, technical orders, and position descriptions (Spirtas et al., 1991). Then, position descriptions with job titles and shops/departments were used as the basis for evaluating chemical exposures. Any job that could not be linked to specific solvents was coded as "mixed solvent" exposure, which consisted of 16 chemicals including tetrachloroethylene (Stewart et al., 1991). Tetrachloroethylene consisted of a dichotomous (yes/no) classification (Radican et al., 2008; Spirtas et al., 1991). Tetrachloroethylene was primarily used to clean fabric in the parachute shop, replacing carbon tetrachloride in the late 1950s. In the accompanying paper in exposures at Hill Air Force Base, Stewart et al. (1991) do not present industrial hygiene monitoring data on tetrachloroethylene concentrations; however, Gold et al. (2008) noted, the arithmetic means for personnel measurements were 13 ppm for <1-hour samples and 1.4 ppm for >1-hour samples for degreasing jobs in the aircraft and parts

industry. Stewart et al. (1991) identified 851 (5.9%) subjects in the Hill Air Force Base cohort as ever exposed to tetrachloroethylene (Stewart et al., 1991).

Observations for tetrachloroethylene and site-specific cancers are limitedly reported in these studies given the study's primary focus on trichloroethylene exposure. Risk estimates for tetrachloroethylene are presented for multiple myeloma and non-Hodgkin lymphoma in Spirtas et al. (1991) and for breast cancer (women), multiple myeloma, and non-Hodgkin lymphoma in Radican et al. (2008). Blair et al. (1998) did not present risk estimates for tetrachloroethylene.

Spirtas (1991) conducted a follow-up of this cohort through 1982. The data in the official personnel folders were supplemented with vital status information, which was ascertained through the Social Security Administration, the U.S. Office of Personnel Management, official personnel folders, Veterans Administration records, motor vehicle bureau records, the National Death Index, interviews with base personnel, and state vital statistics offices. Death certificates were retrieved for all cohort members that died during the follow-up period, and the underlying cause of death was assessed by a nosologist. Follow-up began in 1953 or 1 year after the start of employment, whichever came later. Person-years at risk were stratified by race, sex, 5-year age group, and calendar era. Person-years of exposure were calculated starting 1 year from the date of first exposure or January 1953, whichever came later. Of those who were exposed to any chemical or solvent and died from multiple myeloma, two (33.3%) women and no men were exposed to tetrachloroethylene; of those who were exposed to any chemical or solvent and died from non-Hodgkin lymphoma, two (20%) women and two (9.1%) men were exposed to tetrachloroethylene. SMRs for the cohort of all white civilian employees at Hill Air Force Base and for the tetrachloroethylene subcohort were estimated using Utah death rates as the basis for determining the expected number of deaths. Corresponding 95% CIs were calculated assuming the observed deaths followed a Poisson distribution. Estimates for the full cohort were adjusted for age, sex, and calendar period. These estimates were then stratified by gender and adjusted for age and calendar period only. All calculations were performed on the Caucasian population only, which included those of unknown race. Strengths of this study include its size, analysis of both genders, and use of a variety of mechanisms to assess exposure. Limitations include the lack of adjustment for smoking and employees' exposure to multiple chemicals.

Blair et al. (1998) aimed to better understand the potential relationship between disease risk and trichloroethylene and other organic solvents/chemicals. The follow-up period was extended to December 1990 and conducted through linkage of the cohort with the National Death Index and the Utah Tumor Registry. Person-years for the mortality analyses began January 1, 1953 or 1 year after first employment and ended December 31, 1990, or date of death. Person-years for incidence analyses began January 1, 1973, and ended December 31, 1990, or the date of cancer diagnosis. Deaths were classified according to the International Classification

of Diseases rules. Exposure was assessed using company personnel records from the first job to the end of 1982. Individuals were evaluated as having ever (or never) been exposed to chemicals. All cause mortality in the cohort was 40%, and all cancer mortality was 7%. Overall, of those who died from non-Hodgkin lymphoma, 40 (81.6%) were exposed to any solvent; of those who died from multiple myeloma, 24 (75.0%) were exposed to any solvent; and of those who died from breast cancer, 28 (57.1%) were exposed to any solvent and died from breast cancer. Relative risks and SMRs were estimated based on mortality in Utah. Rate ratios were calculated for mortality and cancer incidence and compared between the exposed and the unexposed using Poisson regression. Regression models adjusted for the following covariates: date of birth, calendar year of death, and sex. The authors do not report strengths of this study. Limitations include the lack of information on tetrachloroethylene, the lack of mutually exclusive exposures, and lack of data on potential lifestyle confounders.

Radican (2008) extended the follow-up period to gain additional information about the health risks associated with workplace exposures. The cohort was linked with the National Death Index using personal identifiers and followed up for the period 1991 to 2000, an addition of 10 years from Blair et al. (1998). Of those women who were exposed to any solvent and died from breast cancer, 1 (2.6%) had been exposed to tetrachloroethylene. Of those who had been exposed to any solvent and died from non-Hodgkin lymphoma, 5 (10%) men and 2 (16.7%) women had been exposed to tetrachloroethylene. Of those who had been exposed to any solvent and died from multiple myeloma, 3 (10%) men and 2 (25%) women had been exposed to tetrachloroethylene. Of those who had been exposed to any solvent and died from nonmalignant respiratory diseases, 46 (9%) men and 4 (51%) women had been exposed to tetrachloroethylene. Cox proportional hazards regression was used to estimate hazard ratios and their corresponding 95% CIs using age as the time variable and race as the covariate. Analyses were stratified by gender. The researchers also examined mortality using the Cox proportional hazards model for a previously conducted study using a different follow-up period to compare the hazard ratios between the two different statistical approaches. This was not performed for tetrachloroethylene, though. Strengths of the study include its size, long follow-up, limited reporting bias due to exposure assessment before the outcome was known, and its use of an internal comparison group to minimize the healthy worker effect. Limitations include the small number of tetrachloroethylene-exposed deaths and reduced statistical power, the inability to estimate risk of one exposure while controlling for exposures to other chemicals, and the potential misclassification of exposure based on job descriptions and other historical information.

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
I.A. Dry-cleaner and laundry worker studies					
Andersen et al. (1999)	Danish, Finnish, and Norwegian cohorts from 1970 Censuses, Swedish cohort from 1960 Census, men and women, 25−64 yr, alive on January 1, 1971; cancer cases from national cancer registries in each country; demographics, occupations, industries from census descriptions provided by the heads of households for all economically active members Proxy—launderers and dry cleaners All cancers (incidence)	Full cohort: 10,101,711 Denmark: 2,346,134 Finland: 2,115,691 Norway: 1,792,817 Sweden: 3,847,069	Follow-up started 1971 and ended with death, emigration, or end of follow-up; Denmark, 1971−1987, linked with Central Population Register; Finland, 1971−1990, linked with Statistics Finland; Norway, 1971−1991, linked with Central Population Register; Sweden, 1971−1989, linked with cause-of-death register	Census descriptions coded according to Nordic Occupational Classification in Finland, Norway, Sweden; Denmark coded according to own standards; researchers then recoded all jobs based on a set of 54 occupational groups based on Nordic Occupational Classification standards; Group 51, Code 95: launderers and dry cleaners 29,333 (0.3%) cohort members, Denmark: 9,873 (0.4%), Finland: 4,949 (0.2%), Norway: 4,061 (0.2%), Sweden: 10,450 (0.3%); Launderers and dry cleaners: 519,844 person-years; Denmark, 159,156; Finland, 94,302; Norway, 78,086; Sweden, 187,580	SIRs, 95% CIs, stratified by cancer site, country, adjusted for age; expected numbers of cases from cancer incidence rates for each population; Poisson distribution assumed for all CIs whose SIRs had <100 cases

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Blair et al. (2003)	Cohort : from dues records of Local No. 161 (St. Louis) of Laundry, Dry Cleaning, Dye House Workers' International Union; male and female dry cleaners, entered union from 1945−1978, worked ≥1 yr; dues records for demographic, employment information, also driver's license records, social security files, health care finance administration records, credit bureaus; excluded if no demographic information; proxy—dry-cleaning tasks All cancers (mortality)	11,062 members identified, 5,369 met inclusion criteria	Extended from Blair et al. (1990), which ended January 1979; started January 1979, ended December 1993 (14 yr); person-years start at entry to union or 1948, whichever later and ended with death or December 1993, whichever came first; deaths from National Death Index	Exposure indices for jobs within dry cleaning: (1) run machines and handle clothes (highest exposure), TWA = 40; (2) pressers, sewers, counter workers, TWA = 7; (3) counter workers at pick-up stations (minimal exposure), TWA = 0; (4) maintenance workers (high, short-term exposures), TWA = 7; entire follow-up period (1948−1993): 220 deaths from cancer among those with little/no exposure (index = 0), 316 deaths from cancer among those with medium/high exposure (index = 7 or 40)	SMRs and 95% CIs to examine relationship between cancer and other causes of death among dry cleaners; Expected numbers based on general U.S. population 5-year age and mortality statistics; 44% deceased at end of entire follow-up period
Cano and Pollan (2001)	Swedish men and women aged 25−64 yr in 1970 Census, employed and counted in 1960, followed 1971−1989. Over 200 occupational codes examined including " launderers and dry cleaners" Non-Hodgkin lymphoma cancer incidence	2,881,315	Followed 1971−1989 or date of death; Swedish Cancer Environment Register linked to population register	Job title reported on 1960 and 1970 Censuses. Eleven of male cases were launderers and dry cleaners (occupational Code 943); no female cases classified as launderers or dry cleaners	Log-linear Poisson models to compare occupations with cohort, adjusted for geographical area; RRs for sectors, occupations, adjusted for age, period, and geographical category

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Chow et al. (1995)	Swedish Cancer Environment Registry, which linked employment and cancer information for all individuals registered in the 1960 Census and National Swedish Cancer Registry; linkage was performed using personal identifiers Esophageal cancer incidence	Not reported	Follow-up: 1961 to 1979	Job title reported on 1960 Census; 3 cases among laundry workers	SIRs, expected numbers based on 5-year birth cohort- and sex-specific rates for esophageal cancer in general Swedish population during time period; only occupations with ≥500 individuals examined; significance evaluated assuming Poisson distribution
Ji et al. (2005a, b); Ji and Hemminki (2006, 2005a, b, c)	Cohort: Swedish males and females in Family-Cancer Database linked national censuses; cancer incidence data from Swedish cancer registry (1961−2000) Ji, et al. [(2005a): bladder cancer] Ji and Hemminki [(2005a): upper aerodigestive tract cancer; (2005b): kidney cancer; (2005c): liver and gallbladder] Additionally, subjects ≥31 yr age and cancer incidence 1961−2002 [Ji and Hemminki (2006): lymphoproliferative diseases; (2005b): leukemia]	1,644,958 employed men (9,255 dry cleaners and launderers) in 1960 Census and 1,154,091 employed women (14,974 dry cleaners and launderers) in 1970 Census	Follow-up from 1961 (1960 Census), 1970 (for 1970 Census or those with same job in 1960 +1970 Censuses), or 1980 (for those with same job in 3 censuses) through 2000 or 2002	Relevant census information (employment status, job title, work industry) coded according to Nordic Occupational Classifications; codes merged into 53 occupational groups, including launderers and dry cleaners; 9,255 (0.6%) male, 14,974 (1.3%) female launderers and dry cleaners	SIRs for each occupation, stratified by gender: (1) adjusted for age period, SES [aerodigestive tract cancers, leukemia/lympho-proliferative diseases, kidney cancer, liver and gall bladder cancer]; (2) smoking-corrected SIR and smoking-corrected 95% CI [based on IARC (2004a): bladder cancer]

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Lindbohm et al. (2009)	Cohort: Finnish males and females participating in the 1970 National Population Census; cancer incidence data from Finnish Cancer Registry Liver cancer incidence	1.2 million men and women who were born between 1906 and 1945	Follow-up from 1971−1995	Industry Code 850, and cumulative exposure for organic solvent class. Cumulative exposure based on exposure for each birth cohort, starting when average age of birth cohort was 20 to end of observation period or age 65 yr and included a 10-yr lag period. If exposure took place before 1960, FINJEM estimated use for 1945−1959 period, otherwise estimated for 1960−1984 period used	SIRs for each occupation, stratified by gender from Poisson regression models adjusted for alcohol consumption, smoking, and socioeconomic status; (smoking and alcohol consumption by occupation obtained for FINJEM from the annual surveys of the Finnish population in 1978−1991)
Lynge and Thygesen (1990)	Cohort: Danish men and women, 20−64 yr, registered in 1970 Census as engaged in laundry and dry-cleaning work; linked to Danish Cancer Registry Site-specific cancer incidence	Cohort: 10,600	Follow-up: 1970−1980 (Lynge and Thygesen, 1990)	Industry Code 860 (laundries, cleaning, and dyeing), occupational Codes 411 (laundry worker, ironer) and 380 (factory hand), and those who reported as self-employed/family workers; 2,434 (23%) self-employed dry cleaners/launderers, 830 (7.8%) family workers, 6,837 (64.5%) laundry workers/ironers, 499 (4.7%) factory hands, 2,886 laundry/dry-cleaning shops in 1970, 695 where dry cleaning was the known predominant activity	SIRs, 95% CIs, assuming Poisson distribution if observed cases ≤30 and normal distribution if >30; expected numbers from multiplying person-years at risk within each 5-year age group with site-specific incidence rates for full 1970 cohort (Lynge and Thygesen, 1990)

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Pukkala et al. (2009)	Cohort: Men and women in 1990 Census or prior census in five Nordic countries (Denmark, Finland, Iceland, Norway, Sweden); dry cleaners and launderers occupational title; incident cases from national cancer registries Site-specific cancer incidence	15 million subjects total, 43,496 dry cleaners and launderers	Person-years started January 1 after the first available census and ended with deaths, emigration, or at end of 2003−2005, whichever came first. (depended on country)	Dry cleaner and launderer (Code 95) according to Nordisk Yrke Klassifisering and International Standard Classification of Occupation	SIRs and 95% CIs; expected number of deaths using national rates
Ruder et al. (2001, 1994); Calvert et al. (2011)	Cohort: from union dry-cleaning records in California, Illinois, Michigan, New York; employed ≥1 yr pre-1960 in dry-cleaning shops using PCE; verified records with visits to shops; National Death Index for deaths that occurred in cohort (site-specific cancer mortality) or REMIS for end-stage renal disease incident cases.	1,704 workers (mortality) 1,296 (end-stage renal disease incidence)	Person-years started January 1, 1940, for mortality or January 1, 1977, for renal disease incidence or after 1 year of employment in unionized shop, whichever came later, and ended with death, loss to follow-up, or end of 2004, whichever came first.	PCE exposure estimated by duration of employment in dry-cleaning shops (1−5 yr or >5 yr) and latency periods (time since first employment <20 yr or 20+ yr); 618 (36%) worked only in shops that only used PCE; 1,086 (64%) worked at shops where PCE use is unable to be verified or where other solvents are known/suspected to be used instead	SMRs (for deaths) and 95% CIs; expected number of deaths estimated using national rates; estimates for each of 4 regions used county and national rates for expected numbers though data not shown SIRs (for end-stage renal disease types) and 95% CIs; expected number of deaths estimated using REMIS rates and national population estimates

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Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Selden and Ahlborg (2011)	Cohort: men and women identified by employer as working between 1973−1983 in dry-cleaning and laundry establishments for a previous study of pregnancy outcome (Ahlborg, 1990a), incident cancers from Swedish National Cancer Registry Site-specific cancer incidence	10,389 employed ≥1 mo identified by employers; 9,440 included in follow-up	Person years started January 1985 and ended with cancer diagnosis, death, emigration, or end of observation period on December 2006, whichever came first.	Jobs assigned to three exposure categories: PCE (dry cleaners or laundries with proportion of dry-cleaning with PCE), laundries (laundering only, no dry cleaning), "other" (dry cleaning with PCE and other solvents)	SIR and 95% CI using site-specific cancer incidence rate of Swedish population
Travier et al. (2002)	Cohort: Men and women reporting work as launderers, dry cleaners, and pressers in 1960 or 1970 Swedish Census, incident cancers from Swedish national cancer registry; all with second primary neoplasms excluded Site-specific cancer incidence	Authors did not report total number included in cohort; 543,036 person-years from 1960 Census and 46,933 person-years from 1970 Census	Person years started January 1971 and ended with cancer diagnosis, death, or loss to follow-up, or December 1989, whichever came first.	Jobs coded by Nordic Classification of Occupations and Swedish Industrial codes; Group 1, launderer, dry cleaner, or presser occupation employed in the laundry, ironing, or dyeing industries in either 1960 or 1970; Group 2, launderer, dry cleaner, or presser occupation employed in the laundry, ironing, or dyeing industries in both 1960 and 1970; Group 3, launderer, dry cleaner, or presser occupation employed in other industries; Group 4, other occupational titles employed in laundry, ironing, or dyeing industries; Group 5, not employed in relevant industries or occupations	Multivariable Poisson regressions, adjusted for age, calendar period, geographic region, urban setting, gender. Analyses also stratified by gender, adjusted for age, calendar period, geographic region, urban setting, and by age in 1960 (<40 yr, 40−59 yr, >59 yr), adjusted for gender, age, calendar period, geographic regions, and urban setting

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Wilson et al. (2008)	Cohort: Swedish men and women employed during 1960 or 1970 Census and still alive in January 1971; cancer information from Swedish Cancer-Environment Registry for 1971−1989 Proxy—laundry, ironing and dyeing industries, laundry workers and clothes pressing occupations Renal pelvis cancer incidence, bladder cancer incidence	4,197, 684 cohort members	Person years began January 1, 1971, and ended with cancer diagnosis, emigration, death, or December 31, 1989, whichever came first. 70,083,912 person-years of follow-up, mean: 16.7 yr	Job titles in censuses coded according to National Swedish Classification of Occupations and Industries standards, laundry workers: occupation Code 943 and clothes pressing: occupation Code 944. Laundry, ironing, and dyeing: industry Code 880; 110 bladder cancer cases and 11 renal pelvic cancer cases with this industry code. Job exposure matrix to assess exposure to indoor work, low physical activity, etc. 25,249 (0.6%) employed in industry Code 880. 16,512 (0.4%) employed in occupation 943, laundry worker	SIRs and 95% CIs for each occupation and industry using expected site-specific cancer incidence rates of total employed Swedish population

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
I.B. Other occupational cohort studies					
Anttila et al. (1995)	Workers with blood:urine biological monitoring; most of TCE in urine; PCE in blood 1974−1983; demographics, date/time sampling, result, workplace, solvent code from FIOH database; excluded if not identified or deceased at start of follow-up Site-specific cancer incidence (pancreas, lung/bronchus, cervix uteri, kidney, nervous system, non-Hodgkin lymphoma, multiple myeloma, and all cancer)	11,534 measurements from 1965−1983, 10,743 (93.1%) linked to personal identifiers, which corresponded to 3,976 workers. Final sample: 3,974 subjects; 849 workers with blood PCE measurements	Follow-up started January 1967 or date of first measurement; ended with emigration, death, or December 1992, whichever came first; used Finnish Cancer Registry, Population Register Center, Central Statistical Office of Finland; overall: 71,800 person-years, averaged 18 yr	Blood measurements: median 0.7 µmol/L in males, 0.4 µmol/L in females, average 3.2 measurements/individual; 849 (21.4%) workers monitored for exposure to PCE, contributed 11,958 person-years; duration not examined for PCE, given its few site-specific cancer cases	SIRs for 8 sites, 95% CIs, expected numbers of cancers from incidence rates of Finnish population; Mantel-Haenszel χ^2 test for significance, assuming observed cases followed Poisson distribution

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Boice et al. (1999)	Cohort: from work history cards, personnel files, retirement records, employed at aircraft manufacturing factories ≥1 yr from 1960 onwards; exclusions: missing work history or incorrect dates; vital status from California death tapes, National Death Index, Pension Benefit Information Files, Social Security Death Index, Health Care Finance Administration files, California Department of Motor Vehicles records, employment work history cards, pension and retirement records, obituaries from 1960−1996; if no vital status information found, assumed alive JEM for PCE exposure Site-specific cancer mortality	113,204 employees, 77,965 (68.9%) included in study	Follow-up: started January 1, 1960 or after 1 year employment and ended with death, age 95 yr or December 1996, whichever came first. 1,889,795 person-years of follow-up; average of >20 yr per person	Exposure assessed via walk-through surveys of factories, interviews with employees, industrial hygiene files/other historical documents; based on job code, job title, job change information; factory jobs only assessed as routine (daily), intermittent (not daily), minimal/no exposure; duration (<1, 1−4, ≥5 yr) based on dates of employment for each job; overall: 5,830 (7.5%) exposed to PCE; 2,631 (5.8%) routine exposure, 3,199 (7.1%) intermittent exposure, 51,214 person-years at risk	SMRs, 95% CIs, assuming observed deaths followed Poisson distribution, expected number of deaths among Caucasians based on race, age, calendar year, sex-specific California rates; expected number of deaths among non-Caucasian based on general U.S. population rates; Poisson regression for duration, adjusted for date of birth, date first employed, date of end of employment, race, sex; tests of trend to examine duration of exposure

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Bond et al. (1990)	Dow Chemical's Midland/Bay City production, research, and headquarters units Nested case-control; cohort: from work history records, 48,521 men and women employed 3+d; Cases: from death certificates, men who died from 1940−1982. Controls: randomly selected from among the cohort of male employees Proxy—factory employment Primary liver cancer, cancer of gallbladder/bile ducts, cancer of liver not specified	44 (0.7%) liver and biliary tract deaths eligible for and included in study; 1,888 (8.8%) controls selected randomly from cohort (n = 21,437 males) Final sample: 44 cases, 1,888 controls	Follow-up period: 1940−1982	Work history records for exposure by work area and exposure to 11 chemicals, including PCE; 6 (13.6%) cases, 213 (11.3%) controls exposed to PCE	Mantel-Haenszel for RRs, adjusted for birth year; Miettinen's method for 95% CI; primary liver cancer and gall bladder/bile duct assessed separately but not presented; duration work exposure failed to reveal any significant trends
Chang et al. (2005; 2003); Sung et al. (2008; 2007)	Cohort: from Bureau of Labor and Insurance's records Proxy—employment in electronics factory in Taiwan Cancer, mortality	Various (see below)	Various (see below)	Various (see below)	Various (see below)

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Chang et al. (2003)	Cohort: identified from 1973−1997, men and women, average age of cohort members: 39.3 yr; linked with National Mortality Database; compared with labor-insurance hospitalization data Site-specific cancer mortality	86, 868 individuals (16,133 men and 70,735 women) who contributed 1,357 deaths, 316 (24%) due to cancer. All-cause mortality rate was 1.56%, and all cancer mortality rate was 0.36%	Person-years started when entered cohort or January 1, 1985, whichever later, and ended with death or December 31, 1997, whichever came first; total of 1,380,354 person-years; average follow-up time: 14.3 yr for men and 16.3 yr for women	Duration of employment: period of employment between the start and end of labor insurance coverage, with assumptions made for those with missing data	SMRs, stratified by underlying cause of death, expected numbers based on general Taiwanese population as a reference. For dose-response assessment, cohort stratified by duration employment: ≤1 year, >1 year but ≤5 yr, >5 yr and calendar year: 1985−1990, 1991−1997
Chang et al. (2005)	Cohort: identified from 1973−1997, men and women, linked with National Cancer Registry and National Mortality Database; compared with labor-insurance hospitalization data Site-specific cancer incidence	86,868 (16,133 men and 70,735 women) who contributed a total of 1,380,354 person-years; 998 incident cancer cases	Follow-up: started January 1, 1979, or date of employment, whichever later, and ended with cancer diagnosis, death, or December 31, 1997, whichever came first	Duration of employment: <1, 1−5, 5−10, and 10+ yr; assumptions made for those with missing start or end dates Period of employment: 1979−1984, 1985−1990, 1991−1997	SIRs comparing exposed to incidence rates in general population of Taiwan by age, calendar year, and sex; trends examined by duration and period of employment Latency periods: <3 mo, 6 mo, and 1, 5, and 10 yr

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Sung et al. (2007)	Cohort: identified from 1973−1992, women worked for 1+ d, cancer diagnosis after employment began; Vital status from Ministry of Interior; cancer diagnoses from Taiwan National Cancer Registry and linked with cohort from 1979−2001 Site-specific cancer incidence: 29 cancer sites	64,000 women employed, 63,982 (99.97%) eligible for and included in the study, contributing 1,403,824 person-years	Follow-up: 1979−2001	Duration of employment (1 mo, 1, 5, 10, 15, and 20 yr) based on date labor insurance started and employment ended; for missing employment information: (1) if missing date labor insurance, and assumed earliest possible age (14 yr); (2) if date employment ended missing assumed factory closure in 1992. Exposure by dates of government regulations: pre-1974 had the highest exposure 8,461 (13.2%) women who started working pre-1974	SIRs and 95% CIs, assuming a Poisson distribution for each cancer site; SIRs and 95% CIs for breast, cervical, colorectal, thyroid cancers, stratified by pre- or post-1974, duration of employment; *t*-tests for breast cancer among those employed pre- or post-1974 latency periods: 5 yr (thyroid/leukemia), 15 yr (breast/cervix uteri), 10 yr (all others)
Sung et al. (2008)	Cohort: identified from 1973−1992, women who worked in a factory, linked to the Taiwan Birth Registration Database from 1978−2001; only first born singletons, demographics from National Birth Registry, children linked with National Cancer Registry from 1979−2001 Childhood cancers	103,506 children born to 47,348 women from 1978−2001, 40,647 children eligible for and included in the study, contributing 639,051 person-years; 11 cancer cases among exposed, 36 cancer cases among nonexposed	Follow-up: 1979−2001	Periconceptional exposure defined as employed at factory during 3 mo prepregnancy and 3 mo after conception; conception calculated by subtracting length of gestation +14 d from the date of birth 8,506 (20.9%) exposed children who contributed a total of 155,121 person-years	Poisson regression for RRs and 95% CIs, adjusted for maternal age, maternal education level, sex, year of birth

Table B-1. Summaries of characteristics of cohort studies (dry-cleaner and laundry workers, and other cohorts) (continued)

Reference	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Spirtas et al. (1991); Blair et al. (1998); Radican et al. (2008)	Cohort: male/female civilian employees, worked 1+ yr at base from 1952−1956, identified from individual earnings records Personnel folders, vital status data from Social Security Administration, U.S. Office of Personnel Management, veterans administration records, motor vehicle records, National Death Index, interviews, state vital statistics; death certificates for cohort members who died during follow-up, underlying cause of death assessed by nosologist Mortality: MM, NHL, breast cancer, nonmalignant respiratory diseases	14,425 with personnel files (99.8% of 14,455 eligible); analysis restricted to 10,461 men and 3,605 women classified as Caucasian.	Follow-up began 1953 or after 1 year of employment Follow-up study through 1982 (Spirtas et al., 1991) or 2000 (Radican et al., 2008)	Job exposure matrix based on industrial hygienists, walkthrough surveys of base, interviews with employees, industrial hygiene files, job descriptions, historical documents. Job titles/shops used as basis for evaluating exposures, which for PCE consisted of ever/never classification; jobs unable to be linked to solvents coded as "mixed solvents". 10,256 ever exposed to mixed solvents, 851 ever exposed to PCE. Of those exposed to any chemical/solvent and died from MM, 2 (33.3%) women, 0 men exposed to PCE; of those who were exposed to any chemical/solvent and died from NHL, 2 (20%) women, 2 (9.1%) men exposed to PCE	SMRs MM and NHL and PCE. All calculations on Caucasian population only, including unknown race (Spirtas et al., 1991) Cox proportional hazards regression for hazard ratios and 95% CIs using age as time variable and race as covariate, stratified by gender (Radican et al., 2008); RR for breast cancer, MM, NHL, nonmalignant respiratory diseases, and PCE

JEM = job-exposure matrices; RR = relative risk.

B.2. Case-Control Studies

Tetrachloroethylene case-control studies have been organized by (1) multiple cancer-site studies and (2) single cancer-site studies. Tables B-2 and B-3 provide summaries of the study characteristics of each paper or group of papers.

B.2.1. Multiple Cancer-Site Studies

A number of case-control studies of multiple cancer-site studies have been conducted by a single research group. These studies are discussed in this section given common methodologies among the studies. The studies are organized by region (British Columbia and Montreal in Canada, Massachusetts in the United States, New Zealand, Germany, and four Nordic countries (Denmark, Finland, Norway, and Sweden).

B.2.1.1. British Columbia (Canada)

B.2.1.1.1. Band et al. (1999), MacArthur et al. (2009)

Band, P.; Le, N.; Fang, R.; Threlfall, W.; Gallagher, R. (1999). Identification of occupational cancer risks in British Columbia. Part II: A population-based case-control study of 1516 Prostatic cancer cases. J Occup Environ Med, 41, 233-247. http://www.ncbi.nlm.nih.gov/pubmed/10224589

MacArthur, A.; Le, N.; Fang, R.; Band, P. (2009). Identification of occupational cancer risk in British Columbia: A population-based case-control study of 2,998 lung cancers by histopathological subtype. Am J Ind Med, 52, 221-232. http://dx.doi.org/10.1002/ajim.20663

Summary: A registry-based case-control study was undertaken to examine occupational risk factors for cancer in British Columbia. Cases were identified through the British Columbia Cancer Registry from 1983 to 1990 and consisted of men aged 20 or older with histologically-confirmed cancer. All cases were mailed a self-administered questionnaire inquiring about lifetime job descriptions, including duration and period of employment, as well as occupation and industry titles. Participants were also asked about their ethnic origin, education, lifetime smoking habits, and alcohol consumption. Data were collected for each cancer site until 1,000 completed questionnaires were returned for that site or until December 31, 1990. If the patient was deceased, the spouse or closest living relative was asked to complete the questionnaire. A total of 25,726 eligible cases were contacted, and 15,463 (60%) participated by returning the questionnaire. Occupations and industries were used as a proxy for exposure and coded according to the Canadian Standard Occupational Classification (SOC) and the Canadian

Standard Industrial Classification (SIC). Laundries and dry cleaners comprised SIC Code 972. The authors did not report the SOC code for dry cleaners. For each occupation and industry, estimates for "ever" (whether or not a job within the given occupation or industry was ever held) and "usual" (job with the longest held lifetime employment in a given occupation or industry) occupations and industries were calculated. Strengths include complete ascertainment of cases and occupational histories, adjustment for confounders, and examination of lung cancer subtypes. Limitations include the lack of information on occupational exposures, small numbers of exposed cases, self-reported lifestyle characteristics, and possible bias due to the use of other cancer cases as controls. There may also be nonrepresentativeness of controls between early and late responders due to the fact that the survey for each cancer site ended at 1,000 cases.

Band et al. (1999) used the data to conduct a matched case-control study examining the occupational risks associated with prostate cancer in British Columbia. Cases consisted of patients with histologically confirmed prostate cancer who returned the questionnaire. A total of 1,519 (9.8%) of the cases in the larger study were prostatic cancer cases. Controls were selected from among the other cancer sites within the larger study, excluding lung cancers and cancers of unknown primary sites, and were matched to cases based on age and year of diagnosis. The final sample consisted of 1,516 cases matched to at least 1 of 4,994 controls. Proxy respondents represented 19.9% of cases and 19.3% of controls. Overall, there were 7 (0.5%) cases who reported "ever" employment and 2 (0.1%) cases who reported "usual" employment in the laundries and cleaners industry. The authors do not report the number of controls that reported "ever" or "usual" employment. Conditional logistic regression was used to estimate odds ratios (ORs) and 90% CIs for each occupation and industry separately for each of two estimates of exposure, adjusted for education, alcohol consumption, smoking duration, and respondent to questionnaire.

MacArthur et al. (2009) evaluated the occupational risks for lung cancer. Of the 5,528 eligible, incident lung cancer cases, 2,998 (54.2%) returned the questionnaire. Controls consisted of all other cancer cases, excluding those with unknown primary sites (708 other cases) and were matched to cases based on age and year of diagnosis. Laundries and dry cleaners comprised Code 972 and contained 10 (0.3%) cases of lung cancer (squamous cell carcinoma, adenocarcinoma, and small cell lung cancer). Matched case-control analyses for industries and occupations with at least three cases were performed to calculate maximum likelihood estimates of ORs and their corresponding 90% CIs for "ever" and "usual" employment. Lung cancer subtypes (squamous cell carcinoma, adenocarcinoma, small cell lung cancer, large cell lung cancer) were also separately assessed. The estimates for all lung cancers combined were adjusted for smoking, questionnaire respondent, alcohol, and education. Lung cancer subtype estimates were separately adjusted for their own set of covariates. All subtypes were adjusted for

questionnaire respondent; squamous cell carcinoma, adenocarcinoma, and large cell lung cancer were adjusted for alcohol consumption status; adenocarcinoma, small cell lung cancer, and large cell lung cancer were each adjusted for smoking duration (years); squamous cell carcinoma was also adjusted for cigarette pack-years, marital status, pipe smoking status, and cigar smoking status. Adenocarcinoma was also adjusted for ethnicity, and small cell lung cancer's additional covariates included ethnicity and cumulative alcohol score. The final covariate for large cell lung cancer was level of education.

B.2.1.1.1.1. Band et al. (2000)

Band, P. R.; Le, N. D.; Fang, R.; Deschamps, M.; Gallagher, R. P.; Yang, P. (2000). Identification of occupational cancer risks in British Columbia: A population-based case-control study of 995 incident breast cancer cases by menopausal status, controlling for confounding factors. J Occup Environ Med, 42, 284-310. http://www.ncbi.nlm.nih.gov/pubmed/10738708

Summary: This study is a population-based case-control study whose objective was to examine the relationship between occupational risk and hormonal factors in breast cancer. Cases were identified through the British Columbia Cancer Registry and consisted of all women under the age of 75 years who were diagnosed with breast cancer between June 1988 and June 1989. In order to be included in the study, cases needed to be Canadian citizens, residents of British Columbia, English-speaking, and have no prior history of breast cancer. Controls were randomly selected from the 1989 British Columbia Provincial Voters List, matched on age, and had no history of breast cancer diagnosis before June 1989.

Participants were mailed a self-administered questionnaire inquiring about demographics, lifetime smoking, lifetime alcohol consumption, current body weight, weight in late teens, age at menarche, parity, age at first birth, history of breast biopsy before 1987, family history of breast cancer, breastfeeding, birth control, estrogen replacement therapy, and lifetime occupational history, including job descriptions, occupation and industry titles, duration, and period of employment. Of the 1,489 eligible cases, 1,018 (68%) returned the questionnaire; of the 1,502 eligible controls, 1,025 (68%) returned the questionnaire. After matching and excluding those with missing information on statistically significant confounders, a total of 995 cases and 1,020 controls were available for the analysis. Occupations and industries were coded according to the Canadian SOC and the Canadian SIC; dry cleaning was included in SOC Code 6162 and SIC Code 9721. Two surrogates of exposure were assessed: "usual" occupation/industry, defined as the job with the longest held lifetime employment in a given occupation or industry, and "ever" occupation/industry, defined as whether a job was ever held in the occupation or industry in question. Overall, there were 12 (1.2%) cases "ever" exposed and 9 (0.9%) cases with "usual" exposure to the laundry and dry cleaning occupation; there were also 23 (2.3%)

cases with "ever" exposure and 10 (1.0%) cases with "usual" exposure to the power laundries and/or dry-cleaners industry. The authors do not report the controls' exposures.

Conditional logistic regression was used to estimate ORs and 90% CIs for all occupations and industries, stratified by menopausal status and "usual"/"ever" occupation. Covariates were individually assessed using a forward methodology. The occupational analyses were adjusted for the following three factors: (1) premenopausal women—cigarette pack-year groups, breast biopsy, and family history of breast cancer in the mother and sisters; (2) postmenopausal women—weights in 1986, family history of breast cancer in a first-degree relative, a history of breast biopsy for benign breast diseases, and cumulative alcohol scores; and (3) all women combined—both the pre- and postmenopausal confounders. Strengths of the study include its population-based design, lifetime occupational history, and stratification by menopausal status. Limitations include a lack of information on actual exposures, small number of cases in each occupational category, chance occurrence, and lack of assessment of duration or intensity of exposure.

B.2.1.1.1.2. Teschke et al. (1997)

Teschke, K.; Morgan, M. S.; Checkoway, H.; Franklin, G.; Spinelli, J. J.; van Belle, G.; Weiss, N. S. (1997). Surveillance of nasal and bladder cancer to locate sources of exposure to occupational carcinogens. Occup Environ Med, 54, 443-451. http://www.ncbi.nlm.nih.gov/pubmed/9245952

Summary: This case-control study examined sources of occupational exposure to known or probable carcinogens in British Columbia, Canada, with the aim of alerting regulatory agencies and industrial health professionals about occupations that warranted occupational hygiene exposure measurement and control. Cases were identified through the British Columbia Cancer Agency and consisted of men and women aged 19 years or older with histologically confirmed nasal cavity/sinus or urinary bladder cancers. Nasal cavity/sinus cancer cases were obtained for the time period from 1990 to 1992, and bladder cancer cases were selected between 1990 and 1991. Bladder cancer cases born before 1916 were excluded from the study, as were carcinomas in situ. Controls consisted of British Columbia residents aged 19 years or older. They were randomly selected from the provincial voter list and matched to cases based on age and sex. Any selected controls that were in prison or in a mental health institution by court order were excluded from the study. Of the 54 eligible nasal cancer cases and 195 eligible nasal cancer controls, 48 (88.9%) cases and 159 (81.5%) controls participated in the study. Of the 119 eligible bladder cancer cases and 173 eligible bladder cancer controls, 105 (88.2%) cases and 139 (80.3%) controls participated in the study. The final sample consisted of 153 cases and 298 controls.

Interviews were conducted with all cases and controls using a structured questionnaire administered by a registered nurse who knew of their case or control status. In-person or telephone interviews were conducted with all subjects who lived within a 6-hour (one-way) drive of Vancouver. Telephone interviews were conducted with all participants residing more than 6 hours away (21% cases and 23% controls). Proxy interviews with relatives were conducted if the individual was deceased, did not speak English well, or if he/she could not accurately remember life events. This occurred with 26 (17%) cases and 41 (13.8%) controls. The questionnaire inquired about occupational, residential, medical, smoking, and exposure histories; a blinded industrial hygienist evaluated all completed interviews and asked the nurse to conduct follow-up, asking clarification questions of the participant when necessary. Occupations and industries were first coded according to standard occupational and industrial classifications and then blindly grouped according to a previously established classification system. Assignment into a group was based on whether the occupation or the industry was more likely to determine the individual's exposure. In the event that both the occupation and the industry determined exposure, the occupation was used. After that, all duties and exposures related to each occupation were reviewed to verify the accuracy of all categorizations, and all groups with less than 20 individuals were reviewed to determine if they could be combined with others. In total, 57 occupational groups were developed. Laundry personnel were part of the "other" category for nasal cancer and contained no cases or controls; on the other hand, laundry personnel were included in the "originally suspect" group for bladder cancer and contained five cases (3.3%) and four (1.3%) controls who reported "ever" employment in the occupation.

Exact methods were used to estimate summary ORs and their corresponding 95% CIs according to Breslow and Day (1980). In the event that nonoccupational risk factors were found to be positively associated with any of the cancers, the odds ratios and their corresponding 95% CIs were estimated using unconditional logistic regression, adjusted for these risk factors. Latency times of 5, 10, and 15 years were also examined, though the results were not shown. All odds ratios were adjusted for sex, age, and smoking. The influence of duration of employment (6 months to 10 years, and 10 years or more) was also examined but only reported if the estimates affected the results. Occupational groups were then assessed for their need for further surveillance based on a set of criteria. Limitations of the study include its small sample size, the grouping of jobs with different duties and exposures, and the exclusion of carcinomas in situ. The authors do not report any strengths associated with the methodology of their study.

B.2.1.2. Montreal (Canada)

B.2.1.2.1. Siemiatycki et al. (1991; 1987), Aronson et al. (1996), Parent et al. (2000)

Siemiatycki, J. (1991). Risk factors for cancer in the workplace. Boca Raton, FL: CRC Press.

Siemiatycki, J.; Wacholder, S.; Richardson, L.; Dewar, R.; Gérin, M. (1987). Discovering carcinogens in the occupational environment: Methods of data collection and analysis of a large case-referent monitoring system. Scand J Work Environ Health, 13, 486-492. http://www.ncbi.nlm.nih.gov/pubmed/3433050

Aronson, K.; Siemiatycki, J.; Dewar, R.; Gérin, M. (1996). Occupational risk factors for prostate cancer: Results from a case-control study in Montréal, Québec, Canada. Am J Epidemiol, 143, 363-373. http://www.ncbi.nlm.nih.gov/pubmed/8633620

Parent, M. E.; Hua, Y.; Siemiatycki, J. (2000). Occupational risk factors for renal cell carcinoma in Montreal. Am J Ind Med, 38, 609-618. http://www.ncbi.nlm.nih.gov/pubmed/11071683

Summary: Siemiatycki (1991) used a population-based case-control design to examine the possible association between occupational exposures and cancer. Cases were identified from hospitals in Montreal and consisted of male residents of Montreal aged 35 to 70 years who were diagnosed or histologically confirmed with any of the following cancers between 1979 and 1985: esophagus, stomach, small intestine, colon, rectum, gall bladder, pancreas, peritoneum, lung, pleura, skin, prostate, penis, testes, bladder, kidney, eye, lymphoid tissue, and multiple myeloma. Brain cancer, buccal cavity cancer, larynx cancer, and leukemia were excluded; due to limited resources, lung cancer was excluded in Years 2, 3, and 6; rectal cancer was excluded in Years 1 and 2; prostate cancer was excluded in Years 4 and 5. All of the large hospitals in Montreal took part, providing 97% population-based case ascertainment. Of the 4,576 cases identified, 3,730 (81.5%) participated in the interview. Response rates for individual cancers varied between 78% and 85%. Two sets of controls were used. Population-based controls were selected through electoral lists and random digit dialing. Of the 541 chosen from electoral lists, 375 (69.3%) were interviewed. Of 199 eligible participants identified through random digit dialing, 158 (79.4%) participated in the interview. Overall, of 740 population controls selected, 533 (72%) were interviewed. The final sample consisted of 99 esophagus cases, 251 stomach cases, 497 colon cases, 257 rectum cases, 116 pancreas cases, 857 lung cases, 449 prostate cases, 484 bladder cases, 177 kidney cases, 103 melanoma cases, and 215 lymphoma cases. In-person interviews were conducted by trained interviewers with cases and controls through a two-part questionnaire. The first section was structured and inquired about demographics; residential history; lifetime consumption of cigarettes, alcohol, coffee, and tea; consumption of food containing carotene; and height and weight. The second part was semi-structured, so as to

acquire detailed information on each of the jobs held during the man's working lifetime. Occupations and industries were coded according to the Canadian Classification and Dictionary of Occupations 1971 and the SIC Manual, respectively. Exposure was classified by a team of blinded chemists and hygienists, who used a checklist of 294 substances to determine the number of potential exposures for each job. All classifications were based on a three-point scale: the degree to which they believed the exposure had actually occurred (possible, probable, definite), the frequency of exposure in a normal workweek (<5, 5−30, and >30%), and the level of the concentration of the exposure (low, medium, high). Nonexposure was evaluated according to the background levels of that particular substance. The 294 substances were combined with 98 occupations and 77 industries to make a total of 469 occupational circumstances. Exposure was assessed as both direct exposure to tetrachloroethylene and proxy exposure through employment as launderers and dry cleaners. There were 6 (1.2%) cases of colon cancer, 7 (0.8%) cases of lung cancer, and 9 (2.0%) cases of prostate cancer that were "ever" exposed to tetrachloroethylene. Similarly, there were 4 (1.6%) cases of stomach cancer, 5 (1.0%) cases of colon cancer, 5 (2.0%) cases of rectum cancer, 12 (1.4%) cases of lung cancer, 9 (2.0%) cases of prostate cancer, 10 (5.6%) cases of kidney cancer, 3 (2.9%) cases of skin melanoma, and 3 (1.4%) cases of non-Hodgkin lymphoma among those who reported "ever" employment as launderers or dry cleaners. The Mantel-Haenszel method was used to estimate odds ratios and their corresponding 90% CIs for "ever" exposure and "substantial" exposure. All estimates were adjusted for age, family income, and cigarette index. Additionally, stomach cancer was adjusted for birthplace; colon and rectum cancers were adjusted for ethnic origin and beer index; lung cancer was adjusted for ethnic origin, alcohol index, and respondent; prostate cancer was adjusted for ethnic origin, Quetelet index, and respondent; and kidney cancer and skin melanoma were adjusted for ethnic origin. Strengths of the study design include its detailed information on potential confounders and occupational histories, its blind exposure assessment, use of histologically confirmed cases and access to two different control groups. Limitations to the study include the possible misclassification of exposure, the small numbers of exposed, the examination of many chemicals and job categories, and the study's goal to identify risk factors for further investigation.

Aronson et al. (1996) and Parent et al. (2000) used the data from Siemiatycki (1991) to further examine associations with selected cancers. Aronson et al. (1996) examined the association between occupations and prostate cancer. Of the 557 prostate cancer cases, 449 (81%) participated in the interview. The cancer controls included all other cancer cases from Siemiatycki et al. (1991) except lung cancer. The final sample consisted of 449 cases, 1,550 nonprostate cancer controls, and 533 population controls. Overall, 55 (27 substances, 11 industries, and 17 occupations) of the 469 occupational circumstances initially reviewed in

Siemiatycki et al. (1991) were examined in this study. Tetrachloroethylene exposure was classified as "unexposed," "nonsubstantial," or "substantial." There were eight participants with "substantial" exposure, but the authors failed to note whether these were cases or controls, precluding a calculation of exposure prevalence. Unconditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs for exposures using partially adjusted and fully adjusted models. The partially adjusted model controlled for age, ethnicity, socioeconomic status, Quetelet index, and self-/proxy respondent status, while the fully adjusted model included the covariates in the partially adjusted model, in addition to the all-core substances with 30 or more exposed cases. Due to the fact that very few differences were found when analyzing the control groups separately, the majority of the results were reported using the pooled group. In the event that two substances were highly correlated, one was removed from the model.

Parent et al. (2000) examined occupation and renal cell cancer. Of the 227 eligible kidney cases, 177 (78%) were interviewed and 142 of the 177 kidney cancers were renal cell carcinoma. There were a total of 1,900 cancer controls, representing a participation rate of 78%. Occupations and industries were assessed as a proxy for exposure and classified as any exposure and duration of exposure >10 years. The laundry and cleaning industry had a total of four cases (2.8%) that were "ever" exposed to the industry. Fewer than 4 cases were exposed for more than 10 years, and the results were not reported. The authors did not report exposure to tetrachloroethylene in this study, although the predecessor study, Siemiatycki et al. (1991) did. Unconditional logistic regression models were used to calculate odds ratios and their corresponding 95% CIs for each occupation and industry, stratified by any exposure and duration of exposure >10 years. Estimates for any exposure were adjusted for respondent status, age, smoking, and BMI.

B.2.1.3. Massachusetts (United States)

B.2.1.3.1. Aschengrau et al. (1998; 1993), Paulu et al. (2002, 1999)

Aschengrau, A.; Ozonoff, D.; Paulu, C.; Coogan, P.; Vezina, R.; Heeren, T.; Zhang, Y. (1993). Cancer risk and tetrachloroethylene-contaminated drinking water in Massachusetts. Arch Environ Health, 48, 284-292. http://www.ncbi.nlm.nih.gov/pubmed/8215591

Aschengrau, A.; Paulu, C.; Ozonoff, D. (1998). Tetrachloroethylene-contaminated drinking water and the risk of breast cancer. Environ Health Perspect, 106, 947-953. http://www.ncbi.nlm.nih.gov/pubmed/9703477

Paulu, C.; Aschengrau, A.; Ozonoff, D. (1999). Tetrachloroethylene-contaminated drinking water in Massachusetts and the risk of colon-rectum, lung, and other cancers. Environ Health Perspect, 107, 265-271. http://www.ncbi.nlm.nih.gov/pubmed/10090704

Paulu, C.; Aschengrau, A.; Ozonoff, D. (2002). Exploring associations between residential location and breast cancer incidence in a case-control study. Environ Health Perspect, 110, 471-478. http://www.ncbi.nlm.nih.gov/pubmed/12003750

Summary: These population-based case-control studies of bladder cancer, kidney cancer, and leukemia evaluated the relationship between various types of cancer and tetrachloroethylene exposure through contaminated drinking water. From 1968 to1980, tetrachloroethylene was used in a vinyl liner for asbestos cement water distribution pipes throughout Massachusetts to improve taste and odor. A substantial amount had been installed in five Upper Cape Cod towns, including Barnstable, Bourne, Falmouth, Mashpee, and Sandwich. In 1980, it was discovered that the tetrachloroethylene, which had been assumed to evaporate during the lining process, had leached into drinking water supplies. Cases were identified from the Massachusetts Cancer Registry and consisted of permanent residents of five Upper Cape Cod towns who were diagnosed with cancer between 1983 and 1986. Controls were identified through three mechanisms: living controls <65 years of age were obtained through random digit dialing, living controls ≥65 years of age were randomly chosen from Health Care Finance Administration lists using stratified sampling, and deceased controls were randomly selected from a Massachusetts Department of Vital Statistics and Research file for the period from 1983−1989. Of the 2,236 controls <65 years, 249 (11.1%) were eligible and contacted; of these, 184 (73.9%) were interviewed. Of the 611 controls ≥65 years, 537 (87.9%) were eligible and contacted; of these, 464 (86.4%) were interviewed. Of the 918 deceased controls, 794 (86.5%) were eligible and ascertained; of these, 723 (91.1%) were interviewed with a proxy respondent. Control groups for each of the cancer sites were selected through a two-step process. First, each cancer site was stratified by age, vital status, year of death (if applicable), and gender. Then, all controls that fell within a stratum with at least one case were chosen. Index years for each control group were determined based on the median year of diagnosis for the case group. Controls that moved to the Upper Cape Cod area after the index year, cases or controls with incomplete residential histories, and controls for which no tetrachloroethylene data were available were subsequently excluded. In-person (14%) and telephone (86%) interviews with participants, conducted by trained interviewers, inquired about a 40-year residential history, demographics, smoking, medical and occupational histories and exposures, bottled water consumption, and usual bathing habits. The articles did not provide estimates of proxy interviews. Cases and controls were similar in race, age, marital status, and religion.

Aschengrau et al. (1998; 1993) assessed exposure through relative delivered dose (RDD) of tetrachloroethylene via contaminated water estimated using Webler and Brown's (1993) algorithm, which was based on a tetrachloroethylene-leaching model by Demond (1982). The algorithm accounted for information about the water pipe that supplied each person's home,

including water flow and pipe characteristics. Inputs were determined using maps from local water suppliers or the Massachusetts Department of Environmental Protection. The exposure for cases and controls was assessed by one individual blinded to the individual's case/control status with a high degree of intraobserver and interobserver agreement. An ordinal estimate of exposure to tetrachloroethylene-contaminated water was defined as the estimated mass of tetrachloroethylene that entered the home through the drinking water during a specified period. The estimates were first categorized as "never exposed" (private wells) and "ever exposed," with the latter further categorized as "low" (up to and including median) and "high" (above the 50th, 75th, and 99th percentiles). The estimates based on Webler and Brown (1993) were recently found to correlate with historically measured tetrachloroethylene levels, demonstrating the algorithm's value in epidemiological research (Spence et al., 2008). RDDs were calculated for those that had more than one exposed residence and were categorized into low ($\leq$50th percentile of cumulative exposure among the exposed women), >50th, >75th, and >90th percentiles.

Aschengrau et al. (1993) evaluated the relationship between tetrachloroethylene-contaminated drinking water and bladder cancer, kidney cancer, and leukemia separately. Cases consisted of men and women of all ages who were diagnosed with incident bladder cancer, kidney cancer, or leukemia. Of the 79 bladder cancer cases, 42 kidney cancer cases, and 44 leukemia cases, 72 (91.1%) bladder cancer, 36 (85.7%) kidney cancer, and 38 (86.4%) leukemia cases were eligible and contacted. Of these, 63 (87.5%) bladder cancer, 35 (97.2%) kidney cancer, and 35 (92.1%) leukemia cases participated in the study. After employing the two-step control selection process and the additional exclusion criteria, the final sample consisted of 61 bladder cancer cases and 852 bladder cancer controls, 35 kidney cancer cases and 777 kidney cancer controls, and 34 leukemia cases and 737 leukemia controls. Industries and job titles were coded according to standard industrial (1987) and occupational (1990) classifications. Occupational exposure to tetrachloroethylene was based on industry and job titles, as well as specific questions posed during the interview. Overall, 34.4% bladder cancer cases, 26.2% bladder cancer controls, 25.7% kidney cancer cases, 25.2% kidney cancer controls, 35.3% leukemia cases, and 25.3% leukemia controls reported occupational exposure to solvents including tetrachloroethylene. Overall, there were 13 (21.3%) bladder cancer cases, 127 (14.9%) bladder cancer controls, 6 (17.1%) kidney cancer cases, 112 (14.4%) kidney cancer controls, 7 (20.6%) leukemia cases, and 94 (12.8%) leukemia controls with any exposure to tetrachloroethylene through drinking water without considering a latency period. Unadjusted odds ratios were estimated for all sites with at least two exposed cases, stratified by bottled water consumption and bathing habits separately. The Fisher exact test was used to estimate corresponding 95% CIs. These analyses were performed with and without the assumption of a latency period of 15 years for bladder and kidney cancer and 5 years for leukemia. Multiple

logistic regression was used to estimate odds ratios adjusted for sex, age at diagnosis for cases or index year for controls, vital status at interview, education, and occupational exposures. Additional potential confounders were included if present in at least three or more cases. This consisted of prior medical treatment with irradiation in the leukemia analysis, usual number of cigarettes smoked, and history of a urinary tract infection or stone in the kidney cancer analysis, and usual number of cigarettes smoked, history of a urinary tract infection or stone, and history of a cancer-associated job in the bladder analysis. Maximum likelihood estimates of the standard errors were used to estimate corresponding 95% CIs. Strengths of this study include its ability to control for a variety of potential confounders, including occupational exposures, and its examination of the effect of latency periods on the different cancers. Limitations to the study include a small bladder cancer sample size to examine the effect of a latency period, unknown levels of exposures, and nonblinded interviews.

Both Aschengrau et al. (1998) and Paulu et al. (2002) examined the relationship between tetrachloroethylene-contaminated drinking water and breast cancer in women diagnosed between 1983 and 1986. Of the 334 breast cancer cases, 295 (88.3%) were eligible, and 265 (89.8%) were interviewed. There were 763 controls identified through the two-step control selection process. After employing the additional exclusion criteria, the final sample consisted of 258 cases and 686 controls.

Aschengrau et al. (1998) reported 36 (14%) exposed cases and 81 (11.8%) exposed controls without considering a latency period. Latency periods of 5, 7, 9, 11, 13, and 15 years were also evaluated. Unadjusted odds ratios and their corresponding 95% CIs examined crude associations and potential modifiers. Multiple logistic regression was used to calculate odds ratios adjusted for age at diagnosis or index year, vital status at interview, family history of breast cancer, age at first live birth or stillbirth, personal history of prior breast cancer and benign breast disease, and occupational exposure to solvents. Maximum likelihood estimates of the standard errors were used to estimate corresponding 95% CIs. A strength of the study is its adjustment for a variety of potential confounders. Limitations include potential for measurement error in exposure estimates, small numbers of women, and possible misclassification due to inaccurate reporting on death certificate of control's address or cause of death.

Paulu et al. (1999) studied the relationship between tetrachloroethylene-contaminated drinking water and colon-rectum, lung, brain, and pancreatic cancer cases between 1983 and 1986. Of the 420 colon-rectum, 326 lung, 42 brain, and 43 pancreatic cancer cases selected, 366 (87.1%) colon-rectum, 272 (83.4%) lung, 40 (95.2%) brain, and 39 (90.7%) pancreatic cancer cases were contacted and eligible. Of these, 326 (89.1%) colon-rectum, 252 (92.6%) lung, 37 (88.1%) brain, and 37 (86.1%) pancreatic cancer cases were interviewed for an overall participation rate of 79%. The final sample consisted of 311 colon-rectum cancer cases and

1,158 colon-rectum cancer controls, 243 lung cancer cases and 1,206 lung cancer controls, 36 brain cancer cases and 703 brain cancer controls, and 36 pancreatic cancer cases and 622 pancreatic cancer controls. Excluding any latent periods, exposure assessments were as follows: colon-rectum cancer had 44 (14.1%) cases and 153 (13.2%) controls; lung cancer had 33 (13.6%) cases and 158 (13.1%) controls; brain cancer had 3 (8.3%) cases and 92 (13.1%) controls; and pancreatic cancer had 3 (8.3%) cases and 81 (13.0%) controls. Due to their low numbers of "ever exposed," unadjusted estimates of odds ratios and their corresponding 95% CIs were calculated for brain and pancreatic cancer cases. Multiple logistic regressions was used to estimate the odds ratios and 95% CIs for colon-rectum and lung-cancer cases, adjusted for age at diagnosis or index year, vital status at interview, sex, and occupational exposure to tetrachloroethylene and other solvents. Colon-rectum cancer was further adjusted for history of polyps, inflammatory bowel disease, and occupational history associated with colon-rectum cancer. Lung cancer was further adjusted for usual number of cigarettes smoked and history of cigar/pipe use, living with a smoker, and occupational history associated with lung cancer. Latency periods of 0, 5, 7, 9, 11, 13, and 15 years were considered in the analyses. Strengths of this study are its adjustment for confounders and consideration of a latency period. Limitations include a lack of measured tetrachloroethylene levels, lack of adjustment for smoking, particularly for lung cancer, and low-exposure prevalence, particularly for brain and pancreatic cancer cases.

