EPA/635/R-09/001F
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TOXICOLOGICAL REVIEW

OF

1,1,2,2-TETRACHLOROETHANE

(CAS No. 79-34-5)

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

September 2010

U.S. Environmental Protection Agency
Washington, DC

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1,1,2,2-TETRACHLOROETHANE (CAS No. 79-34-5)

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTH	adrenocorticotropic hormone
AIC	Akaike's Information Criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BMD	benchmark dose
BMDL	95% confidence limit (lower bound) on the benchmark dose
BMDS	benchmark dose software
BMR	benchmark response
CASRN	Chemical Abstracts Service Registry Number
CHO	Chinese hamster ovary
CNS	central nervous system
CYP	cytochrome P450
DEN	diethylnitrosamine
DF	degrees of freedom
DNA	deoxyribonucleic acid
FEL	frank effect level
FOB	functional observational battery
G6Pase	glucose-6-phosphatase
GD	gestation day
GGT	gamma glutamyltranspeptidase
GST	glutathione S-transferase
Hb	hemoglobin
HED	human equivalent dose
i.p.	intraperitoneal
IU	international units
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
mA	milliampere
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NTP	National Toxicology Program
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
POD	point of departure
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RfV	reference value
RNA	ribonucleic acid
SCE	sister chromatid exchange

SD	standard deviation
SDH	sorbitol dehydrogenase
TWA	time-weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
WBC	white blood cell

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 subchronic and chronic exposure to 1,1,2,2-tetrachloroethane. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 1,1,2,2-tetrachloroethane.

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

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

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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 1,1,2,2-tetrachloroethane. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

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

Development of these hazard identification and dose-response assessments for 1,1,2,2-tetrachloroethane has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk*

Assessment (U.S. EPA, 1991a), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through February, 2010.

Portions of this document were developed under a Memorandum of Understanding with the Agency for Toxic Substances and Disease Registry (ATSDR) as part of a collaborative effort in the development of human health toxicological assessments.

2. CHEMICAL AND PHYSICAL INFORMATION

1,1,2,2-Tetrachloroethane (CASRN 79-34-5) is a synthetic, halogenated hydrocarbon that is a colorless, nonflammable liquid at room temperature. It is highly volatile, somewhat soluble in water, and miscible with many organic solvents. The structure of 1,1,2,2-tetrachloroethane is shown below (Figure 2-1), and the chemical and physical properties are presented in Table 2-1.

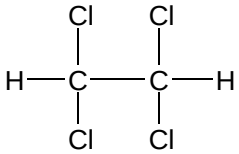


Figure 2-1. Structure of 1,1,2,2-tetrachloroethane.

Table 2-1. Chemical and physical properties of 1,1,2,2-tetrachloroethane

Characteristic	Information	Reference
Chemical name	1,1,2,2-Tetrachloroethane	NLM, 2009; CAS, 1994
Synonym(s)	Acetylene tetrachloride; sym-tetrachloroethane; s-tetrachloroethane; tetrachlorethane; 1,1-dichloro-2,2-dichloroethane	CAS, 1994
Chemical formula	$C_2H_2Cl_4$	CAS, 1994
CASRN	79-34-5	NLM, 2009; CAS, 1994;
Molecular weight	167.85	Lide, 1993; Riddick et al., 1986
Color	Colorless	Hawley, 1981
Freezing point	-43.8°C -36°C	Riddick et al., 1986 Lide, 1993
Boiling point	145.1°C 146.2°C 146.5°C	Riddick et al., 1986 Lide, 1993 Merck Index, 1989
Density at 20°C	1.594 1.595	Riddick et al., 1986 Lide, 1993
Odor threshold: Water Air	 0.50 ppm 1.5 ppm 3–5 ppm	 NLM, 2009; Amoore and Hautala, 1983 Amoore and Hautala, 1983 NLM, 2009
Solubility: Water Organic solvents	 2.87 g/L (20°C) 2.85 g/L (25°C) Miscible with ethanol, methanol, ether, acetone, benzene, petroleum, carbon tetrachloride, carbon disulfide, dimethyl formamide, oils	 Riddick et al., 1986 Merck Index, 1989 NLM, 2009; Merck Index, 1989; Hawley, 1981

Table 2-1. Chemical and physical properties of 1,1,2,2-tetrachloroethane

Characteristic	Information	Reference
Vapor pressure	5.95 mm Hg (25°C) 9 mm Hg (30°C)	Riddick et al., 1986 NLM, 2009; Flick, 1985
Partition coefficients: log K_{ow} log K_{oc}	 2.39 1.66 2.78	 Hansch and Leo, 1985 Chiou et al., 1979 ASTER, 1995
Henry's law constant	4.7×10^{-4} atm-m^3/mol 4.55×10^{-4} atm-m^3/mol 1.80×10^{-3} atm-m^3/mol	Mackay and Shiu, 1981 NLM, 2009 ASTER, 1995
Flash point	None – nonflammable	NLM, 2009; Hawley, 1981
Conversions: ppm to mg/m^3 mg/m^3 to ppm	 1 ppm = 6.87 mg/m^3 1 mg/m^3 = 0.146 ppm	 Calculated Calculated

In the past, the major use for 1,1,2,2-tetrachloroethane was in the production of trichloroethylene, tetrachloroethylene, and 1,2-dichloroethylene (Archer, 1979). 1,1,2,2-Tetrachloroethane has been identified at numerous National Priorities List (NPL) sites (ATSDR, 2008). With the development of new processes for manufacturing chlorinated ethylenes and the availability of less toxic solvents, the production of 1,1,2,2-tetrachloroethane as a commercial end-product in the United States and Canada has steadily declined since the late 1960s, and production ceased by the early 1990s (NLM, 2009; Environment Canada and Health Canada, 1993). 1,1,2,2-Tetrachloroethane may still appear as a chemical intermediate in the production of a variety of other common chemicals. Uses of 1,1,2,2-tetrachloroethane include as a solvent; in cleaning and degreasing metals; in paint removers, varnishes, and lacquers; in photographic films; and as an extractant for oils and fats (Hawley, 1981). Although at one time it was used as an insecticide, fumigant, and weed killer (Hawley, 1981), it presently is not registered for any of these purposes. It was once used as an ingredient in an insect repellent, but registration was canceled in the late 1970s.

3. TOXICOKINETICS

1,1,2,2-Tetrachloroethane is well-absorbed from the respiratory and gastrointestinal tracts in both humans and laboratory animals, and is extensively metabolized and excreted, chiefly as metabolites, in the urine and breath. The metabolism of 1,1,2,2-tetrachloroethane in rats and mice results in the production of trichloroethanol, trichloroacetic acid, and dichloroacetic acid. The dichloroacetic acid is then broken down to glyoxalic acid, oxalic acid, and carbon dioxide. When 1,1,2,2-tetrachloroethane undergoes reductive or oxidative metabolism, reactive radical and acid chloride intermediates, respectively, are produced.

3.1. ABSORPTION

3.1.1. Oral Exposure

There are no known studies that quantify absorption following oral exposure in humans. However, the health effects resulting from ingestion of large amounts of 1,1,2,2-tetrachloroethane in humans (Section 4.1.1) indicate that 1,1,2,2-tetrachloroethane is absorbed following oral exposure.

Observations in animals indicate that the oral absorption of 1,1,2,2-tetrachloroethane is rapid and extensive. Cottalasso et al. (1998) reported hepatic effects only 15–30 minutes following a single oral exposure in rats, including increases in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), a decrease in microsomal glucose-6-phosphatase (G6Pase) activity, and an increase in triglyceride levels. Following a single oral exposure of male Osborne-Mendel rats and B6C3F_1 mice to 150 mg/kg of radiolabeled 1,1,2,2-tetrachloroethane in corn oil, only 4–6% of the activity was recovered in the feces 72 hours postexposure while >90% of the administered activity was found in both species as metabolites, indicating that the compound was nearly completely absorbed in both rats and mice within 72 hours (Dow Chemical Company, 1988). Mitoma et al. (1985) exposed groups of male Osborne-Mendel rats to 25 or 100 mg/kg and B6C3F_1 mice to 50 or 200 mg/kg of 1,1,2,2-tetrachloroethane in corn oil gavage 5 days/week for 4 weeks, followed by a single radiolabeled dose of the compound, and evaluated the disposition of the radiolabeled 1,1,2,2-tetrachloroethane over the next 48 hours. While absorption was not quantified, 79% of the dose was metabolized in rats and 68% was metabolized in mice, suggesting that at least those levels of compound had been absorbed within 48 hours.

3.1.2. Inhalation Exposure

While studies of the systemic toxicity of 1,1,2,2-tetrachloroethane following inhalation in humans are indicative of some level of systemic absorption, comparatively few studies have quantitatively addressed this issue. A study in volunteers was carried out in which a bulb

containing [^{38}Cl]-labeled 1,1,2,2-tetrachloroethane was inserted into their mouths; they immediately inhaled deeply, held their breaths for 20 seconds, and then exhaled through a trap containing granulated charcoal. The study showed that approximately 96% of a single breath of 1,1,2,2-tetrachloroethane was absorbed systemically (Morgan et al., 1970). Two subjects were reported to retain approximately 40–60% of inspired 1,1,2,2-tetrachloroethane after a 30-minute exposure of up to 2,300 mg/m^3 (Lehmann et al., 1936), but additional details were not provided.

The total body burden of 1,1,2,2-tetrachloroethane in male Osborne-Mendel rats and B6C3F$_1$ mice exposed to a vapor concentration of 10 ppm (68.7 mg/m^3) for 6 hours (Dow Chemical Company, 1988) was 38.7 µmol equivalents/kg in rats (9.50 µmol equivalents and using a body weight of 245 g from the study) and 127 µmol equivalents/kg in mice (3.059 µmol equivalents and using a body weight of 24.1 g from the study), indicating that while absorption occurred in both species, mice absorbed proportionally more 1,1,2,2-tetrachloroethane on a per-body-weight basis. Ikeda and Ohtsuji (1972) detected metabolites measured as total trichlorocompounds, trichloroacetic acid, and trichloroethanol, in the urine of rats exposed to 200 ppm (1,370 mg/m^3) 1,1,2,2-tetrachloroethane, indicating that absorption had occurred; however, they did not provide a quantitative estimate of absorption rate or fraction. Similarly, Gargas and Anderson (1989) followed the elimination of 1,1,2,2-tetrachloroethane as exhaled breath from the blood after a 6-hour exposure to 350 ppm (2,400 mg/m^3), but did not provide quantitative estimates of absorption.

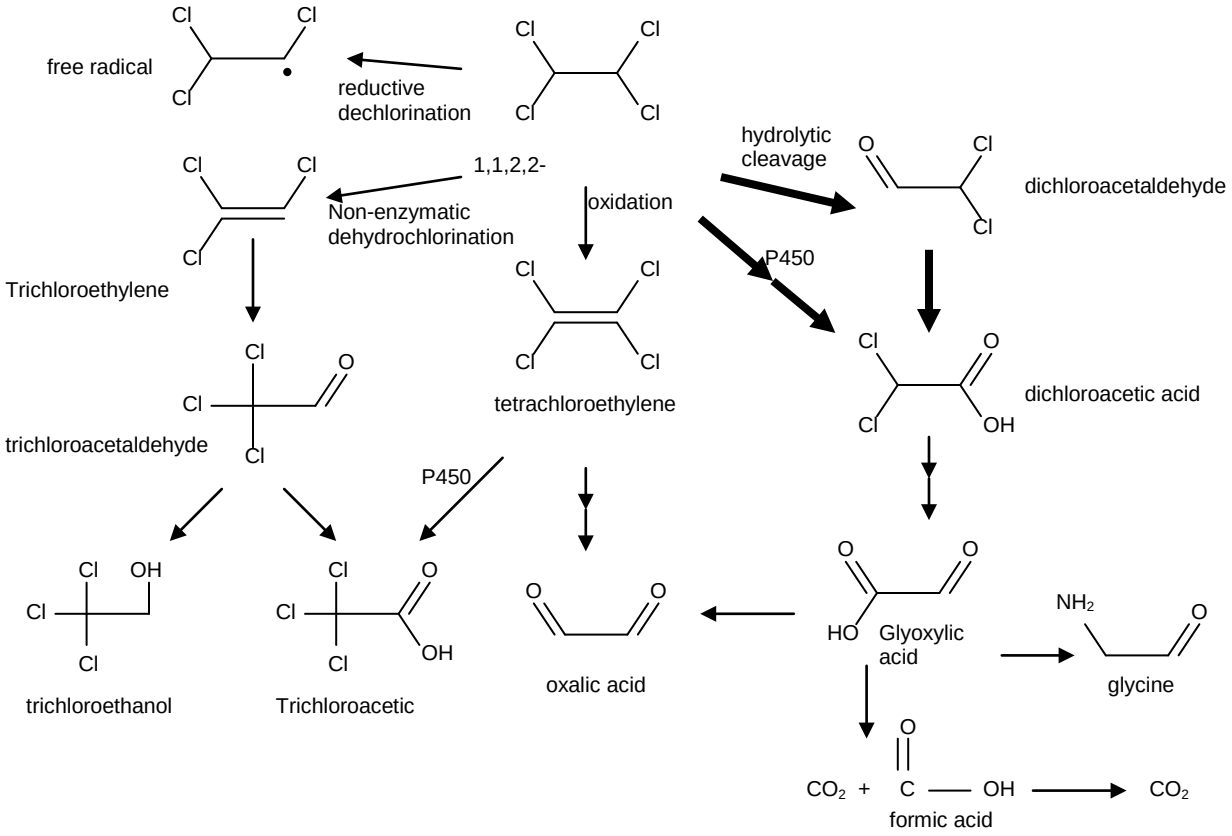
3.2. DISTRIBUTION

No studies measuring the distribution of 1,1,2,2-tetrachloroethane in humans following inhalation or oral exposure were located. Following absorption in animals, 1,1,2,2-tetrachloroethane appears to be distributed throughout the body, but may selectively accumulate to a degree in certain cells and tissues. The human blood-air partition coefficient for 1,1,2,2-tetrachloroethane has been reported to be in the range of 72.6–116 (Meulenberg and Vijverberg, 2000; Gargas et al., 1989; Morgan et al., 1970). The tissue:air partition coefficients for 1,1,2,2-tetrachloroethane in rats have been reported to be 142 (blood), 3,767 (fat), 196 (liver), and 101 (muscle) (Meulenberg and Vijverberg, 2000; Gargas et al., 1989), indicating that 1,1,2,2-tetrachloroethane may partition into fatty tissues, consistent with its low water solubility.