Paulu et al. (2002) examined residential location using GIS-coded information. The 40-year residential history obtained during the interview included full addresses and calendar years of residence. If the complete address was unknown, tax assessors' books were used to help identify the geographical location. All participants were then blindly mapped onto an enlarged version of a U.S. Geological Survey map, which was later converted into a digital format. The Upper Cape Cod area was divided into subregions with two methodologies: the first employed fixed, multiscale grids and coded each participant as ever exposed or unexposed for each grid cell; the second used overlapping circles (adaptive k-smoothing) whose sizes were based on the number of nearby cases and controls. Crude and adjusted odds ratios were estimated for both the grid and k-smoothed methodologies, using map choropleths for visualization. These maps facilitate visualization of "hot spots" for microscale residence. Multiple logistic regression was used to estimate odds ratios for breast cancer, adjusted for age, parity, vital status, family history of breast cancer in a first-degree female relative, age at first live birth or stillbirth, and prior history of breast cancer or benign breast disease. No strengths were reported by the authors for this study; a limitation was the study's lack of individual measurements of household tetrachloroethylene exposures.

B.2.1.3.2. Aschengrau et al. (2003), Vieira et al. (2005)

Aschengrau, A.; Rogers, S.; Ozonoff, D. (2003). Perchloroethylene-contaminated drinking water and the risk of breast cancer: Additional results from Cape Cod, Massachusetts, USA. Environ Health Perspect, 111, 167-173. http://www.ncbi.nlm.nih.gov/pubmed/12573900

Vieira, V.; Aschengrau, A.; Ozonoff, D. (2005). Impact of tetrachloroethylene-contaminated drinking water on the risk of breast cancer: Using a dose model to assess exposure in a case-control study. Environ Health, 4, 3. http://dx.doi.org/10.1186/1476-069X-4-3

Summary: These population-based case-control studies were conducted as a follow-up to Aschengrau et al. (1998) and Paulu et al. (2002) to examine breast cancer and drinking water exposure. Cases were identified through the Massachusetts Cancer Registry and consisted of women diagnosed with breast cancer between 1987 and 1993, a period after that examined in the earlier studies. In contrast to the two-stage control selection process in earlier studies, controls were selected in three ways: (1) random-digit dialing (women ≤64 years), (2) random selection from a Medicare beneficiary roster (≥65 years), or (3) random selection from among death certificates provided by the Massachusetts Bureau of Health Statistics, Research, and Evaluation. Controls were matched to cases based on age and vital status at the time of identification. The final sample consisted of 672 cases and 616 controls, of which 211 (31.4%) cases and 192 (31.2%) controls were nonproxy respondents. Structured interviews were conducted with participants and next of kin to obtain information on demographics, confounders (age at diagnosis, family history of breast cancer, personal history of prior breast cancer, age at first live birth/stillbirth, occupational exposure to tetrachloroethylene, etc.), potential effect modifiers (bathing habits, bottled water, and water filter use), as well as a 40-year residential history. The authors do not state if these were in-person or blinded interviews.

Aschengrau et al. (2003) further examined the hypothesis that tetrachloroethylene exposure via contaminated drinking water increases the risk of breast cancer. Overall, 672 cases (81% selected and eligible cases) and 616 controls (157 [83%] random-digit dialed, 301 [76%] of Medicare roster, and 158 [79%] deceased) were included in the analysis. RDD of tetrachloroethylene via contaminated water was estimated using Webler and Brown's (1993) algorithm, which was based on a tetrachloroethylene leaching model by Demond (1982). The algorithm accounted for information about the water pipe that supplied each person's home, including water flow and pipe characteristics. Inputs were determined using maps from local water suppliers or the Massachusetts Department of Environmental Protection. The exposure for cases and controls was assessed by one individual blinded to the individual's case-control status with a high degree of intraobserver and interobserver agreement. An ordinal estimate of exposure to tetrachloroethylene-contaminated water was defined as the estimated mass of

tetrachloroethylene that entered the home through the drinking water during a specified period. The estimates were first categorized as "never exposed" (private wells) and "ever exposed," with the latter further categorized as "low" (up to and including median) and "high" (with categorization as >50th, >75th, and >99th percentiles). The estimates based on Webler and Brown (1993) were recently found to correlate with historically measured tetrachloroethylene levels, demonstrating the algorithm's value in epidemiological research (Spence et al., 2008). Overall, there were 155 (23.1%) cases and 136 (22.1%) controls exposed to tetrachloroethylene. Data analysis included the following latent periods: 0, 5, 7, 9, 11, 13, 15, 17, and 19 years. Exposure odds ratios and their corresponding 95% CIs estimated crude associations. Multiple logistic regression was used to estimate odds ratios, adjusted for age at diagnosis or index year, vital status at interview, family history of breast cancer, personal history of breast cancer, age at first live birth or stillbirth, and occupational exposure to tetrachloroethylene. Maximum likelihood estimates of the standard error were used to calculate corresponding 95% CIs.

Viera et al. (2005) further studied the associations between tetrachloroethylene exposure and breast cancer. Due to the fact that the majority of the relevant interview information was only collected from nonproxy cases and controls, proxy interviews were excluded from the analyses, though included in comparisons with the total sample. Nonproxy information obtained through the interviews included the daily consumption of tap water or drinks that used tap water (number of drinks), bottled water consumption, and the temperature, frequency, and duration of showers and baths. Data not collected in the interviews, such as inhalation rate, water flow rate, and air exchange rate, were ascertained from the literature. The authors did not provide references for these obtained rates. In contrast to Aschengrau et al. (2003), this study estimated the personal delivered dose (PDD) for each participant by adding the amount inhaled, dermally absorbed, and ingested together for each exposed residence. Inhalation was estimated from reported temperature, frequency, and duration of baths and showers, as well as from the amount of tetrachloroethylene in the bath/shower air. Dermal absorption was estimated according to Fick's first law and used height and weight data to calculate each participant's surface area. Ingestion was based on the volume of tap water the participant drank. RDDs were re-estimated for the nonproxy participants only, and both the RDD and PDD were used to classify each participant into nested exposure levels: ≤50th percentile, >50th, >75th, and >90th percentiles. Latency periods of 0, 5, 7, 9, 11, 13, 15, 17, and 19 years were employed. Without considering a latency period, the full sample contained 155 (23.1%) exposed cases and 136 (22.1%) exposed controls, and the nonproxy sample contained 101 (21.9%) exposed cases and 88 (20.8%) exposed controls. Crude and adjusted analyses were conducted for both the RDD and PDD levels, though adjusted analyses were limited to those with at least three exposed cases and at least three exposed controls. Multiple logistic regression was used to estimate adjusted odds

ratios, controlling for the following confounders, which were identified *a priori:* age at diagnosis or index year, family history of breast cancer, personal history of breast cancer, age at first live birth or stillbirth, and occupational exposure to tetrachloroethylene. Maximum likelihood estimates of the standard errors were employed to estimate corresponding 95% CIs. All nonproxy estimates were subsequently compared to estimates for all subjects. The full sample's adjusted odds ratios (ORs) further controlled for vital status at interview. A goodness-of-fit test compared the RDD and the PDD to ascertain which was a better measure, and a nonparametric rank test evaluated whether RDD and PDD exposures differed significantly from each other. A strength of this study is its incorporation of personal behaviors in estimating exposure, examination of nonproxy respondents, considered to provide more correct information than proxy respondents and to reduce misclassification bias, and comparison of results from only nonproxy respondents to results of all subjects. Limitations include its use of cumulative exposures, which may mask the effect of intensity of exposure, recall bias for behavioral data, and decreased sample size due to the use of nonproxy respondents only.

B.2.1.4. New Zealand

B.2.1.4.1. Corbin et al. (2011), Dryson et al. (2008), 't Mannetje et al. (2008), McLean et al. (2009)

Corbin, M.; McLean, D.; Mannetje, A.; Dryson, E.; Walls, C.; McKenzie, F., . . . Pearce, N. (2011). Lung cancer and occupation: A New Zealand cancer registry-based case-control study. Am J Ind Med, 54, 89-101. http://dx.doi.org/10.1002/ajim.20906

Dryson, E.; 't Mannetje, A.; Walls, C.; McLean, D.; McKenzie, F.; Maule, M., . . . Pearce, N. (2008). Case-control study of high risk occupations for bladder cancer in New Zealand. Int J Cancer, 122, 1340-1346. http://dx.doi.org/10.1002/ijc.23194

't Mannetje, A.; Dryson, E.; Walls, C.; McLean, D.; McKenzie, F.; Maule, M., . . . Pearce, N. (2008). High risk occupations for non-Hodgkin's lymphoma in New Zealand: Case-control study. Occup Environ Med, 65, 354-363. http://dx.doi.org/10.1136/oem.2007.035014

McLean, D.; Mannetje, A.; Dryson, E.; Walls, C.; McKenzie, F.; Maule, M., . . . Pearce, N. (2009). Leukaemia and occupation: A New Zealand Cancer Registry-based case-control Study. Int J Epidemiol, 38, 594-606. http://dx.doi.org/10.1093/ije/dyn220

Summary: The case-control studies of Dryson et al. (2008), Mannetje et al. (2008), McLean et al. (2009), and Corbin et al. (2011) are part of an ongoing series of studies examining the relationship between occupation and cancer in the New Zealand population. Cases were identified through the New Zealand Cancer Registry from 2003 to 2004 in Dryson et al. (2008), 't Mannetje et al. (2008), and McLean et al. (2009) and from 2007 to 2008 in Corbin et al.

(2011). Population-based controls were randomly chosen from the 2003 New Zealand Electoral Roll and matched to cases based on age. Of 1,200 potential controls initially mailed letters of invitation, 1,100 had valid addresses. Of these, 660 were able to be contacted and considered eligible to participate. Overall, 473 controls were interviewed, with an overall response rate of 48%. After excluding controls with missing information for key variables, the final sample consisted of 471 controls. Controls in Corbin et al. (2011) were identified from 2003 to 2008 with letters of invitation mailed to 2,000 individuals, 1,878 of whom had valid addresses. Of these, 1,134 replied, and 796 were interviewed (48% response rate). In Dryson et al. (2008), 't Mannetje et al. (2008), and McLean et al. (2009), cases and controls were similar in occupational class, with the exception of the lowest class, which was more prevalent among cases than controls. In Corbin et al. (2011), "ever" smoking was more frequent among cases than among controls, as might be expected in a study of lung cancer, and the frequency of subjects 71 years of age and older was higher among controls than among cases.

In-person interviews were conducted with a trained interviewer whose background was in occupational health nursing. The questionnaire inquired about demographics, smoking, and occupational history, and more detailed information was obtained on all jobs lasting longer than 1 year. Occupation was assessed as a proxy for exposure, and jobs were blindly coded according to the 1999 New Zealand Standard Classification of Occupations and the Australian and New Zealand SIC. The authors do not report who assigned the codes. Occupation Code 8264 consisted of textile bleaching, dyeing, and cleaning machine operators and was considered *a priori* to be high risk. The authors did not refer specifically to dry-cleaners or laundry workers. McLean et al. (2009) noted workers in this occupational group had similar exposures as laundry and dry cleaning occupations. In addition, Corbin et al. (2011) presented analyses separately for occupational titles of dry cleaner and launderer. Unconditional logistic regression was used to calculate odds ratios and their corresponding 95% CIs for occupations and industries considered *a priori* and *a posteriori* to be high risk. All estimates were adjusted for 5-year age group, sex, smoking ("ever," "ex," "never"), Maori ethnicity, and occupational status. Semi-Bayes adjustments were performed to minimize the risk of false positive results due to multiple comparisons. These adjustments were performed using an estimate of the variation that was determined *a priori*. Strengths of the study design include its population-based design, near complete coverage of both incident cancers and the general population, and adjustment for smoking. Additionally, interviews were conducted in person and obtained detailed occupational histories. The limitations of the study include the lack of an exposure profile, information on duration or length of employment for only certain occupations or chemicals, and possible selection bias due to the low-response rates of cases and controls, though McLean et al. (2009) noted the similarity between the distribution of occupations in the national census and the

sample. Additional limitations include the study's low exposure prevalence and possible exposure misclassification due to the use of broad occupational categories.

Dryson et al. (2008), 't Mannetje et al. (2008), and Corbin et al. (2011) included men and women aged 25 to 70 years who were diagnosed with either bladder cancer or non-Hodgkin lymphoma. The authors did not state if the cases were histologically confirmed. Dryson et al. (2008) studied bladder cancer among selected occupations that may contribute to the risk of bladder cancer. Of the 381 cases in Dryson et al. (2008) identified from the New Zealand Cancer Registry, 232 (60.9%) were able to be contacted by mail and eligible to participate. In total, 213 cases were interviewed for the study, with an overall response rate of 64%. The final sample consisted of 213 cases and 471 controls. Approximately 77% of cases and 47% of controls were male. "Current" and "ever" smoking were more prevalent among cases than controls. There were 3 (1.4%) bladder cancer cases and 10 (2.1%) controls that reported employment in the bleaching, dyeing, and cleaning machine occupations.

't Mannetje et al. (2008) aimed to assess whether previously reported associations (Reif et al., 1989; Pearce et al., 1988; Pearce et al., 1987; Pearce et al., 1985) between occupations and non-Hodgkin lymphoma persist, and to identify other occupations that may also contribute to the risk of non-Hodgkin lymphoma in the New Zealand population. Of the 533 cases identified from the cancer registry, 335 (62.9%) were able to be contacted and eligible to participate. In total, 291 cases were interviewed for the study, with a response rate of 69%. The final sample consisted of 291 cases and 471 controls. Approximately 54% of cases and 47% of controls were male, and current smoking was more common among cases than controls. There were 5 (1.7%) cases and 10 (2.1%) controls that reported employment as a textile bleaching, dyeing, and cleaning machine operators.

McLean et al. (2009) studied the relationship between occupation and leukemia (chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, and other forms of leukemia). Cases consisted of men and women aged 20 to 75 years who were added to the registry between 2003 and 2004. The authors did not state if the cases were histologically confirmed. Of the 391 eligible cases, 225 (57%) participated in the interview; 11 (4.9%) of which were proxy interviews with next of kin. The final sample consisted of 225 cases and 471 controls. Approximately 61% cases and 47% controls were male, and a higher proportion of cases were current smokers than controls. Overall, 6 (2.7%) cases and 10 (2.1%) controls comprised the textile bleaching, dyeing, and cleaning machine occupation.

Corbin et al. (2011) examined lung cancer and occupation to support previously identified risk factors and to identify new risk factors. Of 744 eligible lung cancer cases, aged 20−75 years in Corbin et al. (2011), 458 were interviewed (53% response rate). Among those interviewed, 432 of the 796 cases were by phone, and all interviews were with living subjects.

Face-to-face interviews were carried for the remaining 432 control subjects. Overall, 20 cases and 13 controls were employed as textile bleaching, dyeing, and cleaning machine operators, with 3 of these cases and 4 controls identified as dry cleaners. An additional 9 cases and 5 controls were identified as launderers.

B.2.1.5. Germany

B.2.1.5.1. Pesch et al. (2000a, b)

Pesch, B.; Haerting, J.; Ranft, U.; Klimpel, A.; Oelschlägel, B.; Schill, W. (2000a). Occupational risk factors for renal cell carcinoma: Agent-specific results from a case-control study in Germany. Int J Epidemiol, 29, 1014-1024. http://dx.doi.org/10.1093/ije/29.6.1014

Pesch, B.; Haerting, J.; Ranft, U.; Klimpel, A.; Oelschlägel, B.; Schill, W. (2000b). Occupational risk factors for urothelial carcinoma: Agent-specific results from a case-control study in Germany. Int J Epidemiol, 29, 238-274. http://dx.doi.org/10.1093/ije/29.2.238

Summary: Between 1991 and 1995, a population-based case-control study was conducted in five regions of Germany to independently estimate the risk of urothelial cancer and renal cell cancer as functions of exposure to aromatic amines, polycyclic aromatic hydrocarbons (PAHs), and other chlorinated hydrocarbons. Cases were identified through the large hospitals in each of the regions and consisted of German men and women who were diagnosed with histologically confirmed urothelial or renal cell cancer within the 6 months prior to the start of the study. Controls were randomly selected from local residency registries and matched to cases on age, sex, and region. In order to be included in the study, cases and controls were required to be German nationals; there were no age limits during the recruitment process.

In-person interviews with trained interviewers occurred with cases in the hospital within the first 6 months of diagnosis and with controls in their home. A structured questionnaire inquired about demographics, lifestyle, and occupational exposures. The final sample consisted of 1,970 cases (1,035 urothelial cancer and 935 renal cell cancer) and 4,298 controls, with overall response rates of 84% for cases and 71% for controls. Exposure was assessed based on the participant's reported occupational history, exposure to specific agents during tasks, and average amount of time each day exposed. All jobs held for at least 1 year were coded according to the International Standard Classification of Occupations. Lifetime exposure was calculated as the total number of years spent at a specific job title; task- and agent-specific exposures were estimated as weighted sums of years spent at that task or exposed to the agent in question. Job exposure matrices (JEMs) and job-task exposure matrices (JTEMs) were also used for calculating exposure to specific agents, including tetrachloroethylene. These matrices evaluated the probability and intensity of exposure. The JEM, which assessed exposure based on job title,

used both the British (Pannett et al., 1985) and the German (Robra and Seidler, 1994) versions. The JTEM was developed by the researchers and adjusted for both region and time. Both studies used conditional logistic regression to calculate odds ratios and their corresponding 95% CIs for potential confounders, occupations and tasks, and substances separately, adjusted for age, study center, and smoking. Potential confounders were also stratified by gender, and additional analyses were adjusted for age and study center without smoking. Strengths of these studies include population-based selection of controls and the use of a JEM and a JTEM to assess substance exposure. Limitations include the lower response rate of controls compared to cases and the reliance of self-reported information for exposure assessment.

Pesch et al. (2000a, b) estimated the urothelial cancer risk for occupational exposure to aromatic amines, PAHs, and chlorinated hydrocarbons besides other suspected risk factors. This study sample included 1,035 urothelial cancer cases and 4,298 controls. When tetrachloroethylene was assessed using the German JEM, there were 183 (17.7%) cases with medium exposure, 188 (18.2%) cases with high exposure, and 74 (7.1%) cases with substantial exposure. The JTEM approach, however, only identified 37 (3.6%) cases with medium exposure, 47 (4.5%) with high exposure, and 22 (2.1%) with substantial exposure.

Pesch et al. (2000a, b) examined the possible impact of occupation-related agents on renal cell cancer development. The sample in this study consisted of 935 renal cell cancer cases and 4,298 controls. When tetrachloroethylene was evaluated using the German JEM, there were 166 (17.8%) cases with medium exposure, 138 (14.8%) cases with high exposure, and 54 (5.8%) cases with substantial exposure. The JTEM approach, however, identified only 52 (5.6%) cases with medium exposure to tetrachloroethylene, 45 (4.8%) with high exposure, and 18 (1.9%) with substantial exposure.

B.2.1.6. Nordic Countries

B.2.1.6.1. Lynge et al. (2006)

Lynge, E.; Andersen, A.; Rylander, L.; Tinnerberg, H.; Lindbohm, M. L.; Pukkala, E., . . . Johansen, K. (2006). Cancer in persons working in dry cleaning in the Nordic countries. Environ Health Perspect, 114, 213-219. http://dx.doi.org/10.1289/ehp.8425

Summary: This study of a nested case-control design within a cohort examined eight site-specific cancers (non-Hodgkin lymphoma, esophageal, gastric cardia, liver, pancreatic, cervix uteri, kidney, and bladder), and job title, distinguishing between dry-cleaning workers, a proxy for tetrachloroethylene, and other job titles such as laundry workers. The cohort from which cases and controls arose consisted of 46,768 individuals identified as laundry and dry-cleaning workers in the 1970 Censuses in Denmark, Finland, Norway, and Sweden. All were followed for death, emigration, and incident cancer based on nationwide population, death, and cancer registries.

Relevant cancer cases were identified as those that occurred during the period of November 1970 (Denmark) or January 1971 (Finland, Norway, Sweden) through 1997 to 2001. Controls were randomly selected from the cohort and matched based on country, sex, 5-year age group, and 5-year calendar period at the time of diagnosis. All analyses were conducted at the level of the record rather than person because a subject may have appeared as a case or as a cancer control in the study more than once. Out of 4,014 records from 3,883 persons, 131 subjects were considered both as a case and as a cancer control.

Lynge et al. (2006) used job title and occupational task identified in the 1970 Census to identify tetrachloroethylene exposure potential. Differing recordkeeping systems and record availability in each country necessitated a number of approaches for assigning exposure potential to cases and controls; Johansen et al. (2005) provides an in-depth description of available records for Danish subjects and Lynge et al. (2011) of available records in the four studied countries. In Denmark and Norway, occupational task identified on the 1970 Census form was available and used to identify subjects as (1) dry-cleaners or other workers in dry-cleaning shops with <10 workers, assumed to have high-exposure potential because of the shared work tasks and physical proximity in small dry-cleaning shops; (2) other workers in dry-cleaning shops; (3) unexposed laundry workers and other persons in dry cleaning, and (4) unclassifiable, a category for subjects with missing employment information. Pension data from Denmark and Finland, as well as a Danish biography of dry-cleaning shop owners, were used to identify length of employment, a proxy for cumulative exposure, between 1964 and 1979, and size of workforce, for self-employed subjects. For subjects from Norway and Sweden, a blinded telephone interview was undertaken, given the lack of storage of the 1970 Census forms. The questionnaire asked about occupational task for job title reported on the 1970 Census form, and if dry cleaning, questions sought answers on employment length, number of employees, solvents used, and personal habits of smoking and alcohol consumption. Interviews were obtained with 148 of 258 of cases (57%) and 293 of 457 controls (64%) in Norway; for which 107 cases subjects (72%) and 123 control subjects (42%) were with proxy respondents. For Swedish subjects, interviews were obtained with 369 of 586 cases (63%) and 454 of 756 controls (60%) controls; for which 284 case subjects (77%) and 177 control subjects (39%) were with proxy respondents.

In total, the study included 1,616 cases and 2,398 controls, with roughly two-thirds (68%) of subjects from Denmark and Sweden. There were 695 (17.3%) cases and controls who were exposed due to their work as dry cleaners, 183 (5%) exposed through other work in a dry-cleaning shop, and 716 (18%) for whom information on employment and exposure potential could not be obtained and were identified as “unclassifiable.” The percentage of subjects identified as “unclassifiable” varied by country, with no subjects from Denmark, 41% of all subjects from Finland, 2% of all subjects from Norway, and 35% of all subjects from Sweden.

Lynge et al. (2006) provided some exposure monitoring data, particularly for 1964–1979, the period examined in analyses of exposure duration. Although a large variation in exposure levels was observed in 168 samples from Nordic dry-cleaning shops, the median concentrations over this period were relatively stable and appeared to range from 3–12 ppm. Lynge et al. (2006) reported a mean of 24 ppm from 53 samples of ≥60 minutes in length. Lynge et al. (2011) provided exposure monitoring from dry clean facilities for the period 1947-2000 (Denmark), 1956-1999 (Finland), 1976-2001 (Norway) and 1973-1995 (Sweden). Personal monitoring was not available before 1978; roughly 90% of the stationary air measurements were from the period after 1975The mean of stationary measurements over the monitoring period was 11.92 ppm (95% CI: 10.66, 13.51) and, for personal measurements, 7.27 ppm (95% CI; 6.78, 7.79). Based on the stationary monitoring measurements, the exposure of maintenance workers>dry cleaners>shop assistants, with means of 35.94 ppm (95% CI: 20.92, 55.99), 13.20 ppm (95% CI:, 11.13, 15.24), and 7.50 (95% CI: 6.84, 8.02), respectively. Personal monitoring measurements were lower than stationary monitoring measurements for dry cleaners and shop assistants, no personal monitoring was available for maintenance workers, and suggested job title was not a predictor of exposure intensity. Mean exposures were 7.50 ppm (95% CI: 6.73, 7.94) for dry cleaners and 6.25 ppm (95% CI: 4.09, 8.93) for shop assistants. Exposure by job title of dry cleaner and shop assistant varied in Denmark and Finland, whereas little or no difference was indicated from monitoring data from Sweden and Norway.

Rate ratios (RRs) for dry cleaners versus unexposed controls were estimated using logistic regression. RRs were also calculated for the other persons in dry cleaning and for the unclassifiable persons, although the underlying hypothesis did not include these groups. RRs were estimated for all countries together and for Denmark and Norway together given their lower percentage of unclassifiable subjects compared to that for Finland (41%) or Sweden (35%). The researchers adjusted for the matching criteria, as well as smoking and alcohol use (Norway and Sweden only) in bladder cancer analyses that showed smoking as not greatly affecting observed risk estimates. Strengths of the study include its coverage of the period where tetrachloroethylene was used as the main solvent, its population-based design, its use of a series of nested case-control studies within the cohorts to examine specific cancers, its control for smoking in bladder cancer analysis, and its examination of dry cleaner versus other dry-cleaning tasks. A limitation is a lack of exposure monitoring data on individual subjects as industrial hygiene data from 1964−1979 showed a large variation in tetrachloroethylene concentrations across shops. Additionally, the large number of next-of-kin interviews in cases from Sweden and Norway; a control series which included cases with other cancers of *a priori* interest (8% of case series); assessment of tetrachloroethylene exposure potential for one job, that was held in 1970, versus for the full employment history; and, censoring employment duration to 1979 rather

than for the study's full period, to 1998 or 2001 (depending on country) likely introduces misclassification bias. Finally, a large number of subjects from Sweden and Finland had missing information. If differential reporting of job title and occupational tasks was associated with exposure as a dry cleaner and status as a case or control, then a misclassification bias would be introduced. Lynge et al. (2006) explored the magnitude of this potential bias on esophageal cancer estimates, noting if all unclassified subjects were exposed as dry cleaners, observed odds ratio (0.76; 95% CI: 0.34, 1.69) would increase (to 1.19; 95% CI: 0.67, 2.12), and, if unexposed, would decrease (to 0.66; 95% CI: 0.30, 1.45).

B.2.2. Single Cancer-Site Studies

B.2.2.1. Bladder Cancer

B.2.2.1.1. Burns and Swanson (1991), Swanson and Burns (1995)

Burns, P. B. and Swanson, G. M. (1991). Risk of urinary bladder cancer among blacks and whites: The role of cigarette use and occupation. Cancer Causes Control, 2, 371-379. http://dx.doi.org/10.1007/BF00054297

Swanson, G. M. and Burns, P. B. (1995). Cancer incidence among women in the workplace: A study of the association between occupation and industry and 11 cancer sites. J Occup Environ Med, 37, 282-287. http://www.ncbi.nlm.nih.gov/pubmed/7796194

Summary: This population case-control study is part of the Occupational Cancer Incidence Surveillance Study examining occupation and 11 cancer sites. Burns and Swanson (1991) examined cigarette smoking and occupational title and bladder cancer, with Swanson and Burns (1995) focusing on occupation and cancer in women. The Metropolitan Detroit Cancer Surveillance System (MDCSS) was used to identify cancer cases at 11 sites (lung, colon, rectum, bladder, esophagus, liver, salivary gland, stomach, eye, melanoma, and mesothelioma) among males and females aged 40 to 84 years, diagnosed between 1984−1991. In all, 2,160 bladder cancer cases and 3,979 cancer controls were interviewed by telephone for response rates of 94% and 95% for cases and controls, respectively. Colon and rectal cancer cases from the registry were selected as controls and not matched to cases based on demographic variables. Of those interviewed, 25% of case series and 27.6% of the controls series were proxy or next-of-kin respondents. The high percentage of proxy interviews may introduce potential for recall bias of detailed occupational history. The interview gathered information on complete lifetime occupation history, including occupation and industry titles, lifetime smoking history, medical history, residential history, and demographic information. Occupation and industry data were coded according to the three-digit codes of the 1980 U.S. Census Bureau classification. The paper does not identify if occupational coding was carried out blinded to case or control status.

Exposure prevalence was low for holding an occupation as dry-cleaning worker, 0.4% for cases and 0.4% for controls, or for working in the dry-cleaning or laundry industry, 0.6% for cases and 0.6% for controls. Association with bladder cancer and occupation was examined using unconditional logistic regression adjusted for cigarette smoking habits, race, gender, and age at diagnosis and usual industry or occupation, defined as the longest period of employment.

B.2.2.1.2. Colt et al. (2004)

Colt, J.; Baris, D.; Stewart, P.; Schned, A.; Heaney, J.; Mott, L., . . . Karagas, M. (2004). Occupation and bladder cancer risk in a population-based case-control study in New Hampshire. Cancer Causes Control, 15, 759-769. http://dx.doi.org/10.1023/B:CACO.0000043426.28741.a2

Summary: This population case-control study examined a number of risk factors including occupation exposures for primary bladder cancer among New Hampshire residents, aged 25−74 years. To be eligible for the study, subjects were required to have a listed telephone number and speak English. Six hundred eighteen (n = 618) cases diagnosed over a 4-year period, between July 1, 1994 and June 30, 1998, were identified from the New Hampshire Cancer Registry and histologically confirmed; 459 were subsequently interviewed (74% participation rate). Controls, shared with a study of nonmelanoma skin cancer in the period 1993−1995 and frequency matched based on age and sex, were selected from population lists of the New Hampshire Department of Transportation, if <65 years old, and from New Hampshire Centers for Medicare and Medicaid Services. The study augmented the control group, adding controls for bladder cancer cases diagnosed between July 1, 1995 to June 30, 1997. Interviews were carried out with 665 of the 990 potential controls (67% participation rate). Little age difference existed between cases and controls, although cases were more likely than controls to have a history of cigarette smoking, with current smokers twice as prevalent among the cases as controls.

Subjects who agreed to participate in the study underwent a detailed in-person interview, usually at their home, with questions on sociodemographic information, tobacco use, medical history prior to the diagnosis, and lifetime work history. Each job reported in the occupation history was coded according to the Standard Occupation Classification Manual scheme, with codes of 7658 and 7657 for occupations in dry-cleaning and laundry service. For each occupation, bladder cancer risk was estimated separately for men and women for each job held after age 15 using unconditional logistic regression models adjusted for age and smoking status. Additionally, the authors conducted a separate analysis of *a priori* suspect high-risk occupations, that included dry-cleaner and laundry workers. Only five male case and five male controls reported a job title of dry-cleaner and laundry workers, and the study authors did not report the

associated odds ratio because of the small numbers. The low-exposure prevalence for dry-cleaning and laundry work, as is typical of population case-control studies, greatly reduces the statistical power of this study to examine bladder cancer.

B.2.2.1.3. Colt et al. (2011)

Colt, J. S.; Karagas, M. R.; Schwenn, M.; Baris, D.; Johnson, A.; Stewart, P., . . . Silverman, D. T. (2011). Occupation and bladder cancer in a population-based case-control study in Northern New England. Occup Environ Med, 68, 239-249. http://dx.doi.org/10.1136/oem.2009.052571

Summary: This population case-control study examined occupation and industry as risk factors for urothelial bladder cancer, among residents of Maine, New Hampshire, and Vermont, aged 30−79 years. A focus of the study was exposure to metal working fluids. All residents newly diagnosed with a histologically confirmed carcinoma of the urinary bladder (including carcinoma in situ) between September 1, 2001 and October 31, 2004 (Maine and Vermont) or between January 1, 2002 and July 31, 2004 (New Hampshire) were eligible for study. Cases were identified through a rapid patient ascertainment in each state using data from hospital pathology departments, hospital cancer registries, and state cancer registries. A total of 1,878 eligible cases were identified with in-person interviews obtained from 1,213 (65%). Further pathologic review determined 43 subjects did not have bladder cancer or had nonurothelial carcinoma, leaving 1,170 cases. Controls were randomly selected from state motor vehicle records, if aged 30−64 years, or Medicare or Medicaid records, if aged 65+ years, and frequency matched to cases by state, sex, and age at diagnosis or control selection. Interviews were carried out with 1,418 controls, 594 identified from driver records (65% of eligible) and 824 identified through Medicare/Medicaid roles (65% of eligible).

Case and control subjects were first mailed a questionnaire with follow-up by a home visit where a trained interviewer administered a computer-assisted questionnaire that sought information on all jobs held for at least 6 months since age 16 years, demographic information, tobacco use, and other exposures. For certain occupations held by subjects, a job-specific questionnaire was administered, soliciting detailed information about exposures of interest. Each job was coded blinded to case or control status to the 1980 SOC and the 1987 SIC scheme. Of the 1,170 cases and 1,418 controls, 1,158 cases and 1,402 controls completed both questionnaires.

For each occupation and industry, bladder cancer risk was estimated separately for men and women for each job using unconditional logistic regression models adjusted for age, race, Hispanic ethnicity, state, smoking status, and employment in a high-risk occupation. A high-risk occupation was defined for men and women separately if odds ratios in the current study were

1.5 or higher and 10 or more subjects were employed in the category examined in statistical analyses. Additionally, the authors evaluated smoking effects, replacing smoking status with smoking duration and found minimal changes in the estimated odds ratios; final statistical models were thus adjusted for smoking status only. Interactions between smoking and occupation were tested, adding cross-product terms to the logistic model. Additionally, the authors examined employment duration for occupations and industries with a positive association for "ever"/"never" employed. Tests of linear trend were performed by treating the median duration of employment among controls for each duration category as a continuous variable, with a value of zero assigned to subjects never holding a job in the subject category. For occupations with observed risk estimates that increased with increasing employment duration, an examination of initial year of employment and bladder cancer risk was examined.

Strengths of the study include the population-based design, ascertainment of complete occupational histories from direct interview with study participants, blind assignment of exposure, and ability to adjust for smoking, employment in high-risk occupations, and other risk factors. Limitations include the low prevalence of exposure as laundering and dry-cleaning machine operators (0.5% of all cases) and lower participation rate (65%) for cases and controls. However, the authors concluded that because study participation likely did not differ between cases and controls in an exposure-dependant manner, observations would not be biased. Some exposure misclassification is likely, given limited information for some cases and controls, and the lack of information on specific exposures by using job title. Biases are likely nondifferential and lead to dampened risk estimates.

B.2.2.1.4. Gaertner et al. (2004)

Gaertner, R. R. W.; Trpeski, L.; Johnson, K. C. (2004; 1995). A case-control study of occupational risk factors for bladder cancer in Canada. Cancer Causes Control, 15, 1007-1019. http://dx.doi.org/10.1007/s10552-004-1448-7

Summary: This population-based case control study of bladder cancer in seven Canadian provinces (Newfoundland, Prince Edward Island, Nova Scotia, Manitoba, Alberta, Saskatchewan, and British Columbia) made use of data collected in the Canadian National Enhanced Cancer Surveillance System. The project collected data on cases of various cancers and controls, with the intent of improving knowledge of environmental factors in cancer development. Cases were identified through each province's cancer registry and consisted of men and women aged 20 to 74 years who were diagnosed with histologically confirmed bladder cancers between 1994 and 1997. Controls were randomly selected from the general population of the seven provinces using simple random digit dialing (Newfoundland and Alberta) or sampling from the provincial health insurance plan database (the remaining five provinces), and

frequency matched to cases based on age and sex. Of the 1,499 eligible cases, 887 (59%) completed the mailed questionnaire and participated in the study. The response rate among controls was 62% (n = 2,847) of 4,604 eligible subjects.

The mailed questionnaire sought information on socio-demographics, lifetime smoking history, dietary habits, and occupational history, including information on specific agents. Up to 12 occupations were categorized into SOC codes, and study investigators identified 9 occupations as suspect and of *a priori* interest, including job title of dry cleaner. A total of 9 subjects, 4 cases, and 5 controls, reported "ever" holding an occupation as dry cleaner. Employment duration was calculated from time period reported for each occupational activity over a subject's lifetime. Unconditional logistic regression was used to calculate odds ratios and their corresponding 95% CIs, for males and females separately, adjusted for age, province, race, current smoking status, ex-smoking, consumption of fruit, fried food, and coffee, and "ever" employed in suspect occupations. The authors did not present observations on employment duration and "ever" holding a job as dry cleaner, likely due to the few exposed subjects. The authors reported strengths of the methodology of their study as use of histologically confirmed incident bladder cancer cases and the extensive information on nonoccupational factors. Limitations include the study's small sample size, recall bias among cancer cases, low-response rate among both cases and controls, and use of occupational title as surrogate for tetrachloroethylene exposure potential.

B.2.2.1.5. Kogevinas et al. (2003)

Kogevinas, M.; 't Mannetje, A.; Cordier, S.; Ranft, U.; González, C.; Vineis, P., . . . Boffetta, P. (2003). Occupation and bladder cancer among men in Western Europe. Cancer Causes Control, 14, 907-914. http://dx.doi.org/10.1023/B:CACO.0000007962.19066.9c

Summary: This study used pooled data from 11 previously conducted European case-control studies to examine the association between risk of bladder cancer and occupational exposures in men. The case-control studies were Claude et al. (1988), Cordier et al. (1993), Gonzalez et al. (1989), Hours et al. (1994), Jensen et al. (1987), Pesch et al. (2000a, b), Porru et al. (1996), Rebelakos et al. (1985), Serra et al. (2000), and Vineis and Magnani (1985). These case-control studies were published between 1976 and 1996 and included detailed information on occupation as well as smoking. Cases and controls needed to fall within the 30-to-79-year age range. Cases whose interview occurred more than 2 years after diagnosis were also excluded. Of the 4,101 cases in the pooled dataset, 3,346 (81.6%) met these criteria and were included in the analysis. Of the 7,365 controls in the pooled dataset, 6,840 (92.9%) were included in the analysis. Three of the pooled studies used population controls; one used both hospital and population controls; the remaining seven used hospital controls only. Cases and

controls were matched on 5-year age group and geographic area. All occupational and industrial information were coded according to ISCO-68 and International Standard Industrial Classification of All Economic Activities (ISIC) rev2 standards, respectively. Launderers, dry cleaners, and pressers fell within ISCO Code 56. A total of 19 (0.6%) cases and 30 (0.4%) controls were launderers, dry cleaners, or pressers. The researchers did not consider this occupation to be at high risk *a priori*, though it was identified in other studies to be at high risk.

Unconditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs, adjusted for 5-year age group, smoking, and study center. The interaction between age and study center was found to be significant and was also included in all of the models. Attributable risk for those occupations identified as high risk *a priori* was also calculated, though this did not include launderers, dry cleaners, and pressers. A strength of the study is its pooled nature, which allowed for a high power and the ability to determine whether risks are similar in different populations. A limitation is the low-exposure prevalence among both cases and controls.

B.2.2.1.6. Reulen et al. (2007)

Reulen, R.; Kellen, E.; Buntinx, F.; Zeegers, M. (2007). Bladder cancer and occupation: A report from the Belgian case-control study on bladder cancer risk. Am J Ind Med, 50, 449-454. http://dx.doi.org/10.1002/ajim.20469

Summary: This population-based case control study aimed to add to the data on associations between occupation and bladder cancer, thereby strengthening the case for focused research on specific occupational categories. Cases were identified through the Limburg Cancer Registry and consisted of men and women aged 40 to 96 years who were diagnosed with histologically confirmed transitional cell carcinoma of the bladder between 1996 and 2004. Controls were randomly selected from the general population of Limburg through simple random sampling and consisted of Caucasian men and women over the age of 50 years, with no previous history of bladder cancer. The exclusion of individuals less than 50 years of age was due to the researchers' finding that the majority of controls were over 50 years. Of the 2,230 eligible cases, 202 (9.1%) participated in the study. The response rate among controls was 26% and included 390 participants.

In-person interviews were conducted by three trained interviewers in the participants' homes using a structured questionnaire. Information was obtained on socio-demographics, lifetime smoking history, and lifetime occupational history of all jobs held for at least 6 months. Lifetime occupational history was assessed as a proxy for exposure, and all occupations were blindly coded according to the International Standard Classification of Occupations. Domestic helpers, cleaners, and launderers comprised Code 913 and included a total of 14 (6.9%) cases

and 20 (5.1%) controls. Unconditional logistic regression was used to calculate odds ratios and their corresponding 95% CIs, adjusted for age, sex, current smoking status, years of cigarette smoking, number of cigarettes smoked per day, and education. An interaction of sex and occupation was also included in the model. Only those occupations with 15 or more participants were reported. The authors do not report strengths of their study methodology. Limitations include the study's small sample size, recall bias among cancer cases, and the low-response rate among both cases and controls.

B.2.2.1.7. Schoenberg et al. (1984)

Schoenberg, J. B.; Stemhagen, A.; Mogielnicki, A. P.; Altman, R.; Abe, T.; Mason, T. J. (1984). Case-control study of bladder cancer in New Jersey. I. Occupational exposures in white males. J Natl Cancer Inst, 72, 973-981. http://www.ncbi.nlm.nih.gov/pubmed/6585596

Summary: This population case-control study of New Jersey male residents, 21−84 years of age, examined bladder cancer, including papilloma not specified as benign, and occupation. Newly diagnosed incident bladder cancer cases were identified between 1978−1979 using a mechanism whereby incident cases were reported within 72 hours of diagnosis or by searching hospital pathology records (hospital number and local hospitals not identified in published paper). No overlap occurs between this study and the large National Bladder Cancer Study, which also included cases diagnosed between 1977 and 1978 (Silverman et al., 1990; Silverman et al., 1989a; Silverman et al., 1989b; Smith et al., 1985). Age-stratified random samples of male population controls were identified using random digit dialing, if 21−64 years old or, records of the Health Care Financing Administration, if 65−84 years. Controls were not frequency matched by county. To allow for potential county-specific comparison, additional controls were identified, employed, and stratified by county so that the case-to-control ratio for each age-county group would be at least 1:1. Of the 787 male cases and 1,608 controls meeting the case or control definition, 706 cases (90%) and 1,392 controls (87%) were interviewed; all cases and controls were alive at the time of the interview. Few subjects were non-Caucasian, and analyses were restricted to Caucasian males, 658 cases, and 1,258 controls. Face-to-face interviews were carried out using a structured questionnaire that sought information on demographic, personal, and occupational risk factors. Information on all jobs held ≥6 months was ascertained, and subjects were shown lists of industries, employers, and materials to elicit information not initially recalled. All industry and job title information was coded to the 1970 Census Index System and based upon these codes; 19 employment categories were identified *a priori* as known or suspected occupations or exposures; employment as dry-cleaning workers was one of the 19 categories. Few cases and controls were identified with employment as a dry-

clean worker: 7 cases (1.1%) and 10 controls (0.8%), which limited the statistical power of this study to examine bladder cancer and dry-cleaning employment.

Odds ratios and 95% confidence limits were calculated using logistic regression with a model including either 19 exposure terms or, as in the case of employment as a dry-cleaning worker, a term for the specific exposure category. Statistical analyses were adjusted for age and duration of cigarette smoking, placed into four categories. Other covariates, such as previous bladder or kidney infection, family history of urinary tract cancer, coffee consumption, education, and use of artificial sweeteners did not change the odds ratio estimate by more than 10% and, therefore, were not included in the final logistic regression model.

B.2.2.1.8. Smith et al. (1985), Silverman et al. (1990; 1989a; 1989b)

Smith, E. M.; Miller, E. R.; Woolson, R. F.; Brown, C. K. (1985). Bladder cancer risk among laundry workers, dry cleaners, and others in chemically-related occupations. J Occup Med, 27, 295-297. http://www.ncbi.nlm.nih.gov/pubmed/3998883

Silverman, D. T.; Levin, L. I.; Hoover, R. N.; Hartge, P. (1989a). Occupational risks of bladder cancer in the United States: I. White men. J Natl Cancer Inst, 81, 1472-1480. http://dx.doi.org/10.1093/jnci/81.19.1472

Silverman, D. T.; Levin, L. I.; Hoover, R. N. (1989b). Occupational risks of bladder cancer in the United States: II. Nonwhite men. J Natl Cancer Inst, 81, 1480-1483. http://dx.doi.org/10.1093/jnci/81.19.1480

Silverman, D. T.; Levin, L. I.; Hoover, R. N. (1990). Occupational risks of bladder cancer among white women in the United States. Am J Epidemiol, 132, 453-461. http://www.ncbi.nlm.nih.gov/pubmed/2389750

Summary: These studies used data from the National Bladder Cancer Study (Hartge et al., 1984), which was a large case-control study researching the relationship between occupation and bladder cancer. Cases consisted of men aged 21 to 84 years who were diagnosed with histologically confirmed urinary bladder cancer between 1977 and 1978 in 9 Surveillance, Epidemiology, and End Results (SEER) reporting locations (Connecticut, Iowa, New Mexico, Utah, Atlanta, Detroit, New Orleans, San Francisco, and Seattle) and one rapid reporting system for bladder cancer, which was mandated by state law (New Jersey). Controls were randomly selected from within each of the 10 geographical areas and matched to cases based on 5-year age group and sex. Control selection occurred in two ways: men aged 21 to 64 years were randomly digit dialed, and men aged 65 years and older were obtained from a stratified random sample of Health Care Finance Administration lists. The random digit dialing telephone screening yielded an 88% response rate, and the home interview response rates were 73% for cases and 83% for controls (Hartge et al., 1984). In-person interviews were conducted by a trained interviewer

within 3 months of diagnosis, reducing the need for proxy interviews. All interviews used a structured questionnaire that inquired about artificial sweeteners, smoking, coffee consumption, medical history, and occupational history for all jobs that lasted at least 6 months from 12 years of age onwards. Job histories were coded according to the U.S. Bureau of the Census Index of Industries and Occupations.

Smith et al. (1985) examined bladder cancer risk among individuals employed as laundry workers and dry cleaners and in other occupations and industries with similar chemical exposures and compared it with that of workers in occupations or industries that did not expose them to these chemicals. The authors did not report the final number of cases and controls included in the study. Participants were classified into one of three exposure categories: (1) exposed through employment as laundry/dry-cleaning operatives for at least 6 months (103 participants); (2) exposed through chemicals encountered in other occupations or industries (5,776 participants); and (3) unexposed (1,869 participants). Duration of exposure among those in the laundry/dry cleaning occupation was calculated as the total number of years employed in that profession. Logistic regression was used to separately calculate the relative risks of occupational exposure, adjusted for age and sex, and duration of exposure by age, sex, and smoking status. One strength of this study is its large, population-based design with in-person interviews and matched cases and controls. A limitation is its small exposed population.

Silverman et al. (1989a; 1989b) examined high risk occupations for bladder cancer among and Caucasian and non-Caucasian men. The final sample consisted of 2,100 Caucasian male cases, 126 non-Caucasian male cases, 3,874 male controls, and 383 non-Caucasian male controls. Cases and controls were similar on occupational history variables, with the exception of age at first employment where cases were younger than controls. Occupations were subsequently grouped by their potential to have similar exposures, which aggregated 417 census codes into 163 categories. Workers involved in "processing" within an industry were also grouped together in one category within that industry. Dry cleaners, ironers, and pressers were examined as miscellaneous *a priori* suspect occupations and contained 11 (8.7%) non-Caucasian cases and 12 (3.1%) non-Caucasian controls. Exposure prevalence for occupation as dry cleaners, ironers, and pressers is not presented in the published papers for Caucasian cases and controls. The maximum likelihood method was used to estimate odds ratios for occupations. The estimate for the dry cleaner, ironer, and presser occupation was adjusted for smoking and employment in other high risk occupations. The estimates' corresponding 95% CIs were calculated using Gart's interval estimation procedure. Maximum likelihood was also used to estimate odds ratios for duration of exposure as a dry cleaner, ironer, or presser (<5 years, ≥ 5 years), adjusted for smoking and age, and a Mantel-Haenszel procedure was used to evaluate one-tailed significance tests of trend. Finally, population attributable risks and their

corresponding 95% CIs were calculated according to Whittemore (1983) and adjusted for age, geographic area, and smoking. Due to the large number of analyses performed, only those occupations for which there were a minimum of 15 exposed cases or controls and who met one of three additional criteria (statistically significant risk, *a priori* category, or summary category) were presented. The authors did not note any strengths of their methodology. A limitation of the methodology is the potential for misclassification of exposure when grouping occupations for the purposes of analysis.

B.2.2.1.9. Steineck et al. (1990)

Steineck, G.; Plato, N.; Gerhardsson, M.; Norell, S. E.; Hogstedt, C. (1990). Increased risk of urothelial cancer in Stockholm during 1985-87 after exposure to benzene and exhausts. Int J Cancer, 45, 1012-1017. http://dx.doi.org/10.1002/ijc.2910450605

Summary: This population case-controls study of males residing in the county of Stockholm 1985−1987 and born between 1911 and 1945 and population controls examined occupational exposures and urothelial cancer. The source for identifying cases is not identified in the published paper. Population controls were identified using random sampling of population registers at four periods during case ascertainment. A total of 320 cases and 363 controls were identified of which 256 cases and 287 controls were alive and completed the interview; participation rates were 80% for cases and 79% for controls. Of the 256 cases, 243 were of the urinary bladder, 5 of the renal pelvis, 5 of the ureter, and 3 of multiple sites. An additional two cases had heavy exposure to aromatic amines and were excluded from the case series.

Occupational history was sought from case and control using a questionnaire with an industrial hygienist blinded to case and control status classifying potential exposure to 38 agents or groups of substances, including 17 categories of aromatic amines. Two cases and two controls reported employment as a dry cleaner or in the dry-cleaning industry, with an exposure prevalence of <1% for either cases or controls. The published paper does not discuss other information obtained from the questionnaire, except smoking, for which a subject was categorized as either a current smoker, former smoker, or never smoker. Some residual confounding is likely given the use of these broad categories rather than pack years. Logistic regression was used to estimate an odds ratio adjusted for birth year and smoking.

B.2.2.1.10. Zheng et al. (2002)

Zheng, T.; Cantor, K. P.; Zhang, Y.; Lynch, C. F. (2002). Occupation and bladder cancer: A population-based, case-control study in Iowa. J Occup Environ Med, 44, 685-691. http://www.ncbi.nlm.nih.gov/pubmed/12134533

Summary: This population case-control study used data from a larger study of drinking water by-products to examine the relationship between occupation and bladder cancer. Cases and controls were identified in two phases. In Phase 1, cases consisted of male Iowa residents without a previous diagnosis of neoplasm, aged 40 to 85 years, and who were diagnosed with one of six cancer sites (brain, kidney, pancreas, colon, rectum, and urinary bladder) between 1986 and 1987. Controls were randomly selected from the Iowa residents, and frequency was matched to all cases based on 5-year age group and sex. Control selection occurred in two ways: (1) men aged 64 aged years or younger were randomly digit dialed, and (2) men aged 65 years and older were obtained from a stratified random sample of Health Care Finance Administration lists. The control matching frequency to bladder cancer case series was ~2.3:1. Phase 2 of case and control ascertainment occurred between 1988 and 1989. Cases, aged 40 to 85 years, with in situ and invasive bladder cancer (transitional cell carcinoma and papillary transitional cell carcinoma) were identified among Iowa residents between 1998−1989 with controls frequency matched to cases at a ratio of 1:1. The random digit dialing telephone screening yielded an 85% response rate for cases (1,452): 82% for controls younger than 65 years, and 80% for controls ≥65 years. A total of 1,452 case (1,135 men, 317 women) and 2,434 control (1,601 men, 833 women) participated in the study.

In-person interviews were conducted by a trained interviewer within 3 months of diagnosis, reducing the need for proxy interviews. All interviews used a structured questionnaire that inquired about artificial sweeteners, smoking, coffee consumption, medical history and occupational history for all jobs held for 5 years or longer from 16 years of age onwards. Proxies completed the questionnaires for 156 cases who had died or were not competent to participate. All controls except two completed questionnaires in person. Job titles and industries were reported by Standard Industry Classification and SOC Manual schemes using two-, three-, and four-digit codes. The SOC code for occupation in laundering and dry cleaning was 7,658. Zheng et al. (2002) reported three female cases, and one female control held an occupation in dry cleaning and laundering; however, these authors did not report the number of male cases or controls.

Odds ratios and 95% CIs were calculated using unconditional logistic regression adjusted for age, lifetime pack-years of cigarette smoking, and having a first-degree relative with bladder cancer. Other variables such as education, frequency of strenuous or moderate exercise, duration of living in a residence served by chlorinated surface water, population size of places of

residence, and other cancer in a first-degree relative were also examined in the statistical analysis but did not result in material change to the observed association and were, therefore, not included in the final logistic regression model. Duration of exposure was examined using a dichotomous grouping of <10 years and ≥10 years employment duration.

Certain characteristics of this study strengthen the interpretation and included use of histologically confirmed cases, use of lifetime job-exposure history, and relatively high-response rates from both cases and controls. On the other hand, observations for dry cleaning and laundering occupation are based on small numbers, limiting the study's sensitivity. Additionally, exposure misclassification may have been introduced because the study did not specifically identify tetrachloroethylene exposure intensity for individual subjects and is likely of a nondifferential direction, which would be expected to attenuate the strength of estimated risks. Additionally, use of employment duration is a crude surrogate for cumulative exposure, particularly in light of any temporal changes in intensity.

B.2.2.2. Brain Cancer

B.2.2.2.1. Heineman et al. (1994)

Heineman, E. F.; Cocco, P.; Gomez, M. R.; Dosemeci, M.; Stewart, P. A.; Hayes, R. B., . . . Blair, A. (1994). Occupational exposure to chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. Am J Ind Med, 26, 155-169. http://dx.doi.org/10.1002/ajim.4700260203

Summary: This case-control study explored the potential association of brain cancer with specific solvents, including tetrachloroethylene. Cases consisted of Caucasian men who had died of brain or other central nervous system (CNS) tumors between 1978 and 1980 in Louisiana and between 1979 and 1980 in New Jersey and Pennsylvania. Controls were Caucasian men who had died of other causes, excluding cerebrovascular diseases, epilepsy, suicide, and homicide. Controls were matched to cases based on age, year of death, and study area. Both cases and controls were obtained from death certificates. Of the 741 cases and 741 controls selected, next of kin were found for 654 (88%) cases and 612 (83%) controls. Of these, proxy interviews were performed for 483 cases (74% of those contacted) and 386 (63% of those contacted) controls. After excluding cases for which a hospital diagnosis was not reported and controls whose death may have been associated with their occupation (e.g., lung cancer, liver cancer, leukemia, etc.), the final sample included 300 cases and 320 controls.

Blinded, trained interviewers conducted interviews with next of kin regarding possible risk factors for brain cancer as well as all occupations held by the case or control since the age of 15 years. Information collected included job title, tasks, name and location of the company, type of industry, kinds of products, employment dates, and hours worked. A job exposure matrix

(Gómez et al., 1994) was used to estimate exposures based on reported occupations and industries. Occupations and industries were coded according to four digit U.S. SIC and SOC codes, respectively. All of the four digit codes were assigned exposure estimates of probability (i.e., low, medium, high) and intensity (i.e, 1, 2, 3) *a priori*. Intensity was defined as an average of the concentration and frequency of exposure. Occupations were also assigned a category. Jobs that fell within Category A, such as dry cleaner operators, had sufficient information to be assessed for exposure, independent of their industry. For jobs that fell within Category B, the probability of exposure depended entirely on the industry, and the intensity was weighted by both the occupation and the industry. Those in Category C had their probability and intensity of exposure fully determined by the industry within which the job fell. Time of employment was accounted for in the matrix through a decade indicator. There were 111 (37%) cases and 106 (33.1%) controls "ever" exposed to tetrachloroethylene.

The analysis included maximum likelihood estimates of odds ratios and 95% CIs using Gart (1970), adjusted for age and study area. Linear trends were examined using Mantel (1963), and logistic regression was performed to estimate odds ratios and their corresponding 95% CIs, controlling for age, study area, and employment in electronics-related occupations or industries. A lag time of 10 or 20 years was included. A strength of the study is its blinded exposure classification. Limitations include possible misclassification due to inaccurate reports from proxy respondents, although cases and controls were dead, minimizing potential differential reporting between cases and controls by proxy respondents, misclassification of exposure due to the interchangeability of some solvents, and a high proportion of nonrespondents.

B.2.2.3. Breast Cancer

B.2.2.3.1. Peplonska et al. (2007)

Peplonska, B.; Stewart, P.; Szeszenia-Dabrowska, N.; Rusiecki, J.; Garcia-Closas, M.; Lissowska, J., . . . Blair, A. (2007). Occupation and breast cancer risk in Polish women: A population-based case-control study. Am J Ind Med, 50, 97-111. http://dx.doi.org/10.1002/ajim.20420

Summary: This study used data from a large case-control study in Poland to evaluate the risk of breast cancer by occupation and industry. Cases were identified through a rapid case ascertainment system organized by participating hospitals and were newly diagnosed histologically confirmed in situ or invasive breast cancers in female residents of Warsaw and Lódź, between 20−74 years of age, diagnosed 2000−2003. Population controls were identified from the Polish Electronic System of Population Evidence and matched to cases by city of residence and age within 5-year age groups.

A structured questionnaire administered using in-person interviews collected data on demographic, reproductive, and menstrual history; hormone use history; physical activity; occupation history; smoking and alcohol use; diet; cancer history in female relatives; medical and screening history; prenatal exposures; and history of weight and height development. With respect to occupation history, all jobs held at least 6 months, including job title and possible exposure to a list of chemicals potentially associated with breast cancer, were obtained, and industry and occupation codes were assigned according to the SIC Manual and SOC Manual. Of the 2,275 cases (79% response rate) and 2,424 controls (66% response rate) completing the questionnaire, 28 cases and 32 controls were identified as working in the laundry, cleaning, and garment services industry; exposure prevalence was 1% for cases and 1% for controls. Peplonska et al. (2007) does not report the percentage of subjects with proxy interviews.

Unconditional logistic regression analyses were used to estimate odds ratio and 95% CIs as the measure of association between occupation or industry and breast cancer risk. Multivariate models included adjustment for age, age at menarche (≤12, 13−14, ≥15, missing), menopausal status (premenopausal; postmenopausal), age at menopause among postmenopausal women (<45, 45−54, ≥55, missing), number of full-term births (≤1, 2, ≥3), body mass index (25, 25−30, >30), breast cancer in first-degree relative (yes, no), education (less than high school, high school, some college, professional training, college degree, missing), and city of residence. The influence of oral contraceptive use, marital status, tobacco and alcohol use, age at first full-term birth and breastfeeding, and recreational and occupational physical activity was also evaluated; but, these factors had little impact on risk estimates and were not included in the final models. Additionally, each specific white-collar job, using all other white-collar jobs as the reference group, was analyzed to control for socioeconomic factors that could not be completely captured by adjustment for education level.

Methodological strength of this study includes the size of the studied population and the scope of information on lifetime occupational history that was collected, together with comprehensive data on potential confounders, and effect modifiers including pre- and postmenopausal status. Potential limitations are the small numbers for many occupational groups, multiple jobs, and multiple comparisons.

B.2.2.4. Colon Cancer

B.2.2.4.1. Fredriksson et al. (1989)

Fredriksson, M.; Bengtsson, N. O.; Hardell, L.; Axelson, O. (1989). Colon cancer, physical activity, and occupational exposures: A case-control study. Cancer, 63, 1838-1842. http://www.ncbi.nlm.nih.gov/pubmed/2702592

Summary: This case-control study examined the relationship between occupational exposures and colon cancer in Sweden. Cases consisted of men and women between 30 and 75 years of age who were diagnosed with large bowel cancer adenocarcinoma between 1980 and 1983. Cases were obtained from the Swedish Cancer Registry and needed to be living at the time of the study (1984 to 1986), located within the admissions region of the Department of Oncology in Umeå, and medically able to complete a mailed questionnaire. Controls were selected from the National Population Register, and two controls were matched to each case based on county of residence, sex, and age. Controls were required to be living at the time of the study and medically able to complete the questionnaire. Of the 402 cases and 717 controls identified, 329 cases and 658 controls met inclusion criteria and were contacted with a mailed questionnaire inquiring about occupational histories, occupational exposures, food and drinking habits, previous diseases, and drug intake. Overall, 312 (94.8%) cases and 623 (94.6%) controls participated in the study.

Occupations were assessed as a proxy for exposure by two physicians and one hygienist, who independently classified exposure as either high or low grade. There were 5 (1.6%) female cases and 5 (0.8%) female controls who reported employment in dry cleaning. The authors did not report any dry cleaning information for men. Mantel-Haenszel methods were used to calculate odds ratios, and Miettinen (1976) was used in the estimation of corresponding 95% CIs. These analyses were performed for all occupations, including dry cleaning, stratified by age and physical activity. The authors note that an advantage to limiting their study to living patients only was the lack of information bias due to proxy responders. Although this is a methodological strength, there is a potential bias created if occupational exposure is associated with survival.

B.2.2.5. Liver Carcinoma

B.2.2.5.1. Austin et al. (1987)

Austin, H.; Delzell, E.; Grufferman, S.; Levine, R.; Morrison, A. S.; Stolley, P. D.; Cole, P. (1987). Case-control study of hepatocellular carcinoma, occupation, and chemical exposures. J Occup Med, 29, 665-669. http://www.ncbi.nlm.nih.gov/pubmed/2821204

Summary: This case-control study studied the relationship between hepatocellular carcinoma and occupational factors and chemical exposures encountered at work or in leisure activities. Cases consisted of men and women aged 18 to 84 years, diagnosed with hepatocellular carcinoma at 5 study centers, including the University of Alabama, Duke University, University of Miami, University of Pennsylvania, and the Harvard School of Public Health. The majority (93.0%) of cases were histologically confirmed, and the remainder were

clinically confirmed. Controls consisted of patients admitted to the same hospitals for other conditions that were diagnosed within 3 years of the interview, excluding bronchitis, emphysema, primary liver disease, and the following cancers: lung, oral cavity, esophagus, larynx, bladder, and pancreas. Controls were matched to cases based on gender, age, race, and study center. The final sample consisted of 86 cases and 161 controls. Authors do not report response rates.

Each participant's occupational history related to all jobs held 6 months or longer was ascertained during the interview, and jobs were coded according to SIC and SOC manuals. There were 0 cases and 4 (2.5%) controls who reported employment in the laundering and cleaning industry. The authors did not report this industry's corresponding code. Conditional likelihood methods were used in logistic regression models to estimate odds ratios and their corresponding 95% CIs. Due to the small numbers of "exposed," the authors did not present the results for the laundry and cleaning industry. The authors do not report strengths of their study. A limitation is the small number of exposed, which precluded the analysis of participants employed in the laundry and dry-cleaning industry.

B.2.2.5.2. Hernberg et al. (1988)

Hernberg, S.; Kauppinen, T.; Riala, R.; Korkala, M. L.; Asikainen, U. (1988). Increased risk for primary liver cancer among women exposed to solvents. Scand J Work Environ Health, 14, 356-365. http://www.ncbi.nlm.nih.gov/pubmed/3212412

Summary: This case-control study examined if previously reported findings of an increased risk of primary liver cancer among women exposed to organic solvents (Hernberg et al., 1984) were a true effect, due to chance, or reflective of an undetected systematic error. Cases consisted of men and women diagnosed with primary liver cancer and reported to the Finnish Cancer Register from 1976 to 1978, and also in 1981. The years 1979 and 1980 were excluded from this study because they were previously examined (Hernberg et al., 1984). Two control groups were used in this study: a control series of randomly selected stomach cancer patients identified from the Finnish Cancer Register in 1977; the other included patients whose hospital autopsy records noted that they had died of a coronary infarction in 1977. Coronary infarction controls were matched to cases on sex, age, and hospital of diagnosis. The authors make no mention of matching between cases and stomach cancer controls. All living patients were excluded from the analyses, as were those with untraceable relatives. Of the 526 cases by proxy who met inclusion criteria, 377 (71.7%) returned the questionnaire. After excluding those for whom a diagnosis could not be confirmed, a total of 344 (65.4%) were included in the analysis. Of the 654 stomach cancer controls and 558 coronary infarction controls who met the inclusion criteria, 476 (72.8%) stomach cancer controls and 385 (69.0%) coronary infarction controls

returned the questionnaire. The final sample consisted of 344 cases and 861 controls (476 stomach cancer and 385 coronary infarction).

A questionnaire mailed to proxy-respondents focused on obtaining information on work history, including employers, work sites, jobs held, and calendar years of work. Information on alcohol, tobacco, coffee, tea, medicines, leisure activities, and for women, history of oral contraceptive use, was also obtained. Two occupational hygienists blindly assessed exposure, based on the likelihood of the participants' industries, workplaces, and job titles, including solvents or other agents. Exposures were classified as heavy, moderate, or light; dry-cleaning exposures were based on 1950 records by the Finnish Institute of Occupational Health that noted tetrachloroethylene exposure ranged from 34−600 ppm during that time. Any exposures that could not be determined by the occupational hygienists were followed up with phone calls to the workplace or the proxy respondent. Two cases (0.6%) were identified with possible chlorinated hydrocarbon exposures: (1) a case assessed as having light, possible exposure to chlorinated hydrocarbons in a laundry facility, and (2) another case estimated to have heavy exposure to chlorinated hydrocarbons during 6 years employed as a dry cleaner. Two coronary infarction controls (0.5%) were determined to have light exposure to tetrachloroethylene as a result of employment in the dry-cleaning industry.

Likelihood-based odds ratios and 90% CIs were calculated according to Cornfield (1956) for the association between primary liver cancer and solvent exposure and for the association between primary liver cancer and heavy/moderate alcohol use. Both were stratified by sex using methods by Gart (1970). A latency period of 10 years was included, and, thus, any exposures that occurred before this time were excluded from the analysis. A strength of the study is its use of a blinded exposure assessment. Limitations to the study include the potential for selection bias due to the number of eligible cases and controls whose proxy respondents could not be found or whose proxy respondents did not return the questionnaire. Moreover, misclassification bias is likely, given the high percentage of proxy respondents. The authors also noted the need for information on previous hepatitis B infection in order to control for it as a potential confounder.

B.2.2.5.3. Houten and Sonnesso (1980)

Houten, L. and Sonnesso, G. (1980). Occupational exposure and cancer of the liver. Arch Environ Health, 35, 51-53. http://www.ncbi.nlm.nih.gov/pubmed/7362270

Summary: This study used a hospital-based case-control design to study the occupational associations of patients admitted to Roswell Park Memorial Institute. The 102 cases were men and women with primary liver cancer between 1956 and 1965. Controls consisted of all other cancer patients admitted to the Roswell Park Memorial Institute during the same time frame.

The authors failed to mention how many controls were included in the study. Occupation was assessed as a proxy for exposure, with a total of two cases (2%) employed in the laundry and dry-cleaning industry. The analysis consisted of a χ^2 goodness-of-fit test, where the distribution of the cases was compared to controls by each industry. Limitations to the study include the size of the sample; few exposed cases, which decreased the study's detection sensitivity; the use of other cancer patients as controls; self-reported occupational information; and inadequate reporting of study design and results.

B.2.2.5.4. Stemhagen et al. (1983)

Stemhagen, A.; Slade, J.; Altman, R.; Bill, J. (1983). Occupational risk factors and liver cancer: A retrospective case-control study of primary liver cancer in New Jersey. Am J Epidemiol, 117, 443-454.
http://www.ncbi.nlm.nih.gov/pubmed/6837558

Summary: This study used a case-control design to examine occupational associations with liver cancer. Cases were identified through New Jersey hospital records, the New Jersey State Cancer Registry, and death certificates and consisted of men and women living in New Jersey who were diagnosed with histologically confirmed primary liver cancer between 1975 and 1980. The authors do not note any age restrictions in their methodology, though cases were aged 20 years and older. Controls were chosen from among men and women admitted to the same hospitals as the cases, as well as from death certificates, and matched to cases on age, race, sex, county of residence, and vital status. Potential controls were excluded from the study if they had a history of liver cancer, hepatitis, cirrhosis, or other liver disease. Deceased controls whose cause of death was homicide or suicide were also excluded from the study because of the sensitivity of approaching next of kin. Of the 335 eligible cases, 296 were able to be contacted, and of these, 265 (79.1%) were interviewed. Of the 825 eligible controls, 687 were able to be contacted, and of these, 530 (64.2%) were interviewed. Demographics between cases and controls were similar.

In-person interviews were conducted with all participants or their next of kin to obtain information on lifetime residence, smoking habits, alcohol, medical history, and employment since the age of 12 years. There were 254 (95.8%) proxy case interviews and 508 (95.8%) proxy control interviews. Occupations held for at least 6 months were assessed as a proxy for exposure. All industries and occupations were coded according to the Index of Industries and Occupations standards developed by the Bureau of Census. The laundering, cleaning, and other garment services industry included 10 male cases (3.8%) and 8 male controls (1.5%). The authors further examined the laundry/dry-cleaning industry by occupations, though the results

are not presented. No information was reported on female employment in the laundry, dry cleaning, or garment service industry.

Mantel-Haenszel methods were used to estimate odds ratios and 95% CIs for males employed at least 6 months in selected industries and occupations. The distribution of subjects by calculated level of alcohol consumption were adjusted for age (women) and age and smoking (men), but risk estimates for occupations and industries were not adjusted for potential confounders. There were no differences in alcohol consumption between cases and controls. The authors did not report any strengths in their study. Limitations include possible misclassification of exposure due to proxy respondents, lack of adjustment for confounders such as smoking and alcohol consumption, possible misclassification due to inaccurate information on death certificates, and lack of assessment of intensity or duration of exposure.

B.2.2.5.5. Suarez et al. (1989)

Suarez, L.; Weiss, N. S.; Martin, J. (1989). Primary liver cancer death and occupation in Texas. Am J Ind Med, 15, 167-175. http://www.ncbi.nlm.nih.gov/pubmed/2729281

Summary: This case-control study examined the risk of liver cancer among occupations in the petroleum and chemical industry and other potentially high-risk occupations. Death certificates, which listed usual occupation and business or industry, were obtained from the Texas Bureau of Vital Statistics and used to identify cases and controls for the time period from 1969 to 1980. Cases consisted of men aged 20 years or older who were living in Texas and whose underlying cause of death was liver cancer. Of the 1,771 potential cases, 1,742 were eligible and included in the study. The same number of controls were randomly selected from among the 537,000 death certificates, which represented all other causes of death, excluding neoplasms, liver and gallbladder diseases, infectious hepatitis, and alcoholism. Controls were matched to cases based on 5-year age group, race, ethnicity, and year of death.

Occupation was assessed as a proxy for exposure grouped according to the U.S. Census Classified Index on industrial categories. Groupings were partially based on Hoar et al. (1980), who categorized industries by product or exposure. In addition to the petrochemical industry, 22 other industries or product categories with at least 10 individuals were examined, including dry-cleaning services. Occupations within these categories that had at least 10 individuals were also analyzed and included dry-cleaning operators. There were a total of 11 cases and 12 controls employed in the dry-cleaning industry and 4 cases and 8 controls employed as dry-cleaning operators. The published paper does not provide information regarding the total number of controls included in the final sample (although they state that the number of controls and cases are the same), precluding a calculation of exposure prevalence for this study.

The Mantel-Haenszel method was used to calculate odds ratios, adjusted for race and ethnicity. Corresponding 95% CIs were estimated using Miettinen's method. Limitations to the study include the lack of control for potential confounders that were not included in the death certificate information, such as alcohol consumption or hepatitis B infection, as well as the lack of information on exposure and the possible misclassification of exposure based on occupation and industry information provided on the death certificates. The authors do not report any strengths of their study's methodology.

B.2.2.6. Lung and Upper Respiratory Tract Cancers

B.2.2.6.1. Brownson et al. (1993)

Brownson, R. C.; Alavanja, M. C.; Chang, J. C. (1993). Occupational risk factors for lung cancer among nonsmoking women: A case-control study in Missouri (United States). Cancer Causes Control, 4, 449-454.
http://www.ncbi.nlm.nih.gov/pubmed/8218877

Summary: This study used a population-based case-control design to evaluate the risk of lung cancer in nonsmokers in relation to their specific occupations. Cases were Caucasian females living in Missouri between 30 and 84 years and diagnosed with primary lung cancer between 1986 and 1991. Cases needed to be either lifetime nonsmokers, ex-smokers who had quit for at least 15 years prior to diagnosis, or ex-smokers that had smoked less than one pack per year. The cases were selected from the Missouri Cancer Registry; hospitals participating in the study were also visited to ensure all cases were documented. Of the 429 cases included in the study, 333 (77%) were histologically confirmed. The 1,021 controls were chosen in two ways: (1) through a sample of state driver's licenses of women under the age of 65 years, provided by the Missouri Department of Revenue; and (2) through a roster of Medicare beneficiaries of women aged 65 to 84 years, provided by the Health Care Finance Administration. Controls were matched to the cases by age group at a 2.2:1 ratio. Of the 650 eligible cases, 618 (95%) participated in the telephone interview, and 429 (69%) of these 618 also participated in the second, in-person interview. Of the 429 cases included in the final analysis, 179 (42%) consisted of interviews with the cases, and 250 (58%) involved interviews with the spouse or another relative. Of the 1,527 eligible controls, 1,402 (92%) participated in the telephone interview, and 1,021 (73%) of these 1,402 also participated in the second, in-person interview. Overall, 30 of the cases and 39 of the controls were employed in the dry-cleaning industry.

Both the telephone and in-person interviews were performed by trained interviewers. The telephone interview inquired about residential history, passive smoke exposure, personal and family health histories, and reproductive health history. The in-person interview consisted of questions related to diet and occupation. Occupational risk factors were determined by

28 questions, which were based on a review of the literature and focused on job title as well as exposure. Subjects reported the years in which they worked at each job or with each exposure. Analysis consisted of the calculation of odds ratios and 95% CIs with multiple logistic regression, adjusted for age, active smoking (for ex-smokers), and history of previous lung disease. In their examination of risk based on duration of employment, the researchers ascertained their cutoff points by achieving an approximate equal distribution of controls in "low" and "high" exposure categories.

Strengths of the study include its large sample size and the fact that it pathologically reviewed all cases. On the other hand, the study's retrospective nature has limitations. For example, there was a substantial difference in the proportion of proxy respondents between the cases and controls. Here, 58% of the case interviews were conducted with surrogates, compared with none of the controls. The authors noted the inclusion of proxy respondents would introduce recall bias that would likely bias risk estimates towards the null. Additionally, the researchers lacked information on the intensity and specific types of occupational exposures these women experienced. Limitations include the study's low statistical power, small sample of histologically confirmed cases, difficulty in assessing passive smoking retrospectively, and that not all cases were eligible to be controls. In this study, 91% of cases under the age of 65 years and 100% of controls had a current driver's license, suggesting that the case population may have differed in some characteristics from the control population.

B.2.2.6.2. Consonni et al. (2010)

Consonni, D.; De Matteis, S.; Lubin, J. H.; Wacholder, S.; Tucker, M.; Pesatori, A. C., . . . Landi, M. T. (2010). Lung cancer and occupation in a population-based case-control study. Am J Epidemiol, 171, 323-333. http://dx.doi.org/10.1093/aje/kwp391

Summary: This large population case-control study, part of the Environment And Genetics in Lung cancer Etiology (EAGLE), was designed to explore various etiologic factors for lung cancer risk factors using an integrative approach that combined epidemiologic, clinical, and molecular data in a clearly defined population setting. Cases and controls were identified from the Lombardy area in Italy and were from 5 cities and 216 municipalities. The study included 1,943 incident lung cancer cases, 35−79 years of age, from 2002−2005, identified from 13 hospitals and 2,116 population controls through population databases (not identified in paper) and frequency matched to case by residence, sex, and age. Cases could have any stage of primary cancer of the trachea, bronchus, and lung as well as morphology that was verified with tissue pathology (67%), cytology (28%), or review of clinical records (5%). Response rates were 92.5% for cases and 99.8% for controls. Controls had higher education and held more jobs compared to cases. All subjects underwent a computer-assisted personal interview and blood

sampling (or buccal rinse collection for a small percentage of study subjects), and they completed self-administered questions available on the EAGLE Web site. Lung tissue sample from cases were collected when available. The interview included lifetime history of jobs held for ≥6 months. Industries and job titles were coded blindly by two of the study investigators, following the International Standard Industrial Classification of All Economic Activities and the ISCO. Codes were then translated into occupations as known (List A) or suspected (List B) lung carcinogens. Two (0.2% exposure prevalence) male cases and 3 male controls and 12 (3% exposure prevalence) female cases and 11 controls were identified as launderers, dry cleaners, or pressers (ISCO Code 560). Odds ratios and 95% CIs were calculated using unconditional logistic regression, separately by gender, with covariates for area, age, smoking pack-years, and number of jobs held. Selected analyses were repeated, adding educational level as a surrogate of socioeconomic status.

Major strengths of the study are the enrollment of incident cases, the large sample size, high participation rates, and face-to-face interviews using a structured questionnaire. Jobs are self-reported, potentially introducing bias; however, the authors noted reliability of self-reported job history is usually considered good. The blind coding of job title could introduce misclassification, a source of nondifferential bias. The low exposure prevalence of dry cleaner, laundry worker, or presser, job titles, particularly among males, in this study reduces its sensitivity.

B.2.2.6.3. Pohlabeln et al. (2000)

Pohlabeln, H.; Boffetta, P.; Ahrens, W.; Merletti, F.; Agudo, A.; Benhamou, E., . . . Jockel, K. H. (2000). Occupational risks for lung cancer among nonsmokers. Epidemiology, 11, 532-538. http://www.ncbi.nlm.nih.gov/pubmed/10955405

Summary: This study used a case-control design to investigate the relationship between occupational exposures and lung cancer in nonsmokers in Europe. A total of 12 study centers in 7 countries (France, Germany, Italy, Portugal, Spain, Sweden, UK) participated. Cases and controls up to the age of 75 years were enrolled in the study between 1988 and 1994. Controls were chosen from the community in six centers, from hospitals in five centers, and from both the community and a hospital in one center. All hospital-based controls had diseases not related to smoking. A nonsmoker was defined as an individual who has smoked less than 400 cigarettes during his/her lifetime. The final sample consisted of 650 nonsmoking cases and 1,542 nonsmoking controls. Cases and controls were similar based on sex, age, and most common histological subtype. With the exception of two centers in Germany and one center in Portugal, whose response rates were below 50%, the response rates ranged between 55% and 95%.

Demographics, diet, smoking exposure, smoking history, and occupational history were collected for each participant through an in-person interview. Industry and occupation were blindly assessed as a proxy for exposure and coded according to ISCO and ISIC standards. All jobs that lasted at least 6 months were assessed according to Ahrens and Merletti (1998), who categorized occupations based on either known (List A) or suspected (List B) associations with lung cancer. Launderers and dry cleaners were classified into List B and included 20 (3.1%) cases and 29 (1.9%) controls. Participants were then divided into one of three exposures: "ever" List A, "ever" List B/"never" List A, and "never" List A or B (unexposed). Unconditional logistic regression was used to calculate odds ratios and their corresponding 95% CIs of "ever" working in a List A or List B occupation, adjusted for age and center and stratified by gender. The inclusion of occasional smoking, residence, diet, and exposure to tobacco smoking as confounders did not significantly affect the estimates and was not included in the final model. No differences were found when considering the control groups separately, and the pooled results were provided. A strength of this study is its size, and limitations include possible misclassification of smoking status among very light smokers, different response rates among the centers in Europe, and lack of assessment of duration or intensity of exposure.

B.2.2.6.4. Richiardi et al. (2004)

Richiardi, L.; Boffetta, P.; Simonato, L.; Forastiere, F.; Zambon, P.; Fortes, C., . . . Merletti, F. (2004). Occupational risk factors for lung cancer in men and women: A population-based case-control study in Italy. Cancer Causes Control, 15, 285-294. http://dx.doi.org/10.1023/B:CACO.0000024223.91059.ed

Summary: This population case-control study was conducted in two regions in North Italy, included subjects in a large international case-control study of nonsmoker lung cancer, and was coordinated under the International Agency for Research on Cancer (Pohlabeln et al., 2000). Richiardi et al. (2004) reported observations from the two Italian centers on lung cancers, adding the smoking cases and occupational factors. Cases (n = 1,171) were incident primary histologically or cytologically confirmed lung cancers among residents 75 years of age or younger and identified from all hospitals in the study area. Controls (n = 1,569) were randomly selected from the local population registries and were frequency matched ($\geq$1:1 ratio) with cases by 5-year age groups and sex. The case series included a higher proportion of ever smokers, heavy smokers, and lower education compared to the control series. The enrollment period was 1990−1991 (Eastern Venice) and 1991−1992 (Turin). Response rates for Turin and Venice regions, respectively, were 86, 72, 85, and 74% among cases and controls, respectively.

In-person interviews with a standardized questionnaire gathered information on demographic details, active and passive smoking, and lifetime occupational history for all jobs lasting at least 6 months. No information is presented by the authors regarding the number of

proxy interviews; however, the paper appears to suggest interviews were carried out directly with subjects. Job title and industry were coded blindly to case-control status using the International Standard Classification of Occupations and the International Standard Industrial Classification. The occupational history of each subject was evaluated for employment in occupations and industries *a priori* known (List A) or suspected (List B) to entail exposure to lung carcinogens; List B included dry cleaner and laundry occupations. Three male cases (0.3% exposure prevalence) and 9 female cases (5% exposure prevalence) were identified as holding dry cleaner or laundry occupation.

Odds ratios (ORs) and 95% CIs were estimated using unconditional logistic regression with analyses conducted separately for males and females for lung cancer histological types. Covariates included in the models were age, study area, education, cigarette smoking, consumption of other tobacco products, and total number of jobs.

Strengths of this study include the high-response rate and statistical control in analyses examining occupational title for smoking with any residual confounding related to smoking likely of a small magnitude. There was evidence of selection bias related to a higher socioeconomic status among nonparticipant cases and lower among nonparticipant controls compared to participant cases and controls, although the potential bias may be minimal, because education was a covariate in statistical analyses and did not substantially change risk estimates. The exposure-assessment approach based on job and industry titles is limited as a proxy for cumulative exposure with potential for misclassification bias, usually, nondifferential and of a downward direction.

B.2.2.6.5. Vaughan et al. (1997)

Vaughan, T. L.; Stewart, P. A.; Davis, S.; Thomas, D. B. (1997). Work in dry cleaning and the incidence of cancer of the oral cavity, larynx, and oesophagus. Occup Environ Med, 54, 692-695. http://www.ncbi.nlm.nih.gov/pubmed/9423585

Summary: This study used data collected from two population-based case-control studies to examine whether employment in the dry-cleaning industry and its associated exposure to tetrachloroethylene increased the risk of upper aerodigestive tract cancers. The authors do not provide any references for the studies. Cases were identified through the Fred Hutchinson Cancer Research Center, a population-based cancer registry encompassing 13 counties in Washington state, and consisted of male and female residents within the three largest counties. Cases were between 20 and 74 years of age and diagnosed with cancer of the oral cavity or pharynx, larynx, esophagus, or gastric cardia between 1983 and 1987 or with adenocarcinoma of the esophagus or gastric cardia between 1987 and 1990. The authors do not state if the cancer cases were histologically confirmed. Response rates were 85.2% for the oral cavity, 80.8% for

the larynx, and 82.9% for the esophagus and gastric cardia. Cases of nonepithelial and nonspecified cancers were excluded, as were cases without telephones on the date of their diagnosis. Controls were selected through random digit dialing and matched to cases based on 5-year age group and sex. Of those contacted, 95.4% were screened, and 80.3% of those eligible were interviewed. The final sample included 1,130 cases (491 oral cavity, 235 larynx, 109 esophagus squamous cell, and 295 esophagus adenocarcinoma), of which, 10 had two cancers, and 724 controls.

In-person interviews were conducted to gather detailed information on all occupations that lasted at least 6 months, including employer, type of business, job title, typical activities performed, and dates of employment. History of occupational exposure to solvents was also obtained. Information on demographics, and tobacco and alcohol consumption was also obtained. Proxy interviews with next of kin were conducted in 7.2% of the laryngeal cases, 18.7% of the oral and pharyngeal cases, and 33.2% of the esophageal and gastric cardia cases. Exposure to tetrachloroethylene was assessed blindly by estimating the probability that the solvent was used on the job and the 8-hour time weighted average exposure to tetrachloroethylene on the job. The latter was based on findings in the literature but was not validated within this population. Overall, 16 (1.4%) cases (7 oral cavity, 5 larynx, 2 esophagus squamous cell, and 2 esophagus adenocarcinoma) and 8 (1.1%) controls reported "ever" employment in the dry-cleaning industry. Exposure to tetrachloroethylene was determined to be possible among 15 (1.3%) cases (7 oral cavity, 4 larynx, 2 esophagus squamous cell, and 2 esophagus adenocarcinoma) and 8 (1.1%) controls. Probable exposure to tetrachloroethylene was determined for 8 cases (0.7%) and 3 controls (0.4%). Finally, duration of employment (1−9 years and ≥10 years) in the dry-cleaning industry and cumulative exposure to tetrachloroethylene (1−29 ppm/year and ≥30 ppm/year) were assessed, with the latter being the product of the duration and the 8-hour time weighted average.

Conditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs for those employed in the dry-cleaning industry and those exposed to tetrachloroethylene. All estimates were adjusted for age, sex, education, study period, alcohol consumption, and cigarette smoking. Including race among the potential confounders in the analysis did not change the estimates and was not included in the final model. A strength of the study is its detailed occupational history. Limitations included the low prevalence of exposed cases and controls, the high proportion of proxy respondents, and the lack of information on solvents used.

B.2.2.7. Lymphopoietic cancers

B.2.2.7.1. Blair et al. (1993)

Blair, A.; Linos, A.; Stewart, P. A.; Burmeister, L. F.; Gibson, R.; Everett, G., . . . Cantor, K. P. (1993). Evaluation of risks for non-Hodgkin's lymphoma by occupation and industry exposures from a case-control study. Am J Ind Med, 23, 301-312. http://dx.doi.org/10.1002/ajim.4700230207

Summary: This population-based case-control study examines occupational exposures—particularly agricultural exposure—as risk factors for non-Hodgkin lymphoma. In Iowa, cases were identified through the Iowa State Health Registry and consisted of Caucasian men who were diagnosed with non-Hodgkin lymphoma between 1981 and 1983. In Minnesota, cases consisted of Caucasian men diagnosed between 1980 and 1982 who were identified from a surveillance of participating network hospitals that covered approximately 97% of the state. The cities of St. Paul, Duluth, Minneapolis, and Rochester were excluded from the study. All identified cases underwent pathology review. Controls included Caucasian men without hematopoietic or lymphatic malignancies who were frequency matched by state, age, and year of death for deceased cases. Controls for living cases who were under the age of 65 years at diagnosis were obtained through random-digit dialing, and those for living cases who were 65 years or older at diagnosis were selected from computerized Medicare files from the Health Care Finance Administration. Controls for deceased cases were chosen from state vital records (death certificates). Of the 715 eligible cases, 622 (87.0%) participated in the interview. A total of 1,245 controls (77% of random digit dialing, 79% of Medicare, and 77% of death certificate) participated in the interview, though the authors do not provide the eligible population. Farmers were excluded from the analysis, leaving a total of 546 cases and 1,087 controls.

In-person interviews were conducted by trained interviewers with a structured questionnaire that inquired about sociodemographic characteristics; agricultural exposures; exposures to chemicals through hobbies; residential, medical, and occupational histories; as well as family history of cancer. Occupational histories were ascertained for all jobs held at least 1 year since the age of 18 years, as well as industry, name of employer, products produced, job title, and duties. There were 184 (29.6%) proxy case interviews and 425 (34.1%) proxy control interviews. Industries and occupations were coded according to SIC and the Dictionary of Occupational Titles (DOT), respectively. Exposure was assessed blindly by an industrial hygienist who used a job-exposure matrix to evaluate probability (4-point scale) and intensity (3-point scale) of exposure. Laundry and garment workers comprised Code 721 and included 16 (2.9%) cases and 14 (1.3%) controls.

Polychomotous unconditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs, adjusted for age, state, direct or surrogate respondent, agricultural

use of pesticides, postsecondary education, use of hair dyes, first-degree family member with malignant lymphoproliferative diseases, and tobacco. Analyses were also conducted for the three main types of non-Hodgkin lymphoma (follicular, diffuse, and other) for selected exposures, occupations, and industries. Exposure-response relationships examined the risk of non-Hodgkin lymphoma, or of the subtypes of non-Hodgkin lymphoma, by duration of employment, intensity of employment, and by the probability of exposure. In this instance, unexposed cases and controls consisted of those not employed in that particular occupation or industry, or those who lacked the exposure of interest. A strength of the study is its use of a job-exposure matrix. Limitations include its low-exposure prevalence, possible misclassification due to limited exposure information, and high percentage of proxy respondents.

B.2.2.7.2. Clavel et al. (1998)

Clavel, J.; Mandereau, L.; Conso, F.; Limasset, J. C.; Pourmir, I.; Flandrin, G.; Hémon, D. (1998). Occupational exposure to solvents and hairy cell leukaemia. Occup Environ Med, 55, 59-64. http://dx.doi.org/10.1136/oem.55.1.59

Summary: This study used a retrospective, hospital-based case-control design to examine the relationship between occupational exposures and hairy cell leukemia in men in France. Cases and controls were obtained from 18 hospitals throughout the country; cases included all patients diagnosed between 1980 and 1990 who were still alive at the time of the study. Controls consisted of patients admitted to the hospitals during this same time frame for other reasons. Due to the researchers' need to find a restricted number of cases in the same age range in each city, controls were predominantly chosen from the orthopedic and rheumatological departments. Control exclusion criteria included patients admitted for malignant disease, diseases related to occupations, and work-related accidents. Cases and controls were matched on birth date, sex, admission date, and residence. Of the 378 cases identified, 278 were considered eligible (i.e., still alive at the time of the study). Of these, 226 (81.3%) participated by returning the questionnaire. Of the 809 eligible controls, 465 (57.5%) participated by returning the questionnaire. Of these, 40 were excluded because the case they were initially matched with either died or did not respond, and they could not be matched to other cases. As a result, 425 (52.5%) of the eligible controls were included in the analysis. Efforts were made to match 2 controls with each case; 30% of cases were matched with 1 control, 56% were matched with 2 controls, and 14% were matched with 3 to 5 controls. The final sample consisted of 226 cases and 425 controls.

Self-administered questionnaires were sent to all participants, inquiring about sociodemographic characteristics, tobacco smoking, lifelong occupations, and leisure activities. Additional questionnaires were sent to participants with suspected occupational exposures.

Semi-structured interviews were also conducted to help experts assess exposures for those involved in textile degreasing, among others.

Jobs were coded according to International Labor Organization (ILO) and ISIC standards. Launderers and dry cleaners comprised ILO Code 5.6. There were 1 (0.4%) case and 2 (0.5%) controls who reported employment as launderers or dry cleaners. Exposure was evaluated in two ways. The first consisted of a blinded assessment by two of the study's researchers, which was based on the consistency of the participant's statements, the type of industry in which they worked, their job title, and the type of exposure. From this information, the researchers were able to classify the type of solvent used as well as the intensity of exposure associated with each job. The second method used a job-exposure matrix that was initially developed for a study by the International Agency for Research on Cancer (Ferrario et al., 1988) to assess exposure to solvents. The matrix used ILO and ISIC codes to classify each job into one of seven categories based on the probability, intensity, and frequency of exposure. These categories included unexposed, possibly exposed/unevaluable, probably exposed (<1/3 of exposed subjects, 1/3−2/3 of exposed subjects, or >2/3 exposed subjects), certainly exposed, and certainly highly exposed.

Conditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs, adjusted for smoking and farming. These estimates were ascertained for job titles, including launderers and dry cleaners, occupational tasks, including degreasing, and the main chemical families of organic solvents. A strength of this study is its use of a job-exposure matrix. Limitations include the study's retrospective recruitment, low response rate, lack of verification of self-reported information, and lack of individual solvent assessment. Also, while the study's inclusion of only living cases may have caused confounding by duration of survival, exposure information was obtained directly by the case, precluding the use of proxy respondents, who often do not provide as accurate information as that obtained directly from subjects. This study was inadequately powered to evaluate dry-cleaning exposures, resulting from the low-exposure prevalence among cases and one reported case as a launderer or dry cleaner.

Interviews were conducted by trained interviewers either in-person or by telephone. Of the 430 cases interviewed, 76% were in-person with the cases themselves, 9% over the telephone with the cases themselves, and 16% were proxy-interviews with next of kin when the case was deceased or too ill to participate. Of the 1,683 controls interviewed, 81% were in-person with the cases themselves, 18% were over the telephone with the cases themselves, and less than 1% were proxy interviews with next of kin. The questionnaire inquired about multiple risk factors, including chemical exposures, which were assessed blindly by the researchers and a toxicologist into 20 categories. For statistical reasons, only those exposures with a minimum of 10 exposed cases were analyzed, and this consisted of aliphatic hydrocarbons, aromatic hydrocarbons, chlorinated hydrocarbons, and pesticides. Chlorinated hydrocarbons included dry-cleaning

solvents, though only one case reported exposure to these particular solvents. The questionnaire also inquired about employment in four industries: petroleum, rubber, dry cleaning, and meat processing. Overall, 14 (3.3%) cases and 59 (3.5%) controls reported working for at least 6 months in the dry-cleaning industry.

Unconditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs. Both unadjusted and adjusted odds ratios were calculated for all-respondents and self-respondents only (excluding proxy-respondents); adjusted odds ratios controlled for race, 10-year age group, education, sex, and study site. The authors do not report strengths of their methodology. Limitations include the study's lack of adjustment for smoking and reliance on self-reported or proxy-reported occupational, which may have introduced recall bias. Misclassification of exposure towards the null was possible, given the discrepancy between those who reported working in the dry-cleaning industry and those who reported exposure to dry-cleaning solvents. Misclassification may have also occurred when participants were required to judge what, if anything, needed to be noted in the "other chemicals" portion of the chemical exposures question. Additionally, frequency, intensity, and duration of exposure were missing, which is mostly due to the fact that the questionnaire was developed to assess different risk factors. The study group was enrolled for the purposes of measuring the effect of gene influences on the immune system, rather than exposure to common chemicals. The study also suffered from a small sample size, impacting the statistical power to examine dry-cleaning exposures.

B.2.2.7.3. Fabbro-Peray et al. (2001)

Fabbro-Peray, P.; Daures, J. P.; Rossi, J. F. (2001). Environmental risk factors for non-Hodgkin's lymphoma: A population-based case-control study in Languedoc-Roussillon, France. Cancer Causes Control, 12, 201-212. http://dx.doi.org/10.1023/A:1011274922701

Summary: This population-based case-control study of NHL evaluated medical, occupational, and environmental risk factors and the occurrence of malignant lymphomas. This study was limited to French men and women aged 18 years or older who were living in Languedoc-Roussillon, which is the French county with the highest incidence of non-Hodgkin lymphoma. Cases were diagnosed with malignant lymphomas between 1992 and 1995 from 19 hospitals and a cancer research center. Controls were randomly chosen from electoral lists in a two-phase approach. First, the municipalities were randomly selected based on their size and the distribution of the population in the county. Second, individuals within each of the chosen municipalities were randomly selected. There were two controls assigned to each case, though the nonelectronic nature of the data prevented matching of cases and controls. Of the

627 eligible cases and 1,962 eligible controls, a total of 517 (82.5%) cases and 1,025 (52.2%) controls participated in an interview between 1992 and 1996. Of the 517 cases, 445 cases (86.0%) presented with NHL and 72 cases (13.9%) with Hodgkin lymphoma. Overall, there were more male cases (56.9%) than male controls (44.8%), and cases were older than controls.

Unblinded interviews were conducted by trained interviewers with cases and controls either in-person or over the phone. The questionnaire inquired about general characteristics, medical history, occupational history, environmental and occupational exposure to chemicals, occupational exposure to electromagnetic radiation, and smoking. Age at first exposure, duration of exposure, total number of days exposed, and time since first exposure were assessed for each chemical, including dry-cleaning solvents. There were a total of 35 (6.8%) cases and 77 (7.5%) controls exposed to dry-cleaning solvents.

Mantel-Haenszel methods were used for estimating the odds ratios and 95% CIs examining the effect of sociodemographic characteristics. Unconditional logistic regression using a forward stepwise approach was used to estimate odds ratios and 95% CIs for the effect of chemical exposures, occupational exposures to electromagnetic radiation, and cigarette smoking individually on non-Hodgkin lymphoma, adjusted for age, gender, urban setting, and education level. A lag time of 5 years prior to cancer diagnosis was included. Limitations to the study include the recall bias, given all information was self-reported, a high rate of refusal to participate in the control group, leading to potential selection bias, nondifferential misclassification, and the use of a broad category of dry-cleaning solvents that included tetrachloroethylene and other solvents. No strengths were reported by the authors.

B.2.2.7.4 Gold et al. (2010a; 2010b)

Gold, L. S.; Milliken, K.; Stewart, P.; Purdue, M.; Severson, R.; Seixas, N., . . . De Roos, A. J. (2010a). Occupation and multiple myeloma: An occupation and industry analysis. American Journal of Industrial Medicine, 53(8), 768-779. http://dx.doi.org/10.1002/ajim.20857

Gold, L. S.; Stewart, P. A.; Milliken, K.; Purdue, M.; Severson, R.; Seixas, N., . . . De Roos, A. J. (2010b). The relationship between multiple myeloma and occupational exposure to six chlorinated solvents. Occupational and Environmental Medicine, 68(6), 391-399. http://dx.doi.org/10.1136/oem.2009.054809

Summary: This population-based case-control study examined occupation exposures, particularly solvents exposure, as risk factors for multiple myeloma, and was carried out in two SEER sites, Seattle, WA, and Detroit, MI. Incident multiple myeloma cases (ICD-O-2/3, 9731 [plasmacytoma not otherwise specified] and 9732 [multiple myeloma]) eligible to participate were 35−74 years old and were newly diagnosed between 2000 and 2002. Gold et al. (2010a) reported on occupational and industry, with Gold et al. (2010b) reporting findings on

6 chlorinated solvents: tetrachloroethylene, trichloroethylene, 1,1,1-trichloroacetic acid, methylene chloride, chloroform, and carbon tetrachloride. Of the 365 cases eligible to participate, 64 (18%) had died before they could be contacted, 28 (8%) were unable to be located, and 18 (5%) were patients of physicans who refused to participate (71% participation rate). Population controls were selected from a previous case-control study of NHL undertaken at the same time in the same two SEER reporting sites (Chatterjee et al., 2004) and who (1) had not been previously diagnosed with multiple myeloma, plasmacytoma, NHL, or HIV, (2) were between 35−74 years of age, (3) were identified as residents of the Detroit or Seattle-Puget sound areas between 1998−2002, and (4) spoke English. Controls under 65 years of age were identified using random digit dialing; controls (65−74 years of age) were identified from Medicare roles. Of the eligible 1,133 controls, 481 (52%) participated. Control participation was not associated with study site or generation, but individuals in the 35−50- and 65−74-age groups were less likely to have participated than subjects 51−64 years old.

In-person interviews were conducted using a computer-assisted personal interview program. All interviews were carried out with the case; proxy interviewees could not complete the interview but could aid in recalling details of occupational exposures. Information on all jobs held since the age of 18 years for at least 1 year between 1941, for cases, and 1946, for controls, and the study enrollment dates, was collected. Subjects were additionally administered job-specific questionnaires for 20 occupations with potential solvent exposure. These modules were administered only when participants held the relevant job for at least 2 years. All jobs were coded blinded to case or control status according to the SOC system (Gold et al., 2010a) or assessed for exposure to six chlorinated solvents using job-exposure matrices developed for each decade for specific industries such as the chemical or rubber industries, occupations such as auto mechanics or hair dressers, and tasks such as degreasing, gluing, and painting, through literature reviews for trichloroethylene and tetrachloroethylene (Gold et al., 2008; Bakke et al., 2007). Each job was assigned a score for probability (0−4) based on the percentage of subjects likely to have had exposure, and for jobs with probability scores of 1 or higher, frequency (1−4), and intensity (1−4) scores. All jobs were assigned a score for confidence levels (1−4). Probability was scores as 0 = <1%; 1 = 1 through <10%; 2 = 10 through <50%; 3 = 50 through <90%; 4 + $\geq$ 90%. Frequency was defined as the average hours per week of exposure: 0 = <15 minutes/week; 1 = 15 minutes through <1 hour/week; 2 = 1−10 hours/week; 3 = >10−20 hours/week; 4 = >20 hours/week. The intensity score was the contraction of solvent estimated to have been in the subject's breathing zone over the exposure period (not an 8-hour TWA): 1 = 1−10 ppm, 2 = >10−100 ppm, 3 = >100−200 ppm, 4 = >200 ppm. The confidence level was assigned as 1 = literature contradictory or no information was available; 2 = one metric (probability, frequency, or intensity) was based on the literature or self-report; 3 = two metrics

were based on the literature or self-report; and 4 = all metrics based on the literature or directly from self-report. Of the 180 cases and 481 controls interviewed, 9 (5%) cases and 4 (0.8%) controls were identified as "ever" holding job as textile, apparel, and furnishing machine operator or tender, of whom, 5 cases (3%) and 3 (0.7%) controls were dry cleaners. Regarding specific exposures, 29 cases (19%) and 63 (13%) controls were assigned "ever" exposed to tetrachloroethylene, of whom, 17 (3%) cases and 15 (3%) controls were assigned high cumulative tetrachloroethylene exposure (>7,794 ppm-hours).

Statistical analyses consisted of unconditional logistic regression to estimate odds ratios and their 95% CIs for associations between the risk of multiple myeloma and the exposure surrogate ["ever" employed in occupation or industry (Gold et al., 2010a) or exposed to any of the six chlorinated solvents or to each of the chlorinated solvents (Gold et al., 2010b)]. Other surrogates examined were employment duration, cumulative exposure (for each exposed job, the midpoint of intensity × the midpoint of frequency × total years worked, summed over all exposed jobs), cumulative exposure for all jobs with a probability score of 2 or greater, and all jobs with solvent exposures lagged 10 years. All models adjusted for sex, age, race, education, and SEER site. As a sensitivity analysis, all analyses were repeated, assuming occupations with confidence scores of 1 were considered as unexposed.

A strength of this study is its use of detailed occupational information to improve assessment of solvent exposure compared to analyses based only on job title. Even so, exposure misclassification was likely. Some limitations of this study were relatively low participation rates among cases and controls, the inability to examine race or socioeconomic status, and if associated with occupation and, potentially, solvents exposure, the potential for selection bias, and small numbers of subjects with exposure to individual chlorinated solvents with limited statistical power. Last, the study may reflect relationships between chlorinated solvents and less severe forms of multiple myeloma due to the large proportion of cases who died before they could be contacted or eligible subjects who refused to participate, particularly, if refusal was related to being too ill.

B.2.2.7.4. Hardell et al. (1981)

Hardell, L.; Eriksson, M.; Lenner, P.; Lundgren, E. (1981). Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: A case-control study. British Journal of Cancer, 43(2), 169-176. http://www.ncbi.nlm.nih.gov/pubmed/7470379

Summary: This study used a case-control design to examine the possible relationship between exposure to chemical classes (organic solvents, chlorophenols, and phenoxy acids) and Hodgkin lymphoma and non-Hodgkin lymphoma. Cases consisted of men aged 25 to 85 years

with histologically confirmed malignant lymphoma between 1974 and 1978. Living controls were obtained from the National Population Registry and matched to cases on sex, age, and municipality. Potential living controls were excluded if they did not live in the same municipality at the time the case was diagnosed, if they were deceased, or if they had emigrated. Deceased controls were obtained from the National Registry for Causes of Death and matched on sex, age, municipality, and year of death. Potential deceased controls were excluded if their death had occurred in 1978, was the result of suicide or malignant tumors, or if the date of last employment did not occur within 5 years of the deceased case's last employment. There were initially 8 living controls matched to each living case and 10 deceased controls matched to each deceased case; in each instance, the two controls closest in age to cases were used in the analysis. The final sample consisted of 169 cases (60 with Hodgkin lymphoma and 109 with non-Hodgkin lymphoma) and 338 controls.

Self-administered questionnaires inquired about leisure-time activities, smoking/drug use, exposure to chemicals, and occupational history (including time and place of employment). A blinded individual evaluated each questionnaire and conducted telephone interviews with the participants when information was unclear or incomplete. Exposure to organic solvents, including tetrachloroethylene, was categorized into high grade and low grade (continuous exposure of $\leq$1 week or repeated, brief exposure for $\leq$1 month). There were 10 (5.9%) cases and 31 (9.2%) controls who reported exposure to low-grade organic solvents, though the authors do not report if this included tetrachloroethylene. Of the 40 (23.7%) cases and 47 (13.9%) controls who reported high-grade exposure to organic solvents, only 1 (0.6%) case reported exposure to tetrachloroethylene. Chi-square tests based on Miettinen (1970) were used to calculate χ^2 estimates and odds ratios. Corresponding 95% CIs were determined according to Miettinen (1976). Limitations to the study include its inability to independently evaluate the effect of tetrachloroethylene within the chlorinated solvents category and possible misclassification due to self-reported exposures. The authors do not report any strengths of their methodology.

B.2.2.7.5. Kato et al. (2005)

Kato, I.; Koenig, K. L.; Watanabe-Meserve, H.; Baptiste, M. S.; Lillquist, P. P.; Frizzera, G., . . . Shore, R. E. (2005). Personal and occupational exposure to organic solvents and risk of non-Hodgkin's lymphoma (NHL) in women (United States). Cancer Causes Control, 16, 1215-1224.

Summary: This study used a population-based case-control design to examine whether exposures to solvents were associated with the risk of non-Hodgkin lymphoma in women. Cases were identified through the New York State Cancer Registry and consisted of women aged 20 to 79 years living in New York State and diagnosed with non-Hodgkin lymphoma between 1995

and 1998. Any potential cases with a previous history of hematologic cancers or without a valid driver's license were excluded. Two sets of controls were used. Those under the age of 65 years were obtained from an age-stratified random sample of driver's licenses from the New York Department of Motor Vehicles (DMV), and those 65 years and older were identified from Health Care Finance Administration (HCFA) beneficiary records. All eligible cases and DMV controls were first sent a solicitation letter by the New York Cancer Registry. Only cases and DMV controls that responded to the letter were contacted for an interview. Of the 722 eligible cases, 376 (56%) participated. The participation rates were 30% for DMV controls and 67% for HCFA controls. The authors did not report response rates for cases or controls. The final sample consisted of 376 cases and 463 (248 DMV and 215 HCFA) controls.

Blinded telephone interviews were conducted with both the cases and controls with a structured questionnaire. Nearly 21% of the case interviews and just over 3% of the control interviews were conducted with proxy respondents, in this case, next of kin. This occurred when the participant was either deceased or medically incapable of answering the questions. The median time between the cancer diagnosis and the interview was 1.2 years and ranged between 2 months and 3.3 years. A total of 50 (13.3%) cases and 48 (10.4%) controls reported occupational exposure to degreasers and cleaning solvents, and 7 (1.9%) cases and 8 (1.7%) controls reported occupational exposure to dry-cleaning fluids. To allow for a minimum lag period of 1 year, an index date was determined for each case. Any exposures that occurred after this date were excluded from the analysis.

Unconditional logistic regression estimated odds ratios and 95% CIs for occupational and household exposures to solvents, adjusting for age at index date, family history of hematologic cancer, college education, surrogate status, year of interview, BMI 10 years before interview, average frequency of use of pain-relieving drugs, total number of episodes of systemic antibiotic use, total number of uses of household pesticide products, and duration of work involving pesticide exposures. This study's strength is its questionnaire that examined long-term exposures by asking about the participant's whole personal history. Limitations include its self-reported occupational history and the potential for recall bias in measuring exposures to degreasers/cleaning solvents and dry-cleaning fluids. It also includes a limited number of household products that contained organic solvents, which may have underestimated actual exposure. Recall bias and the low response rate were additional methodological limitations to this study.

B.2.2.7.6. Malone et al. (1989)

Malone, K. E.; Koepsell, T. D.; Daling, J. R.; Weiss, N. S.; Morris, P. D.; Taylor, J. W., Lyon, J. L. (1989). Chronic lymphocytic leukemia in relation to chemical exposures. Am J Epidemiol, 130, 1152-1158. http://www.ncbi.nlm.nih.gov/pubmed/2589308

Summary: This study used previously collected data from a larger study (Koepsell et al., 1987) to examine the relationship between selected occupations or chemical exposures and leukemia. Cases were identified through SEER reporting sites in Washington state, Utah, Michigan, and Georgia and consisted of men and women under the age of 80 years who were diagnosed with chronic lymphocytic leukemia between 1977 and 1981. Of the eligible cases, 82.5% responded, and 430 were interviewed. The authors were unclear regarding the number of eligible cases. Three of the cases were excluded because the interview failed to provide any information on chemical exposures. Controls were randomly selected in one of two ways: (1) random digit dialing in Utah, Michigan, and Georgia and (2) area sampling in Washington state. Controls were matched based on sex, race, and/or age, depending on the location. Of the 2,028 eligible controls, 83% were interviewed. The final sample consisted of 427 cases and 1,683 controls.

B.2.2.7.7. Mester et al. (2006), Seidler et al. (2007)

Mester, B.; Nieters, A.; Deeg, E.; Elsner, G.; Becker, N.; Seidler, A. (2006). Occupation and malignant lymphoma: A population based case control study in Germany. Occup Environ Med, 63, 17-26. http://dx.doi.org/10.1136/oem.2005.020453

Seidler, A.; Mohner, M.; Berger, J.; Mester, B.; Deeg, E.; Elsner, G., . . . Becker, N. (2007). Solvent exposure and malignant lymphoma: A population-based case-control study in Germany. J Occup Med Toxicol, 2, 2. http://dx.doi.org/10.1186/1745-6673-2-2

Summary: A multicentre, population-based case control study was conducted in six regions in Germany that is part of a larger multicountry lymphoma case-control study (the EPILYMPH study). Cases were identified through physicians who played a role in the diagnosis and treatment of malignant lymphoma in patients admitted to hospitals in each of the study areas. Cases consisted of German residents (men and women) aged 18 to 80 years who were diagnosed with either non-Hodgkin or Hodgkin lymphoma. Controls were identified from the population registration office and matched to cases based on sex, region, and age. The participation rate among controls was 44.3%; more than half (51%) of those who did not participate cited reasons related to lack of interest. Additionally, in order to be included in the study, cases and controls

needed to be familiar with the German language. The final sample consisted of 710 cases and 710 controls.

In-person interviews were conducted with trained interviewers and inquired about the participant's medical history, lifestyle behaviors (smoking, alcohol, etc.) and activities, and occupational history. The occupational history obtained information on dates of employment, title, industry, and tasks associated with each job held for at least 1 year. Any participants who reported potentially hazardous jobs (including dry cleaning) were asked additional questions about their job tasks based on Bolm-Audorff et al. (1989).

Mester et al. (2006) aimed to identify occupations suspected to be associated with lymphoma risk and to generate new hypotheses about occupational risks. Occupation was assessed as a proxy for exposure, with job titles and industries blindly coded by two individuals from the Frankfurt Institute for Occupational Medicine according to ISCO-68 and Statistical Classification of Economic Activities in the European Community. Launderers, dry cleaners, and pressers comprised ISCO-68 Code 56 and included 11 (1.5%) cases and 11 (1.5%) controls. Conditional logistic regression was used to calculate odds ratios and their corresponding 95% CIs, adjusted for smoking and alcohol consumption. Unconditional logistic regression was employed to further examine lymphoma subentities (Hodgkin lymphoma, B-non-Hodgkin lymphoma, T-non-Hodgkin lymphoma, B-non-Hodgkin lymphoma and Hodgkin lymphoma, and other lymphomas), estimating odds ratios and their corresponding 95% CIs, adjusted for age, sex, region, smoking, and alcohol consumption. All estimates were stratified by employment duration (≤10 years or >10 years). Additional analyses examined the effect of a latency period of 10 years by only including exposures that occurred up until 10 years before diagnosis, though the data were not reported. Limitations to the study include the low control response rate, possible misclassification in the assessment of exposure through employment in specific industries and occupations, small numbers of exposed, and lack of control for race/ethnicity or immigration status. The authors do not report strengths associated with the methodology of their study.

Seidler et al. (2007) examined the association between exposure to chlorinated hydrocarbons and lymphoma on an in-depth expert assessment of solvent exposure. Intensity and frequency of exposure was assessed by a blinded, trained industrial physician. Intensity was evaluated as low (0.5−5 ppm), medium (>5−50 ppm), or high (>50 ppm). Frequency was calculated as the percentage of weekly working time exposed and was categorized as low (1−5%), medium (>5−30%), or high (>30%). Confidence in the exposure was classified as possible, probable, or certain, and cumulative exposure (ppm-years) to each solvent for each occupation was also calculated. Overall, there were 36 (5.1%) cases and 31 (4.4%) controls exposed to tetrachloroethylene. Conditional logistic regression was used to calculate odds ratios

and 95% CIs, adjusted for smoking and alcohol consumption. The authors reported only those calculations with at least five participants reporting exposures. Tests for trend were analyzed by including specific exposures as continuous variables in the logistic regression model. Unconditional logistic regression was employed to estimate odds ratios and 95% CIs in an unmatched analysis of the most frequent lymphoma subentities (Hodgkin lymphoma, B-non-Hodgkin lymphoma, T-non-Hodgkin lymphoma, B-non-Hodgkin lymphoma and Hodgkin lymphoma, and other lymphomas), adjusted for age, sex, region, smoking, and alcohol. Strengths of the study include blinded exposure assessment, adjustment for potential confounders, and an expert-based estimate of solvent exposure. A limitation of this study is the low-exposure prevalence.