Following a single intravenous injection of radiolabeled 1,1,2,2-tetrachloroethane, Eriksson and Brittebo (1991) reported selective uptake of nonvolatile radioactivity in the mucosal tissues of olfactory and tracheobronchial regions of the respiratory tract and in the mucosae of the oral cavity, tongue, nasopharynx, esophagus, and cardiac region of the forestomach. High levels of radioactivity were also found in the liver, bile, inner zone of the adrenal cortices, and interstitium of the testes, although the levels were not quantified.

3.3. METABOLISM

No studies were located that investigated the metabolism of 1,1,2,2-tetrachloroethane in humans. Information regarding 1,1,2,2-tetrachloroethane metabolism in animals is summarized below, and a suggested metabolic scheme based on in vivo and in vitro data is presented in Figure 3-1.



Source: Adapted from ATSDR (1996).

Figure 3-1. Suggested metabolic pathways of 1,1,2,2-tetrachloroethane.

In vivo and in vitro studies indicate that the metabolism of 1,1,2,2-tetrachloroethane proceeds via multiple pathways in rodents (Mitoma et al., 1985; Casciola and Ivanetich, 1984; Halpert, 1982; Koizumi et al., 1982; Halpert and Neal, 1981; Ikeda and Ohtsuji, 1972; Yllner, 1971). The predominant pathway appears to involve production of dichloroacetic acid, formed as an initial metabolite via stagewise hydrolytic cleavage of 1,1,2,2-tetrachloroethane, yielding dichloroacetyl chloride and dichloroacetaldehyde as intermediates, or by cytochrome P450 (CYP)-based oxidation of 1,1,2,2-tetrachloroethane (Casciola and Ivanetich, 1984; Halpert and Neal, 1981; Yllner, 1971). Dichloroacetic acid was identified as the major urinary metabolite in mice treated with 1,1,2,2-tetrachloroethane by intraperitoneal (i.p.) injection (Yllner, 1971) and in in vitro systems with rat liver microsomal and nuclear CYP (Casciola and Ivanetich, 1984;

Halpert, 1982; Halpert and Neal, 1981). Dichloroacetic acid can be further metabolized to glyoxylic acid, formic acid, and carbon dioxide (Yllner, 1971), with carbon dioxide a potential major component of the end products (Yllner, 1971). Other pathways may involve the formation of trichloroethylene via dehydrochlorination or tetrachloroethylene via oxidation as initial metabolites (Mitoma et al., 1985; Ikeda and Ohtsuji, 1972; Yllner et al., 1971). Trichloroethylene and tetrachloroethylene are further metabolized to trichloroethanol and trichloroacetic acid, and oxalic acid and trichloroacetic acid, respectively (Mitoma et al., 1985; Ikeda and Ohtsuji, 1972; Yllner et al., 1971). 1,1,2,2-Tetrachloroethane may also form free radicals by undergoing reductive dechlorination (ATSDR, 1996). The formation of free radical intermediates during 1,1,2,2-tetrachloroethane metabolism has been demonstrated in spin-trapping experiments (Paolini et al., 1992; Tomasi et al., 1984).

Metabolism of 1,1,2,2-tetrachloroethane is generally extensive, with 68–95% of a total administered dose found as metabolites (Dow Chemical Company, 1988; Mitoma et al., 1985; Yllner, 1971). Mice given a single 0.21–0.32 g/kg i.p. dose of [^{14}C]-labeled 1,1,2,2-tetrachloroethane eliminated 45–61% of the administered radioactivity as carbon dioxide in expired air and 23–34% of the radioactivity in urine in the following 3 days (Yllner et al., 1971). Dichloroacetic acid, trichloroacetic acid, trichloroethanol, oxalic acid, glyoxylic acid, and urea accounted for 27, 4, 10, 7, 0.9, and 2% of the mean urinary radioactivity excreted by the mice in 24 hours, respectively (Yllner et al., 1971). Yllner et al. (1971) also demonstrated that 20–23% of the [^{14}C]-tetrachloroethane was converted to glycine following the simultaneous i.p. injection of [^{14}C]-tetrachloroethane and sodium benzoate and the estimation of [^{14}C]-hippuric acid in the urine. In rats, trichloroethanol appeared to be present as a urinary metabolite at approximately fourfold greater levels than trichloroacetic acid following a single 8-hour inhalation exposure (Ikeda and Ohtsuji, 1972). Several studies have reported that metabolism of 1,1,2,2-tetrachloroethane is greater in mice than in rats, with magnitudes of the reported difference generally in the range of a 1.1–3.5-fold greater metabolic activity, on a per-kg basis, in mice (Dow Chemical Company, 1988; Mitoma et al., 1985).

As indicated above, CYP-based metabolism of 1,1,2,2-tetrachloroethane to dichloroacetic acid has been demonstrated in vitro. Multiple CYP isozymes are likely to be involved, as demonstrated by studies reporting increased metabolism and covalent binding of metabolites following pretreatment with phenobarbital (Casciola and Ivanetich, 1984; Halpert, 1982), xylene (Halpert, 1982), or ethanol (Sato et al., 1980). The isozymes induced by phenobarbital, xylene, and ethanol include members of the CYP2A, CYP2B, CYP2E, and CYP3A) subfamilies (Omiecinski et al., 1999; Nebert et al., 1987).

1,1,2,2-Tetrachloroethane has also been reported to produce inactivation of CYP. 1,1,2,2-Tetrachloroethane effectively inactivated the major phenobarbital-inducible CYP isozyme, but not the major CYP isozyme induced by β-naphthoflavone, in rat liver in vitro (Halpert et al., 1986). Rat liver nuclear CYP levels were reduced following in vitro incubation

with 1,1,2,2-tetrachloroethane and a NADPH-generating system (Casciola and Ivanetich, 1984). In an in vivo study, CYP activity was evaluated in male and female Swiss albino mice 24 hours after a single 0, 300, or 600 mg/kg i.p. dose of 1,1,2,2-tetrachloroethane (Paolini et al., 1992). 1,1,2,2-Tetrachloroethane treatment statistically significantly ($p \leq 0.01$) reduced total CYP activity 44 and 37% in males and females, respectively, at 300 mg/kg and 85 and 74% in males and females, respectively, at 600 mg/kg. Treatment with 600 mg/kg statistically significantly reduced the microsomal activity of CYP isozymes 3A, 2E1, 1A2, 2B1, and 1A1 in both genders, and 300 mg/kg reduced the activity of CYP3A in both sexes and CYP2B1 in males. Heme content was reduced 13 and 33% at 300 and 600 mg/kg, respectively, and may have contributed to the decrease in CYP levels. The 600 mg/kg dose also reduced the activity of glutathione S-transferase (GST) toward 1-chloro-2,4-dinitrobenzene, a general GST substrate, in both genders.

Due to the extensive metabolism of 1,1,2,2 tetrachloroethane to products such as trichloroethylene and dichloroacetic acid, the relevance of 1,1,2,2-tetrachloroethane interactions with GST is important. Studies of human GST-zeta polymorphic variants show different enzymatic activities toward and inhibition by dichloroacetic acid that could reasonably affect the metabolism of 1,1,2,2-tetrachloroethane (Lantum et al., 2002; Blackburn et al., 2001, 2000; Tzeng et al., 2000). Dichloroacetic acid may covalently bind to GST-zeta (Anderson et al., 1999) and inhibit its own metabolism, leading to an increase in the amount of unmetabolized dichloroacetic acid as the dose and/or duration increases (U.S. EPA, 2003).

Data indicate that 1,1,2,2-tetrachlorethane can be metabolized to dichloroacetic acid (ATSDR, 1996; Yllner, 1971), suggesting a potential role for this metabolite in some of the cancer and noncancer effects observed following exposure to 1,1,2,2 tetrachloroethane. Following an intravenous injection of radiolabeled 1,1,2,2-tetrachloroethane, radioactivity could not be extracted from epithelium of the respiratory and upper alimentary tracts, or from the liver, adrenal cortex, or testes (Eriksson and Brittebo, 1991). The presence of tissue-bound metabolites in the epithelial linings in the upper respiratory tract may demonstrate a first-pass effect by the respiratory tract (Eriksson and Brittebo, 1991). In addition, the presence of irreversible tissue-bound metabolites demonstrates the metabolism of 1,1,2,2-tetrachloroethane to reactive metabolites (Eriksson and Brittebo, 1991). However, the identities of the bound metabolites and modified proteins or phospholipids were not identified. The presence of radiolabel in the proteins may have been radiolabeled-incorporated glycine.

Dow Chemical Company (1988) observed radiolabel in hepatic deoxyribonucleic acid (DNA), although the presence of the radiolabel in the hepatic DNA likely represented the incorporation of single [^{14}C]-atoms via normal biosynthetic pathways. Mice were found to have approximately a 1.9-fold greater extent of [^{14}C] activity irreversibly associated with hepatic macromolecules than rats, which the study authors noted was consistent with the greater metabolism, on a per-kg basis, in mice compared to rats. After a 4-week oral exposure to unlabeled 1,1,2,2-tetrachloroethane followed by a single oral dose of labeled

1,1,2,2-tetrachloroethane, Mitoma et al. (1985) also reported greater levels of hepatic protein-binding in the tissue of mice compared to rats, and the differences were on the order of twofold greater binding in mice, which would be consistent both with the Dow Chemical Company (1988) studies and with the observed differences in metabolism of the two species discussed above. This may also be related to the 3.2–3.5-fold greater absorption, on a per-kg basis, of mice compared to rats following inhalation exposure (Dow Chemical Company, 1988).

The kinetic constants of 1,1,2,2-tetrachloroethane metabolism in rats exposed to 350 ppm of the chemical for 6 hours were determined in gas uptake studies performed by Gargas and Anderson (1989). The rate of exhalation of 1,1,2,2-tetrachloroethane was measured and, combined with previously published values for partition coefficients for blood/air, liver/blood, muscle/blood, and fat/blood, allowed the estimation of the disposition of the chemical in rat (Gargas et al., 1989). A K_m of 4.77 μM and a V_{max} of 12 mg/hour (scaled to a l-kg rat) were measured.

3.4. ELIMINATION

Morgan et al. (1970) reported that the urinary excretion rate of 1,1,2,2-tetrachloroethane in humans was 0.015% of the absorbed dose/minute. No other studies measuring the elimination of 1,1,2,2-tetrachloroethane in humans have been reported.

Available animal data indicate that following absorption into the body, 1,1,2,2-tetrachloroethane is eliminated mainly as metabolites in urine, as carbon dioxide, or as unchanged compound in expired air (Gargas and Anderson, 1989; Dow Chemical Company, 1988; Mitoma et al., 1985; Ikeda and Ohtsuji, 1972; Yllner et al., 1971). The patterns of elimination in rats and mice are qualitatively similar (Dow Chemical Company, 1988; Mitoma et al., 1985), although covalent binding is somewhat greater in mice than rats. Elimination is fairly rapid, with significant amounts present in the urine and expired air at 48–72 hours postexposure (Dow Chemical Company, 1988; Mitoma et al., 1985; Ikeda and Ohtsuji, 1972; Yllner et al., 1971).

Only one study quantitatively evaluated the elimination of 1,1,2,2-tetrachloroethane following inhalation exposure. Dow Chemical Company (1988) followed the excretion of 1,1,2,2-tetrachloroethane for 72 hours following exposure of rats and mice to vapor concentrations of 10 ppm (68.7 mg/m^3) [^{14}C]-1,1,2,2-tetrachloroethane for 6 hours. More than 90% of the absorbed dose was metabolized in both species. The percentage of recovered radioactivity reported in rats was 33% in breath (25% as CO_2 and 8% as unchanged compound), 19% in urine, and 5% in feces. In mice, the percentage of recovered radioactivity was 34% in breath (32% as CO_2 and 2% as unchanged compound), 26% in urine, and 6% in feces. Radioactivity in urine and feces was nonvolatile (inferred by the researchers to be product(s) of metabolism), but was not otherwise characterized.