B.2.2.7.8. Miligi et al. (2006; 1999), Costantini et al. (2008; 2001)

Miligi, L.; Costantini, A. S.; Benvenuti, A.; Kriebel, D.; Bolejack, V.; Tumino, R., . . . Vineis, P. (2006). Occupational exposure to solvents and the risk of lymphomas. Epidemiology, 17, 552-561. http://dx.doi.org/10.1097/01.ede.0000231279.30988.4d

Miligi, L.; Seniori, C. A.; Crosignani, P.; Fontana, A.; Masala, G.; Nanni, O., . . . Vineis, P. (1999). Occupational, environmental, and life-style factors associated with the risk of hematolymphopoietic malignancies in women. Am J Ind Med, 36, 60-69. http://dx.doi.org/10.1002/(SICI)1097-0274(199907)36:1<60::AID-AJIM9>3.0.CO;2-Z

Costantini, A. S.; Benvenuti, A.; Vineis, P.; Kriebel, D.; Tumino, R.; Ramazzotti, V., . . . Miligi, L. (2008). Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: Evidence from the Italian Multicenter Case-control study. Am J Ind Med, 51, 803-811. http://dx.doi.org/10.1002/ajim.20592

Costantini, A. S.; Miligi, L.; Kriebel, D.; Ramazzotti, V.; Rodella, S.; Scarpi, E., . . . Vineis, P. (2001). A multicenter case-control study in Italy on hematolymphopoietic neoplasms and occupation. Epidemiology, 12, 78-87. http://www.ncbi.nlm.nih.gov/pubmed/11138825

Summary: These four publications report on a large, population-based case-control study examining pesticide or solvent exposures and hematolymphopoietic malignancies. The studies were conducted in 12 different parts of Italy (Turin, Ragusa, Siena, Alessandria, Forli, Novara, and Vercelli, as well as Florence, Verona, Imperia, Latina, and Varese provinces), but only 11 of the locations had interviews available for analysis. The authors do not note which study site was excluded. Cases consisted of men and women aged 20 to 74 years who were diagnosed with hematolymphopoietic malignancies between 1991 and 1993. Cases were obtained through surveys with public hospitals in the 12 study areas, as well as regional medical centers or university-affiliated hospitals in Milan, Pavia, Rome, and Bologna to ensure complete collection

of cases from all 12 locations. One location (Varese) found all of its cases through the local cancer registry. Controls were randomly selected from the general population of residents in each of the study locations, stratified by sex and 5-year age groups. Of the 3,357 eligible cases, 3,118 were able to be contacted, and 2,737 (88%) were interviewed, including 1,450 non-Hodgkin lymphoma, 365 Hodgkin lymphoma, 652 leukemia, and 270 multiple myeloma cases. Of the 2,391 eligible controls, 2,196 were able to be contacted, and 1,779 (81%) were interviewed.

In-person interviews were conducted to obtain information on education, lifestyle behaviors, occupational history, extraoccupational exposure to solvents and pesticides, hair dye use, lifelong residential history, medical history, and reproductive history. Proxy interviews were conducted with spouses (45%), children (28%), parents (11%), or another relative (16%) for 19% of the cases and 5% of the controls. The occupational history section of the questionnaire was created by industrial hygienists and agronomists and inquired about the participant's full working history as well as exposure to chemicals, solvents, and pesticides. Industrial hygienists from each of the areas blindly assessed occupational exposures, evaluating the probability and intensity of exposures to categories of solvents as well as individual chemicals, including tetrachloroethylene. Probability was rated as low, medium, or high, and intensity was classified as "very low," "low," "medium," and "high." To ensure consistency in assessment, a job exposure matrix was created with the minimum overall consensus for those jobs that were reported most frequently.

Costantini et al. (2001) investigated the associations between occupational exposures and hematolymphopoietic neoplasms. This study used the full sample of 2,737 cases and 1,779 controls within the 11 available study areas. All jobs were coded according to International Standard Classification of Occupations; launderers, dry cleaners, and pressers fell within Code 56. There were 3 (0.2%) male non-Hodgkin lymphoma cases, 1 (0.3%) male Hodgkin lymphoma case, and 2 (0.3%) male leukemia cases who were employed in the dry-cleaning industry. Odds ratios and their corresponding 95% CIs for non-Hodgkin lymphoma, Hodgkin lymphoma, leukemia, and multiple myeloma were calculated using the Mantel-Haenszel approach adjusted for age. The results for men were presented and compared with the results for men and women combined. Only those occupations that had a minimum of five exposed cases in at least one gender were presented. The authors did not specifically mention strengths associated with the methodology; a limitation is its use of occupation as a proxy for examining risks associated with chemical exposures.

Miligi et al. (2006) evaluated the association between solvent exposure in the work environment and non-Hodgkin lymphoma, including chronic lymphatic leukemia, and Hodgkin lymphoma. Due to the fact that the industrial hygienists' exposure assessment was only

completed in 8 areas, there were 1,719 eligible cases of non-Hodgkin lymphoma, 347 eligible cases of Hodgkin lymphoma, and 2,086 eligible controls. The final sample consisted of 1,428 (83%) NHL cases, 304 Hodgkin lymphoma cases (88%), and 1,530 controls (73%). In-person interviews were conducted with 85% of the non-Hodgkin lymphoma cases, 93% of the Hodgkin lymphoma cases, and 97% of the controls. Intensity of exposure was classified as "very low/low" or "medium/high." There were 18 (1.3%) non-Hodgkin lymphoma cases and 29 (1.9%) controls with "very low/low" exposure to tetrachloroethylene and 14 (1.0%) non-Hodgkin lymphoma cases and 15 (1.0%) controls with "medium/high" exposure. Duration of exposure was categorized as having lasted <15 years or >15 years. There were 10 (0.7%) non-Hodgkin lymphoma cases and 10 (0.7%) controls who reported <15 years of exposure to tetrachloroethylene and 3 (0.2%) non-Hodgkin lymphoma cases and 5 (0.3%) controls who reported 15 years or more. Odds ratios and their corresponding 95% CIs were calculated for non-Hodgkin lymphoma, non-Hodgkin lymphoma subtypes, and Hodgkin lymphoma, individually. All were adjusted for sex, age, education, and area. Strengths of the study include its large sample size and the exclusion of participants who were classified as having a low probability of exposure. Limitations include the potential for misclassification of subjects by individual chemical, the low-exposure prevalence to tetrachloroethylene, the high percentage of proxy interviews among the case series, and the small sample of cases for each lymphoma subtype, which for Hodgkin lymphoma, prevented the examination of potential associations by individual chemical.

Costantini et al. (2008) examined the association between solvent exposure and occurrence of leukemia subtypes and multiple myeloma. The final samples consisted of 586 cases of leukemia (acute myeloid leukemia and chronic lymphatic leukemia) and 1,278 controls collected from 7 of the locations, as well as 236 cases of multiple myeloma and 1,100 controls collected from 6 of the sites. Intensity of exposure was classified as "very low/low" or "medium/high." There were 6 (1.0%) leukemia cases, 17 (1.3%) leukemia controls, 3 (1.3%) multiple myeloma cases, and 15 (1.4%) multiple myeloma controls with "very low/low" exposure to tetrachloroethylene and 7 (1.2%) leukemia cases, 12 (0.9%) leukemia controls, 2 (0.8%) multiple myeloma cases, and 12 (1.1%) multiple myeloma controls with "medium/high" exposure. Duration of exposure was categorized as having lasted <15 years or >15 years, though tetrachloroethylene was not specifically reported. Point odds ratios and their corresponding 95% CIs were calculated for leukemia, leukemia subtypes, and multiple myeloma, individually. All were adjusted for gender, age, education, and area. The authors did not report the method used to derive estimates. A linear test for trend was also conducted using the midpoints of all duration categories (0, 7.5, and 35 years). The authors did not note any

strengths or limitations of their methodology, although those identified for Miligi et al. (2006) are relevant for this study.

B.2.2.7.9. Schenk et al. (2009)

Schenk, M.; Purdue, M.; Colt, J.; Hartge, P.; Blair, A.; Stewart, P., . . . Severson, R. (2009). Occupation/industry and risk of non-Hodgkin's lymphoma in the United States. Occup Environ Med, 66, 23-31. http://dx.doi.org/10.1136/oem.2007.036723

Summary: This study used a case-control design to examine the relationship between occupation and development of non-Hodgkin lymphoma. Cases were identified through the SEER registry and consisted of men and women aged 20 to 74 years, living in Iowa or selected parts of California, Michigan, or Washington state and diagnosed with histologically confirmed non-Hodgkin lymphoma between 1998 and 2000. Controls were selected in two ways. Those under the age of 65 years were chosen from random digit dialing, and those 65−74 years were chosen through Medicare files. Controls were matched to cases on 5-year age group, gender, and race within each study center. All HIV-positive individuals were excluded from both the cases and controls, as were controls with a previous diagnosis of non-Hodgkin lymphoma. Of the 2,248 eligible cases, 1,728 (77%) were contacted, and 1,321 participated in the interview, yielding a response rate of 59% and a participation rate of 76%. Of the 2,409 eligible controls, 2,046 (85%) were contacted, and 1,057 participated, yielding a response rate of 44% and a participation rate of 52%. After excluding those cases and controls who were never employed or whose occupations were unknown, the final sample consisted of 1,189 cases (293 follicular, 366 diffuse large B-cell lymphoma, 487 other, 43 unknown) and 982 controls.

Initially, all participants were mailed a self-administered questionnaire inquiring about either family and medical history or diet. Then participants were visited in their homes for a computer-assisted interview. All participants were asked about demographics, hair coloring, residential history since 1970, and occupational history (Chatterjee et al., 2004). The occupational history asked about all jobs lasting 6 months or longer and obtained information on location, dates of employment, job title, and number of hours worked (part-time or full-time). Occupation was assessed as a proxy for exposure, and all jobs were blindly assigned occupation and industry codes according to SOC and SIC conventions. Participants were considered exposed if they had ever been employed in a particular occupation or industry and unexposed if they had not. Launderers and ironers were assigned Code 503 and included a total of 12 (1.0%) cases and 3 (0.3%) controls.

Unconditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs, adjusted for age, gender, ethnicity, and study center. Analyses were also performed, stratified by gender and histological subtype separately. Strengths of the study

include its population-based sample, the large number of cases, its detailed information on multiple risk factors, as well as its ability to examine this disease by multiple histological subtypes and by gender. Limitations to the study include its small number of exposed participants and low power. The study also fails to examine intensity or duration of exposure and may be subject to selection bias in light of its low participation rate.

B.2.2.7.10. Scherr et al. (1992)

Scherr, P. A.; Hutchison, G. B.; Neiman, R. S. (1992). Non-Hodgkin's lymphoma and occupational exposure. Cancer Res, 52, 5503s-5509s. http://www.ncbi.nlm.nih.gov/pubmed/1394164

Summary: This hospital-based case-control study of non-Hodgkin lymphoma examined occupations and exposures associated with increased risk of all NHLs or of specific NHL histological subtypes. The case series were patients diagnosed with NHL from January 1, 1980, to May 31, 1982, treated at any of nine participating Boston hospitals, and residents of the Boston Standard Metropolitan Statistical Area. A total of 379 NHL histologically confirmed cases were identified, of which, 303 interviews with the living case, next of kin, or parent for ages 17 years or younger (80% response rate). A pathology review of the cases confirmed the NHL diagnosis, and this is one of the early studies to classify NHL subtypes according to the Modified Rappaport or Working Formulation Classification, if tumors were nodular or diffuse, or by cell type (B- or T-cell). The control series were randomly selected from residence lists for all Massachusetts towns and, for controls 17 years of age or older, matched to cases based on sex and age. For cases under 18 years of age, possible controls were identified from matching based on the age and sex of a case's parent or guardian and interviewed to determine whether he or she had a child of the same age and sex as the case. Of 423 potential controls, 303 were interviewed (72% response rate). All interviews were carried out with the liver control. No statistically significant differences between cases and controls were found for education, marital status, current family income, and highest family income. Religion was found to differ between cases and controls ($p < 0.05$).

Face-to-face interviews were carried out using a questionnaire that sought information on current or most recent job, job held 15 years previously, major and second major occupation, and exposure to a list of agents that included chlorinated solvents as a category. One-third of cases' responses were from proxy or next-of-kin respondents. Each occupation was categorized by occupation and industry and coded according to the Dictionary of Occupational Titles.
Nine cases (3% exposure prevalence) were identified as holding jobs in laundering, dry cleaning, and leather products fabrication, and 73 cases (24% exposure prevalence) reported exposure to chlorinated solvents.

Statistical analyses were carried out using a hierarchal approach that aggregated histological subtypes into groups with similar histological characteristics and exposure defined as a function of calendar time (1901−1949, 1950−1959, 1960−1969, 1970 and later) or exposure duration (10 years, 20 years). All exposure that showed consistent patterns within histological categories over calendar time or over duration were considered as candidate variables for conditional logistic models with covariates for age and sex.

A strength of the study is its examination of NHL subtypes, although different classification schemes were used, and no attempt was made to harmonize across schemes. The lack of a common scheme to classify NHLs might lead to potential for disease misclassification. Another limitation of the study includes the high percentage of proxy respondents (33% cases, no controls), which may have led to measurement error and misclassification bias in exposure assignment. Last, the statistical analyses, using a hierarchal approach, could identify true positive associations but was not specific, with a greater potential for some false negative findings.

B.2.2.8. Childhood lymphopoietic cancers

B.2.2.8.1. Infante-Rivard et al. (2005)

Infante-Rivard, C.; Siemiatycki, J.; Lakhani, R.; Nadon, L. (2005). Maternal exposure to occupational solvents and childhood leukemia. Environ Health Perspect, 113, 787-792. http://dx.doi.org/10.1289/ehp.7707

Summary: This study used a population-based case-control design to examine the possible relationship between childhood leukemia and maternal exposure to occupational solvents. Cases consisted of children who were diagnosed with acute lymphoblastic leukemia and were being treated in tertiary care centers in Quebec Province, Canada. Between 1980 and 1993, children aged 0 to 9 years were included in the study; between 1994 and 2000, cases were children between 0 and 14 years of age. Controls were selected in two ways. The 1980−1993 controls were obtained from government records indicating all families who had received a stipend for having children living legally in Canada. The 1994−2000 controls were chosen from universal health insurance records for the Quebec Province. Both mechanisms provided the most complete census of children during these time periods. Controls were matched to cases on sex and age at diagnosis. Prospective participants were excluded if children were adopted or lived with foster families, if neither French nor English was spoken, if they did not currently reside in Canada, or if the parents were not available to be interviewed. Of the 848 eligible cases, 790 (93.1%) of parents were interviewed. Of the 916 eligible controls, 790 (86.2%) of parents were interviewed. The final sample consisted of 790 cases and 790 controls.

Telephone interviews with parents were conducted using a structured questionnaire, which inquired about general risk factors, potential confounders, and maternal occupational risk factors. The latter consisted of a complete occupational history (job title, industry, name, address) provided by the mother for the time period, which began when she was 18 years of age and ended with the birth of the child. All jobs held by the mother during the 2 years prior to the birth of the child were further examined using a semi-structured questionnaire to obtain information on the company's activities, raw materials and machines used, goods produced, responsibilities, working conditions, activities of coworkers, and the presence of solvents and other chemicals. Finally, for all jobs for which there was significant possible exposure, including textile dry cleaners, more detailed questionnaires were used to inquire about the specific tasks, the time spent at these tasks, the specific exposures associated with these tasks, and the environment in which the tasks were carried out.

Exposures were classified by a team of blinded chemists and industrial hygienists. All jobs were coded according to standard Canadian industrial titles (3-digit codes) and job titles (7-digit codes). Then, the team determined whether or not participants were exposed to a list of over 300 chemicals, including tetrachloroethylene, based on the information provided by the respondent, previous information on exposures in that geographical area, and the team's knowledge of exposures in the industry in question. All jobs held in the 2-year time period before the pregnancy were coded separately, based on the team's confidence that the exposure had occurred (possible, probable, or definite), the frequency of the exposure during a normal workweek (<5%, 5−30%, or >30% time), and the level of the concentration (low/background, medium, high). This methodology for exposure assessment has been validated and used in other research publications. All chemicals were assigned 3 digit codes based on Siemiatycki (1991); tetrachloroethylene was Code 243. This study did not provide sufficient detail to determine the number of cases exposed to tetrachloroethylene to calculate a prevalence of exposure.

Conditional logistic regression was performed separately for each chemical using two time periods, the 2 years before the child's birth and during pregnancy. Odds ratios and their corresponding 95% CIs were estimated, adjusting for maternal age and level of schooling. A strength of this study is the use of a detailed exposure assessment. Limitations include power due to small sample size and nondifferential misclassification.

B.2.2.8.2. Lagakos et al. (1986), Costas et al. (2002)

Lagakos, S. W.; Wessen, B. J.; Zelen, M. (1986). An analysis of contaminated well water and health effects in Woburn, Massachusetts. J Am Stat Assoc, 81, 583-596.

Costas, K.; Knorr, R. S.; Condon, S. K. (2002). A case-control study of childhood leukemia in Woburn, Massachusetts: The relationship between leukemia incidence and exposure to public drinking water. Sci Total Environ, 300, 23-35. http://dx.doi.org/10.1016/S0048-9697(02)00169-9

Summary: Lagakos et al. (1986) had two aims: (1) to assess the association between access to contaminated water and the incidence rate of childhood leukemia, and (2) to determine whether adverse pregnancy outcomes (fetal wastage, low birth weight, stillbirth, birth defects) were correlated with exposure to water from the contaminated wells. Cases were identified through the state cancer registry and the Dana-Farber Cancer Institute/Children's Hospital cancer registry and consisted of male and female children aged 19 years and younger who were diagnosed with leukemia in Woburn between 1964 and 1983. In total, 20 cases of childhood leukemia were identified. This study was a precursor to a later case-control study of childhood leukemia by Costas et al. (2002).

In 1982, telephone interviews were conducted by blinded, trained interviewers with Woburn residents. Of the 8,109 telephone numbers, 7,134 (88%) were contacted. After excluding for business, second phones, and disconnected numbers, the sample decreased to 6,219 households. Of these, 5,010 (80.6%) were interviewed. The questionnaire used during the interviews inquired about all pregnancies 1960 and 1982, excluding elective and spontaneous abortions, chronic and recurrent child health problems, and the residential history for each family member (current and former) up through, but excluding the current address. Pregnancy information obtained included the date the pregnancy ended, maternal age, and smoking status during pregnancy, offspring vital status at delivery, offspring weight, gender, and congenital anomalies. A 1983 study by Waldorf and Cleary estimated the monthly distribution of water from the contaminated wells for the time the wells were in use (1964−1979). These estimates were used to determine the proportion of annual water supplied by the contaminated wells for each household between 1960 and 1982. Annual exposures for pregnancies were based on the year the pregnancy ended; annual exposures for children were determined starting the first year they lived in Woburn. Cumulative and binary metrics were used to characterize exposure to well water in the study area. Of the 4,396 pregnancies that occurred during the study period and 4,978 children about whom information was obtained, approximately 16% of pregnancies, and 27% of children were estimated to have had some exposure to the contaminated wells.

A Cox hazards regression model was used to estimate whether the distribution of childhood leukemia cases was associated with the contaminated wells. Individual risk sets

consisted of children from the survey of adverse pregnancy outcomes and childhood disorders who were matched to cases on year of birth and were residents of Woburn when the case was diagnosed. These risk sets were used to estimate the expected cumulative exposure for each case. Logistic regression using the maximum likelihood method was employed to estimate odds ratios and their corresponding 95% CIs for adverse pregnancy outcomes including spontaneous abortion, perinatal death, low birth weight, and musculoskeletal, cardiovascular, eye/ear, CNS, chromosomal, and oral cleft anomalies separately. Each adverse outcome was adjusted for its own set of risk factors. Overall, these were maternal age during pregnancy, smoking during pregnancy, year pregnancy ended, and mother's pregnancy history, which included prior spontaneous abortion, prior perinatal death, prior low birth weight, and prior musculoskeletal anomaly. A survival time model with age of diagnosis as the time variable was used to estimate relative risks of childhood disorders, including anemia/blood disorders, allergy/skin disorders, kidney/urinary tract disorders, lung/respiratory tract disorders, neurologic/sensory disorders, learning disabilities, and other disorders separately. These were each adjusted for their own set of risk factors. Overall, these were year the pregnancy ended, age at pregnancy, SES, and sex. A strength of this study is its large sample of pregnancy outcomes and child disorders. A limitation is its use of annual exposure estimates, which may not have been accurate enough to assess intensity of exposure, particularly for pregnancy outcomes. A second limitation included potential bias of omission of families that moved from Woburn prior to 1982. Nonresponse of eligible, omitted households that were not contacted may have introduced bias.

Costas et al. (2002) used a matched case-control design in their follow-up to a Massachusetts Department of Health study (Cutler et al., 1986), which found a cluster of leukemia cases in Woburn, Massachusetts. This study expanded on the initial research by using water distribution models to assign exposure rather than location of residence (Costas et al., 2002) and aimed to determine whether childhood leukemia was associated with exposure to water from Wells G and H (MDPH, 1997). Cases consisted of children who were diagnosed with leukemia before their 19th birthday between 1969 and 1989. Those diagnosed before 1982 were identified through pediatric health professionals and greater-Boston pediatric oncology centers, and those diagnosed from 1982 onwards were obtained through the Massachusetts Cancer Registry. Controls were randomly selected from Woburn Public School records and matched to cases based on race, sex, and date of birth. Two controls were matched to each case. Both cases and controls were required to be Woburn residents at the time of the case's diagnosis. Of 21 eligible cases, 19 (90.5%) participated in the study. Of the 38 controls selected, one was excluded from the study when it became known that they no longer fit the inclusion criteria. The authors do not report response rates for controls. The final sample consisted of 19 cases and

37 controls. Cases and controls were similar on family history of cancer, maternal smoking and alcohol consumption, and potential exposure to 60 Hz electric and magnetic fields.

In-person interviews were conducted with both parents of cases and controls, except for two instances where the father was interviewed via telephone. The maternal questionnaire inquired about demographics, lifestyle characteristics, medical history, environmental and occupational exposures, and use of public drinking water at home. The paternal questionnaire inquired about occupational history and occupational exposures. A detailed residential history was also ascertained for each mother and child for the period 2 years preconception through date of case diagnosis, and all were evaluated for electromagnetic field exposure using a power distribution wire code scheme (Kaune and Savitz, 1994). All estimates were based on well water contaminant levels, which were measured just before the wells' closure in 1979. As a result, exposure was determined by the potential for a residence to receive water from the contaminated wells rather than the actual concentrations of contaminants. A water distribution model developed by Murphy (1990) was used to estimate water distribution patterns through the creation of exposure index values for each neighborhood in Woburn for each month that the wells were in use between 1964 and 1979. Overall, seven cumulative exposure scores were estimated for each participant for the entire etiologic period, preconception, each trimester, overall pregnancy, and period between birth and diagnosis. Two exposures were assessed for each participant: (1) cumulative exposure (summed for all months of residence in a particular location), and (2) average exposure (consisted of water exposure data averaged over time). During the full exposure time frame (2 years preconception through case diagnosis), 16 (84.2%) cases and 24 (64.9%) controls had been exposed to water from the contaminated municipal wells. Of these, 7 cases (36.8%) and 13 controls (35.1%) received the most exposure, and 9 cases (47.4%) and 11 controls (29.8%) received the least.

Conditional logistic regression with a proportional hazards model was used to calculate odds ratios and their corresponding 95% CIs. Unadjusted odds ratios examined the relationship between case-control status and effects of maternal alcohol consumption, breastfeeding, paternal grandfather with cancer, paternal employment in a high-risk industry, and public water as primary beverage. Odds ratios for four exposure time periods (2 years preconception through case diagnosis, 2 years preconception, pregnancy, birth to diagnosis) examined "ever" exposure and subcategories within "ever" exposure (i.e., "most" or "least") separately. Each was then adjusted for a composite covariate according to Tukey (1991), who controlled for socioeconomic status, maternal smoking during pregnancy, maternal age at birth of child, and breastfeeding. Trends related to increasing exposure (i.e., "never," "least," "most") were evaluated for each exposure time period separately using the χ^2 method. Strengths of this study include its adjustment for potential confounders and its use of exposure estimates that were developed

through an investigation of the distribution of municipal water throughout the city. A limitation of the study is its lack of information on actual well water contamination levels during the time the wells were in use and the study's small size, which leads to low statistical power and imprecise estimates of risk.

B.2.2.8.3. Lowengart et al. (1987)

Lowengart, R. A.; Peters, J. M.; Cicioni, C.; Buckley, J.; Bernstein, L.; Preston-Martin, S.; Rappaport, E. (1987). Childhood leukemia and parents' occupational and home exposures. J Natl Cancer Inst, 79, 39-46.
http://www.ncbi.nlm.nih.gov/pubmed/3474448

Summary: This case-control study investigated possible etiologic factors for childhood leukemia. Cases were identified through the Los Angeles County Cancer Surveillance Program and consisted of children aged 10 years or younger at the time of their diagnosis between 1980 and 1984. In order to be included in the study, biological case mothers were required to be available for an interview. Controls were selected in two ways: (1) friends of cases were identified by case mothers and asked to participate, and (2) population-based controls were chosen through random digit dialing when friends were not available. Of the 216 eligible cases, 202 (94%) were able to be contacted. Of them, 159 (79%) mothers were interviewed. There were 154 case fathers also interviewed, of which, 30 cases where mother's provided proxy information on paternal variables. There were five fathers who did not participate in the study. There were 136 control mothers and 130 control fathers who participated in the interview. There were 6 fathers for whom interviews could not be obtained, and 43 of the paternal interviews were by proxy with mothers. The authors do not report control response rates. Controls were matched to cases based on age, sex, race, and Hispanic origin (if the race was "white"), though 3 population-based controls were unable to be matched based on sex and 10 were unable to be matched based on race. After further exclusions (4 cases and 5 controls) for incomplete occupational histories, the final sample consisted of 123 case-control pairs for which complete information was available about both parents.

Telephone interviews were conducted by two, nonblinded, trained interviewers using a structured questionnaire that inquired about family and personal medical histories, alcohol and tobacco use, household and personal products, X-ray exposure, and occupational history (job title, industry, time period worked). The maternal questionnaire also asked about medical complications, use of drugs, and diet during the index pregnancy, as well as the child's medical history and exposure to ionizing radiation. The interviews occurred between 1983 and 1985. Industries and occupations were coded according to 1970 U.S. Census classifications and grouped based on potential hydrocarbon exposure. All occupations and exposures within 1 year

of conception were excluded. There was 1 (0.8%) case father who reported exposure to tetrachloroethylene in the year before pregnancy, 1 (0.8%) case father who reported exposure during pregnancy, and 2 (1.6%) case fathers who reported exposures after delivery. No control fathers were exposed to tetrachloroethylene. The authors did not report information on maternal exposure to tetrachloroethylene. Conditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs. Limitations of the study include its lack of exposure verification and inability to assess intensity of exposure.

B.2.2.8.4. Shu et al. (1999)

Shu, X. O.; Stewart, P.; Wen, W. Q.; Han, D.; Potter, J. D.; Buckley, J. D., . . . Robison, L. L. (1999). Parental occupational exposure to hydrocarbons and risk of acute lymphocytic leukemia in offspring. Cancer Epidemiol Biomarkers Prev, 8, 783-791. http://www.ncbi.nlm.nih.gov/pubmed/10498397

Summary: This case-control study examined the association between parental occupational exposure and the risk of childhood acute lymphocytic leukemia. Potential cases and controls were required to meet the following criteria: have a telephone in their place of residence and have their English-speaking, biological mother available for an interview. Cases consisted of children aged 15 years and under who were diagnosed with acute lymphocytic leukemia between 1989 and 1993 by 1 of 37 participating Children's Cancer Group members or institutions from Australia, California, Canada, Colorado, Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Nebraska, New York, North Carolina, Ohio, Oregon, Pennsylvania, Tennessee, Texas, Washington state, Washington, DC, Wisconsin, and Utah. Of the 2,081 eligible cases, 1,914 (92%) mothers were interviewed. Controls were chosen through random digit dialing and matched to cases based on age, race, and telephone area code and exchange. Of the 2,597 eligible controls, 1,987 (76.5%) mothers were interviewed. After excluding the 72 cases for whom a matched control could not be found, the final sample consisted of 1,842 cases and 1,986 controls.

Telephone interviews were conducted with case mothers and fathers using a structured questionnaire. The maternal questionnaire inquired about demographics; personal habits; household exposures before and during index pregnancy; exposure to environmental hazards; and occupational, medical, reproductive, and family histories. The paternal questionnaire inquired about personal habits; household exposures; and medical, occupational, and family histories. Of the 2,081 eligible cases and 2,597 eligible controls, fathers were interviewed for 1,801 (86.5%) cases and 1,183 (69.8%) controls, yielding 1,618 matched sets. The majority (83.4% cases and 67.7% controls) consisted of direct interviews with fathers; the remainder were proxy interviews with the mothers.

Maternal occupational histories were collected for all jobs that lasted at least 6 months and occurred between the 2 years prior to the pregnancy and the case's diagnosis, while paternal occupational histories were obtained for all jobs lasting at least 6 months from age 18 years onwards. Both maternal and paternal occupational histories inquired about job titles, industries, duties, dates of employment, and exposure to solvents/degreasers/cleaning agents, plastic materials, paints, pigments/thinners, and oil/coal products. Any self-reported exposures that were not included as part of the exposures listed in the questionnaire were blindly assessed by an industrial hygienist and placed into the established exposure categories. Maternal and paternal dates of employment were used to determine whether an exposure occurred during preconception, pregnancy, or the postnatal period; duration of exposure within each of these time frames was calculated and categorized using the control group's median time as the cutoff. Maternal exposures to tetrachloroethylene occurred anytime in 4 (0.2%) cases and 9 (0.5%) controls, during preconception in 3 (0.2%) cases and 2 (0.1%) controls, during pregnancy in 3 (0.2%) cases and 2 (0.1%) controls, and during the postnatal period in 4 (0.2%) cases and 8 (0.4%) controls. Paternal exposures to tetrachloroethylene occurred anytime in 25 (1.4%) cases and 23 (1.9%) controls, during preconception in 21 (1.2%) cases and 22 (1.9%) controls, during pregnancy in 8 (0.4%) cases and 14 (1.2%) controls, and during the postnatal period in 10 (0.6%) cases and 15 (1.3%) controls.

Conditional logistic regression was used to estimate odds ratios and their corresponding 95% CIs for maternal exposures, adjusted for maternal education, race, and family income. Unconditional logistic regression was used to calculate odds ratios and their corresponding 95% CIs for paternal exposures, adjusted for paternal education, race, family income, age, and sex of the case. Tests for trend were conducted by incorporating the categorical variables of exposure as continuous variables in the models. No strengths were reported by the authors. Limitations included self-reported information on exposure based on a list of specific exposures provided to the participant, the lack of information on intensity or level of exposure, the lack of specific information related to additional exposures that prevented their categorization, low prevalence of maternal tetrachloroethylene exposure, and the high proportion of proxy paternal interviews that likely results in an increased potential for misclassification bias.

B.2.2.9. Neuroblastoma

B.2.2.9.1 DeRoos et al. (2001)

De Roos, A.; Olshan, A.; Teschke, K.; Poole, C.; Savitz, D.; Blatt, J., . . . Pollock, B. (2001). Parental occupational exposures to chemicals and incidence of neuroblastoma in offspring. Am J Epidemiol, 154, 106-114. http://dx.doi.org/10.1093/aje/154.2.106

Summary: This case-control study evaluated the effects of parental occupational exposure on neuroblastoma incidence in offspring. Cases consisted of male and female children aged 18 years or under who were diagnosed with neuroblastoma between 1992 and 1994 and registered in one of 139 participating hospitals in either the United States or English-speaking Canada. Of the 741 eligible cases, 538 (73%) were enrolled in the study. A control was selected for each of 504 cases through random digit dialing and matched to cases based on birth date. Approximately 71% of the eligible controls were recruited, and 74% of the households that were screened participated. After excluding those with missing occupational exposures and all proxy interviews, the final sample consisted of 537 case mothers, 405 case fathers, 503 control mothers, and 302 control fathers.

Telephone interviews were conducted with both mothers and fathers and inquired about demographics as well as lifetime occupational history, which included dates of employment, names of employers, occupations, industries, job titles, specific duties, and hours per week. Chemical exposure histories were requested for all jobs held within 2 years of the index child's birth. Interviews were conducted with 537 case mothers, 472 case fathers, 503 control mothers, and 445 control fathers, though 67 (14.2%) of the case father interviews and 141 (31.7%) of the control father interviews were completed by mothers as the proxy respondent. All proxy interviews were subsequently excluded from the analyses. Exposure was assessed in two ways. First, the participant was asked to report his/her possible exposure to any of 65 substances, as well as the form (liquid, gas, dust, smoke, solid) and route (inhalation, dermal, ingestion, clothing) of the exposure, the activities being performed during the exposure, the number of hours per week exposed, and the time frame during which the exposure occurred.

Second, these responses were then reviewed by a blinded industrial hygienist who reclassified any improbable exposures as nonexposed. The hygienist did not review the responses of participants who reported no exposure to any of the possible chemicals during their jobs; as a result, jobs that may have had exposure potential were not reclassified. The substances themselves were classified into five categories: halogenated hydrocarbons, a category which included tetrachloroethylene, nonvolatile hydrocarbons, volatile hydrocarbons, paints/inks/pigments, and metals/alloys/solders. Maternal exposure to halogenated hydrocarbons was reported by 15 (2.8%) cases and 19 (3.8%) controls. After the industrial hygienist's review, this decreased to 6 (1.1%) cases and 8 (1.6%) controls. Among the fathers, 8 (2.0%) cases and 11 (3.6%) controls reported exposure to tetrachloroethylene more specifically; the industrial hygienist's review subsequently decreased this to 4 (1.0%) cases and 6 (2.0%) controls.

Unconditional logistic regression was used to calculate exposure odds ratios and their corresponding 95% CIs for each of the five categories of substances as well as for each of the individual chemicals, adjusted for the child's age, maternal race, maternal age, and maternal

education. Limitations to the study include possible misclassification of self-reported exposures, lack of adjustment for smoking, and recall bias. Additionally, the researchers' focus on correcting false positives means that the study may have included false negatives. No strengths were reported by the authors.

B.2.2.10. Pancreatic Cancer

B.2.2.10.1. Kernan et al. (1999)

Kernan, G. J.; Ji, B. T.; Dosemeci, M.; Silverman, D. T.; Balbus, J.; Zahm, S. H. (1999). Occupational risk factors for pancreatic cancer: A case-control study based on death certificates from 24 U.S. States. Am J Ind Med, 36, 260-270. http://dx.doi.org/10.1002/(SICI)1097-0274(199908)36:2<260::AID-AJIM5>3.0.CO;2-P

Summary: This study used a case-control design to examine the risk of pancreatic cancer by occupation, industry, and exposure to solvents, including tetrachloroethylene. Cases were identified using International Classification of Disease Code 157 (pancreatic cancer) on death certificates in 24 states (Maine, New Hampshire, New Jersey, Rhode Island, Vermont, Indiana, Ohio, Wisconsin, Kansas, Oklahoma, Missouri, Nebraska, Kentucky, Georgia, North Carolina, South Carolina, Tennessee, West Virginia, Colorado, Idaho, Nevada, New Mexico, Utah, and Washington) that also included codes for occupation and industry, based on 1980 Census codes. Controls were chosen from among those who died of nonpancreatic, noncancer causes within the same time frame. Each case was matched to four controls based on state, race, gender, and 5-year age group. For the study period 1984−1993, 63,097 cases and 252,386 controls were selected. JEMs were developed by industrial hygienists for the solvents, including tetrachloroethylene. Indexes of probability and intensity of exposure to tetrachloroethylene were estimated and scored as "low," "medium," and "high." Overall, there were 5,344 participants exposed to "low" levels, 2,187 exposed to "medium" levels, and 903 exposed to "high" levels of tetrachloroethylene. Although not cited in the paper, the author's affiliation with the National Cancer Institute and the identified solvents make it likely that the JEM was that of Gomez et al. (1994) and Dosemeci et al. (1994).

Race and gender-specific mortality odds ratios and their corresponding 95% CIs were estimated for intensity and probability of exposure to solvents, including tetrachloroethylene, adjusted for age, marital status, metropolitan status, and region of residence. A strength of the study is its use of the JEM in exposure assessment. Limitations include the possibility of missing information related to occupation and potential confounders on death certificates, as well as the potential for misdiagnosis of pancreatic cancer.

B.2.2.10.2. Lin and Kessler (1981)

Lin, R. S. and Kessler, I. I. (1981). A multifactorial model for pancreatic cancer in man: Epidemiologic evidence. JAMA, 245, 147-152.
http://www.ncbi.nlm.nih.gov/pubmed/7452829

Summary: This case-control study aimed to collate information of malignant neoplasms whose prevalence was so low as to render investigations in one institution—or even one city—largely impractical. The study was conducted in over 115 hospitals in Buffalo, Detroit, Miami, Minneapolis-St. Paul, and New York City and collected information on 13 (adrenal, gallbladder, kidney, liver, nasopharynx, pancreas, ureter, urethra, breast, penis, testis/scrotum, vagina, and vulva) cancers. Cases were identified through medical records and the pathology departments of each hospital and consisted of men and women aged 15 and over. Controls were randomly chosen from the admissions records of cancer-free patients of the same hospital as the case and matched to cases based on age, sex, race, and marital status. The authors do not report the response rates for cases and controls, though they note that 22% of those eligible were not interviewed due to the extremity of their situation. Once these individuals were excluded, the male and female response rates were 86.2% and 86.3%, respectively. The final sample consisted of 109 case-control pairs (67 male pairs and 42 female pairs).

In-person interviews were conducted by blinded interviewers in the hospital or at the participant's home. The majority took place in the hospital and inquired about demographics, residential history, occupations, toxic exposures, animal contacts, smoking habits, diet, medical history, medications, and family history. The occupational history encompassed all jobs that were held full-time for at least 6 months or part-time for at least 1 year. Men were also asked about their sexual practices and urogenital conditions, and women were questioned about their marital, obstetric, and gynecologic histories. All medical conditions that were diagnosed within 1 year of the cancer diagnosis were excluded. Duration of exposure to dry cleaning and gasoline derivatives was categorized into 0 years, ≤2 years, 3−5 years, 6−10 years, and >10 years. Overall, there were 25 (37.3%) male cases and 23 (34.3%) male controls exposed to either dry-cleaning or gasoline derivatives.

Chi-squares and *t*-tests were used to examine the differences between cases and controls. Odds ratios were calculated to estimate the relative risk for pancreatic cancer among men and women who were exposed to a variety of risk factors, including occupational exposure to dry cleaning. A strength of this study is its detailed questionnaire, inquiring about part-time and full-time jobs, as well as a variety of possible confounders. A limitation is its failure to differentiate between occupational exposures to dry-cleaning and gasoline derivatives, as well as control group biases weighting for diabetics, which might have obscured the observed associations with pancreatic cancer.

B.2.2.11. Renal Cell Cancer

B.2.2.11.1. Asal et al. (1988)

Asal, N. R.; Geyer, J. R.; Risser, D. R.; Lee, E. T.; Kadamani, S.; Cherng, N. (1988). Risk factors in renal cell carcinoma. II. Medical history, occupation, multivariate analysis, and conclusions. Cancer Detect Prev, 13, 263-279. http://www.ncbi.nlm.nih.gov/pubmed/3266567

Summary: This population-based case-control study examined risk factors of renal cell carcinoma. Cases were identified from 29 hospitals in Oklahoma that agreed to participate in the study. The authors do not report details regarding where the hospitals were located, but they do note that they included Tulsa and Oklahoma City. Cases consisted of men and women with renal cell cancer who had a tissue or radiological examination diagnosis between 1981 and 1984. Two sets of controls were used in this study. The first comprised hospital-based controls matched to cases based on age, sex, race, hospital, and date of admission. The authors do not state how they identified the hospital-based controls, and it is not known whether controls were drawn from the same hospitals as cases, though they excluded anyone diagnosed with a kidney disease or a psychiatric illness. The second group consisted of population-based controls from the general Oklahoma population and were chosen through random digit dialing according to Waksberg (1978). These controls were matched to cases based on age and sex. Of 345 identified cases, 315 (91.3%) participated in the study. Those not included in the final sample either refused or were unable to participate or did not notify the study on time. The authors did not provide the response or participation rates for control groups. The final sample included 315 cases, 313 hospital-based controls, and 336 population-based controls.

Interviews were conducted in the hospital with cases and hospital controls and in the home or business with population-based controls, inquiring about medical history, medications, radiation exposure, occupational history for all jobs held at least 1 year, self-reported industrial exposure, tobacco smoking, beverage use, artificial sweeteners, family history of disease, height and weight at age 20, weight most recently, and highest weight. BMI was calculated from reported height and weights, and the occupational history was used to identify the predominant occupation or the job held the longest out of all reported occupations lasting 1 year or more. Employment in occupations and industries were assessed as a proxy for exposure; dry cleaning was examined as a high-risk industry and had 11 (3.5%) cases and 7 (1.1%) controls reporting at least 1 year of employment. Cox linear logistic regression modeling was employed to estimate odds ratios and 95% CIs for lifetime occupations and high-risk industries. All of the predominant lifetime occupations in men were adjusted for age, smoking, and weight. The authors do not report lifetime occupation calculations for women. The industry estimates varied in their adjustment of confounders. Painting and welding only adjusted for age, while chemical

manufacturing, machining, petroleum refining, dry cleaning, and metal degreasing adjusted for age, smoking, and weight. All industry calculations were stratified by gender, though only petroleum refining and dry cleaning reported estimates for both men and women. Strengths of the study include its use of confirmed cases of renal cell carcinoma, its population-based design, its use of two control groups, and its adjustment for smoking. Limitations include its low-exposure prevalence and its inability to distinguish between jobs within the dry-cleaning industry.

B.2.2.11.2. Auperin et al. (1994)

Auperin, A.; Benhamou, S.; Ory-Paoletti, C.; Flamant, R. (1994). Occupational risk factors for renal cell carcinoma: A case-control study. Occup Environ Med, 51, 426-428. http://dx.doi.org/10.1136/oem.51.6.426

Summary: This hospital-based case-control study examined the relationship between occupation and renal cell carcinoma in France between 1987 and 1991. Cases consisted of 138 men and 58 women with histologically confirmed renal cell carcinoma in 1 of 10 hospitals. Two controls, one with a malignant disease and one with a nonmalignant disease (excluding tobacco related diseases), were matched for each case based on sex, age at interview, hospital, and interviewer. Patients with alcohol-related cirrhosis or diabetes were excluded from the study. Eligibility and matching criteria caused some recruitment difficulties, resulting in 151 cases being matched to 2 controls and 45 cases being matched to 1 control. In total, the study consisted of 161 controls with cancer (107 men and 54 women) and 186 controls with nonmalignant disease (128 men and 58 women). Only one of the eligible cases and two of the eligible controls refused to participate in the interview.

Trained interviewers used a standardized questionnaire to obtain information on education, height, weight, smoking habits, beverage consumption, and medication, as well as a complete occupational history. In the occupational history, participants provided their duration of employment for each job held (minimum 1 year). Although interviewers were not blinded to the individual's case or control status, the job history data were coded blindly, according to the International Standard Classification of Occupations. The authors did not report the code for launderers or dry cleaners. The numbers of exposed were not reported for launderers or dry cleaners, though the authors noted that the estimates for laundry workers could not be calculated due to the small numbers of exposed.

Conditional logistic regression was used to estimate odds ratios and their 95% CIs for occupations, including launderers and dry cleaners. Analyses looked at women and men separately, and matched odds ratios were adjusted for the matching criteria (age, hospital, interviewer). Covariates included educational level, cigarette smoking, and the Quetelet index. After similar results were obtained for each of the control groups, the groups were pooled into

one control group. The authors do not report strengths of their study. A limitation is the small number of exposed laundry workers.

B.2.2.11.3. Delahunt et al. (1995)

Delahunt, B.; Bethwaite, P. B.; Nacey, J. N. (1995). Occupational risk for renal cell carcinoma. A case-control study based on the New Zealand Cancer Registry. Br J Urol, 75, 578-582. http://dx.doi.org/10.1111/j.1464-410X.1995.tb07410.x

Summary: This registry-based case-control study investigated the risk for renal cell carcinoma among various occupational groups. Cases consisted of men and women aged 20 years and older who were diagnosed with renal cell carcinoma and registered in the New Zealand Cancer Registry between 1978 and 1986. Controls were randomly selected from among all other cancer cases during this same time frame, excluding those with a primary tumor outside of the urinary tract, and included men and women aged 20 years or older. Cases or controls without active occupational codes in their New Zealand Cancer Registry files were excluded from the analysis. Of the 1,060 identified cases, 914 (86.2%) were eligible for and included in the study. The proportion of female participants with occupational information was low (204 cases); as such, they were excluded from the analysis. The authors did not report any information regarding how many controls were identified for inclusion. The final sample consisted of 710 male cases and 12,756 male controls.

All information was obtained from the New Zealand Cancer Registry, which in 1978, began recording patients' current or most recent occupations and smoking habits. The registry coded all occupations according to the New Zealand Standard Classification of Occupations, and the authors did not delineate the codes used for each of the occupations they examined. All of the occupations included in this study were determined *a priori* due to their previously established or potential association with renal cell carcinoma. This included dry cleaning, which was classified within the occupational category of services. Overall, there were a total of 52 male cases (7.3%) and 737 male controls (5.8%) whose occupation was classified as a service, including catering/lodging, hairdressers, firefighters, and policemen, in addition to dry cleaners. The authors did not provide the numbers of case and control dry cleaners.

The Mantel-Haenszel method was used to estimate relative risks in stratified 10-year age groups for each occupation, including dry cleaning, and Miettinen's approximation method was used to calculate their associated 95% CIs. All were stratified by smoking history and 10-year age groups. A strength of this study is its use of other cancer patients in the registry for the selection of controls, which reduces information and selection bias. Limitations include selection bias if other cancers are associated with the selected occupations and/or their

exposures, the assumption that current or most recent occupation represented lifetime occupation, and the lack of stratification of service jobs in terms of exposure prevalence.

B.2.2.11.4. Dosemeci et al. (1999)

Dosemeci, M.; Cocco, P.; Chow, W. H. (1999). Gender differences in risk of renal cell carcinoma and occupational exposures to chlorinated aliphatic hydrocarbons. Am J Ind Med, 36, 54-59. http://www.ncbi.nlm.nih.gov/pubmed/10361587

Summary: This case-control study evaluated the effects of organic solvents on renal cell carcinoma risk in Minnesota. Cases were identified through the Minnesota Cancer Surveillance System and consisted of Caucasian men and women aged 20 to 85 years who were diagnosed with histologically confirmed renal cell carcinoma between 1988 and 1990 (Chow et al., 1994). Of 796 eligible cases, 690 (87% response rate) were interviewed. Two groups of controls were elicited: the first group included Caucasian men and women between 20 and 64 years of age who were identified through age- and gender-stratified random digit dialing; the second group consisted of Caucasian men and women aged 65 years and older who were identified through an age- and gender-stratified systematic sample of Health Care Financing Administration lists. Overall, 707 (86% response rate) controls were interviewed. The final sample for the occupational analyses consisted of 438 cases and 687 controls.

In-person interviews were conducted with blinded, trained interviewers about demographics, diet, smoking, and drug use, as well as medical, residential, and occupational histories. Of the 690 case interviews completed, 241 (34.9%) were proxy with next of kin. The occupational history inquired about recent and usual job and industry, activities performed, dates of employment, and part-time or full-time status. Duration of employment was also obtained for 13 occupations and industries, as well as 7 occupations with specific exposures. A job exposure matrix (Gómez et al., 1994) was used to estimate exposures based on reported occupations and industries. Occupations and industries were coded according to four digit U.S. SIC and SOC codes, respectively. All of the four digit codes were assigned exposure estimates of probability (i.e., "low," "medium," "high") and intensity (1, 2, 3) *a priori*. Intensity was defined as an average of the concentration and frequency of exposure. Occupations were also assigned a category. Jobs that fell within Category A, such as dry cleaner operators, had sufficient information to be assessed for exposure independent of their industry. For jobs that fell within Category B, the probability of exposure depended entirely on the industry, and the intensity was weighted by both the occupation and the industry. Those in Category C had their probability and intensity of exposure fully determined by the industry within which the job fell. Time of employment was accounted for in the matrix through a decade indicator. Overall, 48 (11%) cases and 76 (11%) controls were identified as potentially "ever" exposed to tetrachloroethylene.

Logistic regression using the Breslow and Day (1980) method was employed to estimate relative risks and their corresponding 95% CIs, adjusted for age, smoking, BMI, and hypertension status and/or use of diuretics and/or antihypertension drugs. All analyses were stratified by gender and did not include subjects with proxy respondents. The authors did not examine duration of employment. The authors did not report any strengths of their methodology; limitations include the small number of exposed participants, potential survival bias, and the lack of a lifetime occupational history.

B.2.2.11.5. Harrington et al. (1989)

Harrington, J. M.; Whitby, H.; Gray, C. N.; Reid, F. J.; Aw, T. C.; Waterhouse, J. A. (1989). Renal disease and occupational exposure to organic solvents: A case referent approach. Br J Ind Med, 46, 643-650. http://dx.doi.org/10.1136/oem.46.9.643

Summary: This case-control study conducted a detailed "blind" exposure assessment to identify occupational risk factors for renal cancer. Cases were identified through the West Midlands Regional Cancer Registry and consisted of living men and women in West Midlands with histologically confirmed renal adenocarcinoma that was diagnosed between May 1984 and April 1985. Controls were randomly selected from among the patient loads of each of the case's general practitioners and matched (one control per case) based on 5-year age group, sex, ethnicity, geographical location, and socioeconomic status. Of the 101 eligible renal cancer cases, 85 (84%) were allowed to be contacted, and of these, 59 (69%) cases agreed to be interviewed. Due to the fact that 5 of the cases were unable to have matched controls, the final sample decreased to 54 cases and 54 controls.

In-person interviews were conducted with each participant, inquiring about personal habits, such as smoking, coffee, and alcohol consumption; medical history; and occupational history. Exposure was assessed blindly by an experienced chemist/occupational hygienist using an independent checklist of exposures to solvents; exposure indices were calculated by a computer program that multiplied the exposure level by the duration of exposure. None of the cases or controls reported exposure to dry-cleaning fluids, but it appears that 9 (16.7%) cases and 12 (22.2%) controls reported exposure to degreasing agents. Paired analyses were conducted to calculate odds ratios and 95% CIs in two exposure categories using Schlesselman (1982) and three exposure categories using Pike et al. (1975). There were no strengths reported by the authors. Limitations to this study include its small sample size (low power), low prevalence of exposure to dry-cleaning fluids, low response rate, unaddressed renal cancer latency, and possible recall bias associated with self-reporting.

B.2.2.11.6. Mandel et al. (1995)

Mandel, J. S.; McLaughlin, J. K.; Schlehofer, B.; Mellemgaard, A.; Helmert, U.; Lindblad, P., . . . Adami, H.-O. (1995). International renal-cell cancer study. IV. Occupation. Int J Cancer, 61, 601-605. http://dx.doi.org/10.1002/ijc.2910610503

Summary: This international, multicenter case-control study evaluated factors possibly related etiologically to renal cell cancer (Mandel et al., 1995). Six centers in five countries (Australia, Denmark, Germany, Sweden, and United States;one center in each country, with the exception of Germany, which had two) participated in the study. Each center had different start dates, which were not provided. Cases were identified through population-based cancer registries in all locations except Germany, where they were obtained through a surveillance of all departments where renal cell cancer was diagnosed or treated. Cases consisted of men and women aged 20 to 79 years (20−75 in Heidelberg) who were diagnosed with histologically or cytologically confirmed renal cell adenocarcinoma between 1989 and 1991. In all centers except in Australia and the United States, participants were required to have been born in their respective countries. Controls were ascertained through the following: population-based registers in Denmark and Sweden, electoral rolls in Australia, residential lists in Germany, and either random digit dialing for American controls <65 years of age or Health Care Finance Administration lists for American controls ≥65 years. All controls were matched to cases based on gender and 5-year age group. The final sample consisted of 1,732 cases (73.2% response rate) and 2,309 controls (74.7% response rate); cases and controls were comparable in terms of demographics: approximately 60% were men, and 62% were over the age of 60 years at the time of their diagnosis or interview.

In-person interviews were conducted by trained interviewers either in the hospital (German cases) or in the participant's home (German controls and all other countries) and inquired about tobacco, diuretics, analgesics, antihypertensive drugs, diet pills, hormones and alcohol, height and weight, physical activity, medical and reproductive histories, family history of cancer, demographics, and occupational history. The two centers in Germany obtained complete occupational histories, and the four other centers asked about industries, occupations, and exposures of interest. Occupations and industries were coded according to various standards, including the International Labour Office (1968, 1988), the UN Department of Economic and Social Affairs (1968, 1971, 1990), the U.S. Department of Commerce (1980), and the U.S. Office of Management and Budget (1987). Only those occupations, industries, or exposures that were commonly reported by all study centers were included in the analysis. The authors do not state if the codes were harmonized or if exposure was assessed blindly. Duration of exposure was assessed as the total number of years worked or exposed and was subsequently divided into tertiles based on the distribution among controls. Exposures to dry-cleaning

solvents were stratified into duration categories of 1−7, 8−25, and 26−60 years. Participants were determined to be "exposed" if they had been employed in the occupation or industry or had been exposed to the chemical of interest for at least 1 year. There were 23 (1.3%) cases and 28 (1.2%) controls who reported "ever" working in the dry-cleaning industry and 302 (17.5%) cases and 265 (11.5%) controls who reported "ever" exposure to dry-cleaning solvents.

Logistic regression was used to estimate odds ratios and their corresponding 95% CIs stratified by gender and adjusted for age, smoking status, BMI, education, and study center. These estimations were performed for industry, occupation, exposure, and duration of exposure based on the categories stated above separately, though only the results for men were presented, as there were fewer cases among women were exposed. Tests of heterogeneity were used to assess differences between centers. Odds ratios and their corresponding 95% CIs were also calculated to assess the effect of education, stratified by gender and adjusted for age, smoking, BMI and hypertension, and study center. A strength of this study is its large sample size and standardized methodology to collect information. Limitations are its failure to verify self-reported data and its inability to examine specific chemical agents, which was carried out by Dosemeci et al. (1999) for a subset of this study's cases from Minnesota.

B.2.2.11.7. McCredie and Stewart (1993)

McCredie, M. and Stewart, J. H. (1993). Risk factors for kidney cancer in New South Wales. IV. Occupation. Br J Ind Med, 50, 349-354. http://www.ncbi.nlm.nih.gov/pubmed/8494775

Summary: This case-control study sought to report the results of a New South Wales study examining the relationship between occupational exposure and renal cell cancer, as well as those pertaining to cancer of the renal pelvis. Cases were identified through urologists and the New South Wales Central Cancer Registry and consisted of men and women aged 20 to 79 years who were diagnosed with renal cell and renal pelvis cancer between 1989 and 1990. In order to be included in the study, cases needed to be registered in the current electoral roll, have a telephone number that could be found, and be able to speak English. Controls were selected through a proportional random sample of electoral rolls. Of the 744 eligible renal cell cancer cases and 200 eligible renal pelvis cancer cases, 503 (68%) renal cell and 149 (75%) renal pelvis cancer cases were interviewed. Of the 725 eligible controls, 535 (74%) participated in the interview. After excluding for those who completed self-administered questionnaires, the final sample included 489 renal cell cancer cases, 147 renal pelvis cancer cases, and 523 controls.

Interviews were conducted by a trained interviewer, and all but 10 case interviews took place within 1 year of diagnosis. Depending on the proximity of the participant to Sydney, interviews consisted of one of three formats: in-person (256 renal cell cases, 71 renal pelvic

cases, and 232 controls), telephone (233 renal cell cases, 76 renal pelvic cases, and 291 controls), and self-administered (14 renal cell cases, 2 renal pelvic cases, and 12 controls). The questionnaire inquired about demographics, chemical exposures, and employment in various occupations and industries. Occupation was assessed as a proxy for exposure, and there were 16 (3.3%) renal cell cases, 8 (5.4%) renal pelvic cases, and 7 (1.3%) controls who reported employment in the dry-cleaning industry.

Logistic regression was used to estimate relative risks and their corresponding 95% CIs, adjusted for age, sex, method of interview, and smoking. Renal cell cancer estimates were also adjusted for BMI, and renal pelvic cancer estimates were further adjusted for education and phenacetin-containing analgesics. A strength of this study is its adjustment for smoking; limitations included the small exposure prevalence, potential recall bias due to self-reported exposures, and the study's lack of detailed occupational information, which prevented any assessment of intensity of exposure.

B.2.2.11.8. Mellemgaard et al. (1994)

Mellemgaard, A.; Engholm, G.; McLaughlin, J. K.; Olsen, J. H. (1994). Occupational risk factors for renal-cell carcinoma in Denmark. Scand J Work Environ Health, 20, 160-165. http://www.ncbi.nlm.nih.gov/pubmed/7973487

Summary: This study used a population-based case-control design to examine the relationship between employment in specific occupations and risk of renal cell carcinoma. Cases were selected from among the Danish Cancer Registry and consisted of men and women aged 20 to 79 years who were born and living in Denmark. Controls were chosen from the Central Population Register and matched to cases based on gender and 5-year age group. After selecting the controls, the researchers found that they failed to account for the structure of the Central Population Register and had obtained an inaccurate representation of certain regions. To address this problem, the researchers randomly removed controls from the regions that had been overrepresented and randomly selected additional controls from the regions that had been underrepresented. Of the 482 eligible cases, 368 (76%) were interviewed. Of the 500 eligible controls, 396 (79%) were interviewed.

In-person interviews were conducted by trained interviewers who inquired about occupation and occupational exposure histories, as well as demographics, smoking, medical history, and diet. Jobs were coded according to the International Standard Classification of Occupation, and industries were coded according to the International Standard Industrial Classification. Although dry cleaning was among those identified *a priori* as a high risk industry, the authors did not provide the specific code used. Exposures were assessed for jobs held at least 1 year and occurred at least 10 years prior to the interview. A total of 4 (1.1%)

cases and 2 (0.5%) controls were employed in the dry-cleaning industry. Unconditional logistic regression was used to estimate odds ratios and 95% CIs for men and women separately, adjusted for age, BMI, and smoking. Strengths of the study are its population-based design and its response rate of nearly 80% for both cases and controls. A limitation to the study is its low number of exposed participants.