With regard to oral exposure, the excretion of 1,1,2,2-tetrachloroethane was followed for 72 hours after oral administration of 150 mg/kg doses to rats and mice (Dow Chemical

Company, 1988). Greater than 90% of the absorbed dose was detected as metabolites in both species. In rats, 41% was excreted in breath (32% as CO_2 and 9% as unchanged compound), 23% in urine, and 4% in feces. In mice, 51% was excreted in breath (50% as CO_2 and 1% as unchanged compound), 22% in urine, and 6% in feces. Radioactivity in urine and feces was nonvolatile (inferred by the researchers to be product(s) of metabolism), but was not otherwise characterized. Mitoma et al. (1985) found that mice given an oral dose of 1,1,2,2-tetrachloroethane excreted about 10% of the dose unchanged in the breath, and the rest was either metabolized and expired in the breath as carbon dioxide (10%), excreted in the urine and feces (30%, measured together), or retained in the carcass (27%) after 48 hours. Rats showed similar patterns of excretion (Mitoma et al., 1985). The most comprehensive study of the metabolism and excretion of 1,1,2,2-tetrachloroethane was an i.p. study in mice using [^{14}C]-labeled 1,1,2,2-tetrachloroethane. Yllner (1971) showed that after 72 hours, about 4% of the radioactivity was expired unchanged in the breath, 50% was expired as carbon dioxide, 28% was excreted in the urine, 1% was excreted in the feces, and 16% remained in the carcass.

Delays in elimination may be the result of covalent binding of 1,1,2,2-tetrachloroethane metabolites, as reflected in high levels of compound detected in the carcasses of animals. Mitoma et al. (1985) reported a 30.75% retention in the carcass of rats and a 27.44% retention in the carcass of mice 48 hours after exposure to a single labeled dose of 25 and 50 mg/kg 1,1,2,2-tetrachloroethane in rats and mice, respectively. Dow Chemical Company (1988) reported 30% retention in the carcass in rats exposed to 10 ppm by inhalation, 25% in mice exposed to 10 ppm by inhalation, 23% in rats exposed to 150 mg/kg by gavage, and 17.3% in mice exposed to 150 mg/kg by gavage. Colacci et al. (1987) reported covalent binding of radiolabeled 1,1,2,2-tetrachloroethane to DNA, ribonucleic acid (RNA), and protein in the liver, kidneys, lung, and stomach of rats and mice exposed to a single intravenous dose and analyzed 22 hours postexposure. In vitro binding to calf thymus DNA was found to be greatest when the microsomal fraction was present, and was inhibited by SKF-525A, indicating that metabolic activation was likely required for DNA binding (Colacci et al., 1987). However, Collaci et al. (1987) did not distinguish between covalent binding and whether the presence of radiolabel in the DNA, RNA, and protein was the result of incorporated radiolabeled carbon into the biomolecules through normal biochemical processes.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

No physiologically based pharmacokinetic (PBPK) models for 1,1,2,2-tetrachloroethane were located for humans. Meulenberg et al. (2003) used saline:air, rat brain:air, and olive oil:air partition coefficients to model 28 chemicals from three distinct chemical classes, including alkylbenzenes, chlorinated hydrocarbons, and ketones. The saline:air, rat brain:air, and olive oil:air partition coefficients derived for 1,1,2,2-tetrachloroethane were 35.6 ± 6.05, 344 ± 21.0, and 10,125 ± 547, respectively. The brain partition coefficients for the 28 chemicals were

predicted with accuracy within a factor of 2.5 for 95% of the chemicals. While the study demonstrates the ability to predict rat brain partition coefficients using a bilinear equation, the utility of the information for this assessment is limited. Similarly, several PBPK investigations of 1,1,2,2-tetrachloroethane exposure in fish (McKim et al., 1999; Nichols et al., 1993) provide little utility for this assessment. In sum, adequate information for PBPK modeling of 1,1,2,2-tetrachloroethane remains a research need.

Chiu and White (2006) presented an analysis of steady-state solutions to a PBPK model for a generic volatile organic chemical metabolized in the liver. The only parameters used to determine the system state for a given oral dose rate or inhalation exposure concentration were the blood-air partition coefficient, metabolic constants, and the rates of blood flow to the liver and of alveolar ventilation. At exposures where metabolism is close to linear (i.e., unsaturated), it was demonstrated that only the effective first order metabolic rate constant was needed. Additionally, it was found that the relationship between cumulative exposure and average internal dose (e.g., areas under the curve [AUCs]) remains the same for time-varying exposures. The study authors concluded that steady-state solutions can reproduce or closely approximate the solutions using a full PBPK model. Section 5.2.2 addresses the applicability of using this model to conduct a route-to-route extrapolation in this assessment.

4. HAZARD IDENTIFICATION

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

4.1.1. Oral Exposure

A number of case reports provide information on the effects of intentional acute exposure to lethal oral doses of 1,1,2,2-tetrachloroethane (Mant, 1953; Lilliman, 1949; Forbes, 1943; Elliot, 1933; Hepple, 1927). Subjects usually lost consciousness within approximately 1 hour and died 3–20 hours postingestion, depending on the amount of food in the stomach. Postmortem examinations showed gross congestion in the esophagus, stomach, kidneys, spleen, and trachea, gross congestion and edema in the lungs, and histological effects of congestion and cloudy swelling in the lungs, liver, and/or kidneys (Mant, 1953; Hepple, 1927). Amounts of 1,1,2,2-tetrachloroethane recovered from the stomach and intestines of the deceased subjects included 12 mL (Hepple, 1927), 25 g (Lilliman, 1949), 48.5 mL (Mant, 1953), and 425 mL (Mant, 1953). Assuming a density of 1.594 g/mL and an average body weight of 70 kg, the approximate minimum doses consumed in these cases are estimated to be approximately 273, 357, 1,100, and 9,700 mg/kg, respectively. No deaths occurred in eight patients (six men and two women) who were accidentally given 3 mL of 1,1,2,2-tetrachloroethane (68 mg/kg, using the above assumptions) or three patients (one young man, one young woman, and one 12-year-old girl) who were accidentally given 2 or 3 mL (98–117 mg/kg, using the density and reported body weights) as medicinal treatment for hookworm (Ward, 1955; Sherman, 1953). These patients experienced loss of consciousness and other clinical signs of narcosis that included shallow breathing, faint pulse, and pronounced lowering of blood pressure.

4.1.2. Inhalation Exposure

The symptoms of high-dose acute inhalation exposure to 1,1,2,2-tetrachloroethane commonly include drowsiness, nausea, headache, constipation, decreased red blood cell (RBC) count, weakness, and at extremely high concentrations, jaundice, unconsciousness, and respiratory failure (Coyer, 1944; Hamilton, 1917).

An experimental study was conducted in which two volunteers self-inhaled various concentrations of 1,1,2,2-tetrachloroethane for up to 30 minutes (Lehmann et al., 1936). The results of this study suggest that 3 ppm (6.9 mg/m^3) was the odor detection threshold; 13 ppm (89 mg/m^3) was tolerated without effect for 10 minutes, while 146 ppm (1,003 mg/m^3) for 30 minutes or 336 ppm (2,308 mg/m^3) for 10 minutes produced irritation of the mucous membranes, pressure in the head, vertigo, and fatigue. No other relevant information was reported.

Minot and Smith (1921) reported that symptoms of industrial 1,1,2,2-tetrachloroethane poisoning (concentrations not specified) included fatigue, perspiration, drowsiness, loss of appetite, nausea, vomiting, constipation, headache, and jaundice. Hematological changes included increased large mononuclear cells, elevated white blood cell (WBC) count, a slight but progressive anemia, and a slight increase in platelet number. Similar symptoms were reported by Parmenter (1921) and Willcox et al. (1915). Horiguchi et al. (1964) reported that in 127 coating workers employed in artificial pearl factories and exposed to 75–225 ppm (500–1,500 mg/m^3) 1,1,2,2-tetrachloroethane (along with other solvents), observed effects included decreased specific gravity of the whole blood, decreased RBC count, relative lymphocytosis, neurological findings (not specified), and a positive urobilinogen test.

Lobo-Mendonca (1963) observed a number of adverse health effects in a mixed-gender group of 380 workers at 23 Indian bangle manufacturing facilities (80% of workers employed at these facilities were examined). In addition to the inhalation exposure, approximately 50% of the examined workers had a substantial amount of dermal exposure to 1,1,2,2-tetrachloroethane. Some of the workers were exposed to a mixture of equal parts acetone and 1,1,2,2-tetrachloroethane. Air samples were collected at several work areas in seven facilities. Levels of 1,1,2,2-tetrachloroethane in the air ranged from 9.1 to 98 ppm (62.5–672 mg/m^3). High incidences of a number of effects were reported including anemia (33.7%), loss of appetite (22.6%), abdominal pain (23.7%), headaches (26.6%), vertigo (30.5%), and tremors (35%). The significance of these effects cannot be determined because a control group of unexposed workers was not examined, and coexposure to acetone was possible. The study authors noted that the incidence of tremors appeared to be directly related to 1,1,2,2-tetrachloroethane exposure concentrations, as the percentage of workers handling tetrachloroethane and displaying tremors increased as the air concentration of 1,1,2,2-tetrachloroethane increased.

Over a 3-year period, Jeney et al. (1957) examined 34–75 workers employed at a penicillin production facility. 1,1,2,2-Tetrachloroethane was used as an emulsifier, and wide fluctuations in atmospheric levels occurred throughout the day. The investigators noted that the workers were only in the areas with high 1,1,2,2-tetrachloroethane concentrations for short periods of time, and gauze masks with organic solvent filters were worn in these areas. During the first year of the study, 1,1,2,2-tetrachloroethane levels ranged from 0.016 to 1.7 mg/L (16–1,700 mg/m^3; 2–248 ppm). In the second year of the study, ventilation in the work room was improved and 1,1,2,2-tetrachloroethane levels ranged from 0.01 to 0.85 mg/L (10–850 mg/m^3; 1–124 ppm). In the third year of the study, the workers were transferred to a newly built facility and 1,1,2,2-tetrachloroethane levels in the new facility ranged from 0.01 to 0.25 mg/L (10–250 mg/m^3; 1–36 ppm). At 2-month intervals, the workers received general physical examinations, and blood was drawn for measurement of hematological parameters, serum bilirubin levels, and liver function tests; urinary hippuric acid levels were measured every 6 months. It appears that workers with positive signs of liver damage, including palpability of

the liver, rise in bilirubin levels, positive liver function tests, and urobilinogenuria, were transferred to other areas of the facility and were not examined further.

In the first year of the study, 31% of the examined workers had "general or gastro-intestinal symptoms." Loss of appetite, bad taste in the mouth, epigastric pain, and a "dull straining pressure feeling in the area of the liver" were reported by 66% of the workers experiencing gastrointestinal symptoms. Other symptoms included headaches, general weakness, and fatigue in 29%, severe weight loss in 4%, and "tormenting itching" in 1%. Enlargement of the liver was observed in 38% of the screened workers. Urobilinogenuria was detected in 50% of the workers, most often following more than 6 months of employment, and 31% of the workers with urobilinogenuria also had palpable livers.

In the second year of the study, there was a decline in the number of symptomatic workers (13% of examined workers) and in workers with positive urobilinogenuria findings (24%). Liver enlargement was observed in 20% of the examined workers. In the third year, the number of workers reporting symptoms decreased to 2%, and positive urobilinogen findings were found in 12%. The investigators noted that the increased urobilinogen levels during the third year of observation may have been secondary to excessive alcohol consumption or dietary excess. Enlarged livers were found in 5% of the examined workers.

During the course of the study, no alterations in erythrocyte or hemoglobin (Hb) levels were found. Leukopenia (defined as leukocyte levels of <5,800 cells/mL) was found in 20% of the workers, but no relationship between the number of cases and duration of 1,1,2,2-tetrachloro-ethane exposure was found. A positive relationship between duration of exposure and frequency of abnormal liver function test results was observed, as statistically significant correlations were found on the thymol and Takata-Ucko liver function tests, but not the gold sol reaction test. The thymol liver function test measures the direct precipitation of both lipids and abnormal lipid protein complexes appearing in liver disease by the addition of a thymol solution (Kunkel and Hoagland, 1947). The Takata-Ucko (or Takata-Ara) test detects an increase in the amounts of the globulin components of the serum, signifying liver disease (Kunkel and Hoagland, 1947). Abnormal hippuric acid levels were only detected in 1% of the examined workers during the first 2 years, and no abnormalities were observed during the third year. Increased serum bilirubin levels (>1 mg/dL) were observed in 20, 18.7, and 7.6% of the workers during the first, second, and third years, respectively. The prevalence of hepatitis was assessed using sickness benefit files. In the 1-year period prior to the study, 21 cases of hepatitis were found (total number of workers not reported). Three cases of hepatitis were found in the first year of the study, eight cases in the second year, and four cases in the third year. The lack of a control group and poor reporting of study design and results precludes using this study for quantitative dose-response analysis.