B.2.2.11.9. Schlehofer et al. (1995)

Schlehofer, B.; Heuer, C.; Blettner, M.; Niehoff, D.; Wahrendorf, J. (1995). Occupation, smoking and demographic factors, and renal cell carcinoma in Germany. Int J Epidemiol, 24, 51-57. http://www.ncbi.nlm.nih.gov/pubmed/7797356

Summary: This population-based case-control study examined the demographic and occupational risk factors, as well as the risk of smoking on the development of renal cell cancer in the Rhein-Neckar-Odenwald area of Germany. Cases consisted of German men and women with histologically confirmed renal cell cancer between 1898 and 1991. Of the 328 cases identified, 277 (84.5%) participated in the study. Controls were randomly selected from the population register of the Rhein-Neckar-Odenwald area and matched to cases based on age and gender. Of the 381 controls identified, 286 (75%) participated in the study.

In-person interviews were conducted by trained interviewers with both cases and controls. The majority (92%) of cases was interviewed in the hospital; all of the control interviews took place at participants' homes. Efforts were made to interview matched cases and controls within 6 months of the case's diagnosis. A standardized questionnaire was used to collect information on demographics, smoking history, occupational history, medical history, family history, physical activity, weight, and diet. Occupational information was obtained on four levels: (1) all industries in which the subject was "ever" employed, (2) occupations in which the subject was trained, (3) activities performed during employment, and (4) exposure to specific substances. An individual was assessed as "exposed" to an industry, occupation, or substance if it occurred for 5 years or more. Industries were coded, and industries, occupations, and activities were grouped into different categories. Of the 51 substances examined for possible exposure, 22 were reported by at least 5% of male subjects and subsequently analyzed. This included chlorinated solvents, which consisted of tetrachloroethylene and tetrachlorocarbonate, and contained a total of 27 cases (14.6%) and 12 (13%) controls. Female exposures were not prevalent and, therefore, not examined in this study.

Unconditional logistic regression was used to calculate odds ratios and their corresponding 95% CIs for demographics, smoking, industry/occupation, and substance exposure separately. Demographic calculations were adjusted for age and smoking; smoking was adjusted for age; industry and occupational groups were adjusted for age, gender, and

smoking; and substance groups were adjusted for age and smoking. Limitations to the study include its inability to independently evaluate the impact of tetrachloroethylene versus tetrachlorocarbonate within the chlorinated solvents category and possible misclassification due to self-reported exposure. The authors do not report any strengths of their methodology.

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
British Columbia					
Band et al. (1999); MacArthur et al. (2009)	Cases: from British Columbia Cancer Registry from 1983−1990, men, ≥20 yr, histologically confirmed cancer; Controls: from all other cancer sites examined, matched to cases on age, year of diagnosis Proxy—laundry and dry cleaner Site-specific cancer incidence (prostate, lung)	25,726 eligible cases contacted, 15,463 (60%) returned questionnaire	Self-administered questionnaire: demographics, lifetime smoking habits, alcohol consumption, occupational history (lifetime job descriptions, duration and period of employment, occupation/industry titles)	Occupations/industries coded according to Canadian SOC and the Canadian SIC; launderers and dry cleaners SIC Code 972, SOC code not reported; assessed "ever" and "usual" (longest employment) occupations/industries	Various (see below)
Band et al. (1999)	Cases: prostate cancer; Controls: excluded lung cancer, cancer of unknown primary site Prostate cancer incidence	Final sample: 1,516 cases and 4,994 controls	See above Proxy respondents for 19.9%, 19.3% controls	7 (0.5) cases ever, 2 (0.1%) cases usual employment in dry-cleaning industry; control exposures not reported	Conditional logistic regression for ORs, 95% CIs for occupations/industries, adjusted for education, alcohol consumption, smoking duration

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
MacArthur et al. (2009) Evaluate occupational risks for lung cancer Case-control	Cases: lung cancer; Controls: excluded unknown primary sites Proxy—launderers and dry cleaners Lung cancer (squamous cell carcinoma, adenocarcinoma, small cell, large cell)	5,528 eligible lung cancer cases, 2,998 (54.2%) returned questionnaire	See above	10 (0.3%) cases of lung cancer in SIC Code 972 (laundries and dry cleaners)	Matched case-control analyses for maximum likelihood estimates for ORs, 90% CIs; lung cancer subtypes separately evaluated Adjustments: all lung cancers—smoking, alcohol, education, questionnaire respondent; subtypes—varied
Band et al. (2000)	Cases: from British Columbia Cancer Registry, women <75 yr, diagnosed with breast cancer from 1988−1989, Canadian citizens, residents of British Columbia, English speaking, no prior history of breast cancer; Controls : randomly selected from 1989 from British Columbia Provincial Voters List, no history of breast cancer before 1989, matched on age Proxy—dry cleaning Breast cancer incidence	1,489 eligible cases and 1,502 eligible controls, 1,018 (68%) cases and 1,025 controls returned questionnaire Final sample: 995 cases, 1,020 controls	Self-administered questionnaire: lifetime job descriptions, duration/period employment, occupation/industry titles, demographics, smoking, alcohol consumption, current body weight, weight in late teens, age at menarche, parity, age at first birth, history of breast biopsy before 1987, family history of breast cancer, breast feeding, birth control, estrogen replacement therapy	Occupations/industries coded according to Canadian SOC and Canadian SIC; dry cleaning: SOC Code 6162 and SIC Code 9721; assessed “usual” and “ever” occupation; 12 (1.2%) cases “ever” exposed, 9 (0.9%) cases “usual” exposure to laundry and dry cleaning occupation; 23 (2.3%) cases “ever” exposed, 10 (1.0%) cases “usual” exposure to power laundries and/or dry-cleaners industry; no information on control exposure	Conditional logistic regression for ORs and 90% CIs for each occupation for each estimate of exposure, stratified by menopausal status and “ever”/“usual” occupation; premenopausal adjustment: cigarette pack years groups, breast biopsy, family history of breast cancer in mother/sisters; Postmenopausal adjustment: weights in 1986, family history of breast cancer in first degree relative, history of breast biopsy for benign breast disease, cumulative alcohol scores; all women combined: both pre- and postmenopausal covariates

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Teschke et al. (1997)	Cases: from British Columbia Cancer Agency, men and women, 19+ yr, histologically confirmed nasal cavity/sinus/urinary bladder cancer from 1990−1992, exclusions: bladder cancer cases born before 1916 and carcinoma in situ; Controls: British Columbia residents, 19+ yr, randomly selected from provincial voter list, matched to cases based on age, sex; exclusions: in prison or mental health institution Proxy—laundry personnel Nasal cavity or sinus cancer, urinary bladder cancer	54 eligible nasal cancer cases and 195 eligible nasal cancer controls, 48 (88.9%) cases and 159 (81.5%) controls interviewed 119 eligible bladder cancer cases and 173 eligible bladder cancer controls, 105 (88.2%) cases and 139 (80.3%) controls interviewed Final sample: 153 cases and 298 controls	In-person or telephone interview by nonblinded RN; proxy interviews if not an English speaker, poor memory of life events, or deceased Structured questionnaire: occupational, residential, medical, smoking, exposure histories Blinded industrial hygienist evaluated interviews and asked follow-up questions when necessary	Occupations and industries coded according to standard classifications, blindly grouped; assignment based on whether occupation or industry more likely to determine exposure; if both, occupation used; all reviewed to verify accuracy; all groups with <20 reviewed for combination with others; In total, 57 occupational groups created No case/control laundry personnel for nasal cancer; 5 cases (3.3%), 4 (1.3%) controls of laundry personnel for bladder cancer	Exact methods for summary ORs and 95% CIs; if nonoccupational risk factors found positively associated, unconditional logistic regression for ORs and 95% CIs, adjusted for risk factors; all adjusted for sex, age, smoking Latency times: 5, 10, 15, 20 yr; only 20 yr reported

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Montreal					
Siemiatyki (1991); Aronson et al. (1996); Parent et al. (2000)	Cases: from hospitals in Montreal, male residents, 35−70 yr, diagnosed with histologically confirmed cancer from 1979−1985, 97% population based case ascertainment; Controls: (1) population controls from electoral lists/random digit dialing, (2) cancer controls from all other cases	4,576 cases identified, 3,370 (81.5%) participated; response rates varied from 78−85% for cancer sites; 740 population controls, 533 (72%) participated	In-person interviews by trained interviewers Structured questionnaire: demographics, residential history, lifetime consumption cigarettes, alcohol, coffee, tea, food with carotene, height, weight Semi-structured questionnaire: detailed occupational history	Occupations/industries coded according to Canadian Classification and Dictionary of Occupations/SIC; blinded chemists, evaluated for confidence exposure occurred, frequency, concentration, 294 substances + 98 occupations + 77 industries = 469 circumstances	See below
Siemiatyki (1991)	See above PCE exposure and proxy—launderers and dry cleaners Site-specific cancer incidence	Cancer cases: 99 esophagus, 251 stomach, 497 colon, 257 rectum, 116 pancreas, 857 lung, 449 prostate, 484 bladder, 177 kidney, 103 melanoma, and 215 lymphoma; 533 controls	See above	6 (1.2%) colon, 7 (0.8%) lung, 9 (2.0%) prostate cases ever exposed to PCE; 4 (1.6%) stomach, 5 (1.0%) colon, 5 (2.0%) rectum, 12 (1.4%) lung, 9 (2.0%) prostate, 10 (5.6%) kidney, 3 (2.9%) skin melanoma, 3 (1.4%) non-Hodgkin lymphoma cases "ever" employed as launderers or dry cleaners	Mantel-Haenszel for ORs, 90% CIs for "ever"/"substantial" exposure; all adjusted for age, family income, cigarette index; stomach cancer also adjusted for birthplace; colon/rectum cancers also adjusted for ethnic origin, beer index; lung cancer also adjusted for ethnic origin, alcohol index, respondent; prostate cancer also adjusted for ethnic origin, Quetelet index, respondent;

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Siemiatyki (1991) (continued)					kidney cancer and skin melanoma also adjusted for ethnic origin
Aronson et al. (1996)	Cases: prostate cancer; Cancer controls: lung cancer excluded PCE exposure Prostate cancer incidence	557 prostate cancer cases, 449 (81%) participated Final sampled: 449 cases, 1,550 cancer controls, 533 population controls	See above	55 (27 substances, 11 industries and 17 occupations) of 469 occupational circumstances; PCE exposure classified as unexposed, nonsubstantial, or substantial (8 subjects with substantial exposure)	Unconditional logistic regress for ORs, 95% CIs for exposures; partially adjusted models controlled for age, ethnicity, socioeconomic status, Quetelet index, respondent status; fully adjusted models also controlled for core substances with ≥30 exposed cases; control groups pooled
Parent et al. (2000)	Cases: renal cell cancer; Controls: (1) population controls from electoral lists/random digit dialing, (2) cancer controls from all other cases Proxy—laundry and dry cleaners Renal cell cancer incidence	227 eligible cases, 177 (78%) participated, 142 renal cell carcinoma; 1,900 cancer controls with 78% participation rate Final sample: 142 cases, 1,900 cancer controls, 533 population controls	See above	4 cases (2.8%) ever employed in laundry/dry-cleaning industry, <4 cases exposed >10 yr and data not reported	Unconditional logistic regression for ORs, 95% CIs for each occupation/industry, stratified by exposure and duration exposure >10 yr, adjusted for respondent status, age, smoking, BMI

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Massachusetts (Cape Cod)					
Aschengrau et al. (1998; 1993); Paulu et al. (2002, 1999)	Cases: permanent residents of 5 Upper Cape Cod towns, diagnosed with cancer from 1983−1986, from Massachusetts Cancer Registry; Controls: (1) living <65 yr—random digit dialing, (2) living ≥65 yr—randomly from Health Care Finance Administration lists, (3) deceased—randomly from Massachusetts Department of Vital Statistics file; method: (1) cancer site stratified by age, vital status, year of death, gender (Aschengrau et al., 1993), (2) all controls in stratum with 1+ case chosen; exclusions: moved after index year, incomplete residential histories, no PCE data Proxy—residence near contaminated water	Controls: 2,236 controls <65 yr, 249 (11.1%) eligible/contacted, 184 (73.9%) interviewed; 611 controls ≥65 yr, 537 (87.9%) eligible/contacted, 464 (86.4%) interviewed; 918 deceased controls, 794 (86.5%) eligible/ ascertained, 723 (91.1%) interviewed via proxy respondent	In-person (14%) and telephone (86%) interviews by trained interviewers Questionnaire: 40-year residential history, demographics, smoking, medical and occupational histories and exposures, bottled water consumption, usual bathing habits	Various (see below)	Various (see below)

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Aschengrau et al. (1993)	Cases: men/women, all ages, diagnosed with bladder cancer, kidney cancer, or leukemia PCE in drinking water: Estimated quantity delivered to residence Site-specific cancer incidence: Bladder cancer, kidney cancer, leukemia	79 bladder cases, 72 (91.1%) eligible and contacted, 63 (87.5%) interviewed; 42 kidney cases, 36 (85.7%) eligible and contacted, 35 (97.2%) interviewed; 44 leukemia cases, 38 (90.5%) eligible and contacted, 35 (92.1%) interviewed Final sample: 61 bladder cancer cases, 852 bladder cancer controls, 35 kidney cancer cases, 777 kidney cancer controls, 34 leukemia cases, 737 leukemia controls	See above	Industries/job titles coded according to standard industrial (1987) occupational (1990) classifications; exposure based on industry, job titles, percentage reporting occupational exposure to solvents including PCE # (%) with any exposure to PCE in drinking water: 34.4% bladder cases, 26.2% bladder controls, 25.7% kidney cases, 25.2% kidney controls, 35.3% leukemia cases, 25.3% leukemia controls Water exposure (Relative Delivered Dose) via Webler and Brown (1993) algorithm, based on leaching model by Demond (1982); blinded assessments; 13 (21.3%) bladder cases, 127 (4.9%) bladder controls, 6 (17.1%) kidney cases, 112 (14.4%) kidney controls, 7 (20.6%) leukemia cases, 94 (12.8%) leukemia controls without latency period	Unadjusted OR for sites with 2+ exposed cases; Fisher exact test for 95% CIs. Analyses with and without latency periods (15 yr: bladder/kidney cancers, 5 yr: leukemia); stratified by bottled water, bathing habits; multiple logistic regression for ORs adjusted for sex, age at diagnosis/index year, vital status at interview, education, job exposures; other confounders if in 3+ cases; maximum likelihood estimates of standard errors for 95% CIs

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Aschengrau et al. (1998)	Cases: women of all ages, diagnosed with incident breast cancer PCE in drinking water: Estimated quantity delivered to residence Breast cancer incidence	334 cases, 295 (88.3%) eligible and contacted. Of these, 265 (89.8%) were interviewed. 2,236 population controls identified by random digit dialing, vital records for deceased controls, and HCFA records if >65 yr. There were 763 controls identified through the two-step control selection process After employing the additional exclusion criteria, the final sample consisted of 258 cases and 686 controls	See above	Water exposure (RDD) via Webler and Brown (1993) algorithm, based on a leaching model by Demond (1982); blinded assessments RDD categorized into low ($\leq 50^{th}$ percentile cumulative exposure), $>50^{th}$, $>75^{th}$, and $>90^{th}$ percentiles 36 (14%) exposed cases, 81 (11.8%) exposed controls without latency period	Unadjusted ORs, 95%CIs for crude associations/modifiers; multiple logistic regression for ORs adjusted for age at diagnosis or index year, vital status at interview, family history of breast cancer, age at first live birth or stillbirth, personal history of prior breast cancer and benign breast disease, occupational exposure to solvents; maximum likelihood estimates of the standard errors for 95% CIs; latency periods of 5, 7, 9, 11, 13, and 15 yr

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Paulu et al. (1999)	Cases: diagnosed with colon-rectum cancer, lung cancer, brain cancer, pancreatic cancer between 1983 and 1986 PCE in drinking water: Estimated quantity delivered to residence Site-specific cancer incidence	420 colon-rectum, 326 lung, 42 brain, and 43 pancreatic cancer cases selected; 366 (87.1%) colon-rectum, 272 (83.4%) lung, 40 (95.2%) brain, and 39 (90.7%) pancreatic cancer cases were contacted and eligible. Of these, 326 (89.1%) colon-rectum, 252 (92.6%) lung, 37 (88.1%) brain, and 37 (86.1%) pancreatic cancer cases were interviewed for an overall participation rate of 79% Final sample: 311 colon-rectum cancer cases, 1,158 colon-rectum cancer controls, 243 lung cancer cases, 1,206 lung cancer controls,	See above	# (%) with any exposure to PCE in drinking water: Excluding any latent periods: 44 (14.1%) colon-rectum cancer cases and 153 (13.2%) controls; 33 (13.6%) lung cancer cases and 158 (13.1%) controls; 3 (8.3%) brain cancer cases and 92 (13.1%) controls; 3 (8.3%) pancreatic cancer cases and 81 (13.0%) controls	Unadjusted ORs, 95% CIs for brain/pancreatic cancer. Multiple logistic regression for ORs, 95% CIs for colon-rectum/lung-cancer, adjusted for age at diagnosis or index year, vital status at interview, sex, occupational exposure to PCE and other solvents. Colon-rectum cancer further adjusted for history of polyps, inflammatory bowel disease, occupational history associated with colon-rectum cancer. Lung cancer further adjusted for usual number of cigarettes smoked and history of cigar/pipe use, living with a smoker, occupational history associated with lung cancer. Latency periods: 0, 5, 7, 9, 11, 13, and 15 yr

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Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Paulu et al. (1999) (continued)		36 brain cancer cases, 703 brain cancer controls, 36 pancreatic cancer cases, 622 pancreatic cancer controls; overall participation rate: 79%			
Paulu et al. (2002)	Cases: women diagnosed with breast cancer PCE in drinking water: GIS analysis Breast cancer incidence	334 cases, 295 (88.3%) eligible/contacted, 265 (89.8%) interviewed Final sample: 258 cases and 686 controls	40 year residential history during interview included full addresses and calendar years of residence; if complete address unknown, tax assessors' books used to identify	All participants blindly mapped onto U.S. Geological Survey map, later converted into digital format; Upper Cape Cod area divided into subregions in 2 ways: (1) fixed, multiscale grids, coding each participant as exposed or unexposed for each grid cell, (2) overlapping circles (adaptive k-smoothing) with sizes based on number of nearby cases/controls	Crude and adjusted ORs for both grid and k-smoothed methods, using map choropleths for visualization Multiple logistic regression for OR, adjusted for age, parity, vital status, family history of breast cancer in a first-degree female relative, age at first live birth or stillbirth, prior history of breast cancer or benign breast disease

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Aschengrau et al. (2003); Viera et al. (2005)	Follow-up to Aschengrau et al., 1998; permanent residents of 8 Cape Cod Towns from 1987−1993; Cases: women diagnosed with breast cancer from 1987−1993; identified via Massachusetts Cancer Registry; Controls: (1) random-digit dialing (≤64 yr); (2) random selection from a Medicare beneficiary roster (≥65 yr), (3) random selection from roster of deceased residents; Controls matched to cases based on age, vital status PCE in drinking water: Estimated quantity delivered to residence Breast cancer incidence	Various (see below)	Structured interviews: demographics, age at diagnosis, family history of breast cancer, personal history of prior breast cancer, age at first live birth/stillbirth, occupational exposure to PCE, etc., bathing habits, bottled water, and water filter use, 40-year residential history; proxy interviews with 211 (31.4%) cases, 192 (31.2%) controls	Various (see below)	Various (see below)

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Aschengrau et al. (2003)	See above	672 cases (81% selected and eligible cases) and 616 controls (157 [83%] random-digit dialed, 301 [76%] of Medicare roster, and 158 [79%] deceased) were included in the analysis	See above	RDD of PCE estimated using Webler and Brown's (1993) algorithm, which was based on PCE leaching model by Demond (1982); algorithm accounted for water flow, pipe characteristics for each home, inputs determined using maps; exposure assessed; estimates categorized as "never exposed" (private wells) and "ever exposed," with "ever" as low ($\leq 50^{th}$percentile) or high ($>50^{th}$, $>75^{th}$, and $>99^{th}$ percentiles)	EOR and 95% CIs for crude associations. Multiple logistic regression for ORs, adjusted for age at diagnosis or index year, vital status at interview, family history of breast cancer, personal history of breast cancer, age at first live birth or stillbirth, occupational exposure to PCE; maximum likelihood estimates of standard error were for 95% CIs Latent periods: 0, 5, 7, 9, 11, 13, 15, 17, 19 yr
Viera et al. (2005)	See above. Also, proxy interviews excluded from analyses, compared with results from total sample; Data not collected in interviews (inhalation rate, water flow rate, and air exchange rate) from literature	Full sample: 672 cases, 616 controls Nonproxy sample: 461 cases, 424 controls	Nonproxy information obtained via interviews: daily number of glasses of tap water or drinks with tap water, bottled water consumption, temperature, frequency, duration of showers/baths	Dose model estimated PDD (inhalation + dermal + ingestion for each exposed residence); inhalation: reported temperature, frequency, duration of baths/showers, and amount of PCE in bath/shower air; dermal: estimated according to Fick's first law, height and weight data to calculate surface area; ingestion: volume of tap water participant drank	Adjusted analyses limited to those with 3+ exposed cases and 3+ exposed controls Multiple logistic regression for ORs, adjusted for age at diagnosis/index year, family history of breast cancer, personal history of breast cancer, age at first live birth/stillbirth, occupational exposure to PCE;

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Viera et al. (2005) (continued)				RDD reestimated for nonproxy participants only, and both RDD and PDD were used to classify into nested exposure levels: $\leq 50^{th}$ percentile, $>50^{th}$, $>75^{th}$, and $>90^{th}$ percentiles. Without latency, full sample: 155 (23.1%) exposed cases, 136 (22.1%) exposed controls, nonproxy sample: 101 (21.9%) exposed cases, 88 (20.8%) exposed controls	Maximum likelihood estimates standard errors for 95% CIs; goodness-of-fit test compared RDD and PDD; nonparametric rank test evaluated whether RDD and PDD exposures differed significantly Latency periods: 0, 5, 7, 9, 11, 13, 15, 17, and 19 yr
New Zealand					
Corbin et al. (2011); Dryson et al. (2008); 't Mannetje et al. (2008); McLean et al. (2009)	Cases: From New Zealand Cancer Registry from 2003−2004 or 2007−2008 (Corbin et al., in press), men and women, 25−70 yr, diagnosed with bladder cancer or non-Hodgkin lymphoma; Controls: randomly chosen from 2003 electoral roll, matched to cases on age Proxy—occupation in textile bleaching, dyeing and cleaning machine operators (all four studies, and dry cleaners and launderers (Corbin et al., in press)	1,200 potential controls, 1,100 valid addresses, 660 contacted and eligible, 473 interviewed for response rate of 48% Final sample: 471 controls	In-person interviews with trained interviewer (occupational health nursing background) Questionnaire: demographics, smoking, occupational history, detailed information on all jobs lasting >1 year.	Jobs blindly coded according to 1999 New Zealand Standard Classification of Occupations and Australian/New Zealand SIC; textile bleaching, dyeing, cleaning machine operators: occupation Code 8264, *a priori* high risk	Unconditional logistic regression for ORs and 95% CIs for occupations/industries considered *a priori* and *a posteriori* to be high risk, adjusted for 5-year age group, sex, smoking, Maori ethnicity, occupational status; Semi-Bayes adjustments to minimize risk of false positives due to multiple comparisons

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Corbin et al. (2011) Identify occupations that may contribute to the risk of lung cancer in the New Zealand population Case-control	See above Lung cancer incidence	Of 1,057 cases, 744 eligible, 458 interviewed. Controls identified from 2003−2008; Of 2,000 potential controls, 1,878 with valid addresses, 1,134 replied, 796 interviewed. Excluding ineligible, case response rate: 53% control response rate 48% Final sample: 457 cases, 792 controls	See above	20 (0.2%) cases, 13 (1.6%) controls reported employment as bleaching, dyeing, cleaning machine operators; 3 cases (0.7%), 4 controls (0.5%) identified as dry cleaner; 9 cases (2.0%), 5 controls (0.6%) identified as launderer	See above
Dryson et al. (2008)	See above Bladder cancer incidence	Of 358 cases, 232 eligible, 213 interviewed. Of 1,200 controls, 660 eligible, 473 interviewed. Excluding ineligible, case response rate: 64% Final sample: 213 cases, 471 controls	See above	3 (1.4%) cases, 10 (2.1%) controls reported employment as bleaching, dyeing, cleaning machine operators	See above

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
't Mannetje et al. (2008)	See above Non-Hodgkin lymphoma incidence	533 cases, 335 contacted/eligible, 291 interviewed for response rate of 69% Final sample: 291 cases, 471 controls	See above	5 (1.7%) cases, 10 (2.1%) controls reported employment as bleaching, dyeing, cleaning machine operators	See above
McLean et al. (2009)	See above Leukemia incidence (chronic lymphocytic leukemia, AML, chronic myeloid leukemia, acute lymphoblastic leukemia, and other forms of leukemia)	391 eligible cases, 225 (57%) participated; 11 (3.7%) proxy with next of kin; 988 eligible controls, 660 contacted, 473 (48%) participated	In-person interviews by trained interviewers with background in occupational health nursing Questionnaire: demographics, smoking, detailed occupational history	All occupations coded according to New Zealand Standard Classification of Occupations; textile, bleaching, dyeing, cleaning machine: Code 8264 designated *a priori* as high risk 6 (2.7%) cases and 10 (1.0%) controls were in textile, bleaching, dyeing, cleaning machine occupation	Unconditional logistic regression for ORs and 95% CIs for "ever" vs. "never" occupation, adjusted for age, gender, smoking; Semi-Bayes adjustments to assess the impact of multiple comparisons
McCredie et al. (1993)	Cases: New South Wales residents, 20 to 79 yr, identified from hospitals and physicians, who were diagnosed with renal cell or renal pelvic cancer between 1989 and 1990, needed to be registered in the current electoral roll, have a telephone number, speak English; Controls: random sample of electoral rolls	744 eligible renal cell cancer cases and 200 eligible renal pelvic cancer cases, 503 (68%) and 149 (75%) interviewed, respectively; 725 eligible controls, 74% participated in interview	In-person (327 cases, 232 controls), telephone (309 cases, 291 controls), self-administered (16 cases, 12 controls) Questionnaire: occupations, industries, chemical exposures, demographics	Exposures quantified in textiles based on distribution in control group, though not provided for PCE or the dry-cleaning industry 16 (3.3%) renal cell cases, 8 (5.4%) renal pelvis cases, 7 (1.3%) controls reported employment in dry-cleaning industry	Logistic regression for RRs and 95% CIs; renal cell cancer adjusted for age, sex, method of interview, smoking, BMI; renal pelvis cancer adjusted for age, sex, method of interview, smoking, education, phenacetin-containing analgesics

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
McCredie et al. (1993) (continued)	Proxy—dry cleaning Renal cell carcinoma and renal pelvic carcinoma incidence	Final sample: 489 renal cell carcinoma, 147 renal pelvic cancer, 523 controls			
Germany					
Pesch et al. (2000a, b)	5 regions in Germany; Cases via hospitals, 1991−1995, German men/women with histologically confirmed urothelial or renal cell cancer within the 6 mo of study start; Controls: randomly selected via local residency registries, matched on age, sex, region; cases and controls required to be German nationals; no age limits JEM/JTEM for PCE Site-specific cancer incidence	Response rates: 84% cases, 71% controls Final sample: 1,970 cases (1,035 urothelial cancer, 935 renal cell cancer) and 4,298 controls	In-person with trained interviewers; cases: hospital within 6 mo diagnosis; controls: home Structured questionnaire: demographics, lifestyle, occupational exposures	Jobs held 1+ yr coded according to ISCO; based on self-reported occupational history, exposure to specific agents during tasks, average amount of time exposed daily (1) Lifetime exposure: total number of years spent at job title; weighted sum of years spent at task or exposed to specific agent; (2) JEM based on job title; (3) JTEM adjusted for region and time; JEM/JTEM evaluated probability, intensity of exposure	Conditional logistic regression for ORs and 95% CIs Adjusted for age, study center, smoking
Pesch et al. (2000b)	See above JEM/JTEM for PCE Urothelial cancer incidence	1,035 urothelial cancer cases, 4,298 controls	See above	PCE by JEM: 183 (17.7%) cases with “medium,” 188 (18.2%) cases with “high,” 74 (7.1%) cases with “substantial” exposure PCE by JTEM: 37 (3.6%) cases with “medium,” 47 (4.5%) cases with “high,” and 22 cases with “substantial” exposure	See above

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Pesch et al. (2000a, b)	See above JEM/JTEM for PCE Renal cell cancer incidence	935 renal cell cancer cases, 4,298 controls	See above	PCE by JEM: 166 (17.8%) cases with "medium," 138 (14.8%) cases with "high," 54 (5.8%) cases with "substantial" exposure PCE by JTEM: 52 (5.6%) cases with "medium" 45 (4.8%) with "high," 18 (1.9%) with "substantial" exposure	See above

Table B-2. Summaries of characteristics of case-control studies: multiple cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Nordic Countries					
Lynge et al. (2006)	Nested case-control study Non-Hodgkin lymphoma, esophageal, gastric cardia, liver, pancreatic, cervix uteri, kidney, and bladder incident cancer cases in cohort of 46,768 individuals with occupational code "laundry and dry-cleaning worker" or industry code "laundry and dry cleaning" in 1970 Censuses in Denmark, Finland, Norway, Sweden. Cases: from 1970 (Denmark) or 1971 (Finland, Norway, Sweden) through 1997 to 2001; ascertained from mortality and cancer registries. Controls: randomly selected from cohort, matched based on country, sex, 5-year age group, 5-year calendar period at the time of diagnosis (1:3 matching except 1:6 for esophageal cancer cases) Proxy—dry cleaner	4,014 records—1,616 cases, 2,398 controls, 131 subjects were both cases and controls. Participation rates: 57% cases, 64% controls in Norway, 63% cases, 60% controls in Sweden	In Denmark and Finland, occupational task identified on the 1970 Census form. For subjects from Norway and Sweden, a blinded telephone interview was undertaken, as 1970 Census forms were unavailable. The questionnaire asked about occupational task for job title reported on the 1970 Census form, and if dry cleaning, questions sought answers on employment length, number of employees, solvents used, and personal habits of smoking and alcohol consumption. Proxy interviews: 76% cases (Norway, 72%; Sweden, 77%), 40% controls (Norway, 42%; Sweden, 39%)	Occupational classification: (1) dry-cleaners or other workers in dry-cleaning shops with <10 workers, assumed to have high-exposure potential as dry cleaners because of the shared work tasks and physical proximity in small dry-cleaning shops; (2) other workers in dry-cleaning shops; (3) unexposed laundry workers and other persons in dry cleaning, and (4) unclassifiable, a category for subjects with missing employment information 695 cases and controls were dry cleaners, 183 were exposed through other work in a dry-cleaning shop, 716 were unclassifiable	Logistic regression for RRs and corresponding 95% CIs, adjusted for matching criteria plus smoking and alcohol use for Swedish and Norwegian cohorts only All analyses were conducted at the level of the record rather than person because a subject may have appeared as a case or as a control in the study more than once

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Bladder cancer					
Burns and Swanson (1991); Swanson and Burns (1995)	Cases: Histologically confirmed urinary bladder cancer cases in men and women, aged 40−84 yr, identified from MDCSS, 1984−1991 Controls: Histologically confirmed colon and rectal cancer cases in men and women, 1984−1991, aged 40−84 yr, identified from MDCSS Proxy—dry cleaning Bladder cancer incidence	2,160 bladder cancer cases interviewed (94%); 3,979 cancer controls (95%)	Telephone interview Questionnaire for lifetime occupational history, lifetime smoking history, medical history, residential history, demographic information Proxy respondents: 25% of cases series; 27.6% of control series	1980 U.S. Census Bureau classification to Code 3-digit level job title and industry; dry-cleaning worker occupation and dry-cleaning and laundry industry 8 cases and 14 controls identified as dry-cleaning workers (0.4% prevalence cases, 0.4% prevalence controls) and 15 cases and 27 controls identified as working in dry cleaners and laundries (0.6% prevalence cases, 0.6% prevalence controls)	Unconditional logistic regression for ORs and 95% CI, adjusted for cigarette smoking, race, gender, and age at diagnosis
Colt et al. (2004)	Cases: Primary bladder cancer cases, diagnosed 1994−1998, in men and women, aged 25−74 yr, identified from New Hampshire Cancer Registry Controls: population controls identified with driver's licenses, if <65 yr age, or from state Medicare and Medicaid roles, if ≥65 yr. Controls series from previous melanoma study (1993−1995) with additional controls identified using same process for period 1995−1997	459 bladder cancer cases interviewed of 618 eligible cases (74%); 665 interviews among 990 eligible controls (67%)	In-person interview Questionnaire for sociodemographic information, tobacco use, medical history, work history since age 15 No proxy interviews	SOC Manual used to code to 2-, 3-, and 4-digit level job title; dry-cleaner and laundry workers, Codes 7657, 7658 5 cases and 5 controls identified as dry-cleaner/laundry worker. Exposure prevalence—cases (1%), controls (0.08%)	Unconditional logistic regression for ORs and 95% CIs, adjusted for 5-year age group and smoking

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Colt et al. (2004) (continued)	Proxy—dry-cleaner/laundry worker Bladder cancer incidence				
Colt et al. (2011)	Cases: Primary bladder cancer cases, diagnosed 2001−2004 (Maine, Vermont) or 2002−2004 (New Hampshire), in men and women, aged 30−79 yr, identified from rapid patient ascertainment systems; Controls: population controls identified with driver's licenses, if <65 yr age, or from state Medicare and Medicaid roles, if ≥65 yr. Controls: series from previous study, and frequency matched to cases by state, sex, and diagnosis age or control selection	1,170 cases (65% participation rate), 1,418 controls (65% participation rate). 1,158 cases and 1,402 controls completed interview	Mailed questionnaire with follow-up, in-person visit to administer a computer-assisted questionnaire for information on all jobs held since age 16 yr, demographic information, tobacco use, and other exposures. For certain occupations, a job-specific questionnaire used for information on exposures of interest	Proxy—ever employed as textile, apparel and furnishings machine operator, or tender (SOC Code, 765) for >6 mo (males, 46 exposed cases, 5%; females, 27 exposed cases, 10%), of which 6 cases were laundering and dry-cleaning machine operators and tenders (SOC Code, 7658) (0.5%) or in laundry, cleaning, and garment services (SIC Code, 721), 24 exposed cases (males, 14 cases, 13%; females, 10 cases, 3%) Each job coded blinded to case or control status to the 1980 SOC and the 1987 SIC scheme	Unconditional logistic regression for males and females separately, adjusted for age, race, Hispanic ethnicity, state, smoking status, and employment in high-risk occupation. Other analyses examined duration of employment, exposure-response using test of linear trend, year of first employment, and potential for interaction between occupation and smoking

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Gaertner et al. (2004)	Cases: from 7 Canadian province cancer registries, men and women, 20−74 yr, histologically confirmed, 1974−1997 Controls: population controls recruited through random digit dialing (2 provinces) or identified from provincial health insurance plan database (5 provinces) Proxy—dry cleaner Bladder cancer incidence	1,499 cases, 887 completed questionnaire (59% response rate); 4,604 controls, 2,847 completed questionnaire (62% response rate)	Mailed questionnaire with telephone follow-up when necessary; questionnaire sought socio-demographic information, occupation history (up to 12 occupations), smoking, specific agent exposures and dietary habits	Proxy—ever employed as dry cleaner for >1 year Up to 12 occupations categorized into SOC codes with employment duration calculated from time period reported for each occupational activity over subject's lifetime; dry cleaner was a suspect occupation Questionnaire sought information on individual agents, if >1 yr exposure 4 (0.7%) male and no female cases were reported with dry cleaner job title	Unconditional logistic regression for males and females, separately, adjusted for age, province, race, smoking, ex-smoking, consumption of fruit, fried food, and coffee, and ever employed in 8 other suspect occupations
Kogevinas et al. (2003)	Pooled data from 11 previous European case-control studies from 1976−1996; cases and controls aged 30−79 yr, cases excluded if interview occurred >2 yr after diagnosis; 3 studies used population controls; 1 used hospital/population controls; 7 used hospital controls; controls matched to cases on 5-year age group, geographic area. Proxy—launderers, dry cleaners, pressers Bladder cancer incidence	4,101 cases in pooled dataset, 3,346 (81.6%) met inclusion criteria; 7,365 controls in pooled dataset, 6,840 (92.9%) met inclusion criteria	None reported	All data coded according to ISCO-68 standards; launderers, dry cleaners, pressers: Code 56 19 (0.6%) cases and 30 (0.4%) controls were launderers, dry cleaners, or pressers	Unconditional logistic regression for ORs and 95% CIs, adjusted for 5-year age group, smoking, study center; interaction between age and study center found significant and included in models; attributable risk for occupations identified *a priori* as high risk calculated, did not include launderers, dry cleaners, pressers

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Reulen et al. (2007)	Cases: from Limburg Cancer Registry, men and women, 40−96 yr, diagnosed with histologically confirmed transitional cell carcinoma of the bladder from 1996−2004; Controls: Caucasian men and women, 50+ yr, no previous history of bladder cancer, randomly selected from general population of Limburg through simple random sampling Proxy—domestic helpers, cleaners, launderers Bladder cancer incidence	2,230 eligible cases, 202 (9.1%) participated in the study; 390 controls (response rate: 26%)	In-person interviews by 3 trained interviewers in homes Structured questionnaire: sociodemographics, lifetime smoking, lifetime occupational history of all jobs lasting 6+ mo	All occupations blindly coded according to ISCO; domestic helpers, cleaners, and launderers: Code 913 14 (6.9%) cases, 20 (5.1%) controls were domestic helpers, cleaners, launderers	Unconditional logistic regression for ORs and 95% CIs, adjusted for age, sex, current smoking status, years of cigarette smoking, number of cigarettes smoked per day, education; interaction term of sex and occupation also included in model; only occupations with 15+ participants reported
Schoenberg et al. (1984)	Cases: men, 21−84 yr, diagnosed with histologically confirmed urinary bladder cancer from 1978−1979 in New Jersey, a rapid reporting location; Controls: (1) 21−64 yr random digit dialed, (2) ≥65 yr from stratified random sample of Health Care Finance Administration lists, matched to cases on age, sex Proxy—dry-cleaner/laundry worker Bladder cancer incidence	787 eligible cases, 706 (90%) participated in study; 1,608 eligible controls, 1,392 (87%) participated in study Analysis restricted to 658 Caucasian male cases and 1,258 Caucasian male controls	In-person interviews using structured questionnaire that sought information on demographic, personal and occupational history (all jobs held ≥6 mo and self-reported list of exposures)	All occupations coded to 1970 Census Index System with 19 *a priori* employment categories, including dry-cleaning and laundering (proxy exposure) 7 cases (1.1%) and 10 controls (0.8%) identified employment in dry cleaning or laundry	Logistic regression for ORs and 95% CIs, adjusted for age and cigarette smoking duration

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Smith et al. (1985); Silverman et al. (1989a; 1989b)	Data from National Bladder Cancer Study (Hartge et al., 1984), Cases: men, 21−84 yr, diagnosed with histologically confirmed urinary bladder cancer from 1977−1978 in 9 SEER reporting locations and 1 rapid reporting location; Controls: (1) 21−64 yr random digit dialed, (2) ≥65 yr from stratified random sample of Health Care Finance Administration lists, matched to cases on age, sex Proxy—laundry, dry cleaning occupation Bladder cancer incidence	Overall study response rates: 75% cases, 84% controls <65 yr, 83% controls 65+ yr	In-person interviews by trained interviewer within 3 mo of diagnosis Structured questionnaire: artificial sweeteners, smoking, coffee consumption, medical history, occupational history for all jobs ≥6 mo from age 12 yr onwards	Occupations/industries coded according to U.S. Census Bureau indices	Various (see below)
Smith et al. (1985)	Cases: transitional or squamous cell carcinoma of urinary bladder	Total: 7,748 # cases and controls not reported	See above	Exposure categories: (1) employed ≥6 mo as laundry or dry-cleaning operative; (2) chemicals in other occupations or industries; (3) unexposed; Duration of exposure: total number of years in profession; Exposed by category: 1: 103 subjects, 2: 5,776 subjects, 3: 1,869 subjects	Logistic regression for RRs of occupational exposure, adjusted for age, sex, and duration of exposure, adjusted for age, sex, smoking

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Silverman et al. (1989a; 1989b)	Cases and controls: non-Caucasian men	Final sample: 126 cases, 383 controls	See above	Occupations grouped by potential to have similar exposures; dry cleaners, ironers, pressers: 11 (8.7%) cases, 12 (3.1%) controls	Maximum likelihood method for OR; Gart's interval estimation procedure for 95% CIs; Mantel-Haenszel for tests of trend; Whittemore (1983) for PARs, 95% CIs All adjusted for smoking; dry cleaners adjusted for smoking, high risk occupation; PAR adjusted for age, geographic area, smoking
Steineck et al. (1990)	Cases: men, born 1911−1945 and residing in a county of Stockholm 1985−1987, source not identified in published paper Controls: population controls randomly sampled at 4 periods from population registers between 1985 and 1987 Proxy—dry-cleaning worker Bladder cancer incidence	254 cases, 287 controls Response rates: 80% cases, 79% controls	Interview method not identified in published paper. Structured questionnaire for information on occupational history and smoking	Self-reported occupational title 2 (0.8%) cases, 2 (0.7%) controls were dry cleaners or worked in dry-cleaning industry	

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Zheng et al. (2002)	Cases: histologically confirmed incident bladder cancer cases, 1986−1989, 40−85 yr, Iowa State Health Registry Controls: population controls frequency-matched (1.7:1) by sex and age and randomly selected from state driver's license records for subject <65 yr age or from HFCA records if ≥65 yr age Proxy—dry-cleaner/laundry worker Bladder cancer incidence	1,452 cases, 2,434 controls Response rates: 85% cases, 82% controls <65 yr, and 80% for controls ≥65 yr	Mailed and telephone interviews with structured questionnaire for information on each job held ≥5 yr, demographic factors, residence, smoking, past medical history, first-degree family history of bladder cancer, and other potential risk factors Proxy respondents for 156 cases (11%) and all controls	Self-reported occupation title and industry coded to SIC and SOC Proxy—laundering and dry cleaning occupation, SOC Code 7658 Employment duration: 10 yr, ≥10 yr	Unconditional logistic regression for ORs, 95% CIs and adjusted for age, lifetime pack-years of cigarette smoking, and having a first-degree relative with bladder cancer. Other variables such as education, frequency of strenuous or moderate exercise, duration of living in a residence served by chlorinated surface water, population size of places of residence, and other cancer in a first-degree relative did not result in material change in association and was not included in final statistical model

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Brain Cancer					
Heineman et al. (1994)	From death certificates in New Jersey, Pennsylvania; cases: Caucasian men, died from brain/other CNS tumors from 1978−1980, exclusions: no hospital diagnosis; Controls: Caucasian men, died from other causes, exclusions: death associated with occupation, epilepsy, suicide, homicide, cerebrovascular diseases, matched to cases based on age, year of death, location JEM for PCE Brain cancer mortality	741 cases, 654 (88%) contacted, 483 (74%) interviewed; 741 controls, 612 (83%) contacted, 386 (63%) interviewed Final sample: 300 cases, 320 controls	Blinded interviews with next of kin by trained interviewers Questionnaire: brain cancer risk factors, occupations held from age 15 yr onward (job title, tasks, company name and location, industry, products, employment dates, hours worked)	Occupations/industries coded according to U.S. standards; all codes assigned *a priori* estimates of probability and intensity of exposure; JEM by Gomez et al., 1994 to estimate exposures to PCE 111 (37%) cases, 106 (33.1%) controls "ever" exposed to PCE	Maximum likelihood estimates for ORs, 95% CIs using Gart (1970); Linear trends using Mantel (1963) Lag time of 10, 20 yr

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Breast cancer					
Peplonska et al. (2007)	Cases: hospital cases newly diagnosed histologically confirmed in situ or invasive breast cancers residents of Warsaw and Lódź, between 20−74 yr of age, 2000−2003; Controls: population controls identified from the Polish Electronic System of Population Evidence and matched to cases by city of residence and age within 5-year age groups. Proxy—laundry, cleaning, and garment services industry Breast cancer incidence	2,275 cases (79% response rate), 2,424 controls (66% response rate)	In-person interview with cases and controls by trained interviewer Questionnaire: known and suspected risk factors for breast cancer, reproductive history, occupations held ≥6 mo	Occupation/industry coded to SIC/SOC Manuals 28 (1%) cases and 32 (1%) controls worked in laundry, dry cleaning, and garment services industry	Unconditional logistic regression for ORs and 95% CIs adjusted for age, age at menarche, age at menopause, number of full-term births, breast cancer in first degree relative, education, and city of residence
Colon cancer					
Fredriksson et al. (1989)	All: alive at time of study, medically able to participate; Cases: from Swedish Cancer Registry, men and women, 30−75 yr, diagnosed with large bowel adenocarcinoma from 1980−1983; Controls: from National Population Register, matched to cases based on county of residence, sex, age Proxy—dry cleaning Colon cancer incidence	402 cases, 329 contacted/eligible, 312 (94.8%) participated; 717 controls, 658 contacted/eligible, 623 (94.6%) participated	Mailed questionnaire: occupational history, occupational exposures, food and drinking habits, previous diseases, drug intake	Occupational exposures assessed by 2 physicians and 1 hygienist as high grade, low grade 5 (1.6%) female cases, 5 (0.8%) female controls reported employment in dry cleaning	Mantel-Haenszel for ORs, Miettinen (1976) for 95% CIs for all occupations, stratified by age, physical activity

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Liver cancer					
Hernberg et al. (1988)	Cases: men and women, diagnosed with primary liver cancer reported to the Finnish Cancer Register in 1976−1978 and 1981; Controls: (1) randomly selected stomach cancer patients reported to Finnish Cancer Register in 1977; (2) patients whose hospital autopsy records noted death in 1977 due to coronary infarction; Coronary infarction controls matched to cases based on sex, age, and hospital of diagnosis; matching criteria for stomach cancer controls not reported in paper; cases excluded if no confirmed diagnosis Proxy—dry cleaning Liver cancer incidence	618 eligible cases, 526 contacted, 377 (71.7%) responded; 772 eligible stomach cancer controls, 654 contacted, 476 (72.8%) responded; 674 eligible coronary infarction controls, 558 contacted, 385 (69.0%) responded Final sample 344 cases, 861 controls	Mailed questionnaire: occupational history (employers, work sites, jobs held, and calendar years of work), alcohol, tobacco, coffee, tea, medicines, leisure activities, and for women, history of oral contraceptive use	2 occupational hygienists blindly assessed exposure, based on industries, workplaces, job titles; exposures not determined. Followed up with phone calls to workplace or proxy respondent; exposure classified as "heavy," "moderate," "light"; Dry-cleaning exposures based on 1950 records by Finnish Institute of Occupational Health, which noted PCE exposure ranged from 34−600 ppm during that time 2 cases (0.6%): possible chlorinated hydrocarbon exposures (laundry facility, dry-cleaning employment); 2 controls (0.5%): light exposure to PCE from dry-cleaning employment	Likelihood-based ORs and 90% CIs according to Cornfield (1956) for association between primary liver cancer and solvent exposure and for association between primary liver cancer and heavy/moderate alcohol use; both stratified by sex using Gart (1970) Latency period 10 yr
Houten et al. (1980)	Cases: men and women diagnosed with primary liver cancer from 1956−1965; Controls: all other cancer patients admitted to Roswell Park Memorial Institute from 1956−1965 Proxy—laundry and dry cleaners Liver cancer incidence	102 cases, number of controls not reported	None reported	Occupation assessed as a proxy for exposure 2 cases (2%) employed in laundry/dry-cleaning industry	χ^2 goodness-of-fit test, where distribution of cases compared to controls by each industry

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Stemhagen et al. (1983)	Cases: from NJ hospital records, NJ State Cancer Registry, death certificates, men and women living in NJ diagnosed with histologically confirmed primary liver cancer from 1975−1980; Controls: men and women admitted to same hospitals as cases and from death certificates, matched to cases based on age, race, sex, county of residence, vital status, excluded if history of liver cancer, hepatitis, liver disease; homicide, suicide Proxy—laundering, cleaning, and other garment services Liver cancer incidence and mortality	335 eligible cases, 296 contacted, 265 (79%) interviewed; 96% cases deceased, so proxy interview with next of kin; 825 eligible controls, 687 contacted, 530 (64.2%) interviewed	In-person interviews Questionnaire: lifetime residence, smoking, alcohol, medical history, and employment from age 12 yr onward	Occupations/industries coded according to Index of Industries and Occupations Standards developed by Bureau of Census; Occupations 6+ mo assessed as proxy for exposure; laundering, cleaning, other garment services industry: 10 (3.8%) male cases and 8 (1.5%) male controls Authors examined laundry/dry-cleaning industry by individual occupations but method not reported; no information for females not reported	Mantel-Haenszel methods for ORs and 95% CIs for men employed 6+ mo in selected industries and occupations; also looked at distribution of subjects by level of alcohol consumption, adjusted for age, smoking

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Suarez et al. (1989)	Death certificates from 1969−1980; Cases: men, 20+ yr, living in Texas, liver cancer cause of death; Controls: randomly selected from population-based group of 537,000 who died of all other causes, excluding neoplasms, liver and gallbladder diseases, infectious hepatitis, alcoholism; matched to cases on 5-year age group, race, ethnicity, year of death Proxy—dry cleaning occupation/industry Liver cancer mortality	1,771 potential cases, 1,742 (98.4%) eligible and included in study; did not report total number of controls	Not applicable	Occupations grouped according to U.S. Census Classified Index, groupings partially based on Hoar et al. (1980) 11 cases, 12 controls employed in dry-cleaning industry; 4 cases, 8 controls employed as dry-cleaning operators; unable to calculate exposure prevalence	Mantel-Haenszel for ORs; Miettinen's method for 95% CIs; adjusted for race, ethnicity Nonpetrochemical categories, occupations within categories with 10+ participants also analyzed
Lung and upper airway cancers					
Brownson et al. (1993)	Cases: from Missouri Cancer Registry and participating hospitals, Caucasian females, 30−84 yr, living in Missouri, diagnosed with primary lung cancer from 1986−1991, nonsmokers/selected ex-smokers; Controls: (1) <65 yr: state driver's licenses, (2) 65−84 yr: Medicare roster, matched to cases on age Proxy—dry cleaning Lung cancer incidence	650 eligible cases, 429 (66%) participated, 1,527 eligible controls, 1,021 (67%) participated Final sample: 429 cases, 1,021 controls	Telephone and in-person interviews by trained interviewers; Telephone: residential history, passive smoke, personal and family health histories, reproductive health history; In-person: diet, occupation	Occupational risk factors determined by 28 questions, based on review of literature, focused on job title and exposure; subjects reported years worked at each job/with each exposure; 30 (7.0%) cases, 39 (3.8%) controls employed in dry-cleaning industry	Multiple logistic regression for ORs and 95% CIs, adjusted for age, active smoking (for ex-smokers), history of previous lung disease

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Consonni et al. (2010)	Cases from 13 hospitals in Lombardy region of Italy, part of EAGLE study, 35−70 yr, 2002−2005; Controls: population controls identified through population databases, frequency matched by residence, sex, and age. Proxy—dry cleaning and laundry occupation Lung cancer incidence	2,100 eligible cases, 1,943 (92.5%) participated, 2,120 eligible controls, 2,116 (99.8%) participated	Computer-assisted questionnaire with in-person interview including lifetime history of jobs ≥6 mo	Occupations/industries blindly coded according to ISCO and ISIC; laundry and dry cleaners included in list of suspected occupations/industries 14 (0.7%) cases, 14 (0.7%) controls employed in laundry and dry cleaning occupation	Unconditional logistic regression for ORs and 95% CIs for "ever" worked in either known or suspected occupations associated with lung cancer, stratified by gender, adjusted for age, area, education, smoking pack-years, and number of jobs held
Pohlabeln et al. (2000)	Cases from 12 study centers in 7 countries, subjects ≤75 yr enrolled from 1988−1994; Controls: community and hospital-based; hospital-based controls had diseases not related to smoking Proxy—Launderers and dry cleaners Lung cancer incidence	650 nonsmoking cases, 1,542 nonsmoking controls; response rates ranged 55−95%, except 2 German centers, 1 Portuguese center (response rates <50%)	In-person interview: demographics, diet, smoking exposure, smoking history, and occupational history (6+ mo duration minimum)	Occupations industries blindly coded according to ISCO and ISIC; laundry and dry cleaners included in list of suspected occupations/industries 20 (3.1%) cases, 29 (1.9%) controls employed in laundry and dry cleaning occupation	Unconditional logistic regression for ORs and 95% CIs for ever worked in either known or suspected occupations associated with lung cancer, stratified by gender, adjusted for age, center; no effect of different sources of controls, so pooled results reported

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Richiardi et al. (2004)	Cases: histologically (74%) or cytologically (26%) confirmed lung cancers from hospitals in Turin and East Venice, Italy, men and women, residents ≤75 yr, 1990−1991 (Venice) and 1991−1992 (Turin) Controls: population control from local registries frequency matched on sex and age (≥1:1 frequency) Proxy—launderers and dry cleaners Lung cancer incidence	1,171 lung cancer cases, 1,569 controls; response rates for Turin and Venice regions, respectively, 86% and 72%, and 85% and 74% among cases and controls Final analyzed sample: 1,132 cases (956 men and 176 women) and 1,553 controls (1,253 men and 300 women)	In-person interview: demographics, diet, smoking exposure, smoking history, and lifetime occupational history for all jobs ≥6 mo	Occupations/industries blindly coded according to ISCO and ISIC; laundry and dry cleaners included in list of suspected occupations/industries 12 (1.1%) cases, 14 (0.9%) controls employed in laundry and dry cleaning occupation	Unconditional logistic regression for ORs and 95% CIs for "ever" worked in either known or suspected occupations associated with lung cancer, stratified by gender, adjusted for age, study center, cigarette smoking, consumption of other tobacco products, education, and total number of jobs

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Vaughan et al. (1997)	Cases from Fred Hutchinson Cancer Research Center (population-based registry), men and women, 20−74 yr, diagnosed with cancer of oral cavity/pharynx, larynx, esophagus/gastric cardia from 1983−1987, with adenocarcinoma of esophagus/gastric cardia from 1987−1990, residents of Washington state; exclusions: nonepithelial and nonspecified cancers, no telephones at diagnosis; Controls: random digit dialing, matched to cases on 5-year age group, sex PCE exposure for dry cleaning Upper aerodigestive tract cancer incidence	Case response rates: 85.2% oral cavity, 80.8% larynx, 82.9% esophagus/gastric cardia. Controls: 95.4% contacted were screened, 80.3% eligible were interviewed Final sample: 1,120 cases, 724 controls	In-person interviews Questionnaire: demographics, tobacco, alcohol consumption, occupational history (6+ mo duration, employer, business, job title, typical activities, dates, solvent exposures) Proxy interviews: 7.2% laryngeal cases, 18.7% oral/pharyngeal cases, 33.2% esophageal and gastric cardia cases	Blindly assessed by estimating probability PCE was used and 8-h time weighted average exposure in the job; Duration of employment and cumulative exposure assessed 16 (1.4%) cases, 8 (1.1%) controls “ever” employed in the dry-cleaning industry; 15 (1.3%) cases, 8 (1.1%) controls “possibly” exposed to PCE, 8 (0.7%) cases, 3 (0.4%) controls “probably” exposed to PCE	Conditional logistic regression for ORs and 95% CIs for those employed in dry-cleaning industry and those exposed to PCE, adjusted for age, sex, education, study period, alcohol consumption, cigarette smoking

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Lymphopoietic cancer					
Blair et al. (1993)	Cases: all pathology reviewed, Iowa: from Iowa State Health Registry, Caucasian men diagnosed with non-Hodgkin lymphoma from 1981−1983, Minnesota: from surveillance of hospitals, Caucasian men diagnosed from 1980−1982; Controls: Caucasian men without hematopoietic or lymphatic malignancies, matched on state, age, year of death, (1) <65 yr from random digit dialing, (2) 65+ yr from Medicare files, (3) deceased from state vital records; farmers excluded Proxy—laundry and garment workers Incidence of non-Hodgkin lymphoma	715 eligible cases, 622 (87.0%) participated; 1,245 controls participated (77% random digit dialing, 79% Medicare, 77% deceased) Final sample: 546 cases, 1,087 controls	In-person interviews with trained interviewers Structured questionnaire: sociodemographic characteristics, agricultural exposures, exposures to chemicals through hobbies, residential history, medical history, family history cancer, occupational history (all jobs held 1+ year from 18 yr onward, industry, employer, products produced, job titles, duties)	Blinded exposure assessment by an industrial hygienist; occupations/industries coded according to DOT and SIC standards; Job-exposure matrix used to evaluate probability (4-point scale) and intensity (3-point scale) exposure; laundry/garment workers: Code 721, 16 (2.9%) cases, 14 (1.3%) controls	*Polychotomous* unconditional logistic regression for ORs and 95% CIs, adjusted for age, state, direct or surrogate respondent, pesticides, tobacco, postsecondary education, hair dye use, first-degree family member with malignant lymphoproliferative diseases; exposure-response relationships for risk of non-Hodgkin lymphoma or subtypes by duration, intensity, probability exposure