Norman et al. (1981) examined the mortality of the employees of 39 chemical processing plants used by the Army during World War II. Ten plants used 1,1,2,2-tetrachloroethane to help

treat clothing, while the others plants used water in the same process. Estimates of exposure levels were not reported, and coexposure to dry-cleaning chemicals was expected. At the time of evaluation, 2,414 deaths were reported in the study cohort. No differences in standard mortality ratios were seen between the tetrachloroethane and water groups for total mortality, cardiovascular disease, cirrhosis of the liver, or cancer of the digestive and respiratory systems. The mortality ratio for lymphatic cancers in the tetrachloroethane group was increased relative to controls or the water group, although the number of deaths was small (4 cases, with an expected number of 0.85). No other differences were seen between the groups.

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

4.2.1. Oral Exposure

4.2.1.1. *Subchronic Studies*

The National Toxicology Program (NTP, 2004) fed groups of male and female F344 rats (10/sex/group) diets containing 0, 268, 589, 1,180, 2,300, or 4,600 ppm of microencapsulated 1,1,2,2-tetrachloroethane for 14 weeks. NTP (2004) reported that the microcapsules containing 1,1,2,2-tetrachloroethane were specified to be no greater than 420 µm in diameter, and were not expected to have any significant effect on the study. The reported average daily doses were 0, 20, 40, 80, 170, or 320 mg/kg-day, and vehicle control (feed with empty microcapsules) and untreated control groups were used for both genders. Endpoints evaluated throughout the study included clinical signs, body weight, and feed consumption. Hematology and clinical chemistry were assessed on days 5 and 21 and at the end of the study; urinalyses were not performed. Necropsies were performed on all animals, and selected organs (liver, heart, right kidney, lung, right testis, and thymus) were weighed. Comprehensive histological examinations were performed on untreated control, vehicle control, and high dose groups. Tissues examined in the lower dose groups were limited to bone with marrow, clitoral gland, liver, ovary, prostate gland, spleen, testis with epididymis and seminal vesicle, and uterus. A functional observational battery (FOB) was performed on rats in the control groups and the 20, 40, and 80 mg/kg-day groups during weeks 4 and 13. Sperm motility, vaginal cytology, estrous cycle length, and percentage of time spent in the various estrus stages were evaluated in control groups and the 40, 80, and 170 mg/kg-day groups.

All animals survived to the end of the study, but clinical signs of thinness and pallor were observed in all animals in the 170 and 320 mg/kg-day groups (NTP, 2004). Final body weights (Table 4-1) were statistically significantly lower than vehicle controls in males at 80, 170, and 320 mg/kg-day (7, 29, and 65% lower, respectively) and females at 80, 170, and 320 mg/kg-day (9, 29, and 56% lower, respectively), with both genders at 320 mg/kg-day losing weight over the course of the study. However, feed consumption by the rats also decreased with increasing dose level (NTP, 2004).

Table 4-1. Final body weights (g) and percent change compared to controls in F344/N rats exposed to 1,1,2,2-tetrachloroethane in feed for 14 weeks

Dose (mg/kg-d)	n	Males		n	Females	
Vehicle control	10	366 ± 5[a]	–	10	195 ± 4[a]	–
20	10	354 ± 9	-3%	10	192 ± 4	-2%
40	10	353 ± 6	-4	10	189 ± 2	-3
80	10	341 ± 6[b]	-7	10	177 ± 2[b]	-9
170	10	259 ± 9[b]	-29	10	139 ± 4[b]	-29
320	10	127 ± 9[b]	-65	10	85 ± 3[b]	-56

[a]Mean ± standard error.
[b]Statistically significant compared to controls ($p \leq 0.05$).

Source: NTP (2004).

Statistically significant increases in absolute liver weights were observed in female rats exposed to 80 mg/kg-day, and statistically significant decreases in absolute liver weight were observed at ≥170 mg/kg-day in males and at 320 mg/kg-day in females (Table 4-2). Statistically significant increases in relative liver weights (Table 4-3) were observed at ≥40 mg/kg-day in males and females (NTP, 2004). Significant alterations in absolute and/or relative weights were also observed in the thymus, kidney, heart, lung, and testes primarily at 170 and 320 mg/kg-day.

Table 4-2. Absolute liver weights (g) and percent change compared to controls in F344/N rats exposed to 1,1,2,2-tetrachloroethane in feed for 14 weeks

Dose (mg/kg-d)	n	Males		n	Females	
Vehicle control	10	12.74 ± 0.26[a]	–	10	6.84 ± 0.17[a]	–
20	10	12.99 ± 0.35	2%	10	7.03 ± 0.12	3%
40	10	14.47 ± 0.44	14	10	7.14 ± 0.16	4
80	10	15.54 ± 0.39	22	10	7.80 ± 0.08[b]	14
170	10	11.60 ± 0.44[b]	-9	10	6.66 ± 0.21	-3
320	10	6.57 ± 0.18[b]	-48	10	4.94 ± 0.12[b]	-28

[a]Mean ± standard error.
[b]Statistically significant compared to controls ($p \leq 0.05$).

Source: NTP (2004).

Table 4-3. Relative liver weight (mg organ weight/g body weight) and percent change compared to controls in F344/N rats exposed to 1,1,2,2-tetrachloroethane in feed for 14 weeks

Dose (mg/kg-d)	n	Males		n	Females	
Vehicle control	10	34.79 ± 0.42[a]	–	10	35.07 ± 0.56[a]	–
20	10	36.72 ± 0.44	6%	10	36.69 ± 0.36	5%
40	10	41.03 ± 0.85[b]	18	10	37.84 ± 0.51[b]	8
80	10	45.61 ± 0.52[b]	31	10	44.20 ± 0.27[b]	26
170	10	44.68 ± 0.45[b]	28	10	48.03 ± 0.89[b]	37
320	10	52.23 ± 1.42[b]	50	10	58.40 ± 1.42[b]	67

[a]Mean ± standard error.
[b]Statistically significant compared to controls ($p \leq 0.05$).

Source: NTP (2004).

Results of the FOB showed no exposure-related findings of neurotoxicity. The hematology evaluations indicated that 1,1,2,2-tetrachloroethane affected the circulating erythroid mass in both genders (Table 4-4). There was evidence of a transient erythrocytosis, as shown by increases in hematocrit values, Hb concentration, and erythrocyte counts on days 5 and 21 at ≥170 mg/kg-day. The erythrocytosis was not considered clinically significant and disappeared by week 14, at which time minimal to mild, dose-related anemia was evident, as shown by decreases in hematocrit and Hb at ≥40 mg/kg-day. For example, although males exposed to 40 mg/kg-day showed a statistically significant decrease in Hb at week 14, the magnitude of the change was small (3.8%). The anemia was characterized as microcytic based on evidence suggesting that the circulating erythrocytes were smaller than expected, including decreases in mean cell volumes, mean cell Hb values, and mean cell Hb concentration in both genders at ≥80 mg/kg-day at various time points. At week 14, there were no changes in reticulocyte counts, suggesting that there was no erythropoietic response to the anemia, which was in turn supported by the bone marrow atrophy observed microscopically. As discussed by NTP (2004), the erythrocytosis suggested a physiological response consistent with hemoconcentration due to dehydration, as well as compromised nutritional status due to the reduced weight gain and food consumption, both of which may have contributed to the development of the anemia.

Table 4-4. Serum chemistry and hematology changes[a] in rats exposed to dietary 1,1,2,2-tetrachloroethane for 14 weeks

Oral dose (mg/kg-d)	Vehicle control	20	40	80	170	320
Males (10/group)						
Serum total protein (g/dL)	7.2 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	6.7 ± 0.1[b]	6.0 ± 0.1[b]
Serum cholesterol (mg/dL)	73 ± 2	74 ± 3	76 ± 2	67 ± 2	68 ± 2	65 ± 2[b]
ALT (IU/L)	48 ± 2	49 ± 2	53 ± 2	69 ± 3[b]	115 ± 8[b]	292 ± 18[b]
ALP (IU/L)	256 ± 7	260 ± 5	248 ± 5	245 ± 6	353 ± 12[b]	432 ± 24[b]
SDH (IU/L)	23 ± 1	27 ± 1[b]	26 ± 2	31 ± 1[b]	47 ± 2[b]	74 ± 4[b]
Bile acids (µmol/L)	29.2 ± 2.9	27.5 ± 2.7	27.2 ± 2.7	35.9 ± 3.9	92.0 ± 16.6[b]	332.4 ± 47.4[b]
Hematocrit (%) (automated)	45.2 ± 0.5	44.9 ± 0.4	44.0 ± 0.9	43.3 ± 0.7	43.1 ± 0.6[b]	39.0 ± 1.1[b]
Hb (g/dL)	15.8 ± 0.1	15.6 ± 0.1	15.2 ± 0.3[b]	14.9 ± 0.1[b]	14.6 ± 0.1[b]	13.6 ± 0.3[b]
Mean cell volume (fL)	50.7 ± 0.1	51.8 ± 0.3	52.3 ± 0.2	51.3 ± 0.2	49.4 ± 0.2	44.4 ± 0.4[b]
Mean cell Hb (pg)	17.7 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	17.7 ± 0.2	16.8 ± 0.1[b]	15.5 ± 0.2[b]
Platelets (10^3/µL)	728.4 ± 12.3	707.0 ± 5.8	727.0 ± 25.2	716.3 ± 9.7	692.8 ± 12.6[b]	773.4 ± 23.2[b]
Females (10/group)						
Serum total protein (g/dL)	7.2 ± 0.1	7.3 ± 0.0	7.3 ± 0.1	6.9 ± 0.1	6.4 ± 0.1[b]	5.6 ± 0.1[b]
Serum cholesterol (mg/dL)	104 ± 4	105 ± 3	98 ± 1	81 ± 2[b]	64 ± 3[b]	55 ± 3[b]
ALT (IU/L)	46 ± 2	42 ± 1	41 ± 2	49 ± 2	112 ± 7[b]	339 ± 18[b]
ALP (IU/L)	227 ± 5	216 ± 4	220 ± 3	225 ± 11	341 ± 7[b]	468 ± 22[b]
SDH (IU/L)	27 ± 1	27 ± 1	28 ± 2	25 ± 1	45 ± 3[b]	82 ± 3[b]
Bile acids (µmol/L)	37.0 ± 7.1	46.6 ± 6.5	39.1 ± 5.6	36.3 ± 3.9	39.3 ± 7.9	321.5 ± 50.6[b]
Hematocrit (%) (automated)	42.8 ± 0.4	43.2 ± 0.4	42.1 ± 0.4	40.1 ± 0.5[b]	42.8 ± 0.7	34.7 ± 0.7[b]
Hb (g/dL)	15.2 ± 0.1	15.3 ± 0.1	14.9 ± 0.1	14.2 ± 0.2[b]	14.5 ± 0.2[b]	12.5 ± 0.2[b]
Mean cell volume (fL)	55.4 ± 0.1	56.1 ± 0.1	55.8 ± 0.1	53.3 ± 0.2[b]	49.0 ± 0.2[b]	44.4 ± 0.4[b]
Mean cell Hb (pg)	19.7 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	18.9 ± 0.1[b]	16.6 ± 0.2[b]	16.0 ± 0.2[b]
Platelets (10^3/µL)	742.1 ± 20.4	725.9 ± 12.7	733.9 ± 8.8	727.4 ± 14.2	639.4 ± 9.9[b]	662.5 ± 19.4[b]

[a]Mean ± standard error.
[b]Statistically significantly different from control value.

ALP = alkaline phosphatase; IU = international units; SDH = sorbitol dehydrogenase

Source: NTP (2004).

Changes in serum clinical chemistry parameters indicative of liver damage were observed in both genders, occurring at all time points (day 5, day 21, and week 14) and increasing in magnitude with increasing dose and time. At week 14 (Table 4-4), these effects included statistically significant increases in ALT and sorbitol dehydrogenase (SDH) activity in males at ≥80 mg/kg-day (41, 134, and 496%, and 15, 74, and 174%, respectively) and females at ≥170 mg/kg-day (167 and 707%, and 67 and 204%, respectively), increases in alkaline phosphatase (ALP) activity in both genders at ≥170 mg/kg-day (36 and 66% in males and 58 and 117% in females), increases in bile acid levels in males at ≥170 mg/kg-day (233 and 1,110%) and females at 320 mg/kg-day (590%), and decreases in serum cholesterol levels in females at

≥80 mg/kg-day (23, 39, and 48%, respectively) and males at 320 mg/kg-day (12%). There were no exposure-related changes in rat serum 5'-nucleotidase activity at week 14, although increases occurred on day 5 in females at ≥20 mg/kg-day and on day 21 in males and females at 80, 170, and/or 320 mg/kg-day.