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Clavel et al. (1998)	All from 18 hospitals in France; cases: patients diagnosed from 1980−1990, still alive at time of study; Controls: patients admitted to hospitals during same time for other reasons, exclusions: malignant disease, diseases related to occupations, work-related accidents, matched to cases based on birth date, sex, admission date, residence Proxy—launderers and dry cleaners Hairy cell leukemia incidence	278 eligible cases, 226 (81.3%) participated; 809 eligible controls, 465 (57.5%) participated Final sample: 226 cases, 425 controls	Self-administered questionnaires: socio-demographic characteristics, tobacco smoking, lifelong occupations, leisure activities; more sent to those with suspected occupational exposures; Semi-structured questionnaires: assess exposures to textile degreasing, among others	Occupations/industries coded according to ILO and ISIC; launderers/dry cleaners: ILO Code 5.6; (1) exposure blindly assessed by 2 researchers, based on responses, industry, job title, exposure; (2) job-exposure matrix for exposure to solvents based on ILO/ISIC codes for probability, intensity, frequency exposure; 1 (0.4%) case, 2 (0.5%) controls occupation as launderers and dry cleaner.	Conditional logistic regression for ORs and 95% CIs for job titles, occupational tasks, chemical families of organic solvents, adjusted for smoking, farming

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Fabbro-Peray et al. (2001)	All participants: French men and women, 18+ yr, living in Languedoc-Roussillon region of France; Cases: from 19 hospitals and 1 cancer research center, diagnosed with malignant lymphomas from 1992−1995, HIV-negative; Controls: randomly chosen from electoral lists in randomly selected municipalities based on size and population distribution and randomly selected individuals within each municipality; not matched to cases Proxy—dry-cleaning solvents Non-Hodgkin lymphoma incidence	627 eligible cases, 517 (82.5%) interviewed; 1,962 eligible controls, 1,025 (52.2%) interviewed Final sample: 445 NHL cases, 1,025 controls	Cases: in-person interviews at hospital; Controls: in-person at home or telephone Questionnaire: general characteristics, medical history, occupational history, environmental and occupational exposures, smoking	Age at first exposure, duration of exposure, and cumulative index of exposure calculated for each chemical for each participant; classified as either not exposed, lower than threshold, higher than threshold 35 (6.8%) cases, 77 (7.5%) controls exposed to dry-cleaning solvents	Mantel-Haenszel methods for ORs and 95% CIs for effect of sociodemographic characteristics, adjusted for age, gender; unconditional logistic regression using forward stepwise approach for ORs and 95% CIs for effect of chemical and other exposures on non-Hodgkin lymphoma, adjusted for age, gender, urban setting, education level Lag time 5 yr

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Gold et al. (2010a; 2010b)	Cases: Males and females, 35−74 yr, reported to Seattle-Puget Sound, 2000−2002, WA, and Detroit, MI SEER registry, alive at time of interview; Controls: population control identified previously for NHL study, random-digit dialing (for <65 yr) and Medicare roles (65+ yr) and frequency matched to NHL cases, resident of two areas, 1998−2004, 35−74 yr old, spoke English PCE exposure Proxy—dry cleaner or launderer Multiple myeloma incidence (ICO-O-2/3, 9731, 9732, plasmacytoma not otherwise specified or multiple myeloma)	255 eligible cases, 181 participated (71%) and 180 interviewed; 1,133 eligible controls, 481 participated and interviewed (52%)	Cases and controls: in-person interviews using a computer-assisted personal interview program Questionnaire: all jobs held for at least 1 year between 1941 (cases) or 1946 (controls) and enrollment date. Job-specific module for solvent exposures when participant held relevant job for ≥2 yr	Occupation, industry, ever exposed to 6 chlorinated solvents or to individual solvent (PCE, TCE, methylene chloride, 1,1,1-trichloroacetic acid, chloroform, carbon tetrachloride), exposure duration, cumulative exposure 9 (5%) cases, 4 (0.8%) controls with occupation as textile, apparel and furnishing machine operator and tender, of whom, 5 cases (3%) and 3 controls (0.7%) were dry cleaners; 29 cases (19%) and 63 controls (13%) "ever" exposed to PCE, of whom 17 (3%) cases and 15 (3%) controls with high cumulative PCE exposure (≥7,794 ppm-hours)	Unconditional logistic regression for ORs and 95% CIs adjusted for sex, age, race, education, and SEER site. Sensitivity analysis considered all occupations with confidence score ≥1 and repeated all analyses (Gold et al., 2010b) Lag time 10 yr (Gold et al., 2010b)

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Hardell et al. (1981)	Cases: men, 25−85 yr, histologically confirmed malignant lymphoma from 1974−1978. Controls: (1) living: from National Population Registry, exclusions: not in same municipality at case diagnosis, deceased, emigrated, (2) deceased: from National Registry for Causes of Death, exclusions: died in 1978, suicide, malignant tumor, date of last employment >5 yr from case; living matched on sex, age, municipality; deceased matched on sex, age, municipality, year of death Exposure to organic solvents, including PCE Hodgkin **lymphoma**, non-Hodgkin lymphoma incidence	Final sample: 169 cases (60 with Hodgkin lymphoma and 109 with non-Hodgkin lymphoma), 338 controls	Self-administered Questionnaire: leisure activities, smoking/drug use, chemical exposures, and occupational history (including time/place of employment) Blinded reviewer telephone interviews with participants when information unclear/incomplete	Exposure to organic solvents, including PCE, categorized into "high-grade" and "low-grade" 10 (5.9%) cases, 31 (9.2%) controls reported "low-grade" exposure to organic solvents, PCE-specific exposure not reported 40 (23.7%) cases, 47 (13.9%) controls reported "high-grade" exposure to organic solvents, only 1 case (0.6%) reported exposure to PCE	χ^2 tests based on Miettinen (1970) for χ^2 estimates and ORs; Miettinen (1976) for 95% CIs

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Kato et al. (2005)	Cases: women, 20−79 yr, no prior history of hematologic cancer, living in upstate New York, diagnosed with non-Hodgkin lymphoma from 1995−1998; Controls: <65 yr from age-stratified random sample of driver's licenses, ≥65 yr from Health Care Finance Administration records Proxy—worker exposure to degreasers/cleaning solvents or dry-cleaning fluids Non-Hodgkin lymphoma incidence	722 eligible cases, 376 (56%) cases, 248 (30%) DMV controls, 215 (67%) HCFA controls	Blinded telephone interviews with cases and controls with structured questionnaire 21% case interviews and >3% of control interviews were conducted with proxy respondents Median time between the cancer diagnosis and the interview was 1.2 yr and ranged between 2 mo and 3.3 yr	Occupational: hours exposed, year of first and last exposure, and total number of years/months of exposure. Cumulative exposure hours based on hours per time unit and total exposure duration 50 (13.3%) cases, 48 (10.4%) controls reported exposure to degreasers/cleaning solvents, 7 (1.9%) cases, 8 (1.7%) controls reported exposure to dry-cleaning fluids	Unconditional logistic regression for ORs and 95% CIs, for occupational, household exposures to solvents, adjusted for age at index date, family history of hematologic cancer, college education, surrogate status, year of interview, BMI 10-yr preinterview, average frequency of use of pain-relieving drugs, total number of episodes of systemic antibiotic use, total number of household pesticides, duration of work involving pesticide exposures Lag period 1 yr

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Malone et al. (1989)	Cases: from SEER reporting sites in Washington state, Utah, Michigan, Georgia, men and women, <80 yr, diagnosed with chronic lymphocytic leukemia from 1977−1981; Controls: random digit dialing in Utah, Michigan, Georgia, random area sampling in Washington; controls matched to cases based on sex, race, and/or age, depending on location Chlorinated hydrocarbons (including, but not limited to dry-cleaning solvents) and proxy (dry-cleaning industry) Leukemia incidence	83% eligible cases responded, 430 interviewed (total eligible not stated and unclear if responded equals interviewed); Of 2,028 eligible controls, 83% interviewed Final sample: 427 cases, 1,683 controls	In-person or telephone interviews by trained interviewers Questionnaire: chemical exposures, other risk factors, employment in petroleum, dry cleaning, rubber, meat processing industries Cases: 76% in-person, 9% telephone, 16% next of kin; Controls: 81% in-person, 18% telephone, <1% next of kin	Chemical exposures assessed blindly by researchers and toxicologist into 20 categories; exposures with 10+ cases analyzed; chlorinated hydrocarbons (dry-cleaning solvents included) assessed; 1 case reported dry-cleaning solvent exposure 14 (3.3%) cases, 59 (3.5%) controls reported working for 6+ mo in dry-cleaning industry	Unconditional logistic regression for ORs and 95% CIs for all respondents and nonproxy respondents only; adjusted odds ratios controlled for race, 10-year age group, education, sex, study site
Mester et al. (2006) Siedler et al. (2007)	Part of EPILYMPH study; 6 regions in Germany; Cases: from physicians, diagnosed 1998−2003, German men and women, 18−80 yr, diagnosed with non-Hodgkin or Hodgkin lymphoma; Controls: from population registration office, matched to cases on sex, region, age; Exclusions: subjects who did not speak German . Lymphoma incidence	Participation rate among controls: 44.3%; participation rate among cases not reported Final sample: 710 cases, 710 controls	In-person with trained interviewers Questionnaire: lifestyle, medical history, occupational history (dates of employment, title, industry, tasks) for each job ≥1 year	Various (see below)	Conditional logistic regression for ORs and 95% CIs, adjusted for smoking, alcohol consumption Unconditional logistic regression for ORs and 95% CIs in unmatched analysis of most frequent lymphoma subentities, adjusted for age, sex, region, smoking, alcohol

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Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Mester et al. (2006)	Proxy—launderers, dry cleaners, pressers	See above	See above	Job titles/industries blindly coded according to ISCO-68 and Statistical Classification of Economic Activities in the European Community; launderers, dry cleaners, pressers: ISCO-68 Code 56 11 (1.5%) cases, 11 (1.5%) controls were launderers, dry cleaners, pressers	All estimates stratified by duration of employment (≤10 yr, >10 yr) Latency period of 10 yr, though data not reported
Seidler et al. (2007)	Chlorinated solvent, PCE exposure	See above	See above	Blinded, trained industrial physician Intensity: "low" (0.5−5 ppm), "medium" (>5−50 ppm), "high" (>50 ppm); frequency: percentage weekly working time exposed: "low" (1−5%), "medium" (>5−30%), or "high" (>30%); confidence in exposure: "possible," "probable," "certain"; cumulative exposure: ppm-years 36 (5.1%) cases, 31 (4.4%) controls exposed to PCE	Tests for trend used exposures as continuous variables in logistic regression

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Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Miligi et al. (2006; 1999); Costantini et al. (2008; 2001)	12 sites in Italy, 11 used for analysis; Cases: from hospitals, medical centers, local cancer registry, men and women, 20−74 yr, diagnosed with hematolymphopoietic malignancies from 1991−1993, Controls: randomly selected from general population in each site. Stratified by sex and age (5-year groups)	3,357 eligible cases, 3,118 contacted, 2,737 (88%) responded; 2,391 eligible controls, 2,196 contacted, 1,779 (81%) responded Final sample: 2,737 cases and 1,779 controls	In-person interviews and proxy with next of kin (19% cases, 5% controls) Questionnaire: residential, medical, reproductive, and occupational histories, behaviors, education, solvent exposure	Industrial hygienists blindly assessed probability ("low," "medium," "high") and intensity ("very low," "low," "medium," "high") of occupational exposures; job-exposure matrix created with consensus for jobs reported most frequently	Various (see below)
Costantini et al. (2001)	Information from 11 sites Proxy—launderers, dry cleaners, and pressers Incidence: NHL, HD, leukemia, MM stratified by sex, age	Final sample: 2,737 cases (1,450 NHL, 365 HD, 652 leukemia, 270 MM) and 1,779 controls	See above	Jobs coded according to International Standard Classification of Occupations; launderers, dry cleaners, pressers: Code 56: 3 (0.2%) NHL, 1 (0.3%) HD, 2 (0.3%) leukemia cases	Mantel-Haenszel method for ORs, 95% CIs, adjusted for age; reported results for men and compares with total sample; reported jobs with 5+ exposed cases

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Miligi et al. (2006)	Information from 8 sites PCE exposure NHL, HD incidence	1,719 eligible NHL cases, 1,428 (83%) responded; 347 eligible HD cases, 304 (88%) responded; 2,086 eligible controls, 1,530 (73%) responded Final sample: 1,732 cases (**285 small lymphocytic NHL, 100 follicular NHL, 308 diffuse NHL, 315 other NHL, 304 HD),** 1,530 controls	In-person interviews with 85% NHL cases, 93% HD cases, 97% controls; proxy interviews with remaining cases and controls	Intensity of exposure to PCE (NHL): 18 (1.3%) cases, 29 (1.9%) controls with "very low/low"; 14 (1.0%) cases, 15 (1.0%) controls with "medium/high"; duration of exposure to PCE (NHL): 10 (0.7%) cases, 10 (0.7%) controls with <15 yr; 3 (0.2%) cases, 5 (0.3%) controls with 15+ yr	ORs and 95% CIs calculated separately for non-Hodgkin lymphoma, non-Hodgkin lymphoma subtypes, and Hodgkin lymphoma; Adjusted for sex, age, education, area
Costantini et al. (2008)	Information from 6 sites PCE exposure Leukemia subtypes, MM	586 leukemia cases and 1,278 controls; 236 multiple myeloma cases and 1,100 controls Final sample: 822 cases and 2,378 controls	See above	Intensity of exposure to PCE: "Very low/low": leukemia—6 (1.0%) cases, 17 (1.3%) controls, MM—3 (1.3%), cases, 15 (1.4%) controls, "Medium/high": leukemia—7 (1.2%) cases, 12 (0.9%) controls, MM—2 (0.8%) cases, 12 (1.1%) controls	Individual point ORs, 95% CIs for leukemia, leukemia subtypes, and multiple myeloma, adjusted for gender, age, education, area; Linear test for trend using duration category midpoints

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Schenk et al. (2009)	Cases: men and women 20 to 74 yr and diagnosed with non-Hodgkin lymphoma between 1998 and 2000, living in Iowa, California, Michigan, or Washington state and in SEER registry; Controls: random digit dialing for <65 yr, Medicare files for ≥65 yr Matched based on 5-year age group, gender, and race within each study center Proxy—launderers and ironers Non-Hodgkin lymphoma incidence	2,248 eligible cases, 1,728 (77%) contacted, 1,321 (59%) interviewed; 2,409 eligible controls, 2,046 (85%) contacted and 1,057 (44%) interviewed Final sample: 1,189 cases (293 follicular, 366 diffuse large B-cell lymphoma, 487 other, 43 unknown), 982 controls	Mailed, self-administered questionnaire: family, medical history, diet; computer-assisted questionnaire in home: demographics, hair coloring, residential history since 1970, occupational history	Jobs blindly assigned occupation/industry codes according to standard conventions; launderers and ironers: occupation Code 503, 12 (1.0%) cases, 3 (0.3%) controls	Unconditional logistic regression for ORs, 95% CIs, adjusted for age, gender, ethnicity, study center; stratified by gender and histological subtype separately

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Scherr et al. (1992)	Cases: men and women, including children diagnosed with NHL between 1980 and 1982, histologically confirmed residents of Boston Standard Metropolitan Statistical Area, treated in one of nine participating hospitals; Controls: randomly selected from town and precinct population lists and, if over 17 yr of age, matched based on sex and age, or, if case ≤17 yr, one parent or guardian matched based on age and sex to adult resident (with interview to determine whether child was living in household of same age and sex as case) Proxy—occupation in laundry, dry cleaner, leather products fabrication industries; chlorinated solvents as a category NHL classified using Rapapport or Working Formulation classification system, diffuse or nodular tumors, or B- or T-cell	202 cases, 303 controls Response rates, 80% cases, 72% controls	In-person interview Questionnaire: current or most recent job, job held 15 yr prior, major and second major occupation, exposure to 10 specific agents or chemical classes (including chlorinated solvents) Proxy respondents: 33% cases, none for controls	Occupation and industries coded according to standard classification, or to any of 10 specific agents 3% of cases reported employment in laundering, dry cleaning, leather products fabrication industries; 24% reported exposure to chlorinated solvents	Hierarchal approach that aggregated histological subtypes into groups with similar histological characteristics and exposure defined as a function of calendar time (1901−1949, 1950−1959, 1960−1969, 1970 and later) or exposure duration (10 yr, 20 yr). All exposure that showed consistent patterns within histological categories over calendar time or over duration were considered as candidate variables for conditional logistic models with covariates for age and sex

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Childhood lymphopoietic cancer					
Costas et al. (2002)	Follow-up to a study (Cutler et al., 1986) that found a cluster of leukemia cases in Woburn, MA. Cases: pre-1982 cases from pediatric health professionals/pediatric oncology centers; post-1982 cases from Massachusetts Cancer Registry, children, diagnosed with leukemia ≤19-yr-old from 1969−1989; Controls: randomly selected from Woburn Public School records, matched on race, sex, and date of birth; excluded if not Woburn resident at case diagnosis Proxy—drink contaminated water Leukemia incidence	21 eligible cases, 19 (90.5%) participated; 38 controls selected, 1 excluded; no control response rates reported Final sample: 19 cases, 37 controls	In-person interviews with parents of cases and controls, except 2 fathers via telephone Questionnaires: Maternal—lifestyle, demographics, medical history, environmental/ occupational exposures, public drinking water at home; (2) Paternal—occupational history/exposures; Residential history for each mother/child for 2 yr preconception to case diagnosis	Based on well water contaminant levels from before 1979 closure; determined by potential for residence to receive water from contaminated wells using distribution model by Murphy (1990); 2 exposures assessed (cumulative and average); 16 (84.2%) cases, 24 (64.9%) controls "ever" exposed; 7 cases (36.8%), 13 controls (35.1%) "most" exposure, 9 cases (47.4%), 11 controls (29.8%) "least" exposure	Conditional logistic regression with proportional hazards model for ORs, 95% CIs; unadjusted ORs for effect of possible confounders; adjusted ORs for "ever" and "most"/"least"/"never" exposure, adjusted with composite covariate controlling for socioeconomic status, maternal smoking during pregnancy, maternal age at birth of child, breastfeeding; trends evaluated with χ^2 method

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Infante-Rivard et al. (2005)	Cases: children 0−14 yr, diagnosed with acute lymphoblastic leukemia, treated in tertiary care centers in Quebec Province, 1980−2000; Controls: (1) 1980−1993 from family stipend records; (2) 1994−2000 from health insurance records; Exclusions: adopted/foster, French/English not spoken at home, not in Canada, parents unavailable; matched to cases on sex, age Proxy—occupational exposure to PCE Leukemia incidence	848 eligible cases, 790 (93.1%) parents interviewed; 916 eligible controls, 790 (86.2%) parents interviewed Final sample: 790 cases, 790 controls	Telephone interviews Questionnaires: (1) structured: risk factors and confounders, maternal job history from 18 yr to child's birth; (2) semi-structured: company activities, raw materials or machines, goods, responsibilities, working conditions, coworker activities, solvents/ chemical presence, etc; (3) detailed tasks: time, exposures, environment	Blind classification by chemists and industrial hygienists; Jobs coded according to Canadian industrial titles (3-digit) and job titles (7-digit); assessed exposure to chemicals through interview responses, geographical information, previous knowledge industry exposures; then chemicals assigned codes based on Siemiatycki (1991), PCE Code 243; jobs 2 yr before birth, coded separately based on confidence that exposure occurred, frequency and concentration of exposure Not enough detail to calculate exposure prevalence	Conditional logistic regression for ORs and 95% CIs for each chemical, stratified by time period, adjusted for maternal age, education

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Lowengart et al. (1987)	Cases: from Los Angeles County Cancer Surveillance Program, ≤10 yr at diagnosis from 1980−1984, biological mothers required availability for interview; controls: (1) friends of cases identified by case mothers, (2) population-based controls via random digit dialing, matched to cases on age, sex, race, Hispanic origin (if "white"); additional exclusions if incomplete occupational history PCE exposure Leukemia incidence	216 eligible cases, 202 (94%) contacted, 159 (79%) case mother interviews; 154 case father interviews; 136 control mother interviews, 130 control father interviews; 30 case and 43 control paternal interviews proxy with mothers; control response rates not reported Final sample: 123 case-control pairs	Telephone interviews by 2 nonblinded, trained interviewers Structured questionnaire: family and personal medical histories, alcohol and tobacco use, household and personal products, X-ray exposure, occupational history (job title, industry, time period worked); maternal questionnaire also asked about use of drugs, medical complications, diet during index pregnancy, child's medical history, child's exposure to ionizing radiation	Industries/occupations coded according to 1970 U.S. Census classifications, grouped based on hydrocarbon exposure; occupations/exposures within 1 year conception excluded; 4 case fathers reported exposure to PCE 1 year before pregnancy (1 case), during pregnancy (1 case), or after delivery (2 cases); no control fathers reported exposure to PCE; maternal exposure to PCE not reported	Conditional logistic regression for ORs and 95% CIs

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Shu et al. (1999)	Cases: children ≤15 yr, diagnosed with acute lymphocytic leukemia from 1989−1993 by Children's Cancer Group member or institution, exclusions: no matched control; Controls: random digit dialing, matched to cases on age, race, telephone area code and exchange; Excluded if no telephone residence; no biological, English-speaking mother available PCE exposure Leukemia incidence	2,081 eligible cases and 2,597 eligible controls; Mothers: 1,914 (92%) case mothers and 1,987 (76.5%) control mothers interviewed Final sample: 1,842 cases and 1,986 controls Fathers: 1,801 (86.5%) case fathers and 1,183 (69.8%) control fathers interviewed; 16.6% cases and 32.3% control interviews were proxy with mothers Final sample: 1,842 cases, 1,986 controls	Telephone interviews with parents Structured questionnaire: (1) Maternal—demographics, personal habits, household exposures before/during pregnancy, environmental hazards exposure, medical/family/reproductive/job histories; (2) Paternal—personal habits, household exposures, medical/family/job histories; occupational history: job titles, industries, duties, employment dates, exposures	Maternal—all jobs 6+ mo from 2 yr prepregnancy through cancer diagnosis; Paternal—all jobs 6+ mo from age 18 yr onwards; Self-reported exposures not on exposure list blindly assessed by industrial hygienist; timing of exposure (preconception, pregnancy, postnatal): dates of employment; duration of exposure: control group's median time as cut-off; Maternal exposures to PCE: anytime: 4 (0.2%) cases, 9 (0.5%) controls; preconception: 3 (0.2%) cases, 2 (0.1%) controls; pregnancy: 3 (0.2%) cases, 2 (0.1%) controls; postnatal: 4 (0.2%) cases, 8 (0.4%) controls; Paternal exposures to PCE: anytime: 25 (1.4%) cases, 23 (1.9%) controls; preconception: 21 (1.2%) cases, 22 (1.9%) controls; pregnancy: 8 (0.4%) cases, 14 (1.2%) controls, postnatal: 10 (0.6%) cases, 15 (1.3%) controls	Maternal exposures: Conditional logistic regression for ORs, 95% CIs, adjusted for maternal education, race, family income; Paternal exposures: Unconditional logistic regression for ORs, 95% CIs, adjusted for paternal education, race, family income, age, sex of case; Tests for trend: add categorical variables of exposure as continuous variables in the models

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
DeRoos et al. (2001)	Cases: male and female children, ≤18 yr, diagnosed with neuroblastoma from 1992−1994, registered in participating hospital in United States or English-speaking Canada; Controls: random digit dialing, matched to cases on birth date; all proxy interviews excluded Halogenated hydrocarbons, including PCE Neuroblastoma incidence	741 eligible cases, 538 (73%) enrolled; 71% recruited; 74% screened households participated Final sample: 538 neuroblastoma cases, 504 controls	Telephone interviews with mothers/fathers; proxy interview with mothers if father unavailable Questionnaire: demographics, occupational history, occupational exposure history of all jobs within 2 yr of child's birth Interviews with 537 case mothers, 472 case fathers (14.2% proxy), 503 control mothers, 445 control fathers (31.7% proxy)	Blinded industrial hygienist reclassified improbable exposures; did not review participants who failed to report any exposures; classified substances into 5 categories, included halogenated hydrocarbons, which included PCE Maternal exposure to halogenated hydrocarbons: 15 (2.8%) cases, 19 (3.8%) controls, after review, decreased to 6 (1.1%) cases, 8 (1.6%) controls. Paternal exposure: 8 (2%) cases, 11 (3.6%) controls, after review, decreased to 4 (1.0%) cases, 6 (2.0%) controls	Unconditional logistic regression for ORs and 95% CIs for each category and individual chemicals, adjusted for child's age, maternal race, maternal age, maternal education

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Pancreatic cancer					
Lin and Kessler (1981)	>115 hospitals in Buffalo, Detroit, Miami, Minneapolis-St. Paul, New York City; Cases: from medical records and hospital pathology departments, men and women ≥15 yr; Controls: randomly chosen from admissions records of cancer-free patients of same hospital as case, matched to cases based on age, sex, race, marital status Proxy—unspecified occupational exposure to PCE and gasoline derivatives combined Pancreatic cancer incidence	Response rates not reported but 22% eligible not interviewed due to extreme illness; once excluded, response rates: 86.2% men and 86.3% women Final sample: 109 case-control pairs (67 male pairs and 42 female pairs)	Blinded, in-person interviews in hospital (most) or at home Questionnaire: demographics, residential history, occupations (all jobs held full-time 6+ mo or part-time for 1+ year), toxic exposures, animal contacts, smoking habits, diet, medical history, medicines, family history; sexual practices (men), urogenital conditions; marital, obstetric, gynecologic histories (women)	Duration of exposure to dry-cleaning and gasoline derivatives categorized into 0, ≤2, 3−5, 6−10, >10 yr; 25 (37.3%) male cases, 23 (34.3%) male controls exposed to either dry-cleaning or gasoline derivatives	χ^2s and *t*-tests to examine differences between cases and controls; ORs for relative risk for pancreatic cancer among men and women exposed to a variety of risk factors, including occupational exposure to dry cleaning
Kernan et al. (1999)	1984−1993; Cases: from death certificates, all International Classification of Disease Code 157 in 24 states, included occupation/industry codes based on 1980 Census. Controls: from death certificates, nonpancreatic, noncancer causes, matched to cases based on state, race, gender, 5-year age group PCE Pancreatic cancer mortality	63,097 cases and 252,386 controls were selected	Not applicable	JEM developed by industrial hygienists for solvents, probability and intensity of exposure estimated and scored as "low," "medium," or "high"; 5,344 exposed to "low" levels, 2,187 exposed to "medium" levels, and 903 exposed to "high" levels of PCE	Race and gender-specific mortality odds ratios for intensity and probability of exposure to PCE, adjusted for age, marital status, metropolitan status, region of residence

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Renal cancer					
Asal et al. (1988)	Cases: from 29 hospitals in Oklahoma; men and women with renal cell cancer diagnosed from 1981−1984; Controls: (1) hospital-based: excluded kidney disease/psychiatric illness, matched to cases based on age, sex, race, hospital, date of admission, (2) population-based: random digit dialing, matched to cases based on sex, age Proxy—dry cleaning Renal cell cancer incidence	345 identified cases, 315 (91.3%) participated; control response/participation rates not reported Final sample: 315 cases, 313 hospital-based controls, 336 population-based controls	In-person interviews in hospital with cases and hospital-based controls, home with population-based controls Questionnaire: medical history, medications, radiation exposure, occupational history for all jobs held ≥1 year, industrial exposures, tobacco smoking, beverage use, artificial sweeteners, family history disease, height, weight	Dry cleaning examined as high-risk industry: 11 cases (3.5%), 7 controls (1.1%)	Cox linear logistic regression for ORs, 95% CIs for lifetime occupations and high-risk industries, adjusted for age, smoking, weight
Auperin et al. (1994)	Cases: men and women with histologically confirmed renal cell carcinoma identified at 10 selected hospitals; Controls: (1) with a malignant disease, (2) with a nonmalignant disease, excluded tobacco-related diseases, matched for each case based on sex, age at interview, hospital, and interview. Patients with alcohol-related cirrhosis or diabetes excluded from study. Proxy—laundry workers Renal cell carcinoma incidence	151 cases matched to two controls, 45 cases matched to 1 control; 161 controls with cancer and 186 with nonmalignant disease	Unblinded, trained interviewers Questionnaire: education, height, weight, smoking habits, beverage consumption, and medication, complete occupational history, including duration of employment for each job held. Interviewers were not blinded to the case or control status	Blinded exposure assessment; coded according to International Standard Classification of Occupations; minimum of 1 year employment for each job held; numbers of exposed laundry workers not reported	Conditional logistic regression for ORs, 95% CIs, stratified by gender; pooled control group; adjusted for age, hospital, interview, educational level, cigarette smoking, and the Quetelet index

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Delahunt et al. (1995)	Cases and controls: men and women in New Zealand Cancer Registry from 1978−1986; Cases: men and women, 20 yr and older, diagnosed with malignant neoplasm of kidney; Controls: men and women, 20 yr or older, diagnosed with primary tumor outside of urinary tract; women subsequently excluded Proxy—dry cleaning Renal cell carcinoma incidence	1,060 eligible cases, 914 (86.2%) information on occupations Final sample: 710 cases, 12,756 controls	New Zealand Cancer Registry for current/most recent occupations, smoking habits	Occupations coded according to New Zealand Standard Classification of Occupations, selected *a priori* 52 (7.3%) cases and 737 (5.8%) controls in service occupation, including but not limited to dry cleaners	Mantel-Haenszel method for RRs for each occupation; Miettinen's approximation method for 95% CIs; all stratified by smoking history, 10-year age groups
Dosemeci et al. (1999)	Cases: from Minnesota Cancer Surveillance System, Caucasian men and women, 20−85 yr, diagnosed with histologically confirmed renal cell carcinoma from 1988−1990; Controls: Caucasian men and women, (1) 20−64 yr from random digit dialing, (2) ≥65 yr from systematic sample of Health Care Financing Administration lists PCE Renal cell carcinoma incidence	796 eligible cases, 690 (87% response rate) interviewed, 241 (34.9%) proxy with next of kin; 707 (86% response rate) controls interviewed Final sample: 438 cases, 687 controls	In-person interviews with blinded, trained interviewers Questionnaire: demographics, diet, smoking, drug use, medical/residential histories, occupational histories recent and usual job and industry, activities, employment dates, part-time/full-time status. Duration of employment for specific industries, occupations, exposures	Occupations/industries coded according to SOC and SIC; linked with JEM by Gomez et al., 1994; 11% cases, 11% controls exposed to PCE	Logistic regression using Breslow and Day (1980) method for RRs and 95% CIs Adjusted for age, smoking, BMI, and hypertension status, and/or use of diuretics and/or antihypertension drugs All stratified by gender

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Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Harrington et al. (1989)	Cases: from West Midlands Regional Cancer Registry, men and women, living in West Midlands, diagnosed with renal adenocarcinoma from 1984−1985; Controls: randomly selected from patients of general practitioners, matched based on 5-year age group, sex, ethnicity, geographical location, socioeconomic status; excluded if no matching controls Proxy—dry-cleaning fluids, degreasing agents Renal cancer incidence	101 eligible renal cancer cases, 85 (84%) contacted, 59 (69%) interviewed Final sample:54 cases, 54 controls	In-person interviews Questionnaire: personal habits (smoking, coffee, and alcohol consumption), medical history, occupational history	Exposure assessed blindly by chemist/occupational hygienist using checklist of exposures; exposure indices calculated by computer program (exposure level × duration exposure) No cases or controls reported exposure to dry-cleaning fluids; 9 (16.7%) cases, 12 (22.2%) controls reported exposure to degreasing agents	Paired analyses for ORs and 95% CIs for 2 exposure categories using Schlesselman (1982) and 3 exposure categories using Pike et al. (1975)

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Mandel et al. (1995)	6 centers: Australia, Denmark, Germany (2 centers), Sweden, United States; Cases: from population-based cancer registries except Germany (surveillance of diagnosis/treatment departments), men and women, 20−79 yr, diagnosed with histologically or cytologically confirmed renal cell adenocarcinoma from 1989−1991; required in-country birth (except Australia/United States); Controls: Denmark/Sweden: population-based registers, Australia: electoral rolls, Germany: residential lists, United States: Health Care Finance Administration lists and random digit dialing, matched on gender, 5-year age group Proxy—dry-cleaning industry, dry-cleaning solvents Renal cell cancer incidence	Final sample: 1,732 cases (73.2% response rate) and 2,309 controls (74.7% response rate)	In-person interviews by trained interviewers in hospital (German cases) and homes (German controls/all others) Questionnaire: tobacco, diuretics, analgesics, antihypertensive drugs, diet pills, hormones, and alcohol, height and weight, physical activity, medical/reproductive histories, family history cancer, demographics, occupational history	Industries/occupations coded according to International Labour Office (1968, 1988), UN Department of Economic/Social Affairs (1968, 1971, 1990), U.S. Department of Commerce (1980), U.S. Office of Management Budget (1987); Duration: total number years worked/exposed; tertiles based on control distribution; dry-cleaning solvents duration: 1−7, 8−25, 26−60 yr; 23 (1.3%) cases, 28 (1.2%) controls in dry-cleaning industry; 302 (17.5%) cases and 265 (11.5%) controls exposed to dry-cleaning solvents	Logistic regression for ORs and 95% CIs for industry, occupation, exposure, stratified by gender, adjusted for age, smoking status, BMI, education, study center; only men reported Only industries, occupations, exposures reported by all centers analyzed; tests of heterogeneity to assess differences between centers

Table B-3. Summaries of characteristics of case-control studies: single cancer-site studies (continued)

Reference, objective/hypothesis, study type	Study design	Sample size	Data collection	Exposure assessment	Statistical approach
Mellemgaard et al. (1994)	Cases: from Danish Cancer Registry, women and men 20−79 yr, born and living in Denmark; Controls: from Central Population Register; matched to cases on gender, 5-year age group Proxy—dry cleaning Renal cell carcinoma incidence	482 eligible cases, 368 (76%) interviewed; 500 eligible controls, 396 (79%) interviewed	In-person interviews with trained interviewers Questionnaire asked about occupation, occupational exposures, medical history, diet, smoking, demographics	Occupations and industries coded according to ISCO and ISIC; dry cleaning identified *a priori* as high risk (code not provided); exposures assessed for jobs held 1+ year and occurred 10+ yr prior to interview	Odds ratios and 95% CIs were calculated for men and women separately Adjusted for age, BMI, and smoking
Schlehofer et al. (1995)	Cases: German men and women, histologically confirmed renal cell cancer, from 1988−1991; Controls: randomly selected from population register of the Rhein-Neckar-Odenwald area Matched on age, gender Chlorinated solvents Renal cell cancer incidence	Of the 328 cases identified, 277 (84.5%) participated in the study Of the 381 controls identified, 286 (75%) participated in the study	In-person interviews by trained interviewers; 92% case interviews in hospital; 100% control interviews at home Questionnaire: medical, smoking family, weight, diet, demographics, physical activity, occupational history (industry, occupation, activities, chemical exposures)	Industries coded; industries, occupations, activities grouped into different categories; 51 possible substances, 22 reported by ≥5% male subjects and analyzed, including chlorinated solvents (PCE and tetrachlorocarbonate); exposed if 5+ yr duration 27 (14.6%) male cases, 12 (13%) male controls exposed to chlorinated solvents; female exposures not examined	Unconditional logistic regression for ORs and 95% CIs for smoking, age, and sex.

HD = Hodgkin lymphoma.

B.3. Geographically Based And Other Studies

The following papers examined tetrachloroethylene using geographically based (ecological) and other study designs. Summaries of the study characteristics of each paper are provided in Table B-4.

B.3.1. Cohn et al. (1994)

Cohn, P.; Klotz, J.; Bove, F.; Berkowitz, M.; Fagliano, J. (1994). Drinking water contamination and the incidence of leukemia and non-Hodgkin's lymphoma. Environ Health Perspect, 102, 556-561.
http://www.ncbi.nlm.nih.gov/pubmed/9679115

Summary: This ecological incidence rate study examined the following four hypotheses: (1) the incidence of leukemia is associated with exposure to trichloroethylene and/or tetrachloroethylene; (2) childhood leukemia is associated with trichloroethylene and/or tetrachloroethylene; (3) non-Hodgkin lymphoma is associated with trichloroethylene and/or tetrachloroethylene; and (4) gender may be an effect modifier. Cases were identified through the New Jersey State Cancer Registry and consisted of men and women residing in 1 of 75 municipalities diagnosed with primary leukemia (acute lymphocytic, chronic lymphocytic, acute myelogenous, chronic myelogenous, other specified, and unspecified) or non-Hodgkin lymphoma (low-grade, intermediate-grade, intermediate-grade/diffuse large cell/reticulosarcoma, high-grade, and high-grade NHL/non-Burkitt's) between 1979 and 1987. Information was supplemented with death certificates; any cases that were determined exclusively through death certificates were excluded. Municipalities were chosen on the basis of their water supply. Only those where at least 80% of the population received their water from a public water supply were selected. In total, 1,190 cases of leukemia (118 acute lymphocytic, 354 chronic lymphocytic, 276 acute myelogenous, 146 chronic myelogenous, 61 other specified, 235 unspecified) and 1,658 cases of non-Hodgkin lymphoma (434 low-grade, 708 intermediate-grade, 402 intermediate-grade/diffuse large cell/reticulosarcoma, 69 high-grade, 51 high-grade/non-Burkitt's) were included in the study.

Tetrachloroethylene exposure potential was based on water monitoring data—averages from 1984−1985 by the New Jersey Department of Environmental Protection and Energy. Although the authors do not explicitly state these were the same municipalities, due to the mandatory nature of the monitoring data, this may be assumed. Samples were taken from water treatment plants as well as tap sites within the distribution system. Tetrachloroethylene exposures were categorized as <0.1, 0.1−5, and >5 ppb, based on EPA standards. Surveys conducted by the New Jersey Department of Environmental Protection and Energy and the

Department of Health between 1978 and 1983 were used to provide corroborating evidence for use of the 1984−1985 estimates. The authors do not state if these were the same municipalities. The latter data were used as the primary source of exposure estimation because the earlier surveys were conducted primarily in response to known contamination, and the quality assurance and quality control of the latter mandatory monitoring were reported to be better. The 1978−1983 data are further described elsewhere (Cohn et al., 1994).

There were 440 cases of leukemia (37%) and 662 cases of non-Hodgkin lymphoma (39.9%) that were assessed as exposed due to their residence in a municipality with tetrachloroethylene levels between 0.1 and over 5 ppb. The remainder were determined to have <0.1-ppb exposure. Log-linear regression models with the Poisson distribution were used to estimate incidence rate ratios and their corresponding 95% CIs, adjusted for age and stratified by sex with subjects, identified <0.1 ppb as referents. The rate ratios were also stratified by sex and type of leukemia or non-Hodgkin lymphoma. The authors do not report strengths of their methodology. Limitations included the lack of adjustment for possible confounders, potential misclassification of exposure that biases the result away from the null, and the ecological study design, which measured exposure and disease at the same time, assigned exposure and leukemia incidence at the municipal level, and lacked information on individual exposure potential.

B.3.2. Lee et al. (2003)

Lee, L.; Chung, C.; Ma, Y.; Wang, G.; Chen, P.; Hwang, Y.; Wang, J. (2003). Increased mortality odds ratio of male liver cancer in a community contaminated by chlorinated hydrocarbons in groundwater. Occup Environ Med, 60, 364-369. http://dx.doi.org/10.1136/oem.60.5.364

Summary: Exposure potential to chlorinated hydrocarbons was assigned in this community case-control study of liver cancer in males >30 years of age using residency as coded on death certificates obtained from local household registration offices. No information is available to assess the completeness of death reporting to the local registration office. Of the 1,333 deaths between 1966 and 1997 in two villages surrounding a hazardous waste site, an electronics factory operating between 1970 and 1992 in Taoyuan, Taiwan, 1,266 cancer deaths were identified; 53 liver cancer deaths, 39 stomach cancer deaths, 26 colorectal deaths, and 41 lung cancer deaths. Controls were identified from 344 deaths due to cardiovascular and cerebrovascular diseases, without arrhythmia; 286 were included in the statistical analysis. Residents from a village north and northeast of the plant were considered exposed and residents living south considered unexposed to chlorinated hydrocarbons. Additionally, death certificates were obtained from the registration offices in two villages near the factory. These records contained information on gender, age, date of birth and death, address, and cause of death. The

underlying cause of death was then blindly assessed by a team of nosologists. Cases consisted of any individual whose cancer was coded by the nosologists as either an underlying cause of death or as a significant condition. All individuals were also linked to the Taiwan National Cancer Registry to verify the accuracy of coded cancer cases.

Residence was assessed as a proxy for exposure, with exposed cases considered exposed if living downstream of the factory and unexposed if living upstream of the factory. Geographical exposure was confirmed through well water sampling. Between 1999 and 2000, 74 groundwater samples were collected from off-site residential wells near a factory whose soil and groundwater had been previously found to be contaminated with chlorinated hydrocarbons, including tetrachloroethylene. Overall, 20 (45.5%) of the wells downstream had concentrations above the maximum contaminant level for tetrachloroethylene. No upstream wells were found to be contaminated with tetrachloroethylene. Death certificates were also linked to the Labour Insurance Bureau to ascertain those who had previously worked in the factory.

The Mantel-Haenszel method was used to estimate mortality odds ratios and their corresponding 95% CIs, adjusted for age. Multiple logistic regression was also conducted, adjusted for age and time period. The Cochran-Armitage test for trend calculated the effect of time period for downstream and upstream villages. A latency period of 10 years was also included.

One strength of this study is its linkage with the National Cancer Registry, which located an additional 12 cancer cases that had not been recorded in the death certificates. Limitations to the study include possible selection bias due to the use of only cardiovascular and cerebrovascular deaths as controls, possible misclassification due to the use of residence as a proxy for exposure status, and the lack of control for potential confounders such as Hepatitis C virus, which was of high prevalence in this area.

B.3.3. Ma et al. (2009)

Ma, J.; Lessner, L.; Schreiber, J.; Carpenter, D. O. (2009). Association between residential proximity to PERC dry cleaning establishments and kidney cancer in New York City. J Environ Public Health, 2009, 183920. http://dx.doi.org/10.1155/2009/183920

Summary: The hypothesis tested in this study was living in an area with a high density of tetrachloroethylene dry cleaners increases tetrachloroethylene exposure and the risk of kidney cancer. Subjects were individuals 45 years of age or older with a principal or other diagnosis of kidney cancer and identified from a New York State register of hospital discharges between 1993 and 2004. The database used to identify subjects did not include personal identifiers leading to an inability to distinguish multiple hospital discharges by a single individual. A subject had the

potential for multiple entries for this reason. The inclusion criteria were restricted to subjects whose residence at the time of discharge was in a New York City zip code having a median household income from $17,864 to $142,926. No information is provided in the paper on how temporal a change as zip code's median income may have affected the inclusion criteria. Of the total of 181 zip codes in New York City, 164 zip codes met the inclusion criteria; six zip codes were not considered because population or income information was unavailable, and 10 zip codes had median household incomes either below or above the inclusion criteria. A total of 674,519 discharges with a diagnosis of cancer, of which 10,916 were of kidney cancer, were identified with a residence at the time of discharge within the 164 eligible zip codes. Population estimates, year not identified by authorsm, by zip code were derived from U.S. Census data and stratified by age, race, and sex.

Dry-cleaning establishments were identified from a listing maintained by the New York State Department of Environmental Conservation. This listing included dry-cleaning establishments who were required under state statutes to report their usage of tetrachloroethylene. The authors do not provide information as to whether the statute identified a minimal usage level that would lead to an underreporting of the number of dry-cleaning establishments. The density of dry cleaners by zip code (number of dry cleaners per km^2) was estimated for each zip code from the number of dry-cleaning establishment and the population density, based upon a zip code's population estimate and area. The authors do not provide information in the paper for the source for estimating area of individual zip codes.

A negative binomial model was fit to the data to examine the rate of discharge rate for a principal or other diagnosis for kidney cancer as functions of the densities of dry-cleaning businesses. For each of the exposure strata, the authors examined different variables as possible effect modifiers, and these included median household, age, and sex, with a finding that effect modifiers differed for each exposure strata. The authors also used a Poisson regression model but did not report findings because of an inadequate fit to the observed data.

This study is ecological in design, with associated limitations known as "ecological fallacy" because variables of exposure and outcome measured on an aggregate level do not represent association at the individual level. A significant shortcoming of this study is the potential for a subject to have multiple discharges, inflating the numerator, but not the denominator, for estimating the discharge rate, and its use of a crude exposure surrogate. The authors did not validate how well the density of dry-cleaning businesses predicted atmospheric concentrations of tetrachloroethylene for individual zip codes or potential exposure to individual subjects. The authors noted New York City zip code densities varied by boroughs, particularly Staten Island, which had large areas but lower population densities and lead to large variation in the exposure surrogate. Furthermore, risk ratios from the negative binomial model are difficult

to interpret because each exposure strata's rate ratio was based upon a different set of covariates. On the other hand, the study was able to characterize disease distribution geographically, as well as dry-cleaning business location.

B.3.4. Mallin (1990)

Mallin, K. (1990). Investigation of a bladder cancer cluster in northwestern Illinois. Am J Epidemiol, 132, S96-106. http://www.ncbi.nlm.nih.gov/pubmed/2356842

Summary: This ecological study examined the incidence of bladder cancer to determine if the high mortality rates also reflect high incidence rates, and if high incidence rates were found in areas of known groundwater contamination. Incident cancer cases were identified through medical records in 8 of the 9 counties within a region of northwestern Illinois. Resident cases diagnosed or treated in the bordering states of Iowa or Wisconsin were also ascertained through the Iowa State Health Registry and the Wisconsin Cancer Reporting System. Cases consisted of men and women who were diagnosed with histologically confirmed bladder cancer between 1978 and 1985. Deaths due to bladder cancer during this time period were also ascertained. Incidence data were stratified by county and zip code. After significantly higher risks of bladder cancer incidence and mortality were found only in Winnebago County, the researchers searched Illinois EPA and Department of Energy and Natural Resources documents and found well water in this county was contaminated with tetrachloroethylene and other compounds.

Indirect standardization was used for the estimation of standardized incidence ratios and SMRs, both adjusted for age. Expected numbers of cases were derived from age-specific rates for 1978−1991 and 1982−1985. Their corresponding 95% CIs were calculated according to Miettinen's exact limits; significance was evaluated using a χ^2 distribution. Where the expected counts were less than 5, Fisher exact test limits were used for the estimation of 95% CIs, and a Poisson distribution was assumed for the significance tests. The authors did not report any strengths of their study. Limitations include the lack of control for potential confounders, ecological design causing chance associations, lack of survival data, lack of medical treatment data, and no data on water consumption among Winnebago inhabitants.

B.3.5. Morton and Marjanovic (1984)

Morton, W. and Marjanovic, D. (1984). Leukemia incidence by occupation in the Portland-Vancouver metropolitan area. Am J Ind Med, 6, 185-205. http://www.ncbi.nlm.nih.gov/pubmed/6475965

Summary: An examination of occupational risks using incident leukemia cases identified over a 15-year period, 1963−1977, was carried out in the Portland-Vancouver Metropolitan area, Oregon. Cases [n = 1,622] were identified through a search of 24 hospitals in the four-county

area of Portland-Vancouver and included a Veteran's Administration hospital and two closed hospitals (whose records were accessible in storage). In addition, death certificates that mentioned leukemia from the same period were searched, adding 244 cases, for a total of 1,866 leukemia cases. The finding of additional cases using death certificates suggests hospital records may have been incomplete. Associations with job title as coded to usual occupation and leukemia cases aged 16−74 years were carried out, examining age-standardized rates based on the direct method of age standardization using the 1970 U.S. population census (midpoint of case ascertainment period). Given census records group population estimates for individuals aged 65 or older, occupation-specific analyses truncated case inclusion at age 67. The determination of significance of a deviation of an occupational leukemia rate from its respective area-wide rate for all women or all men was based on the assumption that a rate is a mean for a distribution of binomial events, and the distribution of such means was regarded as approximately normal. Age-adjusted incidence rates for separate occupations were calculated for all leukemia, all lymphatic leukemia, and all nonlymphatic leukemia. Lymphatic leukemia cases, including chronic lymphatic leukemia, are now classified as subtypes of non-Hodgkin lymphoma (Morton et al., 2005). One female dry cleaner and launderer was identified with both lymphatic and nonlymphatic leukemia subtypes and was counted twice in the statistical analyses.

Occupations were broadly grouped into over 20 categories and included dry cleaners and launderers, a grouping that contained 313 males and 1,298 females with associated exposure prevalences (based on the 1970 population) of 0.1% and 0.3%, respectively. Morton and Marjanovic (1984) do not identify the source for job title information and stated the trained coder could identify "usual occupation," but little else. The lack of information on full job history, in addition to possible misclassification of occupation on death certificates, suggests an incomplete occupation history.

This study is less sensitive for identifying cancer hazard because case ascertainment may be incomplete and because of possible selection bias associated with hospital records, inability to identify specific exposures, and use of age-adjusted incidence rates, rather than a relative risk estimate.

B.3.6. Vartiainen et al. (1993)

Vartiainen, T.; Pukkala, E.; Rienoja, T.; Strandman, T.; Kaksonen, K. (1993). Population exposure to tri- and tetrachloroethene and cancer risk: Two cases of drinking water pollution. Chemosphere, 27, 1171-1181. http://dx.doi.org/10.1016/0045-6535(93)90165-2

Summary: This parallel standardized incidence ratio study had three aims: (1) to find out whether inhabitants in the villages of Oitti and Hattula had been exposed to tetrachloroethylene

by analyzing urinary excretion; (2) to examine which compound(s) would provide the best index of exposure to low levels of tetrachloroethylene; and (3) to determine whether the cancer incidence was increased in the municipalities in question. The first part of this parallel study consisted of residents of two villages (Oitti and Hattula) in Finland who consumed contaminated drinking water. Of the 116 possible participants, 8 were excluded because they had not drunk any contaminated water. The reference population was divided into two groups: (1) ground water controls included volunteers residing in a nearby town whose drinking water came from ground water, and (2) surface water controls included volunteers whose drinking water came from bank-filtrated surface water. The final sample consisted of 108 exposed (87 from Oitti and 21 from Hattula) and 60 unexposed (45 ground water and 15 surface water). All participants were interviewed about their source of drinking water and the approximate amount of water they consumed each day. Gas chromatography was used to detect tetrachloroethylene in the urine samples. These results were then compared among all four locations (2 exposed villages and 2 unexposed villages), though the authors do not report the methods they used to conduct the comparison. The second part of the parallel study used the Finnish Cancer Registry to identify the number of all cancers, liver cancer, non-Hodgkin lymphoma, multiple myeloma, and leukemia in Hausjarvi (municipality where Oitti is located) and Hattula. Overall, there were 1,931 cancer cases identified (972 from Hausjarvi and 959 from Hattula). Standardized incidence ratios, along with their corresponding 95% CIs, were calculated assuming a Poisson distribution. Expected numbers of cancer cases were estimated based on annual age- and sex-specific numbers for the whole of Finland for each year between 1953 and 1991. A strength of the latter part of this parallel study was use of the Finnish Cancer Registry, which contained all cancer cases since 1953 and increased the confidence in calculated estimates. Limitations overall included the extended latency time between exposure to tetrachloroethylene and cancer diagnosis, as well as the ambiguity related to the time period within which cases were exposed. Exposure misclassification bias is likely, given the ecologic design of this study, and exposure assignment to both cases and controls is not validated, given the lack of monitoring data for the examined time period. Furthermore, the presentation of incidence rates for a presumed unexposed population provide little insight on site-specific cancer incidence in the two presumed exposed towns, given direct comparison of SIRs has methodological limitations due to differences in population age structure.

Table B-4. Summaries of characteristics of geographically based and other studies

Reference	Study design[b]	Sample size	Data collection	Exposure assessment[c]	Statistical approach[d]
Cohn et al. (1994)	Cases: identified from New Jersey State Cancer Registry, men and women, diagnosed with primary leukemia or non-Hodgkin lymphoma from 1979−1987, living in 1 of 75 municipalities, information supplemented with death certificates; only municipalities where ≥80% population received public water supply selected Proxy exposure surrogate—PCE Leukemia incidence (acute lymphocytic, chronic lymphocytic, acute myelogenous, other specified, unspecified), NHL (low-grade, intermediate-grade, high-grade)	1,190 cases leukemia (118 acute lymphocytic, 354 chronic lymphocytic, 276 acute myelogenous, 146 chronic myelogenous, 61 other specified, 235 unspecified), 1,658 cases of NHL (434 low-grade, 708 intermediate-grade, 402 intermediate-grade/diffuse large cell/ reticulosarcoma, 69 high-grade, 51 high-grade/non-Burkitt's)	Records based—cancer registry data and summary data from state of average drinking water concentration in each municipality	PCE exposure based on water monitoring data (average from 1984−1985 by NJ Department of Environmental Protection and Energy), calculated for each municipality based on water measurements and proportion of water purchased elsewhere; samples from treatment plants and tap sites within distribution system; PCE exposures categorized as <0.1, 0.1−5, and >5 ppb, based on EPA standards; surveys from 1978−1983 used to corroborate 1984−1985 estimates; 440 (37%) cases of leukemia, 662 (39.9%) cases of NHL exposed due to residences in a municipality with PCE levels from 0.1 through >5 ppb	Log-linear regression models assuming Poisson distribution for incidence rate ratios, 95% CIs, adjusted for age, stratified by sex and type of leukemia or non-Hodgkin lymphoma
Lee et al. (2003)	Death certificates from 2 villages near factory from 1966−1997; nosologists blindly assessed cause of death; Cases: cancer cause of death, linked to Taiwan National Cancer Registry; Controls: cardiovascular or cerebrovascular disease cause of death; Exclusions: arrhythmia, all noncancer diseases also used as controls Cancer mortality (liver, stomach, colorectal, lung, all cancers)	1,333 decedents: 266 cancer cases; 344 cardiovascular-cerebrovascular controls	Not applicable	Proxy—residence near factory. Exposed lived downstream of factory; unexposed: lived upstream Death certificates linked to Labour Insurance Bureau to find previously employed in factory	Mantel-Haenszel for mortality ORs, 95% CIs, adjusted for age Multiple logistic regression for exposure effect, adjusted for age, time period Cochran-Armitage test for trend for effect of time period Latency period of 10 yr

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Table B-4. Summaries of characteristics of geographically based and other studies (continued)

Reference	Study design[b]	Sample size	Data collection	Exposure assessment[c]	Statistical approach[d]
Ma et al. (2009)	Cases discharged from hospital with diagnosis of kidney cancer, 1993−2004, for New York City zip codes with median household income of $17,864−$142,926. Population estimate by zip code from U.S. Department of Census. Proxy exposure surrogate: density of dry-cleaning businesses (number of dry cleaners/zip code area in square kilometers) Renal cell cancer prevalence	10,916 discharges, 1,458 discharges in lowest exposure category (referent group) Unit of analysis is discharge, and a subject could be counted as many times as discharged from hospital	Discharge information obtained from New York Statewide Planning and Research Cooperative System	Density of dry cleaners per zip code (number of dry cleaners/zip code area) proxy surrogate for PCE exposure. Proxy exposure surrogate not validated with ambient monitoring data	Negative binomial regression for prevalence rate ratios, 95% CIs, adjusted for population density, age, race, and interactions specific to individual exposure level
Mallin (1990)	Cases: identified from medical records in 8 counties of northwest Illinois, men and women, diagnosed with histologically confirmed bladder cancer from 1978−1985, those diagnosed or treated in Iowa or Wisconsin also identified from Iowa State Health Registry, Wisconsin Cancer Reporting System; deaths from bladder cancer obtained Proxy—residence near contaminated well water Bladder cancer incidence and mortality	Cases and residence from medical records and cancer registries 712 bladder cancer cases among Caucasian men and women	Not applicable	Incidence data stratified by county and zip code; Winnebago county later found to have well water contaminated with PCE and other compounds	Indirect standardization for SIRs and SMRs, each adjusted for age; 95% CIs using Miettinen's exact limits; χ^2 tests for significance except when expected counts <5: Fisher exact test for 95% CIs, significance assumed Poisson distribution

Table B-4. Summaries of characteristics of geographically based and other studies (continued)

Reference	Study design[b]	Sample size	Data collection	Exposure assessment[c]	Statistical approach[d]
Morton and Marjanovic (1984)	Cases: Men and women leukemia cases, diagnosed 1963−1977, residing in Portland-Vancouver Metropolitan Area, 16−74 yr old, Referents: 1970 age-specific leukemia rates for U.S. population, direct method of age standardization Proxy—occupational title on hospital record or on death certificate Leukemia incidence and mortality, including subtype (lymphatic, nonlymphatic)	975 leukemia cases among males, 336,850 population 703 leukemia cases among females, 102,310 population	Record-based information—Cases ascertained from hospital records and death certificates. Record source for usual occupational title not reported	Occupations grouped into 20 categories, 313 males and 1,298 females identified as dry cleaner or launderer (0.1% and 0.3%, respectively)	Comparison of directly standardized age-adjusted incidence rates
Vartiainen et al. (1993)	1) Cases: 2 villages who consumed contaminated water, 2 reference groups: drank ground water, drank surface water 2) Identified from Finnish Cancer Registry, all cancers, liver cancer, non-Hodgkin lymphoma, multiple myeloma, leukemia in 1 exposed village and 1 exposed municipality Proxy—residence in exposed village Cancer incidence (liver, non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, leukemia, all cancers combined)	1) 116 identified cases, 108 exposed; 60 unexposed references 2) 1,931 cases in exposed villages	1) Interviewed about drinking water source and daily water consumption, urine samples collected 2) Not applicable	1) Exposed: drank contaminated water; gas chromatography to detect PCE in urine samples 2) Residence in exposed village or municipality	1) Compare urine levels in villages; methodology not reported 2) SIR, 95% CIs, assuming Poisson distribution; expected numbers based on age- and sex-specific number of cancer cases for Finland each year 1953−1991

Table B-4. Summaries of characteristics of geographically based and other studies (continued)

[a]A study's hypothesis is included here if explicitly stated; otherwise, only the objective is included.
[b]Study design includes the overall approach, study population, relevant dates, type of exposure, and endpoint measured. For type of exposure, when a proxy is not explicitly stated for PCE, an attempt was made to identify proxies based on relevant industries and occupations, including laundry/dry cleaners and textile industry, and to some extent, metal industry, aerospace industry, appliance industry, automotive industry, and manufacturing of chloroflourocarbons (though not used).
[c]Exposure assessment includes exposure assignment (e.g., was coding conducted and by whom), exposure approach (e.g., what kind of coding was used), and exposure-assessment metrics (e.g., length of exposure).
[d]Statistical approach includes adjustment for covariates, latency, or lag period, and documentation of statistical analysis and observations.