A summary of histopathological alterations following 1,1,2,2-tetrachloroethane exposure is presented in Table 4-5. Hepatic cytoplasmic vacuolization was noted in males exposed to ≥20 mg/kg-day and in females exposed to ≥40 mg/kg-day. Although incidence of this alteration was high in affected groups, severity was only minimal-to-mild and only increased with dose from 20 to 40 mg/kg-day in males and 40 to 80 mg/kg-day in females. Females exposed to ≥80 mg/kg-day showed an increase in the incidence of hepatocyte hypertrophy with an increase in severity and incidence with increasing exposure level, and males showed similar results at exposures ≥170 mg/kg-day. A statistically significant increase in the incidence of hepatocellular necrosis was observed in male and female rats at 170 and 320 mg/kg-day, accompanied by an increased severity with an increase in dose. At ≥170 mg/kg-day, additional effects in the liver in both genders were hepatocyte pigmentation and mitotic alteration and mixed cell foci, with bile duct hyperplasia observed in females only. Pigmentation of the spleen was statistically significantly increased in male rats exposed to ≥80 mg/kg-day and in female rats exposed to ≥170 mg/kg-day. Other histological effects included statistically significantly increased incidences of atrophy (red pulp and lymphoid follicle) in the spleen of males at 170 and 320 mg/kg-day and the spleen of females at 320 mg/kg-day. A statistically significant increase in atrophy of bone (metaphysis) and bone marrow, prostate gland, preputial gland, seminal vesicles, testes (germinal epithelium), uterus, and clitoral gland, as well as an increase in ovarian interstitial cell cytoplasmic alterations, was observed in females at ≥170 mg/kg-day and in males at 320 mg/kg-day.

Table 4-5. Incidences of selected histopathological lesions in rats exposed to dietary 1,1,2,2-tetrachlorethane for 14 weeks

Dose (mg/kg-d)	Vehicle control	20	40	80	170	320
Males (10/group)						
Hepatocyte cytoplasmic vacuolization	0[a]	7[b] (1.3)	9[b] (2.0)	10[b] (1.9)	8[b] (1.4)	0
Hepatocyte hypertrophy	0	0	0	1 (1.0)	9[b] (1.3)	10[b] (3.2)
Hepatocyte necrosis	0	0	0	0	8[b] (1.0)	10[b] (1.6)
Hepatocyte pigmentation	0	0	0	0	7[b] (1.0)	10[b] (1.9)
Hepatocyte mitotic alteration	0	0	0	0	0	6[b] (2.0)
Mixed cell foci	0	0	0	0	3	5[b]
Bile duct hyperplasia	0	0	0	0	0	10[b] (1.7)
Spleen pigmentation	0	0	1 (1.0)	9[b] (1.0)	9[b] (1.0)	9[b] (1.6)
Spleen red pulp atrophy	0	0	0	0	5[b] (1.0)	9[b] (1.4)
Spleen lymphoid follicle atrophy	0	0	0	0	0	5[b] (1.0)

Table 4-5. Incidences of selected histopathological lesions in rats exposed to dietary 1,1,2,2-tetrachlorethane for 14 weeks

Dose (mg/kg-d)	Vehicle control	20	40	80	170	320
Females (10/group)						
Hepatocyte cytoplasmic vacuolization	0[a]	0	10[b] (1.7)	10[b] (2.2)	4[b] (1.3)	0
Hepatocyte hypertrophy	0	0	0	4[b] (1.0)	10[b] (1.7)	10[b] (2.8)
Hepatocyte necrosis	0	0	0	1 (1.0)	7[b] (1.0)	10[b] (1.1)
Hepatocyte pigmentation	0	0	0	0	10[b] (1.3)	10[b] (2.0)
Hepatocyte mitotic alteration	0	0	0	0	3 (2.0)	10[b] (1.9)
Mixed cell foci	0	0	0	0	8[b]	1
Bile duct hyperplasia	0	0	0	0	5[b] (1.0)	10[b] (1.9)
Spleen pigmentation	1 (1.0)	0	0	4 (1.0)	8[b] (1.1)	8[b] (1.3)
Spleen, red pulp atrophy	0	0	0	0	0	9[b] (1.6)
Spleen lymphoid follicle atrophy	0	0	0	0	0	3 (1.0)

[a]Values represent number of animals with the lesion, with the severity score in parentheses; severity grades are as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[b]Significantly different from vehicle control group.

Source: NTP (2004).

Epididymal spermatozoal motility was statistically significantly decreased at ≥40 mg/kg-day, with statistically significant decreases in epididymis weight at ≥80 mg/kg-day and cauda epididymis weight at 320 mg/kg-day. Exposed female rats spent more time in diestrus and less time in proestrus, estrus, and metestrus than control rats (see Section 4.3.1).

In summary, the NTP (2004) 14-week rat study provides evidence that the liver is a primary target of 1,1,2,2-tetrachloroethane toxicity. At the lowest dose tested, 20 mg/kg-day, there was a significant increase in the incidence of hepatic cytoplasmic vacuolization in males. At 40 mg/kg-day, significant increases in relative liver weights were observed in both males and females. Hepatocellular hypertrophy and spleen pigmentation were observed at 80 mg/kg-day in both males and females, although these changes were generally of minimal severity. Increases in serum ALT and SDH, were observed at 80 mg/kg-day in males and at 170 mg/kg-day in females. Decreases in serum cholesterol levels were observed in females at 80 mg/kg-day and at 320 mg/kg-day in males. A decrease in body weight (>10%) was observed at 170 mg/kg-day in both males and females. Increases in serum ALP activity and bile acid levels, hepatocellular necrosis, bile duct hyperplasia, hepatocellular mitotic alterations, foci of cellular alterations, and liver pigmentation occurred at 170 and/or 320 mg/kg-day. A no-observed-adverse-effect level (NOAEL) of 20 mg/kg-day and a lowest-observed-adverse-effect level (LOAEL) of 40 mg/kg-day was identified by EPA for increased relative liver weight in male and female rats. NTP (2004) identified a NOAEL of 20 mg/kg-day in rats based on survival and body weight changes and increased lesion incidences. There were no clinical signs of neurotoxicity at doses as high as

320 mg/kg-day or exposure-related findings in the FOB at doses as high as 80 mg/kg-day (highest tested dose in the FOB), indicating that the nervous system may be less sensitive than the liver for subchronic dietary exposure.

NTP (2004) also exposed groups of male and female B6C3F$_1$ mice (10/sex/group) to diets containing 0, 589, 1,120, 2,300, 4,550, or 9,100 ppm of microencapsulated 1,1,2,2-tetra-chloroethane for 14 weeks, with vehicle and untreated control groups for each gender. The reported average daily doses were 0, 100, 200, 370, 700, or 1,360 mg/kg-day for males and 0, 80, 160, 300, 600, or 1,400 mg/kg-day for females. Endpoints evaluated throughout the study included clinical signs, body weight, and feed consumption. Clinical chemistry was assessed at the end of the study, but hematological evaluations and urinalyses were not performed. Necropsies were conducted on all animals and selected organs (liver, heart, right kidney, lung, right testis, and thymus) were weighed. Comprehensive histological examinations were performed on untreated control, vehicle control, and high dose groups. Tissues examined in the lower dose groups were limited to the liver, spleen, thymus, preputial gland (in males only), and lungs (in females only). An FOB (21 parameters) was performed on mice in both control and 160/200, 300/370, and 600/700 mg/kg-day (1,120, 2,300, and 4,550 ppm, respectively) dose groups during weeks 4 and 13. Sperm motility, vaginal cytology, estrous cycle length, and percentage of time spent in the various estrus stages were evaluated in both control and 160/200, 600/700, and 1,360/1,400 mg/kg-day (1,120, 2,300, and 4,550 ppm, respectively) dose groups.

All mice survived to the end of the study (NTP, 2004). Thinness was observed clinically in male mice (3/10, 9/10, 10/10) at 370, 700, and 1,400 mg/kg-day, respectively, and in female mice (1/10, 2/10, 10/10) at 300, 600, and 1,360 mg/kg-day, respectively. Final body weights were statistically significantly lower than vehicle controls in male mice at 370, 700, and 1,360 mg/kg-day (12, 16, and 23%, respectively) and female mice at 600 and 1,400 mg/kg-day (11 and 12%, respectively) (Table 4-6). Feed consumption was less than controls in males at ≥700 mg/kg-day, but similar to controls in females.

Table 4-6. Final body weights (g) and percent change compared to controls in B6C3F$_1$ mice exposed to 1,1,2,2-tetrachloroethane in feed for 14 weeks

Dose (mg/kg-d)	n	Males	
Vehicle control	10	30.1 ± 0.6[a]	–
100	10	30.6 ± 0.6	2%
200	10	30.0 ± 0.3	0
370	10	26.5 ± 0.4[b]	-12
700	10	25.2 ± 0.2[b]	-16
1,360	10	23.1 ± 0.5[b]	-23
		Females	
Vehicle control	10	24.3 ± 0.5[a]	–
80	10	24.2 ± 0.2	0%
160	10	24.3 ± 0.6	0
300	10	23.3 ± 0.4	-4
600	10	21.7 ± 0.2[b]	-11
1,400	10	21.5 ± 0.6[b]	-12

[a]Mean ± standard error.
[b]Statistically significant compared to controls ($p \leq 0.05$).

Source: NTP (2004).

Statistically significant increases in absolute liver weights were observed in the male mice exposed to 200 and 370 mg/kg-day (16 and 10%, respectively), but not at higher doses, and in female mice exposed to ≥80 mg/kg-day (11, 29, 27, 22, and 32%, respectively) (Table 4-7). Statistically significant increases in relative liver weights were observed in male mice at ≥200 mg/kg-day (16, 24, 24, and 38%, respectively) and in female mice at ≥80 mg/kg-day (11, 28, 33, 36, and 49%, respectively) (Table 4-8). Other organ weight changes (increased kidney weights in males at ≥370 mg/kg-day and decreased thymus weights in both genders at 1,360/ 1,400 mg/kg-day) were considered to be secondary to the body weight changes. Results of the FOBs showed no exposure-related neurotoxicity.

Table 4-7. Absolute liver weights (g) and percent change compared to controls in B6C3F$_1$ mice exposed to 1,1,2,2-tetrachloroethane in feed for 14 weeks

Dose (mg/kg-d)	n	Males	
Vehicle control	10	1.467 ± 0.020	–
100	10	1.557 ± 0.039	6%
200	10	1.701 ± 0.020[b]	16
370	10	1.607 ± 0.038[b]	10
700	10	1.531 ± 0.052	4
1,360	10	1.558 ± 0.045	6
		Females	
Vehicle control	10	1.048 ± 0.028	–
80	10	1.160 ± 0.022[b]	11%
160	10	1.356 ± 0.058[b]	29
300	10	1.336 ± 0.037[b]	27
600	10	1.277 ± 0.030[b]	22
1,400	10	1.386 ± 0.047[b]	32

[a]Mean ± standard error.
[b]Statistically significant compared to controls ($p \leq 0.05$).

Source: NTP (2004).

Table 4-8. Relative liver weights (mg organ weight/g body weight) and percent change compared to controls in B6C3F$_1$ mice exposed to 1,1,2,2-tetrachloroethane in feed for 14 weeks

Dose (mg/kg-d)	n	Males	
Vehicle control	10	48.84 ± 1.17	–
100	10	50.94 ± 0.93	4%
200	10	56.82 ± 0.63[b]	16
370	10	60.63 ± 1.20[b]	24
700	10	60.71 ± 1.76[b]	24
1,360	10	67.43 ± 1.83[b]	38
		Females	
Vehicle control	10	43.26 ± 1.05	–
80	10	47.90 ± 0.85[b]	11%
160	10	55.54 ± 1.17[b]	28
300	10	57.39 ± 0.84[b]	33
600	10	58.73 ± 1.23[b]	36
1,400	10	64.42 ± 1.14[b]	49

[a]Mean ± standard error.
[b]Statistically significant compared to controls ($p \leq 0.05$).

Source: NTP (2004).

Clinical chemistry findings in the mice are summarized in Tables 4-9 and 4-10 and included statistically significant decreases in total serum protein levels in males at ≥200 mg/kg-day, total serum protein levels in females at ≥300 mg/kg-day, and serum albumin levels in females at 1,400 mg/kg-day (NTP, 2004). Decreased serum albumin levels could not fully account for the decreased total protein levels, suggesting that other factors (e.g., changes in other protein fractions, hydration status, and/or hepatic function) contributed to the hypoproteinemia (NTP, 2004). A statistically significant increase of serum SDH activity in females was observed at ≥80 mg/kg-day (22, 111, 444, 575, and 1,181%, respectively) and in males at ≥200 mg/kg-day (38, 424, 424, and 715%, respectively). A statistically significant decrease in serum cholesterol levels was observed in females at ≥160 mg/kg-day (22, 38, 41, and 16%, respectively), and a statistically significant increase in ALT activity was observed in females at ≥160 (30, 278, 294, and 602%, respectively) and in males at ≥370 mg/kg-day (234, 177, and 377%, respectively). Total bile acid levels increased statistically significantly in females at ≥160 mg/kg-day (18, 69, 97, and 290%, respectively) and in males at ≥370 mg/kg-day (148, 178, and 377%, respectively). A statistically significant increase in ALP activity was observed in males at ≥370 mg/kg-day (67, 83, and 136%, respectively) and in females at 300 mg/kg-day (19, 28, 55%, respectively), and a statistically significant increase in 5'-nucleotidase was observed in males at ≥370 mg/kg-day (88, 131, and 288%, respectively).

Table 4-9. Selected clinical chemistry changes in male mice exposed to dietary 1,1,2,2-tetrachloroethane for 14 weeks

Dose (mg/kg-d)	Vehicle control	100	200	370	700	1,360
Serum total protein (g/dL)	5.4 ± 0.1[a]	5.2 ± 0.1	5.1 ± 0.1[b]	5.1 ± 0.1[b]	5.1 ± 0.1[b]	5.1 ± 0.1[b]
Serum cholesterol (mg/dL)	131 ± 7	125 ± 4	94 ± 3[b]	110 ± 5	112 ± 4	126 ± 5
ALT (IU/L)	66 ± 8	62 ± 19	74 ± 8	207 ± 18[b]	172 ± 18[b]	296 ± 24[b]
ALP (IU/L)	85 ± 2	78 ± 2	89 ± 2	130 ± 3[b]	143 ± 7[b]	184 ± 11[b]
SDH (IU/L)	55 ± 3	53 ± 2	76 ± 3[b]	288 ± 20[b]	288 ± 29[b]	448 ± 25[b]
5'-Nucleotidase (IU/L)	18 ± 1	16 ± 1	18 ± 0	30 ± 2[b]	37 ± 3[b]	62 ± 7[b]
Bile acids (µmol/L)	25.3 ± 1.2	22.8 ± 1.5	24.8 ± 0.6	56.5 ± 5.1[b]	63.3 ± 7.5[b]	108.7 ± 8.1[b]

[a]Mean ± standard error.
[b]Statistically significantly different from control value.

Source: NTP (2004).

Table 4-10. Selected clinical chemistry changes in female mice exposed to dietary 1,1,2,2-tetrachloroethane for 14 weeks

Dose (mg/kg-d)	Vehicle control	80	160	300	600	1,400
Serum total protein (g/dL)	5.6 ± 0.1[a]	5.6 ± 0.1	5.5 ± 0.0	5.4 ± 0.1[b]	5.4 ± 0.0[b]	5.1 ± 0.1[b]
Serum cholesterol (mg/dL)	109 ± 2	109 ± 3	85 ± 3[b]	68 ± 2[b]	64 ± 3[b]	92 ± 4[b]
ALT (IU/L)	34 ± 5	50 ± 15	65 ± 5[b]	189 ± 33[b]	197 ± 21[b]	351 ± 35[b]
ALP (IU/L)	131 ± 5	126 ± 2	139 ± 5	150 ± 3[b]	161 ± 7[b]	195 ± 6[b]
SDH (IU/L)	36 ± 1	44 ± 3[b]	76 ± 4[b]	197 ± 15[b]	243 ± 23[b]	461 ± 59[b]
5'-Nucleotidase (IU/L)	59 ± 3	71 ± 2	84 ± 5[b]	62 ± 2	62 ± 3	83 ± 4[b]
Bile acids (µmol/L)	27.2 ± 1.2	26.1 ± 1.9	30.9 ± 1.1[b]	44.2 ± 3.9[b]	51.5 ± 3.6[b]	101.7 ± 12.0[b]

[a]Mean ± standard error.
[b]Statistically significantly different from control value.

Source: NTP (2004).

The histopathological results in the B6C3F$_1$ mice are summarized in Table 4-11. A statistically significant increased incidence of minimal to moderate hepatocyte hypertrophy was observed at ≥160 mg/kg-day in females and ≥200 mg/kg-day in males. The incidence of hepatocellular necrosis was statistically significantly increased in male mice at ≥370 mg/kg-day and in female mice at ≥300 mg/kg-day. A statistically significant increased incidence of pigmentation and bile duct hyperplasia occurred at ≥300 mg/kg-day in females and ≥370 mg/kg-day in males. Additionally, the histological findings included an increased incidence of preputial gland atrophy in males in the 100, 700, and 1,360 mg/kg-day dose groups (Table 4-11), but this effect did not appear dose-related. Based on the increase in serum SDH activity and increased absolute and relative liver weights at 80 mg/kg-day in female mice, as well as serum chemistry changes at ≥160 mg/kg-day and clear evidence of histopathology at higher doses, a LOAEL of 80 mg/kg-day was identified based on liver toxicity.

Table 4-11. Incidences of selected histopathological lesions in mice exposed to dietary 1,1,2,2-tetrachloroethane for 14 weeks

Males (10/group)						
Oral dose (mg/kg-d)	**Vehicle control**	**100**	**200**	**370**	**700**	**1,360**
Hepatocyte hypertrophy	0[a]	0	7[b] (1.0)	10[b] (2.2)	10[b] (2.8)	10[b] (3.1)
Hepatocyte necrosis	0	0	1 (2.0)	8[b] (1.1)	8[b] (1.0)	9[b] (1.0)
Liver focal pigmentation	0	0	0	10[b] (1.2)	10[b] (1.4)	8[b] (1.3)
Bile duct hyperplasia	0	0	0	7[b] (1.4)	9[b] (1.3)	10[b] (2.0)
Preputial gland atrophy	0	4[b] (2.0)	2 (1.0)	0	4[b] (2.5)	5[b] (2.2)
Females (10/group)						
Oral dose (mg/kg-d)	**Vehicle control**	**80**	**160**	**300**	**600**	**1,400**
Hepatocyte hypertrophy	0[a]	2 (1.5)	9[b] (1.0)	10[b] (1.9)	10[b] (2.5)	10[b] (3.0)
Hepatocyte necrosis	0	0	0	3 (1.0)	7[b] (1.0)	4[b] (1.0)
Liver focal pigmentation	0	0	2 (1.0)	9[b] (1.0)	8[b] (1.0)	7[b] (1.1)
Bile duct hyperplasia	0	0	0	8[b] (1.0)	10[b] (1.4)	10[b] (2.0)

[a]Values represent number of animals with the lesion, with the severity score in parentheses; severity grades are as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[b]Significantly different from vehicle control group.

Source: NTP (2004).

4.2.1.2. *Chronic Studies*

Information on the chronic oral toxicity of 1,1,2,2-tetrachloroethane is available from a bioassay in rats and mice. The National Cancer Institute (NCI, 1978) exposed groups of 50 male and 50 female Osborne-Mendel rats to 1,1,2,2-tetrachloroethane in corn oil via gavage 5 days/week for 78 weeks. Vehicle and untreated control groups (20 animals/sex/species) were also used. The initial low and high doses used for rats of both genders were 50 and 100 mg/kg-day. At week 15, the doses were raised to 65 mg/kg-day for low-dose males and 130 mg/kg-day for high dose males. At week 26, the doses were decreased to 40 mg/kg-day for the low-dose females and 80 mg/kg-day for the high-dose females. Beginning at week 33, intubation of all high-dose rats was suspended for 1 week followed by 4 weeks of dosing, and this cyclic pattern of dosing was maintained for the remainder of the treatment period. Low-dose rats were not subject to this regimen. The reported time-weighted average (TWA) doses were 62 and 108 mg/kg-day for male rats and 43 and 76 mg/kg-day for female rats. The exposure period was followed by a 32-week observation period in which the rats were not exposed to 1,1,2,2-tetrachloroethane. Clinical signs, survival, body weight, food consumption, gross pathology, and histology (32 major organs and tissues as well as gross lesions) were evaluated.

There were no clear effects on survival in the male rats. In females, survival in the vehicle control, low-dose, and high-dose groups at the end of the study was 70, 58, and 40%, respectively. Although there was a statistically significant association between increased mortality and dose in the females, the increased mortality was affected by the deaths of 10 high-

dose females, 8 with pneumonia and 2 with no reported lesions, during the first 5 weeks of the study. The study authors also stated that there was no evidence that the early deaths were tumor-related. The male and female rats also demonstrated an increased incidence of endemic chronic murine pneumonia. Respective incidences of chronic murine pneumonia in the vehicle control, low-, and high-dose groups were 40, 68, and 76% in females and 55, 50, and 65% in males. Clinical observations included squinted or reddened eyes in all control and treated groups of both genders, but these effects occurred with greater frequency in the exposed rats. There was a low or moderate incidence of labored breathing, wheezing, and/or nasal discharge in all control and treated groups during the first year of the study; near the end of the study these signs were observed more frequently in the exposed animals.

Dose-related decreases in body weight gain were observed. However, as the study approached termination (weeks 100–110), the differences in body weight across the dose groups decreased.

Histopathological effects included a dose-related increased incidence of hepatic fatty metamorphosis in high-dose males (2/20, 0/20, 2/50, and 9/49 in the untreated control, vehicle control, low-dose, and high-dose groups, respectively). In addition, inflammation, focal cellular changes, and angiectasis were observed in male and female rats but were not statistically significant or biologically relevant. NCI (1978) stated that the inflammatory, degenerative, and proliferative lesions observed in the control and dosed animals were similar in incidence and type to those occurring in naturally-aged rats.

A statistically significant increase in tumor incidence was not observed in the rats; however, two hepatocellular carcinomas, which are rare tumors in male Osborne-Mendel rats (NCI, 1978), as well as one neoplastic nodule, were observed in the high-dose males (Table 4-12). A hepatocellular carcinoma was also observed in an untreated female control. Although interpretation of this study is complicated by the chronic murine pneumonia, it is unlikely to have contributed to the fatty metamorphosis observed in the liver of male rats.

Table 4-12. Incidence of neoplasms in male Osborne-Mendel rats exposed to 1,1,2,2-tetrachloroethane in feed for 78 weeks

Neoplasm	Dose (mg/kg-d)			
	Control	Vehicle control	62	108
Papilloma, stomach	0/20	0/20	0/50	1/48
Squamous cell carcinoma, stomach	0/20	0/20	0/50	1/48
Neoplastic nodule/carcinoma, liver	0/20	0/20	0/50	3/49
Follicular-cell carcinoma, thyroid	1/19	3/20	0/49	2/48
Hemangiosarcoma, all sites	0/20	0/20	2/50	3/49
Adenocarcinoma, mammary gland	1/20	2/20	2/50	0/49
Fibroadenoma, mammary gland	1/20	1/'20	1/50	0/49
Chromophode adenomas, pituitary	2/20	5/14	5/48	5/48
Islet-cell adenomas, pancreatic islets	0/20	2/20	2/49	2/49
Fibroma, subcutaneous tissue	0/20	1/20	2/50	2/49

Source: NCI (1978).

In addition, one papilloma of the stomach, one squamous-cell carcinoma of the stomach, two follicular-cell carcinomas of the thyroid, and three hemangiosarcomas were each observed in high-dose males (Table 4-12). In the low-dose males, two mammary gland adenocarcinomas (2/20 in vehicle controls) and two hemangiosarcomas (0/20 in vehicle control) were observed. Adenomas were observed as follows: pituitary chromophobe adenomas in the vehicle control (5/14) and low- and high-dose males (5/48 and 5/48, respectively); pancreatic islet-cell adenomas in the vehicle control (2/20) and low- and high-dose males (2/49 and 2/49, respectively); mammary gland fibroadenomas in the vehicle control (1/20) and low-dose males (1/50); and subcutaneous tissue fibromas in the vehicle control (1/20) and low- and high-dose females (2/50 and 2/49, respectively). In male rats, the incidence of chromophobe adenomas, islet-cell adenomas, and follicular-cell carcinomas in the vehicle controls was significantly increased over the incidence in historical controls (NCI, 1978).

In the female rats (Table 4-13), one follicular-cell carcinoma was observed in both the low- and high-dose groups. One mammary gland adenocarcinoma was observed in a low-dose female, and two were observed in the high-dose group. One hemangiosarcoma was observed in a low-dose female. Adenomas were observed as follows: pituitary chromophobe adenomas in the vehicle control (3/20) and low- and high-dose females (11/49 and 6/48, respectively); one pancreatic islet-cell adenoma in a low-dose female; mammary gland fibroadenomas in the vehicle control (9/20) and low- and high-dose females (13/50 and 11/50, respectively); and subcutaneous tissue fibromas in the vehicle control (1/20) and low- and high-dose females (2/50 and 1/50, respectively). The incidence of fibroadenomas of the mammary gland in the vehicle control group was statistically significantly increased over the incidence in historical controls (NCI, 1978).

Table 4-13. Incidence of neoplasms in female Osborne-Mendel rats exposed to 1,1,2,2-tetrachloroethane in feed for 78 weeks

Neoplasm	Dose (mg/kg-d)			
	Control	Vehicle control	43	76
Adenocarcinoma, mammary gland	2/20	0/20	1/50	2/50
Fibroadenoma, mammary gland	2/20	9/20	13/50	11/50
Hemangiosarcomas, uterus	0/20	0/20	1/50	0/50
Chromophode adenomas, pituitary	6/19	3/20	11/49	6/48
Islet-cell adenomas, pancreatic islets	1/20	0/20	1/50	0/50
Follicular-cell carcinoma, thyroid	0/20	0/20	1/49	1/50
Fibroma, subcutaneous tissue	0/20	1/20	2/50	1/50

Source: NCI (1978).