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APPENDIX C. CONSISTENCY OF TETRACHLOROETHYLENE AND TRICHLOROACETIC ACID HEPATOCARCINOGENICITY

Trichloroacetic acid (TCA), a metabolite of tetrachloroethylene, is associated with hepatocarcinogenicity in male and female mice (DeAngelo et al., 2008; Bull et al., 2002; Pereira, 1996; Ferreira-Gonzalez et al., 1995; Daniel et al., 1993; JISA, 1993; Bull et al., 1990; Herren-Freund et al., 1987; NTP, 1986; NCI, 1977). There has been some suggestion that TCA does not account for all of the toxicity observed with tetrachloroethylene exposure (Clewell et al., 2005; Buben and O'Flaherty, 1985), while others have suggested that TCA can account for liver tumors induced by tetrachloroethylene (Sweeney et al., 2009). The purpose of this investigation is to examine quantitatively what fraction of tetrachloroethylene hepatocarcinogenicity may be associated with TCA, using tetrachloroethylene and TCA bioassay data along with updated physiologically based pharmacokinetic (PBPK) model-based predictions.

C.1. Methods

C.1.1. Response Data

Because of the more robust liver tumor response in males, and because the PBPK modeling was calibrated exclusively to data in male mice, only response data in male mice were considered. Table C-1 provides the hepatocellular adenoma or carcinoma incidence data from the two tetrachloroethylene inhalation bioassays considered in this assessment, National Toxicology Program (NTP, 1986) and Japan Industrial Safety Association (JISA, 1993) (for convenience, the studies will be referred to in the remainder of this appendix as the NTP and JISA studies). These were previously described in Section 5, and so are not discussed further here.

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.

Table C-2 summarizes data from the available TCA studies considered for carrying out dose-response modeling. A number of these TCA studies lack information for a complete comparison of hepatocarcinogenicity between tetrachloroethylene and TCA, either in terms of exposure (i.e., drinking water intake not reported so total intake of TCA cannot be calculated) or

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in terms of responses. In particular, 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. Only the chronic (104-week) study of DeAngelo et al. (2008) reported data on both total TCA intake as well as combined incidences of adenomas and carcinomas, and only this study was considered further for comparing with the tetrachloroethylene bioassays in male mice. However, one significant limitation of the DeAngelo et al. (2008) study is the high incidence of liver tumors in control animals as compared to other TCA studies and as compared to the historical background rates of these tumors in $B6C3F_1$ mice. This raises concerns about the representativeness of the DeAngelo et al. (2008) study for comparison to other studies. Nonetheless, because this is the only available chronic-duration study that reports all the data needed, it was used for this analysis.

Table C-1. Incidence of hepatocellular adenomas and carcinomas in male $B6C3F_1$ 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

[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.

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Table C-2. TCA drinking water studies in male mice: incidence of hepatocellular adenomas and carcinomas

Source	Weeks of exposure	TCA exposure (g/L)	Average TCA intake (mg/kg-day)	*N*	Incidence of adenomas	Incidence of carcinomas	Incidence of adenomas or carcinomas	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[b] 11	NR NR	3[b] 8	NR NR	0.19 0.73
DeAngelo et al. (2008)	104	0 0.06[c] 0.7[c]	0 6.7[d] 81.2[d]	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 wk. Those exposures were converted to mg/kg-day by multiplying by (1,000 mg/g)/(7 d/wk * 52 wk).
[b] Estimated from the reported proportion responding by selecting the smallest group size and incidence value consistent with the precision of the reported proportion.
[c] Measured concentrations—nominal concentrations were 0.05 and 0.5 mg/L.
[d] Calculated based on measured concentrations (different than those reported in the manuscript, which were based on nominal concentrations).

NR = not reported.

C.1.2. Exposure-Level Conversions

TCA bioassay exposures were generally reported in terms of water concentration, in mg/L or mmol/L. Table C-2 provides the exposure levels as reported by each set of authors. Some reports provided mg/kg-day equivalents. To account for TCA bioavailability, the best estimates of bioavailability from Chiu (2011) were used. These are modeled as an "effective" concentration C_{eff} that changes as a function of actual concentration C: $C_{eff} = C_{max} \times C/(C_{½} + C)$, where C_{max} is a "maximal" effective concentration, and $C_{½}$ is the actual concentration where the effective concentration is half the maximal value. Thus, the rate of TCA absorption will be given by C_{eff} × Drinking water intake rate = (C_{eff}/C) × TCA intake. The best fit values from Chiu (2011) of the parameters are C_{max} = 1.34 mg/L and $C_{½}$ = 1.82 mg/L. The area under the curve (AUC) of TCA in the liver is also calculated using the Chiu (2011) PBPK model for TCA. This model is based on a model previously calibrated to TCA kinetic data from trichloroethylene exposure and TCA oral gavage and i.v. exposures, and subsequently updated by Chiu (2011) with TCA kinetic data from drinking water exposures [the same data used by Sweeney et al. (2009)]. The Chiu and Ginsberg (2011) PBPK model was used to estimate the AUC of TCA in the liver corresponding to the bioassay exposures in the NTP and JISA studies. As shown in Table C-3, accounting for reduced TCA bioavailability, the exposures in the DeAngelo et al. (2008) study lead to internal doses that are within the range of the internal doses predicted for the tetrachloroethylene inhalation bioassays.

Table C-3. PBPK model-estimated TCA internal dose measures for tetrachloroethylene and TCA bioassays used in analysis

Study	*N*	Exposure group	Proportion with adenomas or carcinomas	TCA absorbed[a] (mg/kg-day)	TCA produced from PCE[b] (mg/kg-day)	AUC of free TCA in plasma[a,b] (mg-hr/L-day)	AUC of TCA in liver[a,b] (mg-hr/L-day)
NTP (1986)	49	0 ppm PCE	0.35	–	0	0	0
	47	100 ppm PCE	0.66	–	29	454	487
	50	200 ppm PCE	0.82	–	53	834	895
JISA (1993)	46	0 ppm PCE	0.28	–	0	0	0
	49	10 ppm PCE	0.43	–	4.5	73	78
	48	50 ppm PCE	0.40	–	16	260	280
	49	250 ppm PCE	0.82	–	64	1,043	1,121
DeAngelo et al. (2008)	56	0 g/L TCA	0.55	0	–	0	0
	48	0.06 g/L TCA	0.44	5.9	–	74	58
	51	0.7 g/L TCA	0.71	53	–	666	526

[a] Calculated using PBPK model of Chiu (2011), using posterior mean parameter estimates and best-fit estimate of the fractional absorption from drinking water (88% at 0.6 g/L and 66% at 0.7 g/L).
[b] Calculated using PBPK model of Chiu and Ginsberg (2011), using highest posterior mode parameter estimates.

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C.1.3. Dose-Response Modeling and Statistical Analysis

Due to significantly different tumor incidences in control groups across the TCA and tetrachloroethylene data sets, combined analysis using standardized software such as BMDS is not feasible. Therefore, to test the consistency of the dose-response relationships, a standard statistical approach for binomial data is used. In particular, logistic regression was applied to the various data sets, with potential independent variables of dose, chemical, study, and their interactions (products). For a k-length vector of independent variable $\boldsymbol{x}$, and a probability of effect $p(\boldsymbol{x})$, the logistic model is defined by

$$p(\boldsymbol{x}) = 1/(1 + \exp[-z(\boldsymbol{x})]) \quad \text{(C-1)}$$

$$z(\boldsymbol{x}) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k \quad \text{(C-2)}$$

with parameters $\beta_j, j = 1 \ldots k$. This is equivalent to linear regression with binomial variance of the variable z, which is the natural log of the odds of a effect $\ln[p/(1\text{-}p)]$.

For the purposes of this analysis, β_0 is called the "intercept," β_1 is called the "slope" with respect to the dose metric x_1, $x_2 = 0$ or 1 is either the study (NTP = 0, JISA = 1, for tetrachloroethylene data) or the chemical (tetrachloroethylene = 0, TCA = 1) with β_2 the corresponding regression coefficient (i.e., "different intercepts"), and $x_3 = x_1 \times x_2$ is the interaction term (i.e., "different slopes"). In this analysis, all β coefficients are unconstrained.

This model was implemented using the "glm" function in the R statistical package (version 12.2.1), which reports optimized estimates, standard errors, and p-values for each regression parameter. In addition, analysis of variance (ANOVA) using the "anova" function in R (with a chi-squared test statistic) can also be used to determine whether additional regression parameters produce significant improvement in model fit.

Of particular interest is the "interaction term" with regression coefficient β_3. In the event that this parameter is statistically significant (p-value < 0.05, confirmed by ANOVA), then this would be evidence that tetrachloroethylene and TCA have different dose-responses as a function of internal TCA dose (after accounting for possible differences in control incidences). In the event that this parameter is not statistically significant, it is well known that failure to reject a null hypothesis of no effect may simply be the result of low statistical power. While there is a large literature on "postexperiment power calculations," Hoenig and Heisey (2001) show that such an approach is fundamentally flawed and yield no further insights than confidence intervals (CIs). Therefore, the CI of the β_3 parameter is used as an indication of the range of possible contributions of TCA to tetrachloroethylene hepatocarcinogenesis.

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C.2. Results

C.2.1. Logistic Model Fits to Individual Data Sets

Each bioassay was fit individually (using an intercept β_0 and a slope β_1) and goodness-of-fit evaluated using the chi-squared test on the residuals (see Table C-4, Figures C-1A and C-2A). All p-values were ≥0.15, suggesting the logistic model is an adequate description of the data for the purposes of this comparison.

C.2.2. Consistency of NTP and JISA Data

Analysis was conducted on the NTP and JISA studies to see if they are consistent, which would increase statistical power in the subsequent analysis of the contribution of TCA. Three logistic models were fit (see Table C-4, Figures C-1B, C-1C, and C-1D). Neither the study intercept β_2 nor the study-dose interaction term β_3 was statistically significant (CIs overlapped with 0, p-values from ANOVA were 0.17 and 0.14, respectively). Thus, the null hypothesis that the two bioassays for tetrachloroethylene have a common slope and intercept cannot be rejected.

As suggested by Hoenig and Heisey (2001), looking at the CIs gives insight into the power to reject this null hypothesis. The difference in intercepts (β_2) has a 95% confidence region (based on ± 1.96 × standard error) of (−0.81, 0.14), implying that the odds of a tumor in JISA control animals is between 0.44- and 1.16-fold that of NTP control animals. The difference in slopes (β_3) has a 95% confidence region of (−1.60, 0.23), implying that the odds ratio for JISA-exposed animals is between 0.20- and 1.26-fold that of NTP animals with equivalent AUCs of TCA in the liver. These ranges are quite large—up to twofold difference in background odds, and up to a fivefold difference in exposed/unexposed odds ratios cannot be ruled out by the available data. Nonetheless, for the purposes of further analysis, the NTP and JISA studies are combined, as these provide greater statistical power for determining the extent to which the TCA bioassay is consistent with the tetrachloroethylene bioassays on the basis of TCA internal dose.

Table C-4. Logistic regression model fits—beta coefficients and standard errors[a]

Data analyzed	β_0 (Intercept)	1,000 × β_1 (Dose coefficient)	β_2 (Study or chemical intercept)	1,000 × β_3 (Dose × [study or chemical])	Chi-squared goodness-of-fit *p*-value[b]	Figure
NTP (1986)	–0.599 ± 0.280	2.43 ± 0.53	Not included	Not included	0.75	C-1A
JISA (1993)	–0.763 ± 0.199	1.97 ± 0.39	Not included	Not included	0.37	C-1A
DeAngelo et al. (2008)	–0.028 ± 0.207	1.64 ± 0.72	Not included	Not included	0.15	C-2A
NTP (1986) + JISA (1993)	–0.696 ± 0.162	2.21 ± 0.32	Not included	Not included	0.47	C-1B
	–0.482 ± 0.223	2.14 ± 0.32	*–0.334 ± 0.244*[c]	Not included	0.77	C-1C
	–0.709 ± 0.162	2.59 ± 0.42	Not included	*–0.683 ± 0.467*[c]	0.80	C-1D
NTP (1986) + JISA (1993) + DeAngelo et al. (2008)	–0.428 ± 0.125	1.95 ± 0.28	Not included	Not included	0.068	C-2B
	–0.665 ± 0.155	2.12 ± 0.29	0.556 ± 0.211	Not included	0.40	C-2C
	–0.696 ± 0.162	2.21 ± 0.32	0.668 ± 0.262	*–0.572 ± 0.790*[c]	0.36	C-2D

[a] Logistic model: proportion responding = $p(\boldsymbol{x}) = 1/(1 + \exp[-z(\boldsymbol{x})])$, $z(\boldsymbol{x}) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2$,.
where
x_1 = dose
x_2 = study (NTP = 0, JISA = 1) for analysis of NTP (1986) + JISA (1993)
x_2 = chemical (PCE [NTP or JISA] = 0, TCA [DeAngelo] = 1) for analysis of NTP (1986) + JISA (1993) + DeAngelo et al. (2008).

[b] Chi-squared percentage point at the sum-squared residuals (weighted by inverse binomial variance), with degrees of freedom equal to the number of data points minus the number of parameters.

[c] Parameters in *italics* are not significant ($p > 0.05$) either by regression CI or by ANOVA.

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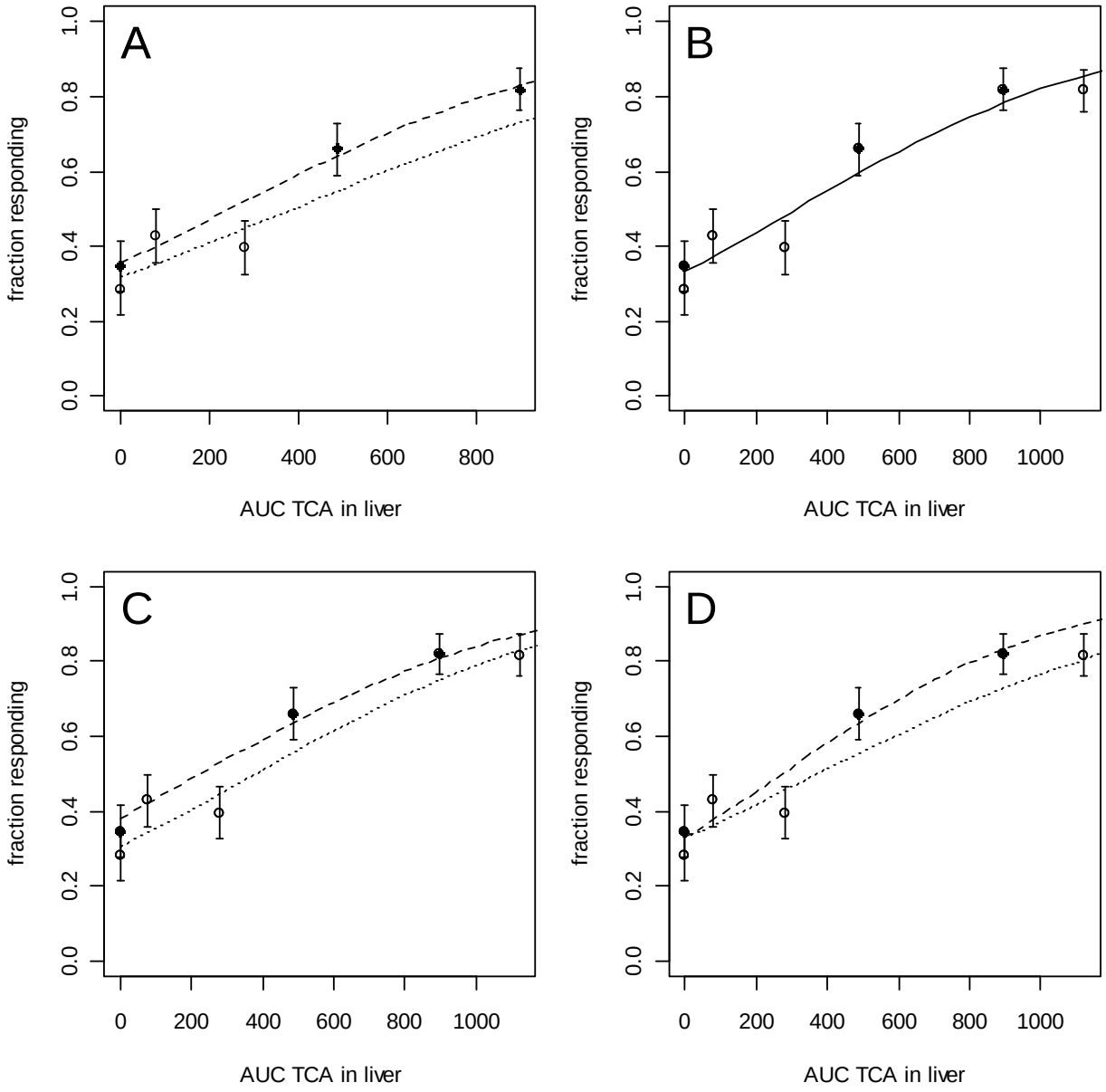

Figure C-1. Logistic regression dose-response fits to tetrachloroethylene data [open circle: JISA (1993); filled circle: NTP (1986)].
A: separate model fits to each dataset; B: single model fit to both data sets; C: model with separate intercepts and common slope; D: model with common intercept and separate slopes.
See Table C-4 for parameter values, standard errors, and goodness-of-fit *p*-values.

C.2.3. Consistency of Tetrachloroethylene and TCA Data

Analysis was conducted on the combined NTP and JISA studies and the DeAngelo et al. (2008) TCA study to see if they are consistent. Three logistic models were fit (see Table C-4, Figures C-2B, C-2C, and C-2D). The chemical intercept term β_2 was statistically significant (parameter CI did not overlap with 0, p-value from ANOVA was 0.009). Thus, the null hypothesis that the two bioassays for tetrachloroethylene have a common intercept is rejected. The chemical slope term β_3 was not statistically significant (parameter CI overlapped with 0, p-value from ANOVA was 0.46). Thus, the null hypothesis that the TCA and tetrachloroethylene bioassays have a common slope (after accounting for different background rates) cannot be rejected.

As suggested by Hoenig and Heisey (2001), looking at the CIs gives insight into the power to reject this null hypothesis. The difference in slopes (β_3) has a 95% confidence region of −2.12, 0.98, implying that the odds ratio for TCA exposed/unexposed animals is between 0.12- and 2.65-fold that of tetrachloroethylene-exposed/unexposed animals with equivalent AUCs of TCA in the liver. These ranges are quite large—up to an eightfold difference in exposed/unexposed odds ratios cannot be ruled out by the available data. Moreover, these CIs assume that the tetrachloroethylene data reflect a common dose-response—relaxing this assumption would lead to wider CIs for the relative odds ratios between TCA and tetrachloroethylene bioassays.

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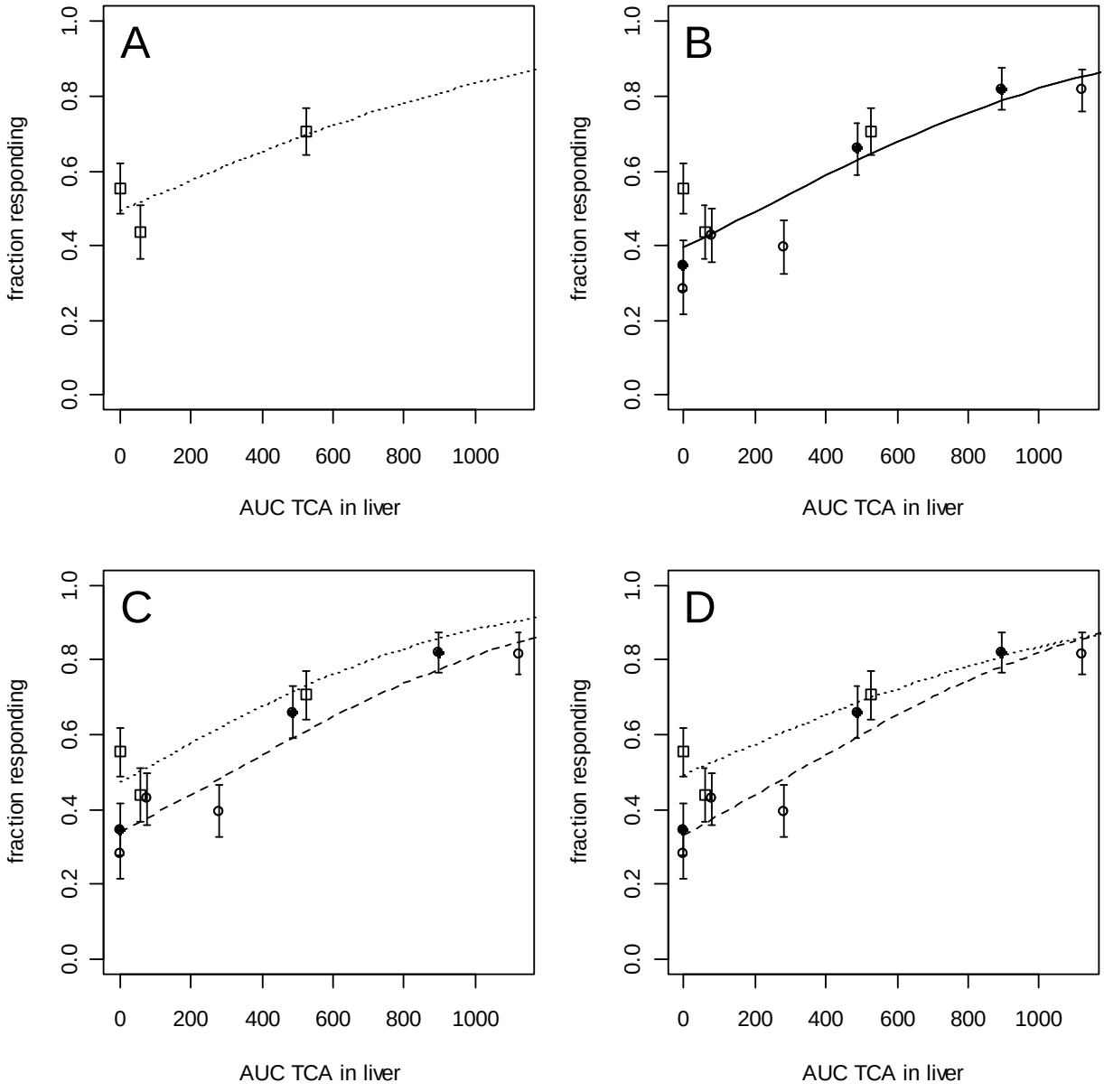

Figure C-2. Logistic regression dose-response fits to TCA
[open square: DeAngelo et al. (2008)] and combined TCA and tetrachloroethylene data [open circle: JISA (1993); filled circle: NTP (1986)]. A: model fit to TCA data only; B: single model fit to all data sets; C: model with chemical-specific intercepts and common slope; D: model with chemical-specific intercepts and chemical-specific slopes. See Table C-4 for parameter values, standard errors, and goodness-of-fit *p*-values.

C.3. CONCLUSIONS

This analysis suggests that TCA might explain the incidence of carcinomas observed in the available tetrachloroethylene bioassays but that a wide range of possible contributions cannot be ruled out by the available data. Specifically, a contribution of TCA from as little as 12 up to 100% cannot be ruled out, under the assumptions that the tetrachloroethylene NTP and JISA bioassay data can be combined, and using the Chiu and Ginsberg (2011) PBPK model for tetrachloroethylene and the Chiu (2011) PBPK model for TCA and TCA bioavailability. If either of these assumptions is relaxed—i.e., given that residual uncertainties of about twofold exist in the PBPK model predictions for TCA internal dose and that there may be some underlying differences between the NTP and JISA bioassays—then the CIs will be greater. Furthermore, the high control tumor incidence reported in the TCA bioassay of DeAngelo et al. (2008) raise questions as to the representativeness of that bioassay for comparison to tetrachloroethylene bioassays. Overall, as discussed in Chiu (2011) with regards to the contribution of TCA to TCE-induced hepatomegaly, factors such as study-to-study experimental variability in kinetics (e.g., metabolism, bioavailability) or in dynamics (e.g., background tumor rates), different analytical methods used to quantify TCA in blood and tissues and uncertainty in TCA dosing patterns in drinking water studies further limit the ability to discern the quantitative contribution of TCA. A more precise quantitative measure of the relative contribution of TCA to tetrachloroethylene-induced liver tumors requires an appropriately designed experiment to better control for these factors.

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APPENDIX D. CANCER DOSE-RESPONSE MODELING

D.1. Model Selection Details for Tumor Sites from JISA (1993)

Table D-1. Model predictions for hepatocellular tumors in male mice (JISA, 1993)[a], using several dose metrics and multistage cancer model

Model stages	Goodness of fit			BMD_{10}	$BMDL_{10}$	Conclusion
	p-value[b]	Largest standardized residual(s)	AIC			
Total liver oxidative metabolism (mg/kg$^{0.75}$-day)						
One	**0.24**	**1.1, low-dose −1.2, mid-dose**	**239.7**	**2.9**	**2.1**	All three fits were adequate by conventional criteria.[b] There was no statistical improvement in adding higher-order coefficients (using likelihood ratio test); one-stage fit was selected.
Two	0.16	−0.7, control 1.1, low-dose	240.8	6.4	2.2	
Three	0.18	−0.7, control 1.0, low-dose	240.6	6.5	2.2	
TCA AUC in liver (mg-hr/L-day)						
One	**0.25**	**1.0, low-dose −1.2, mid-dose**	**239.7**	**97.1**	**68.8**	All three fits were adequate by conventional criteria.[b] There was no statistical improvement in adding higher-order coefficients (using likelihood ratio test); one-stage fit was selected.
Two	0.17	−0.7, control 1.1, low-dose	240.8	209.9	72.8	
Three	0.19	−0.7, control 1.0, low-dose	240.6	213.9	73.8	
Administered tetrachloroethylene concentration (ppm)						
One	**0.27**	**1.2, low-dose −1.0, mid-dose**	**239.5**	**3.9**	**2.7**	All three fits were adequate by conventional criteria.[b] There was no statistical improvement in adding higher-order coefficients (using likelihood ratio test); one-stage fit was selected.
Two	0.16	−0.8, control 1.1, low-dose	240.9	9.0	2.8	
Three	0.17	−0.8, control 1.1, low-dose	240.8	8.2	2.9	

[a] Incidence data and human equivalent continuous exposure estimates provided in Table 5-13.
[b] Goodness-of-fit *p*-values <0.05 for a preferred model, or <0.10 when considering many models, fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within ±2 units) are considered. Best-fit model is highlighted in bold; output for best-fit models provided in following pages.
AIC = Akaike's Information Criteria, BMD = benchmark dose, BMDL = lower bound benchmark dose.

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D.1.1. Modeling Output for Male Mice, Hepatocellular Tumors (JISA, 1993)

D.1.1.1. With total oxidative metabolism in liver as dose metric

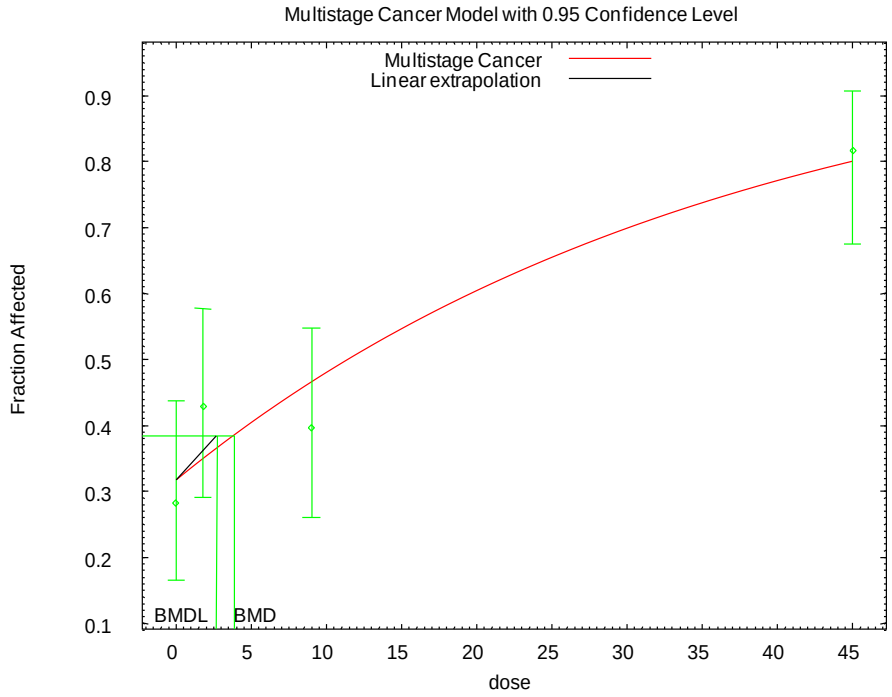

Figure D-1 One-degree multistage model fit to hepatocellular tumors in male mice (JISA, 1993), with BMD and BMDL at 10% extra risk, using total oxidative metabolism in liver (mg/kg$^{0.75}$-day).

```
=================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)

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

   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 = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1
```


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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 = 0.285739
Beta(1) = 0.0395068

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.53
Beta(1)	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.301268	*	*	*
Beta(1)	0.0361674	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-116.442	4			
Fitted model	-117.844	2	2.80477	2	0.246
Reduced model	-132.99	1	33.0977	3	<.0001

AIC: 239.688

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3013	13.858	13.000	46	-0.276
2.2500	0.3559	17.438	21.000	49	1.063
8.3000	0.4825	23.158	19.000	48	-1.201
33.6000	0.7927	38.844	40.000	49	0.408

Chi^2 = 2.81 d.f. = 2 P-value = 0.2448

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.91314

BMDL = 2.06187

BMDU = 4.49484

Taken together, (2.06187, 4.49484) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0484996

D.1.1.2. With TCA AUC in liver as dose metric

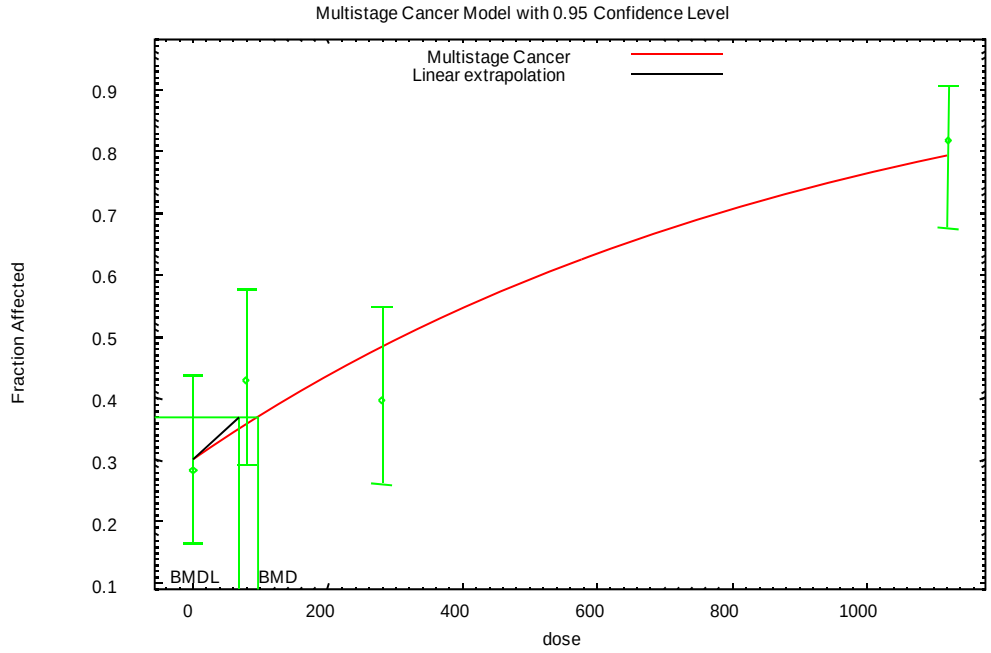

Figure D-2. One-degree multistage model fit to hepatocellular tumors in male mice (JISA, 1993), with BMD and BMDL at 10% extra risk, using TCA AUC as dose metric (mg-hr/L-d).

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)

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

  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 = Response
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                   Background =     0.283935
                      Beta(1) =   0.00118591


          Asymptotic Correlation Matrix of Parameter Estimates
```

	Background	Beta(1)
Background	1	-0.53
Beta(1)	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.299803	*	*	*
Beta(1)	0.0010848	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-116.442	4			
Fitted model	-117.833	2	2.78303	2	0.2487
Reduced model	-132.99	1	33.0977	3	<.0001

AIC: 239.666

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2998	13.791	13.000	46	-0.255
78.4900	0.3570	17.491	21.000	49	1.046
279.7000	0.4831	23.186	19.000	48	-1.209
1121.1000	0.7925	38.832	40.000	49	0.411

Chi^2 = 2.79 d.f. = 2 P-value = 0.2477

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 97.1242

BMDL = 68.7915

BMDU = 149.76

Taken together, (68.7915, 149.76) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00145367

D.1.1.3. With administered tetrachloroethylene concentration (ppm) as dose metric

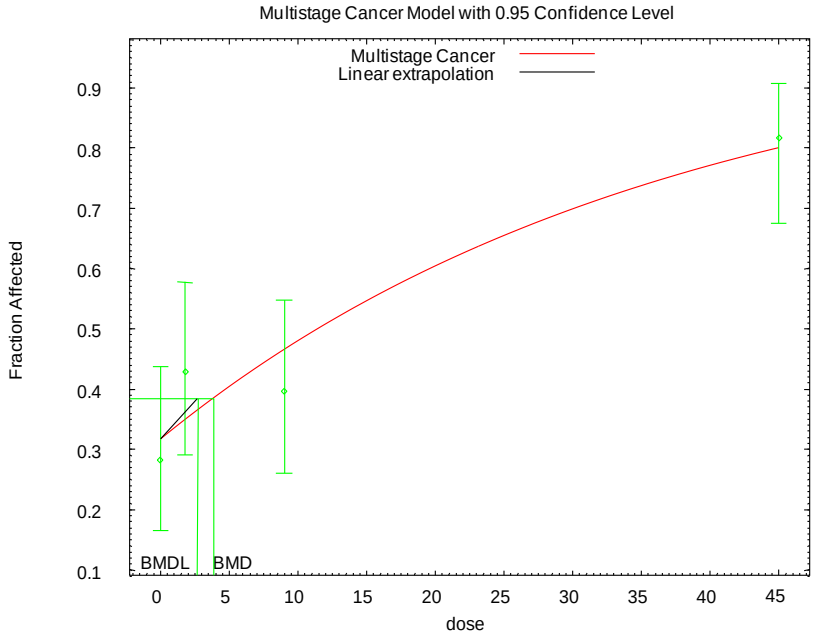

Figure D-3. One-degree multistage model fit to hepatocellular tumors in male mice (JISA, 1993), with BMD and BMDL at 10% extra risk, using administered tetrachloroethylene concentration (ppm).

```
 ================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
================================================================

   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 = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =     0.307193
                        Beta(1) =    0.0290723
```

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.48
Beta(1)	-0.48	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.316506	*	*	*
Beta(1)	0.0273229	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-116.442	4			
Fitted model	-117.738	2	2.59226	2	0.2736
Reduced model	-132.99	1	33.0977	3	<.0001

AIC: 239.476

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3165	14.559	13.000	46	-0.494
1.8000	0.3493	17.116	21.000	49	1.164
9.0000	0.4655	22.344	19.000	48	-0.968
45.0000	0.8001	39.206	40.000	49	0.284

Chi^2 = 2.62 d.f. = 2 P-value = 0.2704

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.85613

BMDL = 2.70709

BMDU = 5.98909

Taken together, (2.70709, 5.98909) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.03694

Table D-2. Model predictions for hepatocellular tumors in female mice (JISA, 1993), using several dose metrics[a] and multistage cancer model

Model stage	Goodness of fit			BMD_{10}	$BMDL_{10}$	Comments	Conclusions
	p-value[b]	Largest standardized residual(s)	AIC				
Total liver oxidative metabolism ($mg/kg^{0.75}$-day)							
One-stage	0.14	-1.4, mid-dose	154.9	3.7	2.8	Adequate fit	Selected two-degree multistage, based on likelihood ratio test.
Two-stage	**0.82**	**-0.18, low-dose**	**152.8**	**8.4**	**4.0**	Adequate fit	
Three-stage	0.82	-0.18, low-dose	152.8	8.4	3.9	Adequate fit	
TCA AUC in liver (mg-hr/L-day)							
One-stage	0.13	-1.4, mid-dose	155.1	129	98	Adequate fit	Selected two-degree multistage, based on likelihood ratio test.
Two-stage	**0.82**	**-0.18, low-dose**	**152.9**	**292**	**141**	Adequate fit	
Three-stage	0.82	-0.18, low-dose	152.9	292	139	Adequate fit	
Administered tetrachloroethylene concentration (ppm)							
One-stage	**0.36**	**−1.1, mid-dose**	**153.0**	**5.0**	**3.8**	Adequate fit	Selected one-degree multistage; no statistical improvement in adding higher order parameters.
Two-, three-stage	0.83	−0.1, low-dose	152.8	9.7	4.3	Identical fits resulted from both models	

[a] Incidence data provided in Table 5-13, and dose metrics provided in Table 5-17; both are included in following output.

[b] Values <0.05 for a preferred model, or <0.10 when considering a suite of models, fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within ±2 units) are considered. Best-fit model is highlighted in bold; output for best-fit models provided in following pages.

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D.1.2. Modeling Output for Female Mice, Hepatocellular Tumors (JISA, 1993)

D.1.2.1. With total oxidative metabolism in liver as dose metric

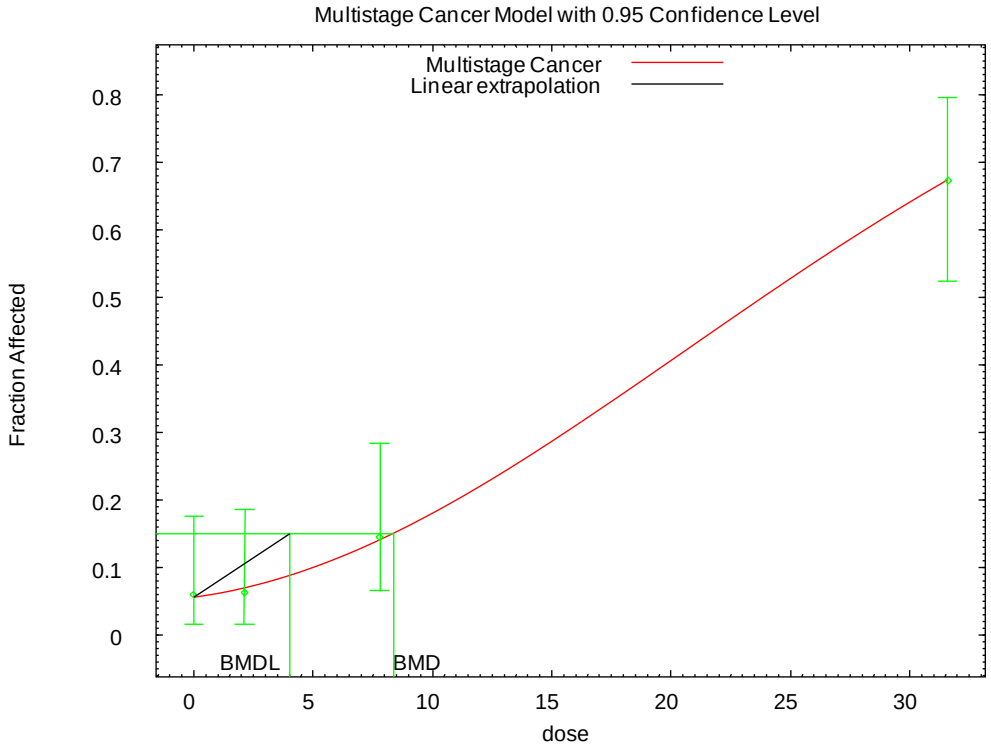

Figure D-4. **Two-degree multistage model fit to hepatocellular tumors in female mice (JISA, 1993), with BMD and BMDL at 10% extra risk.**

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\Usepa\BMDS21\msc_JISA1993_MF_HepAC_oxmet_Perc3_MultiCanc2_0.1.(d)
=================================================================

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values
Background = 0.0554081
Beta(1) = 0.00569729
Beta(2) = 0.000883583

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)	Beta(2)
Background	1	-0.69	0.59
Beta(1)	-0.69	1	-0.97
Beta(2)	0.59	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0566119	*	*	*
Beta(1)	0.00500318	*	*	*
Beta(2)	0.000907152	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-73.398	4			
Fitted model	-73.4233	3	0.050713	1	0.8218
Reduced model	-106.26	1	65.7232	3	<.0001

AIC: 152.847

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0566	2.831	3.000	50	0.104
2.1300	0.0704	3.311	3.000	47	-0.177
7.8000	0.1414	6.789	7.000	48	0.087
31.6000	0.6744	33.048	33.000	49	-0.015

Chi^2 = 0.05 d.f. = 1 P-value = 0.8230

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 8.36661

BMDL = 4.02336

BMDU = 11.6726

Taken together, (4.02336, 11.6726) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0248549

D.1.2.2. With TCA AUC in liver as dose metric

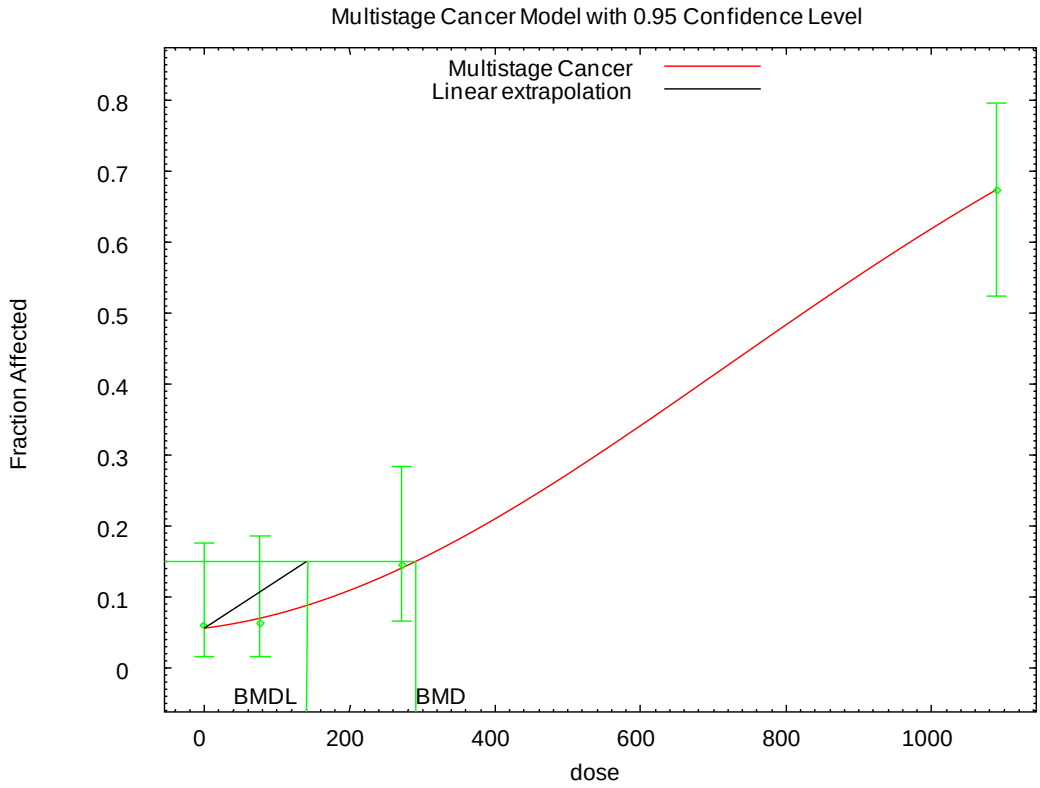

Figure D-5. Two-degree multistage model fit to hepatocellular tumors in female mice (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Usepa\BMDS21\msc_JISA1993_MF_HepAC_tcaAUC_Perc3_MultiCanc2_0.1.(d)
=================================================================

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0553149
                        Beta(1) =  0.000156854
```

Beta(2) = 7.50947e-007

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)	Beta(2)
Background	1	-0.69	0.6
Beta(1)	-0.69	1	-0.97
Beta(2)	0.6	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0565811	*	*	*
Beta(1)	0.000135812	*	*	*
Beta(2)	7.71737e-007	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-73.398	4			
Fitted model	-73.4249	3	0.0538645	1	0.8165
Reduced model	-106.26	1	65.7232	3	<.0001

AIC: 152.85

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0566	2.829	3.000	50	0.105
76.9500	0.0706	3.320	3.000	47	-0.182
271.8000	0.1412	6.776	7.000	48	0.093
1089.6000	0.6745	33.051	33.000	49	-0.016

Chi^2 = 0.05 d.f. = 1 P-value = 0.8177

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 291.833

BMDL = 141.409

BMDU = 402.749

Taken together, (141.409, 402.749) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000707168

D.1.2.3. With administered tetrachloroethylene concentration (ppm) as dose metric

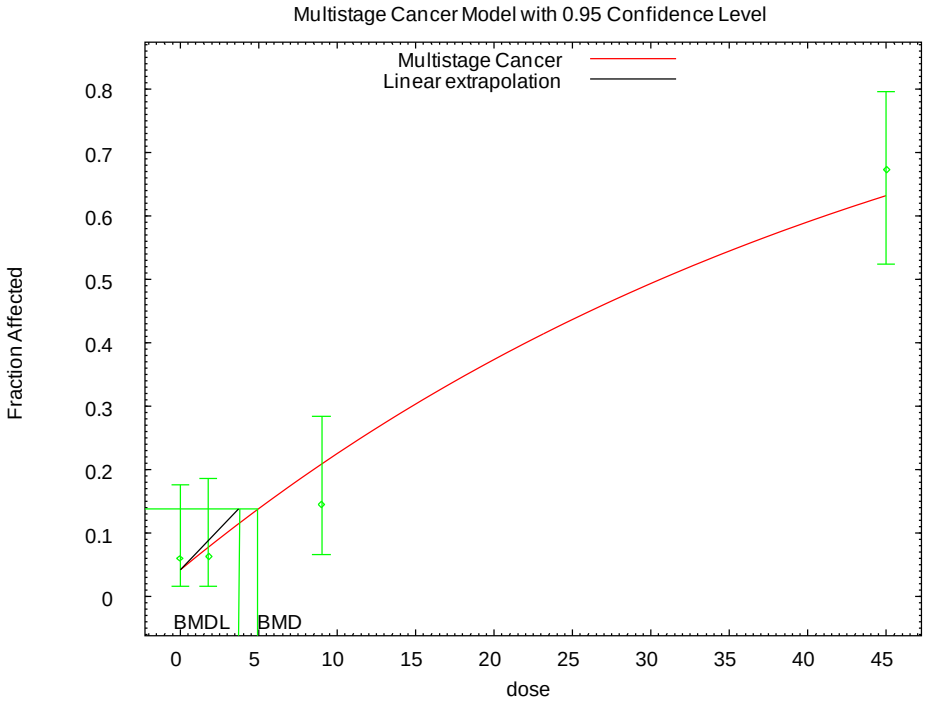

Figure D-6. One-degree multistage model fit to hepatocellular tumors in female mice (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)

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

   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 = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0124442
```

Beta(1) = 0.0242761

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.47
Beta(1)	-0.47	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0427836	*	*	*
Beta(1)	0.0212108	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-73.398	4			
Fitted model	-74.4575	2	2.11904	2	0.3466
Reduced model	-106.26	1	65.7232	3	<.0001

AIC: 152.915

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0428	2.139	3.000	50	0.602
1.8000	0.0786	3.696	3.000	47	-0.377
9.0000	0.2091	10.038	7.000	48	-1.078
45.0000	0.6315	30.942	33.000	49	0.610

Chi^2 = 2.04 d.f. = 2 P-value = 0.3609

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.96731

BMDL = 3.75394

BMDU = 6.8242

Taken together, (3.75394, 6.8242) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0266387

Table D-3. Model predictions for hemangiomas or hemangiosarcomas in male mice (JISA, 1993), using tetrachloroethylene AUC in blood and administered tetrachloroethylene concentration as dose metrics[a] and multistage cancer model

Model stage	Goodness of fit			BMD_{10}	$BMDL_{10}$	Conclusions
	p-value[b]	Largest standardized residual(s)	AIC			
Tetrachloroethylene AUC in blood (mg-hr/L-day)						
One-, two-, three-stage	**0.38**	**−1.0, low-dose, 0.9, mid-dose**	**142.0**	**63.0**	**34.3**	Fits for all three models were the same; only the first order term was >0.
Administered tetrachloroethylene concentration (ppm)						
One-, two-, three-stage	**0.38**	**−1.0, low-dose, 0.9, mid-dose**	**142.0**	**24.4**	**13.3**	Fits for all three models were the same; only the first order term was >0.

[a] Incidence data and human equivalent continuous exposures provided in Table 5-13 and in the output below.
[b] Values <0.05 for a preferred model, or <0.10 when considering suite of models, fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within $\pm$2 units) are considered. The best-fit model is highlighted in bold; the output for best-fit models is provided in following pages.

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D.1.3. Modeling Output for Male Mice, Hemangiomas or Hemangiosarcomas (JISA, 1993)

D.1.3.1. With tetrachloroethylene AUC in blood as dose metric

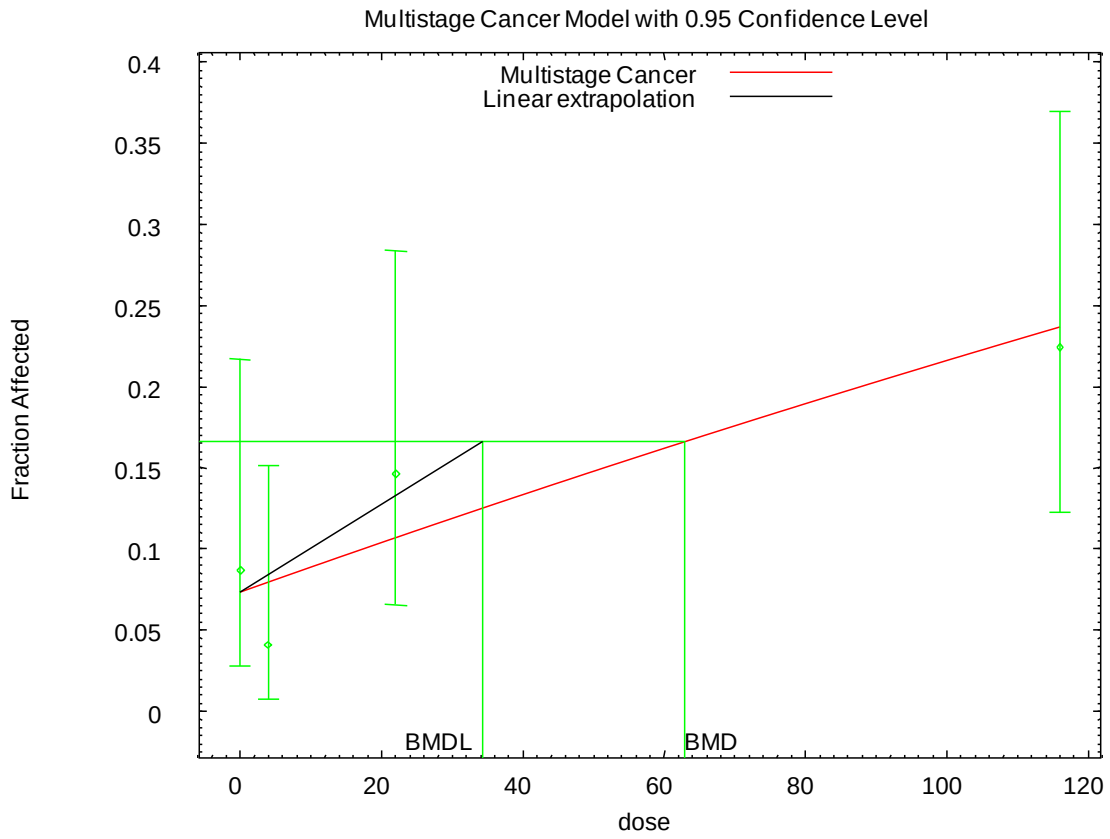

Figure D-7. One-degree multistage model fit to hemangioma or hemangiosarcoma incidence in male mice (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)

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


   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 = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
```


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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 = 0.0779832
Beta(1) = 0.00154747

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.6
Beta(1)	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0731517	*	*	*
Beta(1)	0.00167339	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-67.9801	4			
Fitted model	-69.0102	2	2.06035	2	0.3569
Reduced model	-72.3399	1	8.71962	3	0.03326

AIC: 142.02

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0732	3.365	4.000	46	0.360
4.0000	0.0793	3.887	2.000	49	-0.998
22.0000	0.1067	5.119	7.000	48	0.879
116.0000	0.2367	11.597	11.000	49	-0.201

Chi^2 = 1.94 d.f. = 2 P-value = 0.3794

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 62.9622

BMDL = 34.3348

BMDU = 191.7

Taken together, (34.3348, 191.7) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0029125

D.1.3.2. With administered tetrachloroethylene concentration (ppm) as dose metric

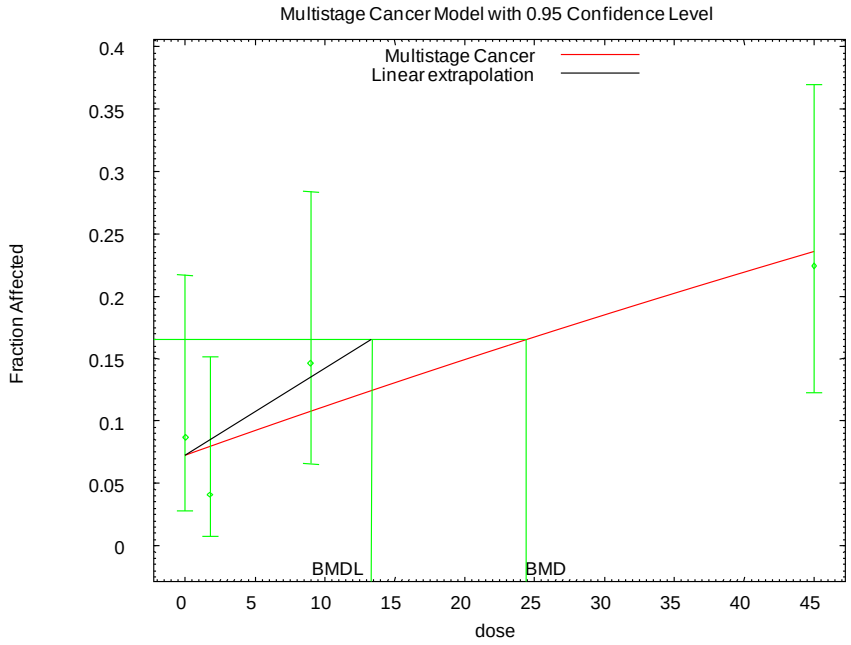

Figure D-8. One-degree multistage model fit to hemangioma or hemangiosarcoma incidence in male mice (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)

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

  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 = Response
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                    Background =    0.0770402
```

Beta(1) = 0.00401128

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.6
Beta(1)	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0723269	*	*	*
Beta(1)	0.00432149	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-67.9801	4			
Fitted model	-69.001	2	2.04183	2	0.3603
Reduced model	-72.3399	1	8.71962	3	0.03326

AIC: 142.002

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0723	3.327	4.000	46	0.383
1.8000	0.0795	3.896	2.000	49	-1.001
9.0000	0.1077	5.170	7.000	48	0.852
45.0000	0.2363	11.577	11.000	49	-0.194

Chi^2 = 1.91 d.f. = 2 P-value = 0.3843

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 24.3806

BMDL = 13.3404

BMDU = 73.8608

Taken together, (13.3404, 73.8608) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00749601

D.1.3.3. Modeling Output for Male Mice (JISA, 1993), Combined Risk of Hepatocellular Tumors or Hemangiomas/Hemangiosarcomas, at 10% Extra Risk, using Administered Concentration and Multistage Modeling (Discussed in Section 5.3.4.1)

```
================================================================
        MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
        Input Data File: C:\Usepa\BMDS220\Data\SessionFiles\New.(d)

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

[For separate model fits of hepatocellular tumors and hemangiomas/hemangiosarcomas
using administered concentration, refer to Sections D.1.1.3 and D.1.3.2, respectively.
Duplicate output from MS_COMBO was omitted here.]