NCI (1978) also exposed groups of 50 male and 50 female B6C3F_1 mice to 1,1,2,2-tetrachloroethane in corn oil via gavage 5 days/week for 78 weeks. Initial dose levels were 100 and 200 mg/kg-day in both genders. In week 19, the doses were increased to 150 and 300 mg/kg-day, respectively. Three weeks later, the doses were increased to 200 and 400 mg/kg-day, respectively. In week 27, the doses were decreased to 150 and 300 mg/kg-day, respectively. The reported TWA doses were 142 and 284 mg/kg-day for male and female mice, respectively. The exposure period was followed by a 12-week observation period in which the mice were not exposed to 1,1,2,2-tetrachloroethane. Vehicle and untreated control groups (20 animals/sex) and a pooled vehicle control were also used. The pooled vehicle control group comprised the vehicle controls from the studies of 1,1,2,2-tetrachloroethane and chloropicrin. Clinical signs, survival, body weight, food consumption, gross pathology, and histology (32 major organs and tissues as well as gross lesions) were evaluated.

A statistically significant association between mortality and dose was observed, as survival was markedly decreased in the high-dose male and female mice. Terminal survival data were not reported for the males, although acute toxic tubular nephrosis was determined to be the apparent cause of death in 33 high-dose males dying between weeks 69 and 70. Survival in the vehicle control, low-dose, and high-dose females at the end of the study was 75, 74, and 34%, respectively, but the cause of death in the high-dose females was not reported. The male and female mice also demonstrated an increased incidence of endemic chronic murine pneumonia. Respective incidences of chronic murine pneumonia in the vehicle control, low-, and high-dose groups were 11, 0, and 2% in males and 5, 13, and 18% in females.

A high incidence (approximately 95%) of pronounced abdominal distension, possibly resulting from liver tumors, was observed in the high-dose females beginning in week 60 and continuing throughout the recovery period. Nodular hyperplasia and organized thrombus were

observed in male and female mice, but the incidences were not statistically significant. Nonneoplastic lesions observed included hydronephrosis (16/46) and chronic inflammation in the kidneys (5/46) in high-dose females and chronic inflammation in the kidneys in the low- (13/39) and high-dose (10/47) males (Table 4-14). In addition, acute toxic tubular nephrosis was observed and was the apparent cause of death, as identified by the study authors, in high-dose male mice that died during weeks 69 and 70.

Table 4-14. Incidence of nonneoplastic kidney lesions observed in male and female $B6C3F_1$ mice exposed to 1,1,2,2-tetrachloroethane in feed for 78 weeks

Lesion	Dose (mg/kg-d)			
	Control	Vehicle control	142	284
Males				
Chronic inflammation – kidney	7/19	5/18	13/39	10/47
Females				
Hydronephrosis	0/19	0/20	0/46	16/46
Chronic inflammation	0/19	0/20	0/46	5/46

Source: NCI (1978).

Statistically significant increases in the incidences of hepatocellular carcinomas occurred in both sexes and at both dose levels (Table 4-15). The incidences in the vehicle control, pooled vehicle control, 142, and 284 mg/kg-day groups were 1/18, 3/36, 13/50, and 44/49, respectively, in males and 0/20, 1/40, 30/48, and 43/47, respectively, in females. Information on the progression from preneoplastic pathology to hepatocellular carcinoma is not available due to the lack of interim sacrifices. The hepatocellular carcinomas varied in microscopic appearance, with some tumors composed of well-differentiated cells and a relatively uniform rearrangement of cords, and other tumors composed of anaplastic cells with large hyperchromatic nuclei with eosinophilic inclusion bodies and/or vacuolated pale cytoplasm. In addition, a decrease in the time to tumor for the hepatocellular carcinomas was also evident in both genders of mice. The spontaneous tumor rate for hepatocellular carcinoma in the historical vehicle controls at the testing laboratory was 74/612 (12%) for male $B6C3F_1$ mice and 8/560 for female $B6C3F_1$ mice.

Table 4-15. Incidence of hepatocelluar carcinomas in male and female B6C3F$_1$ mice exposed to 1,1,2,2-tetrachloroethane in feed for 78 weeks

Hepatocellular carcinoma	Dose (mg/kg-d)			
	Vehicle control	Pooled vehicle control	142	284
Males				
Incidence	1/18	3/36	13/50[a]	44/49[a]
Time to first tumor (wks)	72	NA	84	52
Females				
Incidence	0/20	1/40	30/48[a]	43/47[a]
Time to first tumor (wks)	NA	NA	58	53

[a]Significantly different from control groups.

Source: NCI (1978).

In addition to the liver tumors, alveolar/bronchiolar adenomas in the lung were observed in the male matched vehicle controls (1/18), male and female pooled-vehicle controls (1/36 and 1/40, respectively), low-dose males and females (2/39 and 1/46, respectively), and high-dose males and females (2/47 and 1/44, respectively) (Table 4-16). Lymphomas were observed in low- and high-dose males (4/50 and 3/49, respectively), in female pooled vehicle controls (2/40), and in low- and high-dose females (7/48 and 3/47, respectively).

Table 4-16. Incidence of additional neoplasms in male and female B6C3F$_1$ mice exposed to 1,1,2,2-tetrachloroethane in feed for 78 weeks

Neoplasm	Dose (mg/kg-d)			
	Matched control	Pooled vehicle control	142	284
Males				
Alveolar/bronchiolar adenomas, lung	1/18	1/36	2/39	2/47
Lymphomas, multiple organ	0/18	0/36	4/50	3/49
Females				
Alveolar/bronchiolar adenomas, lung	0/20	1/40	1/46	1/44
Lymphomas, multiple organ	0/20	2/40	7/48	3/47

Source: NCI (1978).

For chronic inflammation in the kidneys of male mice, a LOAEL of 142 mg/kg-day was selected. A NOAEL was not identified. For hydronephrosis and chronic inflammation in the kidneys in females, a NOAEL of 142 mg/kg-day and a LOAEL of 284 mg/kg-day were selected.

4.2.2. Inhalation Exposure

4.2.2.1. *Subchronic Studies*

Truffert et al. (1977) exposed groups of female Sprague-Dawley rats (55/dose) to 1,1,2,2-tetrachloroethane vapor at reported calculated atmospheric concentrations of 0 or 560 mL/m^3 5 days/week for 15 weeks (78 exposures). The daily exposure duration was 6 hours for the first 8 exposures and 5 hours for the remaining 70 exposures. There is uncertainty regarding the actual concentration employed due to the unusual unit of exposure (i.e., mL/m^3). It is assumed that mL/m^3 is a volume/volume vapor concentration, so the reported concentration is equivalent to 560 ppm (3,909 mg/m^3). Interim sacrifices were conducted after 2, 4, 9, 19, 39, and 63 exposures, although the number of animals killed at each time period was not reported.

This study is limited by poor reporting quality and minimal quantitative data. Pronounced prostration was observed "after the first exposures to 1,1,2,2-tetrachloroethane, followed by recovery". Body weight gain was decreased at the end of the study, but the magnitude of the change was not reported. Increases in relative liver weights were observed beginning 15 days after exposure initiation, but were not quantified. Hematological alterations consisting of a decrease in hematocrit "confirmed by the joint RBC and WBC counts" were observed at the end of the study, but were not quantified. A marked increase (313%) in thymidine uptake in hepatic DNA was observed after four exposures, but by the ninth exposure the thymidine uptake had decreased to levels similar to controls. Histological alterations were observed in the liver after nine exposures and included granular appearance, cytoplasmic vacuolization, and evidence of hyperplasia (increase in the number of binucleated cells and the appearance of mitosis), but the alterations regressed after 19 exposures and were no longer observed after 39 exposures. Incidences and severity of the liver lesions were not reported. Considering the lack of incidence and severity data and other inadequately reported results, lack of information on dose-response due to the use of a single exposure level, and uncertainty regarding the exposure concentration, a NOAEL or LOAEL cannot be identified from this study.

Horiuchi et al. (1962) exposed one adult male monkey (*Macaca cynomolga Linné*) to 1,1,2,2-tetrachloroethane for 2 hours/day, 6 days/week for a total of 190 exposures in 9 months. The exposure level was 2,000–4,000 ppm (13,700–27,500 mg/m^3) for the first 20 exposures, 1,000–2,000 ppm (6,870–13,700 mg/m^3) for the next 140 exposures, and 3,000–4,000 ppm (20,600–27,500 mg/m^3) for the last 30 exposures. The TWA concentration was 1,974 ppm (13,560 mg/m^3). The authors noted that the monkey was weak after approximately seven exposures and had diarrhea and anorexia between the 12th and 15th exposures. Beginning at the 15th exposure, the monkey was "almost completely unconscious falling upon his side" for 20–60 minutes after each exposure. The authors noted a gradual increase in body weight during months 3–5 followed by a gradual decrease until the study was terminated. Hematological parameters demonstrated sporadic changes in hematocrit and RBC and WBC counts, but the significance of these findings cannot be determined because there were no clear trends, only one

monkey was tested, and there was no control group. Histological alterations consisted of fatty degeneration in the liver and splenic congestion, and no effects were observed in the heart, lung, kidneys, pancreas, or testes. This study cannot be used to identify a NOAEL or LOAEL for subchronic exposure due to the use of a single animal without a control.

A 6-month inhalation study in rats was performed by the Mellon Institute of Industrial Research (1947). Groups of 12 male and 12 female albino rats were exposed to 0 or 167 ppm (1,150 mg/m^3) of 1,1,2,2-tetrachloroethane for 7 hours/day on alternate days for the 6-month study period. A statistically significant increase (15%) in kidney weight was observed in the 1,1,2,2-tetrachloroethane-exposed rats. The rats also appeared to develop lung lesions following exposure to tetrachloroethane; however, the study authors stated that the pathology reported for tetrachloroethane must be discounted due to approximately 50% of the control animals demonstrating major pathology of the kidneys, liver, or lung. Meaningful interpretation of these results is precluded by the observed endemic lung infection, which resulted in significant early mortality in all of the rats (57 and 69% mortality in the control and tetrachloroethane-exposed groups, respectively). This study also included one mongrel dog that followed the same study design and evaluation as the rats. Serum phosphatase activity levels, mean of 33 units/100 mL, and blood urea nitrogen levels, mean of 20.66%, were increased in the treated dog compared to control values of 5.72/100 mL and 14.94%, respectively. The dog survived the 6-month exposure with effects that included cloudy swelling of the liver and of the convoluted tubules of the kidneys, and light congestion of the lungs. Identification of a LOAEL or NOAEL is precluded by poor study reporting, high mortality in the rats, and the use of a single treated animal in the dog study.

Kulinskaya and Verlinskaya (1972) examined effects of 1,1,2,2-tetrachloroethane on the blood acetylcholine system in Chinchilla rabbits exposed to 0 or 10 mg/m^3 (0 or 1.5 ppm) 3 hours/day, 6 days/week for 7–8.5 months. The animals were immunized twice, at 1.5–2 and 4 months, subcutaneously with a 1.2 and 1.5 billion microbe dose of typhoid vaccine in an attempt to reveal changes in the immunological reactivity following 1,1,2,2-tetrachloroethane exposures. The exposed group contained six animals, and the size of the control group was not specified. In comparison with both initial and control levels, serum acetylcholine levels were decreased after 1.5 months, significantly increased after 4.5 months, and significantly decreased at the end of the study. The concentration of acetylcholine in the blood was increased following the first immunization. No changes in serum acetylcholinesterase activity were reported, although serum butyrylcholinesterase activity was reduced after 5–6 months of exposure. This is a poorly reported study that did not examine any other relevant endpoints. A NOAEL or LOAEL could not be identified because the changes in acetylcholine levels were inconsistent across time and incompletely quantified, and the biological significance of the change is unclear.

4.2.2.2. *Chronic Studies*

In a chronic inhalation study by Schmidt et al. (1972), groups of 105 male rats were exposed to 0 or 0.0133 mg/L (13.3 mg/m^3) 1,1,2,2-tetrachloroethane for 4 hours daily for up to 265 days. Subgroups of seven treated and seven control rats were killed after 110 or 265 days of exposure and 60 days after exposure termination, with the remaining animals observed until natural death. There were no significant alterations in survival. Weight gain in exposed rats was 2.1, 11.6, and 12.2% less than controls on study days 110, 260, and 324, respectively, although the only statistically significant decreases in body weight gain occurred between days 90 and 170. Other statistically significant changes compared to controls included increased leukocyte (89%) and β_1-globulin (12%) levels after 110 days, and an increased percentage of segmented nucleated neutrophils (36%), decreased percentage of lymphocytes (17%), and increased percentage of liver total fat content (34%) after 265 days. There was a statistically significant decrease in γ-globulin levels (32%) at 60 days postexposure and a decrease in adrenal ascorbic acid content (a measure of pituitary adrenocorticotropic hormone [ACTH] activity) at all three time periods (64, 21, and 13%, respectively). This study is insufficient for identification of a NOAEL or LOAEL for systemic toxicity because the experimental design and results were poorly reported, and histological examinations were not conducted.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

Gulati et al. (1991a) exposed timed-pregnant CD Sprague-Dawley rats (8–9 animals/group) to diets containing 0, 0.045, 0.135, 0.27, 0.405, or 0.54% microencapsulated 1,1,2,2-tetrachloroethane from gestation days (GDs) 4 through 20. Based on body weight and food consumption data, the reported estimated doses of 1,1,2,2-tetrachloroethane were 0, 34, 98, 180, 278, or 330 mg/kg-day. Dams were sacrificed and litters were evaluated on GD 20. Evaluations included maternal body weight, feed consumption and clinical signs, uterine weight, and numbers of implantations, early and late resorptions, live fetuses, and dead fetuses. Necropsies were performed on the maternal animals, but fetuses were not examined for malformations.