**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                    -186.73874141530868

  Combined Log-likelihood Constant            169.44438524661712


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       3.32952

            BMDL =        2.4128
```


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Table D-4. Model predictions for male rat mononuclear cell leukemia (MCL) (JISA, 1993), using tetrachloroethylene AUC in blood and administered tetrachloroethylene concentration as dose metrics[a] and multistage model

Model	Goodness of fit			BMD_{10}	$BMDL_{10}$	Conclusions
	p-value[b]	Largest standardized residual(s)	AIC			
Tetrachloroethylene AUC in blood (mg-hr/L-day)						
One-, two-, three-stage	**0.52**	**1.0, mid-dose**	**254.9**	**46.1**	**29.7**	Fits for all three models were the same; only the first order term was >0.
Administered tetrachloroethylene concentration (ppm)						
One-, two-, three-stage	**0.52**	**1.0, mid-dose**	**254.9**	**20.5**	**13.2**	Fits for all three models were the same; only the first order term was >0.

[a] Incidence data and human equivalent continuous exposures provided in Table 5-15 and in the output below.
[b] Values <0.05 for a preferred model, or <0.10 when considering suite of models, fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within $\pm$2 units) are considered. Best-fit model is highlighted in bold; output for best-fit models provided in following pages.

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D.1.4. Modeling Output for Male Rats, MCL (JISA, 1993)

D.1.4.1. With tetrachloroethylene AUC in blood as dose metric

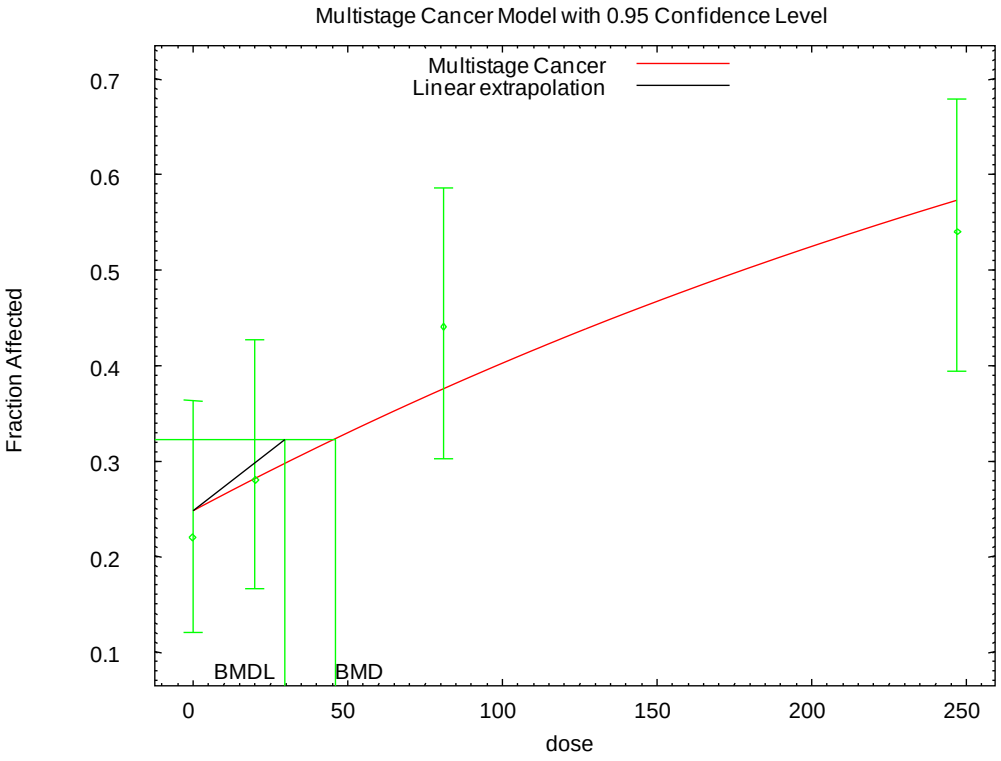

Figure D-9. One-stage model fit to MCL incidence in male rats (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\Usepa\BMDS21\msc_JISA1993_RM_MCL_percAUC_Perc3_MultiCanc1_0.1.(d)
=================================================================

   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 = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.263087
Beta(1) = 0.00204647

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.63
Beta(1)	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.24777	*	*	*
Beta(1)	0.0022863	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-124.787	4			
Fitted model	-125.442	2	1.31081	2	0.5192
Reduced model	-131.791	1	14.0088	3	0.002893

AIC: 254.884

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2478	12.388	11.000	50	-0.455
20.0000	0.2814	14.070	14.000	50	-0.022
81.0000	0.3749	18.747	22.000	50	0.950
247.0000	0.5723	28.617	27.000	50	-0.462

Chi^2 = 1.32 d.f. = 2 P-value = 0.5158

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 46.0834

BMDL = 29.6814

BMDU = 90.5076

Taken together, (29.6814, 90.5076) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00336911

D.1.4.2. With administered tetrachloroethylene concentration (ppm) as dose metric

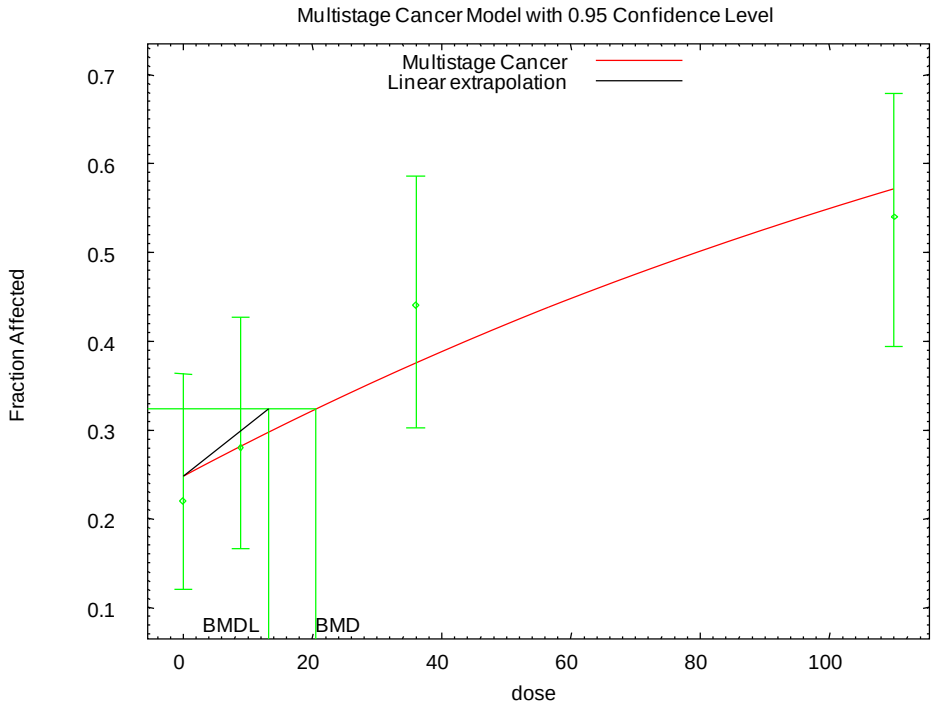

Figure D-10. One-stage model fit to MCL incidence in male rats (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=====================================================================

          Multistage Cancer Model. (Version: 1.5;  Date: 02/20/2007)
=====================================================================

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = mcl
   Independent variable = hecdose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                    Background =     0.263191
                       Beta(1) =   0.00459397
                       Beta(2) =            0
                       Beta(3) =            0
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(2) -Beta(3)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(1)
Background	1	-0.63
Beta(1)	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.247855	*	*	*
Beta(1)	0.00513336	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-124.787	4			
Fitted model	-125.445	2	1.3173	2	0.5175
Reduced model	-131.791	1	14.0088	3	0.002893

AIC: 254.891

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2479	12.393	11	50	-0.456
8.9000	0.2814	14.072	14	50	-0.023
36.0000	0.3748	18.738	22	50	0.953
110.0000	0.5724	28.618	27	50	-0.463

Chi^2 = 1.33 d.f. = 2 P-value = 0.5141

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 20.5247

BMDL = 13.2172

BMDU = 55.2398

Taken together, (13.2172, 55.2398) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00756592

Table D-5. Model predictions for female rat MCL (JISA, 1993),[a] using administered tetrachloroethylene concentration (ppm)[c] and multistage model

Model	Goodness of fit			BMD_{10}	$BMDL_{10}$	Conclusion
	p-value[b]	Largest standardized residual(s)	AIC			
Tetrachloroethylene AUC in blood (mg-hr/L-day)						All three models provided identical fits, with only the first-order parameter >0. However, model did not adequately estimate responses at control and low-dose. **Multistage model not selected** (output shown for administered concentration only)**.**
One-, two-, three-stage	0.34	−1.0, control 1.0, low-dose	249.4	136	61	
Administered tetrachloroethylene concentration (ppm)						
One-, two-, three-stage	0.34	−1.0, control 1.0, low-dose	249.4	60.4	26.8	

[a] Incidence data and human equivalent continuous exposure estimates provided in Table 5-15.
[b] When there is no preferred model, values <0.10 fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within ±2 units) are considered.
[c] Due to the proportionality of tetrachloroethylene AUC in blood, the preferred dose metric, to administered concentration, only administered concentration was used as the dose metric until the final model selection was made.

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Table D-6. Comparison of model predictions for female rat MCL (JISA, 1993),[a] using administered tetrachloroethylene concentration as dose metric[b]

Model	Goodness of fit			BMD_{10}	$BMDL_{10}$	Comments	Conclusion
	p-value[c]	Largest standardized residual(s)	AIC				
All dose groups							
Michaelis-Menten	0.55	(0.0, control) −0.5, mid-dose	249.6	5.3	NA	Best visual fit (refer to output)	
LogLogistic	0.34	−1.0, control 1.0, low-dose	249.4	56.2	NA	Slope parameter unrestricted[c]	Poor fit to control and low-dose responses.
Gamma, Weibull	0.34	−1.0, control 1.0, low-dose	249.4	60.4	NA	Power parameters unrestricted[c]	
Probit	0.33	−1.1, control 1.0, low-dose	249.5	67.6	35.5	—	
Logistic	0.33	−1.1, control 1.0, low-dose	249.5	68.4	36.5	—	
LogProbit	0.26	−1.0, control 1.0, low-dose	250.0	88.0	NA	—	
Highest dose group dropped							
Weibull	0.83	(0.0, control) 0.2, low-dose	181.0	NA	NA	Power parameter unrestricted[d]; step-function	Implausible fit
LogLogistic	0.17	−0.7, control 1.1, low-dose	182.7	26.5	NA	Slope parameter unrestricted[d]	Poor fit to control and low-dose responses
Multistage (one-degree)	0.17	−0.8, control 1.1, low-dose	182.7	28.3	10.3	No statistical improvement with higher order models	
Probit	0.16	−0.8, control 1.1, low-dose	182.8	31.5	13.7	—	
Logistic	0.16	−0.9, control 1.1, low-dose	182.8	31.9	14.1	—	
LogProbit	0.09	Inadequate					
Gamma, Michaelis-Menten	Insufficient degrees of freedom						
Highest two dose groups dropped							
Multistage	Fit statistics not relevant			4.9	2.3	Adequate fit to control data	
Other models	Insufficient degrees of freedom						

[a] Incidence data and human equivalent continuous exposures provided in Table 5-15.
[b] Due to the proportionality of tetrachloroethylene AUC in blood, the preferred dose metric, to administered concentration, only administered concentration was used as the dose metric until the final model selection was made.
[c] When there is no preferred model, values <0.10 fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within ± 2 units) are considered. Best-fit model is highlighted in bold; output for best-fit models provided in following pages.
[d] Slope or power parameters were initially limited to be ≥ 1 to avoid infinite slopes at zero dose. However, fits with restricted parameters may not provide BMDLs with correct statistical coverage. Fits to these data with unrestricted power or slope parameters did not provide BMDLs, effectively 0.

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D.1.5. Modeling Output for Female Rats, MCL (JISA, 1993), with administered tetrachloroethylene concentration as dose metric

D.1.5.1. Multistage model fit

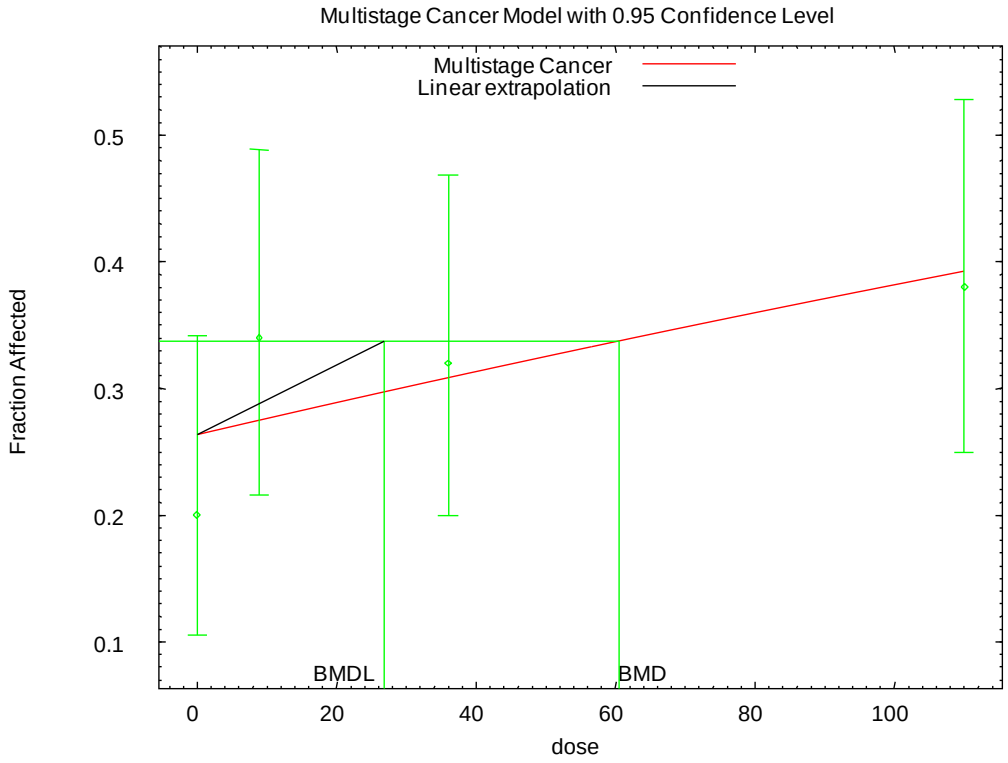

Figure D-11. One-stage multistage model fit to MCL incidence in female rats (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\Usepa\BMDS21\msc_JISA1993_RF_MCL_admc_Perc3_MultiCanc1_0.1.(d)
======================================================================

   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 = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1
```

```
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =     0.269118
                        Beta(1) =   0.00160326


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.66

   Beta(1)        -0.66            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background         0.263768            *                 *                   *
        Beta(1)       0.00174342           *                 *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -121.619         4
   Fitted model         -122.71         2       2.18339      2          0.3356
  Reduced model         -123.82         1       4.40312      3          0.2211

           AIC:         249.421


                                 Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.2638        13.188    10.000          50       -1.023
    8.9000     0.2751        13.755    17.000          50        1.028
   36.0000     0.3086        15.428    16.000          50        0.175
  110.0000     0.3922        19.612    19.000          50       -0.177

 Chi^2 = 2.17      d.f. = 2        P-value = 0.3387


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        60.4331

            BMDL =        26.8451


BMDU did not converge for BMR = 0.100000
BMDU calculation failed
            BMDU = Inf
```

D.1.5.2. Michaelis-Menten model fit

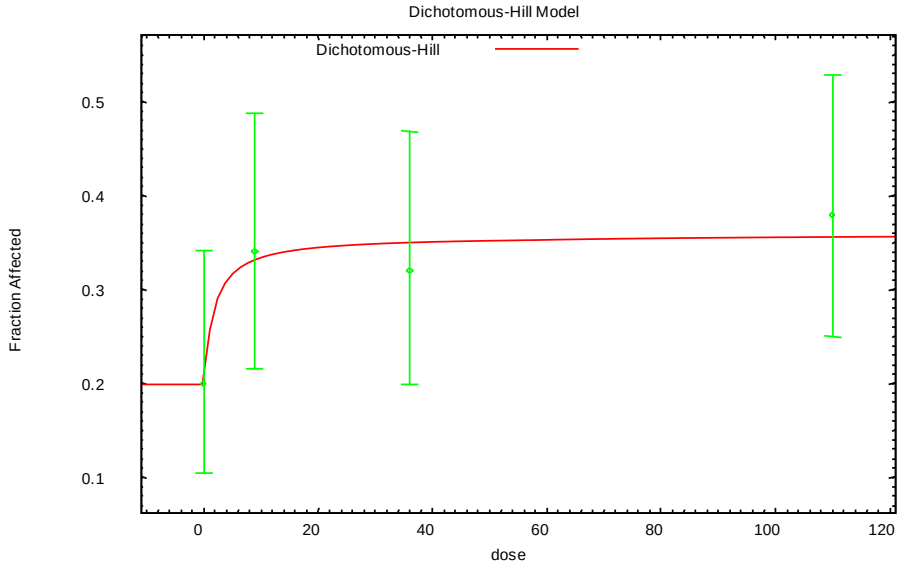

Figure D-12. Michaelis-Menten model (dichotomous Hill model with exponent fixed at 1) fit to MCL incidence in female rats (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
 ================================================================
         Dichotomous Hill Model. (Version: 1.0; Date: 09/24/2006)
         Input Data File: C:\Usepa\BMDS21\dhl_JISA1993_RF_MCL_admc_DichHill_slope1_0.1.(d)
================================================================

   The form of the probability function is:

   P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

        where: 0 <= g < 1, 0 < v <= 1

               v is the maximum probability of response predicted by the model,

               and v*g is the background estimate of that probability.


   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is set to 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  User Inputs Initial Parameter Values
                              v =          0.6
```


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```
                        g =         0.38
                intercept =           -4
                    slope =        -9999   Specified


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                     v           g    intercept

        v            1       -0.53       -0.76

        g        -0.53           1        0.32

intercept        -0.76        0.32           1


                              Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
             v         0.358282        0.0647079            0.231457            0.485107
             g         0.558478         0.186157            0.193618            0.923338
     intercept        -0.531551          4.74243            -9.82655             8.76345


                      Analysis of Deviance Table

      Model      Log(likelihood)  Deviance  Test d.f.   P-value
    Full model         -121.619
   Fitted model        -121.795     0.353046      1          0.5524
  Reduced model         -123.82      4.40312      3          0.2211

          AIC:          249.59


                   Goodness  of  Fit

                                                                  Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000      0.2001        10.005          10           50    -0.001636
    8.9000      0.3329        16.645          17           50       0.1067
   36.0000      0.3511        17.557          16           50      -0.4613
  110.0000      0.3559        17.794          19           50       0.3563

 Chi^2 = 0.351193     d.f. = 1        P-value = 0.5534


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       1.74056
```

Table D-7. Model predictions for combined male and female rat MCL (JISA, 1993),[a] using administered tetrachloroethylene concentrations as dose metric

Model	Goodness of fit			BMD_{10}	$BMDL_{10}$	Comments	Conclusions
	p-value [b]	Largest standardized residual(s)	AIC				
Administered Tetrachloroethylene Concentration (ppm)							
Michaelis-Menten	**0.68**	**−0.3, mid-dose**	**503.6**	**7.7**	**1.4**	**Best-fit to combined data.**	
LogLogistic	0.35	−0.9, control	503.4	5.1	0.003	Unrestricted slope parameter[c]	Fit at control response not useful.
Multistage, one-stage,	0.28	−1.1, control	504.0	32.0	20.9	No statistical improvement from higher order stages	
Gamma	0.96		503.4	4.5	0.001	Unrestricted power parameter	
Weibull	0.98		503.4	4.8	0.002	Unrestricted power parameter	
Probit	0.20	−1.3, control	504.7	40.7	29.7	—	
Logistic	0.19	−1.3, control	504.8	41.6	30.5	—	
LogProbit	0.07	−1.6, control	506.8	55.2	38.6	Inadequate overall fit	
Tetrachloroethylene AUC in blood (mg-hr/L-day)[d]							
Michaelis-Menten[e]	**0.68**	**−0.3, mid-dose**	**503.6**	**17.4**	**3.0**	Best-fit above repeated with preferred dose metric.	

[a] Incidence data and human equivalent continuous exposures provided in Table 5-15 and in output below.
[b] Values <0.10 fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within $\pm$2 units) are considered. Best-fit model is highlighted in bold; output for best-fit models provided in following pages.
[c] Lower limit for slope or power parameters is $\geq$1 to avoid infinite slopes at zero dose. However, fits with restricted parameters may not provide BMDLs with adequate statistical coverage. Fits with unrestricted power or slope parameters did not provide a usable BMDL.
[d] Due to the proportionality of tetrachloroethylene AUC in blood, the preferred dose metric, to administered concentration, administered concentration was used as the dose metric until the final model selection was made.
[e] Dichotomous-Hill model with slope fixed at 1.

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Analyses to evaluate combining the male and female rat MCL data:

1. Following the strategy of Stiteler et al. (1993) the data sets were evaluated for statistical compatibility by applying the generalized likelihood ratio method to the results of fitting a common dose-response model (Michaelis-Menten) to the separate and combined data sets (using administered concentration as dose metric):

	Female Rat MCLs	Male Rat MCLs	Females + Males
Maximum log-likelihoods (LLs), and sum	−121.795 (3 df)	-124.841 (3 df)	−246.636 (6 df)
Overall LL from combined data set			−248.79 (3 df)
X^2 = 2 × absolute difference in LLs.			X^2 = 2.15 (p = 0.54)

2. Logistic regression was used to test whether the datasets differed significantly between males and females. The advantage of this approach is that it does not require the assumption of a specific functional form to represent the dose response relationship. Dose and sex were treated as categorical variables using PROC LOGISTIC in SAS:

Effect	DF	Wald Chi-Square	Pr > ChiSq
dose	3	14.6302	0.0022
sex	1	1.6634	0.1971

The p-value of 0.197 for sex indicates no significant relationship of sex in the pattern of responses.

D.1.6. Modeling Output for Male and Female Rats, MCL (JISA, 1993)

D.1.6.1. With administered tetrachloroethylene concentration (ppm) as dose metric

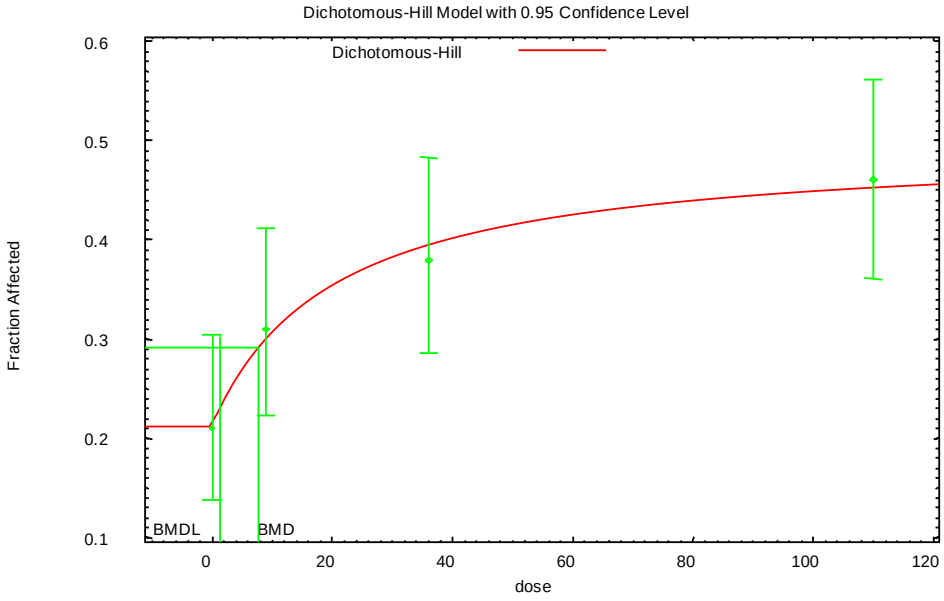

Figure D-13. Michaelis-Menten model (dichotomous-Hill model with exponent fixed at 1) fit to MCL incidence in male and female rats (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=====================================================================
        Dichotomous Hill Model. (Version: 1.0; Date: 09/24/2006)
=====================================================================

  The form of the probability function is:

  P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

       where: 0 <= g < 1, 0 < v <= 1

              v is the maximum probability of response predicted by the model,

              and v*g is the background estimate of that probability.


  Dependent variable = Response
  Independent variable = Dose
  Slope parameter is set to 1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                User Inputs Initial Parameter Values
                            v =          0.6
```

```
                        g =         0.38
                intercept =           -4
                    slope =        -9999   Specified


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                     v            g    intercept

        v            1         -0.6        -0.86

        g         -0.6            1          0.3

intercept        -0.86          0.3            1


                                Parameter Estimates

                                                          95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
             v         0.496794        0.0951495            0.310304            0.683283
             g         0.428222         0.100989            0.230288            0.626157
     intercept         -3.00428          1.19179            -5.34015           -0.668408


                        Analysis of Deviance Table

      Model      Log(likelihood)  Deviance  Test d.f.   P-value
    Full model         -248.707
   Fitted model         -248.79     0.167701      1          0.6822
  Reduced model        -256.414      15.4153      3        0.001494

          AIC:         503.581


                   Goodness  of  Fit

                                                                 Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.2127        21.274          21          100     -0.06691
    8.9000      0.2997        29.970          31          100       0.2249
   36.0000      0.3948        39.479          38          100      -0.3025
  110.0000      0.4528        45.278          46          100       0.1451

 Chi^2 = 0.167615     d.f. = 1        P-value = 0.6822


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =         7.7341

            BMDL =       1.35558
```

D.1.6.2. With tetrachloroethylene AUC in blood as dose metric

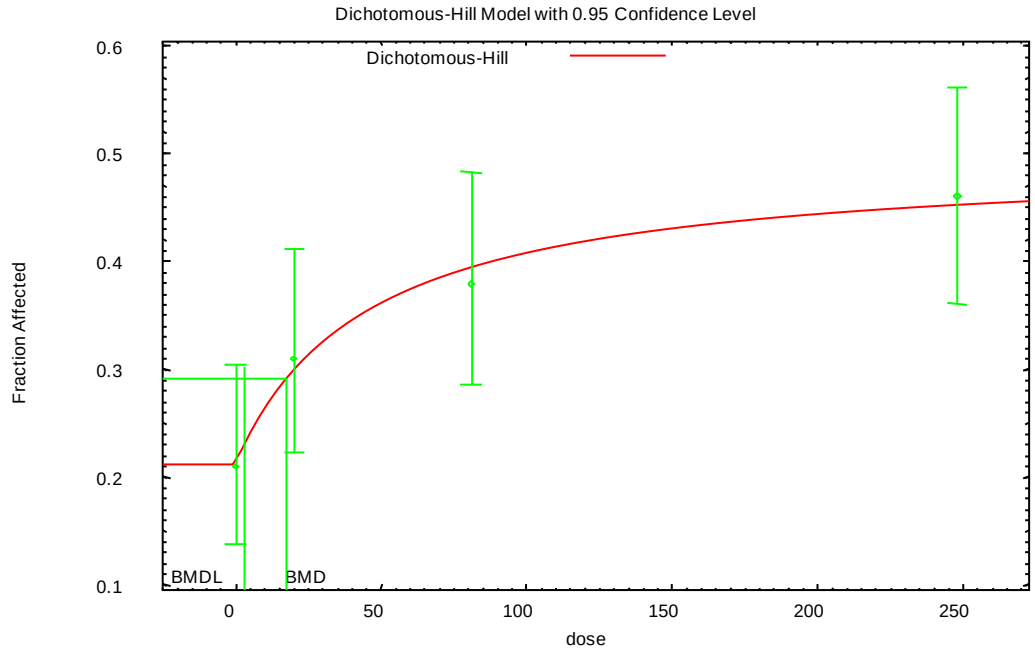

Figure D-14. Michaelis-Menten model (dichotomous-Hill model with exponent fixed at 1) fit to MCL incidence in male and female rats (JISA, 1993), with BMD and BMDL at 10% extra risk.

```
=============================================================
         Dichotomous Hill Model. (Version: 1.0; Date: 09/24/2006)
         Input Data File: C:\Usepa\BMDS21\dhl_JISA1993_RMF_MCL_percAUC_DichHill_slope1_0.1.(d)
 =============================================================

   The form of the probability function is:

   P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

        where: 0 <= g < 1, 0 < v <= 1

               v is the maximum probability of response predicted by the model,

               and v*g is the background estimate of that probability.


   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is set to 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  User Inputs Initial Parameter Values
                              v =          0.6
                              g =         0.38
                      intercept =           -4
```

slope = -9999 Specified

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	v	g	intercept
v	1	-0.6	-0.86
g	-0.6	1	0.3
intercept	-0.86	0.3	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
v	0.496603	0.0949265	0.31055	0.682655
g	0.428392	0.100947	0.230539	0.626245
intercept	-3.81314	1.19145	-6.14833	-1.47795

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test d.f.	P-value
Full model	-248.707			
Fitted model	-248.791	0.168004	1	0.6819
Reduced model	-256.414	15.4153	3	0.001494

AIC: 503.581

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2127	21.274	21	100	-0.06697
20.0000	0.2997	29.969	31	100	0.225
81.0000	0.3948	39.480	38	100	-0.3028
248.0000	0.4528	45.277	46	100	0.1453

Chi^2 = 0.167918 d.f. = 1 P-value = 0.6820

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 17.382

BMDL = 3.04513

D.2. Model Selection Details for Male Rat Tumors (NTP, 1986)

Table D-8. Model predictions for male rat tumors (NTP, 1986),[a] using administered tetrachloroethylene concentration as dose metric and multistage model

Model	Goodness of fit			BMD_{10}[c] (ppm)	$BMDL_{10}$[c] (ppm)	Conclusions
	p-value[b]	Largest standardized residual(s)	AIC			
Kidney tumors						
One-, two-stage	0.75	0.3, low-dose	64.1	110	50	No statistical improvement from adding higher-order parameter; one-stage model selected
Brain gliomas						
One-stage	**0.11**	-1.3, low-dose	**45.7**	**180**	**73**	No statistical improvement from adding higher-order parameter; one-stage model selected.
Two-stage	0.18	-1.3, low-dose	44.6	138	45	
Testicular interstitial cell tumors						
One-, two-stage	0.40	0.7, low-dose	155.8	13.0	6.1	No statistical improvement from adding higher-order parameter; one-stage model selected
MCL						
One-, two-stage	0.18	1.1, low-dose	184.8	12.1	6.5	Only a one-stage model resulted.

[a] Incidence data and human equivalent continuous exposures provided in Table 5-15 and in output below.
[b] Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c] The highest response in these data sets was less than 10% extra risk; however, because the best-fit models were linear, use of BMD_{10} and $BMDL_{10}$ was equivalent to using a BMR within the data range.

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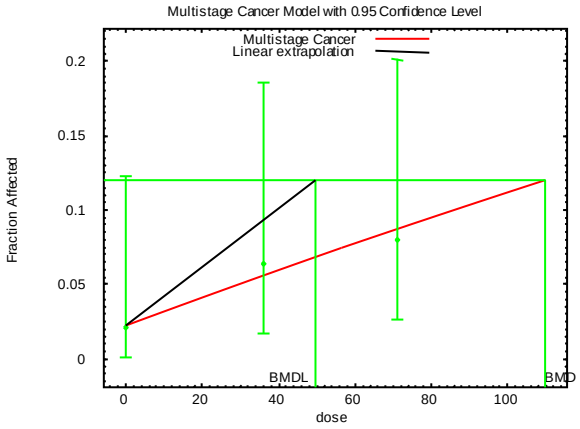
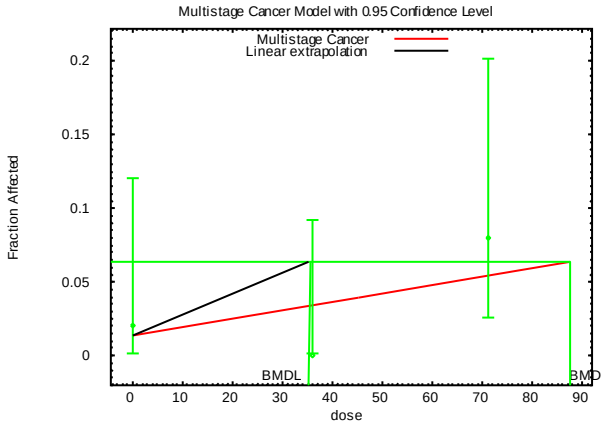
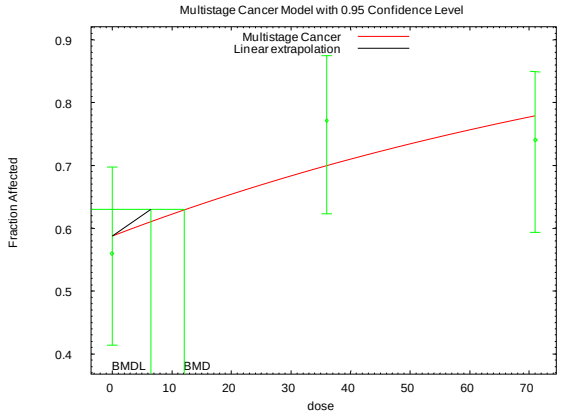


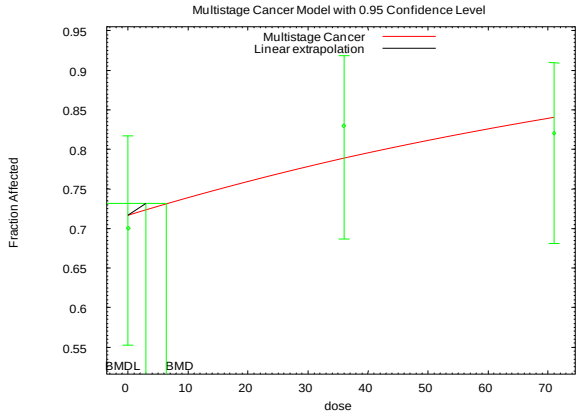
a. One-stage model fit to kidney tumors. Refer to section D.2. for model output.



b. One-stage model fit to brain gliomas. See section D.2. for model output.



d. One-stage model fit to testicular interstitial cell tumors. See section D.2. for model output.



d. One-stage model fit to MCLs. See section D.2. for model output.

Figure D-15. Multistage model fits to tumor incidences at multiple sites in male rats—kidney tumors, brain gliomas, testicular interstitial cell tumors, and MCL (NTP, 1986).
Graphs show BMD and BMDL at 10% extra risk.

D.2.1. Modeling Output for Male Rats (NTP, 1986): MCLs, Brain Gliomas, Kidney Tumors, Testicular Interstitial Cell Tumors and Combined Tumors at 10% Extra Risk, Using Administered Concentration and Multistage Modeling (Discussed in Section 5.3.4.1)

D.2.2. Kidney Tumors

```
 ================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
         Input Data File: C:\Usepa\BMDS21\New.(d)
  ================================================================
   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 = kidney
   Independent variable = hec

 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 =    0.0247493
                       Beta(1) =  0.000885764


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.78

   Beta(1)        -0.78            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background        0.0220312            *                *                   *
       Beta(1)      0.000959208            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model         -29.9768         3
   Fitted model         -30.025         2     0.0964903      1          0.7561
  Reduced model          -31.01         1       2.06651      2          0.3558
```

```
          AIC:           64.05

 Log-likelihood Constant             25.932650402661093


                                 Goodness  of  Fit
                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.0220         1.080     1.000          49       -0.077
   36.0000     0.0552         2.596     3.000          47        0.258
   71.0000     0.0864         4.321     4.000          50       -0.161

 Chi^2 = 0.10      d.f. = 1        P-value = 0.7533


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        109.841

            BMDL =        49.5786

            BMDU =   5.15876e+007

Taken together, (49.5786, 5.15876e+007) is a 90     % two-sided confidence
interval for the BMD
```

D.2.3. Brain Gliomas

```
 ===================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
         Input Data File: C:\Usepa\BMDS21\New.(d)
  ===================================================================
   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 = gliomas
   Independent variable = hec

 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 =   0.00303212
                        Beta(1) =  0.000882936


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)
```

```
Background            1         -0.7

   Beta(1)         -0.7            1


                              Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0135755           *               *                   *
        Beta(1)        0.0005865           *               *                   *

* - Indicates that this value is not calculated.


                       Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -18.8404         3
   Fitted model        -20.8724         2       4.06391      1         0.04381
  Reduced model        -21.8534         1       6.02604      2         0.04914

          AIC:         45.7448

 Log-likelihood Constant             16.259161091237132


                                 Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0136         0.679     1.000          50        0.393
   36.0000     0.0342         1.641     0.000          48       -1.303
   71.0000     0.0538         2.690     4.000          50        0.821

 Chi^2 = 2.53      d.f. = 1        P-value = 0.1119


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        179.643

            BMDL =        72.6378

            BMDU =   2.38388e+101

Taken together, (72.6378, 2.38388e+101) is a 90     % two-sided confidence
interval for the BMD
```

D.2.4. Testicular Tumors

```
 ===================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
         Input Data File: C:\Usepa\BMDS21\New.(d)
===================================================================


   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 = testtumor
Independent variable = hec

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 = 0.728853
Beta(1) = 0.00723542

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.71
Beta(1)	-0.71	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.717295	*	*	*
Beta(1)	0.00809268	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-75.5554	3			
Fitted model	-75.9206	2	0.730362	1	0.3928
Reduced model	-77.0228	1	2.93491	2	0.2305

AIC: 155.841

Log-likelihood Constant 69.650407128412709

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.7173	35.865	35.000	50	-0.272
36.0000	0.7887	37.071	39.000	47	0.689
71.0000	0.8409	42.043	41.000	50	-0.403

Chi^2 = 0.71 d.f. = 1 P-value = 0.3990

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

```
Confidence level =           0.95

             BMD =        13.0192

            BMDL =         6.0591

            BMDU =   8.43867e+014

Taken together, (6.0591 , 8.43867e+014) is a 90     % two-sided confidence
interval for the BMD
```

D.2.5. MCLs

```
 ======================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
         Input Data File: C:\Usepa\BMDS21\New.(d)
  ====================================================================

   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 = M_mcl
   Independent variable = hec

 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 =     0.612363
                        Beta(1) =   0.00746075


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.72

   Beta(1)        -0.72            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background         0.588403            *                *                   *
       Beta(1)       0.00873731            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

```
     Full model        -88.7862         3
   Fitted model        -89.6897         2       1.80709      1          0.1789
  Reduced model        -91.7227         1       5.87302      2         0.05305

           AIC:         183.379

 Log-likelihood Constant             82.552836454191464


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.5884        29.420    28.000          50       -0.408
   36.0000     0.6995        33.575    37.000          48        1.078
   71.0000     0.7787        38.933    37.000          50       -0.659

 Chi^2 = 1.76      d.f. = 1        P-value = 0.1843


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        12.0587

             BMDL =        6.54184

             BMDU =   1.67846e+007

Taken together, (6.54184, 1.67846e+007) is a 90     % two-sided confidence
interval for the BMD
```

D.2.6. Combined BMD and BMDL for Male Rat Tumors

```
**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                    -216.50768590007982

  Combined Log-likelihood Constant            194.39505507650239


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        5.73369

             BMDL =        3.48718
```

D.2.7. Modeling Output for Male Rats (NTP, 1986): MCLs, Brain Gliomas, Kidney Tumors, Testicular Interstitial Cell Tumors and Combined Tumors at 10% Extra Risk, Using Administered Concentration and Multistage Modeling (Discussed in Section 5.3.4.1)

Note: For numerical stability, doses were modeled in units of g-hr/L-d rather than mg-hr/L-d. Refer to Figure 5-15 for corresponding graphs.

D.2.8. Brain Gliomas

```
 ================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
 ================================================================
   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 = Effect
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =   0.00285487
                        Beta(1) =     0.387786


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1         -0.7

   Beta(1)         -0.7            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background        0.0134603            *                *                   *
       Beta(1)         0.257592            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -18.8404         3
   Fitted model        -20.8459         2       4.01101      1          0.0452
  Reduced model        -21.8534         1       6.02604      2         0.04914

           AIC:         45.6919

 Log-likelihood Constant             16.259161091237132
```

```
                                Goodness  of  Fit
                                                                        Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0135         0.673     1.000          50        0.401
    0.0810     0.0338         1.624     0.000          48       -1.296
    0.1640     0.0543         2.713     4.000          50        0.803

 Chi^2 = 2.49      d.f. = 1        P-value = 0.1148


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.409021

            BMDL =       0.166066

            BMDU = Inf
```

D.2.9. Kidney Tumors

```
=====================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
=====================================================================
   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 = Effect
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0251361
                        Beta(1) =     0.381986


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.78

   Beta(1)        -0.78            1
```

```
                              Parameter Estimates

                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background        0.0222199            *                *                   *
       Beta(1)         0.416419            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model         -29.9768         3
   Fitted model        -30.0318         2      0.110125      1           0.74
  Reduced model          -31.01         1       2.06651      2         0.3558

          AIC:         64.0637

 Log-likelihood Constant             25.932650402661093


                                 Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0222         1.089     1.000          49       -0.086
    0.0810     0.0547         2.569     3.000          47        0.277
    0.1640     0.0868         4.338     4.000          50       -0.170

 Chi^2 = 0.11      d.f. = 1        P-value = 0.7368


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.253015

            BMDL =       0.113761


BMDU did not converge for BMR = 0.100000
BMDU calculation failed
            BMDU =   2.05051e+023
```

D.2.10. MCLs

```
=================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
=================================================================

   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 = Effect
```

Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.614571
Beta(1) = 3.18843

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.72
Beta(1)	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.59006	*	*	*
Beta(1)	3.75951	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-88.7862	3			
Fitted model	-89.7301	2	1.88778	1	0.1695
Reduced model	-91.7227	1	5.87302	2	0.05305

AIC: 183.46

Log-likelihood Constant 82.552836454191464

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5901	29.503	28.000	50	-0.432
0.0810	0.6977	33.489	37.000	48	1.104
0.1640	0.7787	38.936	37.000	50	-0.659

Chi^2 = 1.84 d.f. = 1 P-value = 0.1750

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

```
             BMD =      0.0280251

            BMDL =      0.0151104

            BMDU =       0.160002

Taken together, (0.0151104, 0.160002) is a 90     % two-sided confidence
interval for the BMD
```

D.2.11. Testicular Tumors

```
 ======================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
 ======================================================================

   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 = Effect
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =     0.730195
                        Beta(1) =       3.0992


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.71

   Beta(1)        -0.71            1


                               Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background         0.718314            *               *                   *
       Beta(1)          3.48419            *               *                   *

* - Indicates that this value is not calculated.


                       Analysis of Deviance Table
```

```
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -75.5554         3
    Fitted model       -75.9393         2      0.767742      1          0.3809
  Reduced model        -77.0228         1       2.93491      2          0.2305

           AIC:         155.879

 Log-likelihood Constant             69.650407128412709


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.7183        35.916    35.000          50       -0.288
    0.0810     0.7876        37.016    39.000          47        0.707
    0.1640     0.8409        42.046    41.000          50       -0.405

 Chi^2 = 0.75      d.f. = 1        P-value = 0.3874


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =      0.0302396

            BMDL =      0.0139853


BMDU did not converge for BMR = 0.100000
BMDU calculation failed
            BMDU = Inf
```

D.2.12. Combined BMD and BMDL for Male Rat Tumors

```
**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                    -216.54708443096888

  Combined Log-likelihood Constant            194.39505507650239


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =      0.0133069

            BMDL =     0.00805821
```

D.3. Comparison of PODs Resulting from the Use of Models Alternative to the Multistage Model, for Tumor Sites in the JISA (1993) Bioassay

Note: Refer to Section D.1 for alternative modeling for female rat MCLs and combined male and female rat MCLs.

Table D-9. Comparison of model predictions for hepatocellular tumors in mice (JISA, 1993), using administered tetrachloroethylene concentration (ppm) as the dose metric,[a] across a range of dichotomous models

Model	Goodness of fit[b]			BMD_{10}	$BMDL_{10}$	Comments[c]
	p-value	Largest standardized residual(s)	AIC			
Male mice (input data in Table 5-13)						
Gamma	0.14	−1.0, control 1.1, low-dose	241.1	10.2	0.4	Power parameter unrestricted
Weibull	0.15	−1.0, control 1.1, low-dose	241.0	9.5	0.7	Power parameter unrestricted
Michaelis-Menten[d]	0.13	−1.0, control 1.1, low-dose	241.0	2.5	1.3	—
LogLogistic	0.14	−1.0, control 1.1, low-dose	241.1	10.6	1.5	Slope parameter unrestricted
LogProbit	0.14	−1.1, control 1.0, low-dose	241.1	11.0	2.1	—
Multistage	0.27	1.2, low-dose −1.0, mid-dose	239.5	3.9	2.7	Lowest residual at control (−0.5)
Logistic	0.35	−0.7, control 1.1, low-dose	238.9	6.0	4.7	—
Probit	0.36	−0.7, control 1.1, low-dose	238.9	6.1	4.8	—
Female mice (input data in Table 5-13)						
Multistage	0.36	−1.1, mid-dose	152.9	5.0	3.8	—
Gamma	0.96	−0.04, low-dose	152.8	9.5	4.3	—
Weibull	0.93	−0.07, control	152.8	9.5	4.3	—
LogLogistic	0.98	0.02, control	152.8	9.5	4.7	Slope parameter unrestricted
Michaelis-Menten		0.01, control	154.8	9.5	4.7	—
LogProbit	0.95	0.04, low-dose	152.8	9.5	5.0	—
Probit	0.91	0.4, mid-dose	151.0	11.8	9.7	—
Logistic	0.84	0.5, mid-dose	151.2	13.1	10.6	—

[a] Only one dose metric used due to near proportionality of relevant dose metrics.
[b] Goodness-of-fit *p*-values <0.05 for a preferred model, or <0.10 when considering suite of models, fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within ±2 units) are considered.
[c] Lower limit ≥1for slope or power parameters avoids biologically implausible infinite slopes at zero dose. However, fits with restricted parameters may not provide BMDLs with nominal (95%) statistical coverage.
[d] Dichotomous-Hill model with slope fixed at 1

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Table D-10. Comparison of model predictions for male mice, hemangiomas, or hemangiosarcomas (JISA, 1993),[a] using administered tetrachloroethylene concentration as dose metric,[b] across a range of dichotomous models

Model	Goodness of fit[c]			BMD_{10}	$BMDL_{10}$	Comments[d]
	p-value	Largest standardized residual(s)	AIC			
Michaelis-Menten[e]	0.21	−1.0, low-dose	143.7	16.1	4.1	Better fit than restricting or unrestricting slope parameter
LogLogistic	0.19	−1.0, low-dose	141.9	20.7	5.4	Slope parameter unrestricted
LogProbit	0.21	−1.0, low-dose	143.7	19.2	5.4	
Weibull	0.18	−1.0, low-dose	143.9	21.3	5.5	Power parameter unrestricted
Gamma	0.18	−1.0, low-dose	143.9	21.7	5.6	Power parameter unrestricted
Multistage	0.38	−1.0, low-dose 0.9, mid-dose	142.0	24.4	13.3	Lowest residual at control (0.4)
Probit	0.32	−1.1, low-dose 1.0, mid-dose	142.4	30.6	20.9	—
Logistic	0.31	−1.1, control 1.0, low-dose	142.4	31.6	22.1	—

[a] Incidence data and human equivalent continuous exposure estimates provided in Table 5-13.
[b] Only one dose metric used, due to proportionality of relevant dose metrics.
[c] Goodness-of-fit *p*-values <0.05 for a preferred model, or <0.10 when considering suite of models, fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within ±2 units) are considered.
[d] Lower limit ≥1 for slope or power parameters avoids biologically implausible infinite slopes at zero dose. However, fits with restricted parameters may not provide BMDLs with nominal (95%) statistical coverage.
[e] Dichotomous-Hill model with slope fixed at 1.

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Table D-11. Comparison of model predictions for MCL in male rats (JISA, 1993),[a] using administered tetrachloroethylene concentration as dose metric,[b] across a range of dichotomous models

Model	Goodness of fit			BMD_{10}	$BMDL_{10}$	Comments
	p-value[c]	Largest standardized residual(s)	AIC			
Gamma	0.51	−0.4, low-dose 0.4, mid-dose	256.0	6.9	0.062	Power parameter unrestricted; BMDL quite low
Weibull	0.54	−0.4, low-dose 0.4, mid-dose	256.0	7.1	0.11	Power parameter unrestricted
LogLogistic	0.59	0.4, mid-dose	255.9	7.8	0.18	Slope parameter unrestricted
LogProbit	0.63	0.4, mid-dose	255.8	8.5	0.28	—
Michaelis-Menten[d]	0.74	(0.1, control) −0.2, low-dose	255.7	8.6	2.2	—
Multistage	0.51	1.0, mid-dose	254.9	20.5	13.2	—
Probit	0.34	−0.8, control 1.2, mid-dose	255.7	29.3	21.6	—
Logistic	0.32	−0.8, control 1.2, mid-dose	255.8	30.0	22.1	—

[a] Incidence data and human equivalent continuous exposure estimates provided in Table 5-15.
[b] Only one dose metric used for comparing model predictions. Ouputs for the Michaelis-Menten model using either administered concentration or tetrachloroethylene AUC in blood follow this table.
[c] Goodness-of-fit *p*-values <0.05 for a preferred model, or <0.10 when considering suite of models, fail to meet conventional goodness-of-fit criteria. In addition, visual fit and residuals (within $\pm$2 units) are considered.
[d] Dichotomous-Hill model with slope fixed at 1.

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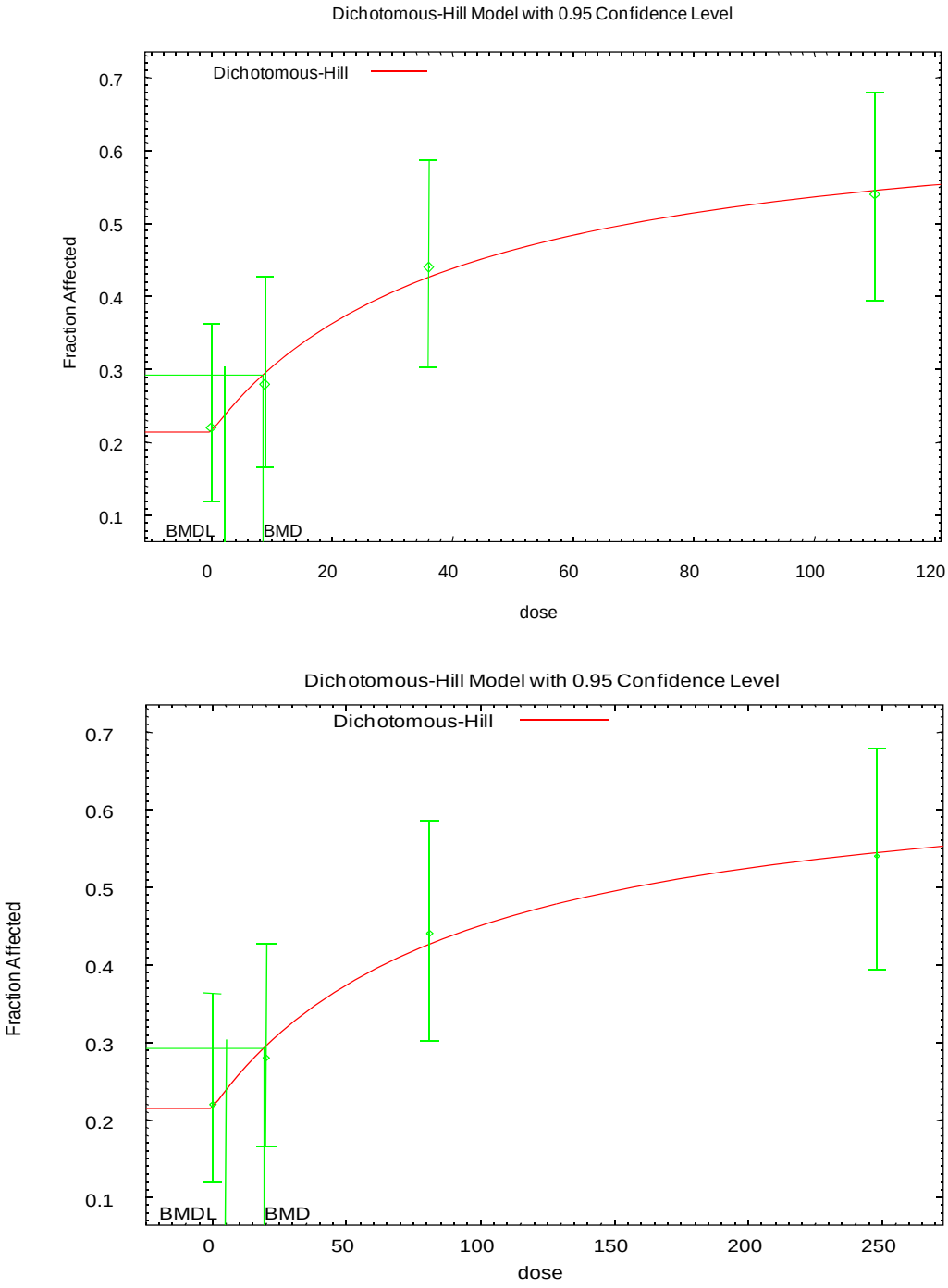

Figure D-16. Michaelis-Menten model (dichotomous-Hill model with exponent fixed at 1) fit to MCL incidence in male rats (JISA, 1993), with BMD and BMDL at 10% extra risk; with administered concentration as dose metric (top) or tetrachloroethylene AUC in blood (bottom)

Output for administered concentration:

```
================================================================
         Dichotomous Hill Model. (Version: 1.0; Date: 09/24/2006)
================================================================

   The form of the probability function is:

   P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

        where: 0 <= g < 1, 0 < v <= 1

               v is the maximum probability of response predicted by the model,

               and v*g is the background estimate of that probability.


   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is set to 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 User Inputs Initial Parameter Values
                             v =          0.6
                             g =         0.38
                     intercept =           -4
                         slope =        -9999   Specified


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                        v            g    intercept

         v              1        -0.71         -0.9

         g          -0.71            1         0.47

 intercept           -0.9         0.47            1


                                 Parameter Estimates

                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
              v         0.668921         0.205885            0.265395             1.07245
              g          0.32044         0.110376            0.104107            0.536774
      intercept         -3.72152          1.21769            -6.10815             -1.3349


                          Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test d.f.   P-value
     Full model         -124.787
   Fitted model         -124.841     0.108028      1          0.7424
  Reduced model         -131.791      14.0088      3        0.002893

           AIC:          255.682
```

```
                    Goodness  of  Fit

                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.2143         10.717         11           50      0.09737
    8.9000      0.2949         14.745         14           50       -0.231
   36.0000      0.4260         21.299         22           50       0.2005
  110.0000      0.5448         27.239         27           50     -0.06785

 Chi^2 = 0.107657     d.f. = 1        P-value = 0.7428


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        8.63516

            BMDL =       2.20116
```

Output for tetrachloroethylene AUC in blood:

```
 ================================================================
         Dichotomous Hill Model. (Version: 1.2; Date: 12/11/2009)
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

        where: 0 <= g < 1, 0 < v <= 1

               v is the maximum probability of response predicted by the model,

               and v*g is the background estimate of that probability.


   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is set to 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                User Inputs Initial Parameter Values
                          v =          0.6
                          g =         0.38
                  intercept =           -4
                      slope =        -9999   Specified


          Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	v	g	intercept
v	1	-0.7	-0.9
g	-0.7	1	0.47
intercept	-0.9	0.47	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
v	0.668365	0.205061	0.266453	1.07028
g	0.320713	0.110275	0.104578	0.536849
intercept	-4.53004	1.21639	-6.91411	-2.14596

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-124.787	4			
Fitted model	-124.841	3	0.107785	1	0.7427
Reduced model	-131.791	1	14.0088	3	0.002893

AIC: 255.681

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2144	10.718	11.000	50	0.097
20.0000	0.2949	14.744	14.000	50	-0.231
81.0000	0.4260	21.300	22.000	50	0.200
248.0000	0.5448	27.239	27.000	50	-0.068

Chi^2 = 0.11 d.f. = 1 P-value = 0.7431

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 19.411

BMDL = 4.94596

D.4. References

JISA (Japan Industrial Safety Association). (1993). Carcinogenicity study of tetrachloroethylene by inhalation in rats and mice. Hadano, Japan.

NTP (National Toxicology Program). (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies). (NTP TR 311). Research Triangle Park, NC: U.S. Department of Health and Human Services, National Toxicology Program. http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr311.pdf.

Stiteler, WM; Knauf, LA; Hertzberg, RC; Schoeny, RS. (1993). A statistical test of compatibility of data sets to a common dose-response model. Regul Toxicol Pharmacol 18: 392-402. http://dx.doi.org/10.1006/rtph.1993.1065.