All dams survived to study termination on GD 20. Compared to controls, maternal body weight was statistically significantly decreased 9, 11, 14, and 24% at 98, 108, 278, and 330 mg/kg-day, respectively, and demonstrated a dose-dependent and time-dependent decrease in all dose groups. However, an increase in maternal body weight on day 20, compared to body weight on day 4, was apparent for all dose groups. Daily food consumption was significantly decreased in all dose groups, and this may have contributed to the decreased body weights observed in the study. Four out of nine rats in the 278 mg/kg-day dose group had slightly rough fur beginning on GD 10, while rough fur was present in all animals in the 330 mg/kg-day dose group. No statistically significant changes were observed in the numbers of live fetuses/litter,

dead fetuses/litter, resorptions/litter, or implants/litter. One dam in the 98 mg/kg-day group and four of nine dams in the 330 mg/kg-day group completely resorbed their litters. At scheduled sacrifice, average fetal weights were statistically significantly decreased 3.9, 12.7, 10.5, and 20.6% in the 98, 180, 278, and 330 mg/kg-day dose groups, respectively (Table 4-17). Gravid uterine weight was statistically significantly reduced only in the 330 mg/kg-day animals. Small but statistically significant decreases were seen in maternal body weight and average fetal weight at ≥98 mg/kg-day. Using statistical significance and a 10% change as the criterion for an adverse change in maternal body weight, a NOAEL of 34 mg/kg-day and LOAEL of 98 mg/kg-day were selected for changes in maternal body weight. A NOAEL of 34 mg/kg-day and LOAEL of 98 mg/kg-day were selected for developmental toxicity based on the lowest dose that produced a statistically significant decrease in fetal body weight.

Table 4-17. Fetal body weight in CD Sprague-Dawley rats exposed to microencapsulated 1,1,2,2-tetrachloroethane on GDs 4–20

Dose (mg/kg-d)	n	Mean	SD	% change
0	9	2.28	0.12	–
34	8	2.17	0.11	4.8
98	8	2.19	0.08	3.9
180	9	1.99	0.15	12.7
278	9	2.04	0.42	10.5
330	5	1.81	0.26	20.6

Source: Gulati et al. (1991a).

Gulati et al. (1991b) exposed timed-pregnant Swiss CD-1 mice (n = 5–11) to diets containing 0, 0.5, 1, 1.5, 2, or 3% microencapsulated 1,1,2,2-tetrachloroethane from GDs 4 through 17. Based on body weight and food consumption data, the reported estimated doses of 1,1,2,2-tetrachloroethane were 0, 987, 2,120, 2,216, or 4,575 mg/kg-day; an average dose could not be calculated for the 3% group due to early mortality. Dams were sacrificed, and litters were evaluated on GD 17. Evaluations included maternal body weight, feed consumption and clinical signs, uterine weight, and numbers of implantations, early and late resorptions, live fetuses, and dead fetuses. Necropsies were performed on the maternal animals, but fetuses were not examined for malformations.

All animals (9/9) in the 3% group died prior to the end of the study. Mortality was 0/11, 0/9, 2/10, 4/5, and 5/7 in the 0, 987, 2,120, 2,216, or 4,575 mg/kg-day groups, respectively, and the mortality in the higher dose groups affected the statistical power of the study for those groups. Maternal body weights were statistically significantly decreased compared to controls at ≥2,120 mg/kg-day beginning on study day 9, although the day 17 data were not statistically significantly different from controls for any treatment group. Average daily feed consumption

was statistically significantly decreased in all treated groups except in the 987 mg/kg-day animals. Gross hepatic effects were reported in dams from all groups except the 987 mg/kg-day group and included pale or grey and/or enlarged livers and a prominent lobulated pattern. Complete litter resorption occurred in 1/11, 0/9, 2/8, 1/1, and 1/2 dams in the 0, 987, 2,120, 2,216, and 4,575 mg/kg-day groups, respectively. No changes in developmental endpoints were noted in the 987 or 2,120 mg/kg-day groups. The 2,120 and 4,575 mg/kg-day groups had too few litters, due to maternal toxicity, to permit statistical analysis of the findings. The high mortality in the exposed mice precluded the identification of a NOAEL or LOAEL for this study.

NTP (2004) conducted a 14-week study in which groups of 10 male and 10 female F344 rats were fed diets containing microencapsulated 1,1,2,2-tetrachloroethane at reported average daily doses of 0, 20, 40, 80, 170, or 320 mg/kg-day. The main part of this study is summarized in Section 4.2.1.1. Reproductive function (fertility) was not evaluated. Endpoints relevant to reproductive toxicity included histology (testis with epididymis and seminal vesicle, preputial gland, prostate gland, clitoral gland, ovary, and uterus) and weights (left cauda epididymis, left epididymis, and left testis) of selected reproductive tissues in all control and treated groups. Sperm evaluations and vaginal cytology evaluations were performed in animals in the 0, 40, 80, and 170 mg/kg-day dose groups. The sperm evaluations consisted of spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The vaginal cytology evaluations consisted of measures of estrous cycle length.

Sperm motility was 17.1, 14.9, and 24.0% lower than in vehicle controls at 40, 80, and 170 mg/kg-day, respectively. Other statistically significant effects in the males included reductions in absolute epididymis weight at ≥80 mg/kg-day and absolute left cauda epididymis weight at 170 mg/kg-day, and statistically significant increases in the incidences (90–100%) of minimal to moderate atrophy of the preputial and prostate gland, seminal vesicle, and testicular germinal epithelium at 320 mg/kg-day. Effects in the females included statistically significant increases in incidences of minimal to mild uterine atrophy (70–90%) at ≥170 mg/kg-day and clitoral gland atrophy (70%) and ovarian interstitial cell cytoplasmic alterations (100%) at 320 mg/kg-day. The vaginal cytology evaluations indicated that the females in the 170 mg/kg-day group spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the vehicle controls. Body weight loss and reduced body weight gain at the lower dose levels may have contributed to the atrophy and other effects observed in both genders (NTP, 2004).

NTP (2004) also tested groups of 10 male and 10 female B6C3F_1 mice that were similarly exposed to 1,1,2,2-tetrachloroethane for 14 weeks at reported average daily dietary doses of 0, 100, 200, 370, 700, or 1,360 mg/kg-day (males) or 0, 80, 160, 300, 600, or 1,400 mg/kg-day (females). The main part of this study is summarized in Section 4.2.1.1. Reproductive function (fertility) was not evaluated, and toxicity endpoints in reproductive organs are the same as those evaluated in the rat part of the study summarized above. The sperm and

vaginal cytology evaluations were performed in the 200-, 700- and 1,360-mg/kg-day male groups and the 160-, 600- and 1,400-mg/kg-day female groups.

Effects observed in the male mice included statistically significant increases in the incidence of preputial gland atrophy at 100, 700, and 1,360 mg/kg-day (incidences in the control to high dose groups were 0/10, 4/10, 2/10, 0/10, 4/10, and 5/10, respectively), decreased absolute testis weight at ≥700 mg/kg-day and absolute epididymis and cauda epididymis weights at 1,360 mg/kg-day, and decreased epididymal spermatozoal motility at 1,360 mg/kg-day (3.1% less than vehicle controls). In female mice, the length of the estrous cycle was significantly increased at 1,400 mg/kg-day (8.7% longer than vehicle controls). The pronounced decreases in body weight gain or body weight loss were similar to those observed in rats.

4.3.2. Inhalation Exposure

Male rats were exposed to 0 or 15 mg/m^3 (2.2 ppm) 1,1,2,2-tetrachloroethane 4 hours/day for up to 8 days in a 10-day period (Gohlke and Schmidt, 1972; Schmidt et al., 1972). Reproductive function was not tested, but evaluations included histological examinations of the testes in groups of seven control and seven treated males following the second, fourth, and eighth exposures, as detailed in Schmidt et al. (1972) in Section 4.2.2.2. This study is limited by imprecise and incomplete reporting of results. It was noted that testicular histopathology, described as atrophy of the seminal tubules with strongly restricted or absent spermatogenesis, was observed in five exposed animals following the fourth exposure; data for the other time periods and the control group were not reported.

The Schmidt et al. (1972) chronic inhalation study, summarized in Section 4.2.2.2, included a limited reproductive function/developmental toxicity assessment. Male rats were exposed to 0 or 13.3 mg/m^3 (1.9 ppm) 1,1,2,2-tetrachloroethane 4 hours/day for 265 days, as well as during the mating period. One week before the end of the exposure period, seven control and seven exposed males were each mated with five unexposed virgin females. Dams were permitted to deliver and the offspring were observed for 84 days and were examined macroscopically for malformations. The percentage of mated females having offspring, littering interval, time to 50% littered, total number of pups, pups/litter, average birth weight, postnatal survival on days 1, 2, 7, 14, 21, and 84, sex ratio, and average body weight on postnatal day 84 were also measured. No macroscopic malformations or significant group differences in the other indices were found, indicating that 13.3 mg/m^3 was a NOAEL for male reproductive toxicity.

No effects attributable to 1,1,2,2-tetrachloroethane were reported in rats exposed to 5 or 50 ppm (34.3 or 343 mg/m^3, respectively) 7 hours/day for 5 days in a dominant lethal test (McGregor, 1980). A viral infection may have resulted in increased numbers of early deaths in all groups, including the control group, possibly affecting study sensitivity. The frequency of sperm with hook abnormalities was statistically significantly increased in the 343 mg/m^3 group, but not at 34.3 mg/m^3.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute Studies (Oral and Inhalation)

4.4.1.1. *Oral Studies*

Oral (single-dose gavage) median lethal dose (LD_{50}) values of 250–800 mg/kg have been reported in rats (NTP, 2004; Schmidt et al., 1980b; Gohlke et al., 1977; Smyth et al., 1969). Cottalasso et al. (1998) described a series of experiments evaluating the effect of a single gavage dose of 1,1,2,2-tetrachloroethane on the liver of exposed rats. In the first experiment, male Sprague-Dawley rats (5/group) were given a single gavage dose of 0, 143.5, 287, 574, or 1,148 mg/kg in mineral oil and five animals from each group were sacrificed 5, 15, 30, or 60 minutes later. Sixty minutes after treatment, statistically significant, dose-related increases in serum activity levels of AST (66, 129, and 201%, respectively) and ALT (54, 88, and 146%, respectively) were observed at ≥287 mg/kg. The increase in rat serum activities of AST and ALT were also increased in a time-dependent manner. Serum AST increased 13–130% from 5 to 60 minutes in rats at 574 mg/kg-day and serum ALT increased 8–88% from 5 to 60 minutes. A statistically significant decrease in hepatic microsomal G6Pase activity (19, 36, and 47%, respectively) was observed at ≥287 mg/kg. A statistically significant decrease in levels of dolichol, a polyisoprenoid compound believed to be important in protein glycosylation reactions, in the liver (41 and 56%, respectively) and a statistically significant increase in triglyceride levels in liver homogenate (60 and 83%, respectively) were observed at ≥574 mg/kg. A statistically significant increase in the trigylceride levels in liver microsomes (46, 65, and 97%, respectively) was observed at ≥287 mg/kg. See Table 4-18 for a summary of these acute liver toxicity results. A time-dependent effect was observed in the decrease in G6Pase, in the increase in triglyceride levels, and in the decrease in levels of dolichol in the liver at 574 mg/kg from 5 to 60 minutes.

Table 4-18. Liver function and other effects observed in Sprague-Dawley rats 60 minutes after gavage exposure to 1,1,2,2-tetrachloroethane

Dose (mg/kg)	Serum AST (IU/L)	Serum ALT (IU/L)	Microsomal G6Pase (nmol/min/mg protein)	Homogenate triglycerides (mg/g liver)	Microsomal triglycerides (mg/g liver)	Homogenate total dolichol levels (ng/mg protein)
0	62 ± 9	26 ± 4	361 ± 29	14.5 ± 2.0	1.61 ± 0.12	335 ± 0.28
143.5	80 ± 10	32 ± 6	342 ± 43	15.9 ± 2.3	1.95 ± 0.21	302 ± 53
287	103 ± 21[a]	40 ± 7[a]	291 ± 39[a]	19.7 ± 3.2	2.35 ± 0.30[a]	268 ± 45
574	143 ± 13[a]	49 ± 6[a]	230 ± 18[a]	23.2 ± 2.8[a]	2.65 ± 0.35[a]	197 ± 25[a]
1,148	187 ± 24[a]	64 ± 9[a]	191 ± 31[a]	26.5 ± 3.4[a]	3.17 ± 0.42[a]	147 ± 21[a]

[a]Significantly different from control.

Source: Cottalasso et al. (1998).

Schmidt et al. (1980b) administered 0 or 100 mg/kg doses of 1,1,2,2-tetrachloroethane in corn oil by gavage to groups of 10 male Wistar rats, followed immediately by increased environmental temperatures, and evaluated hepatic effects 20–22 hours post administration. Statistically significant increases in serum leucine aminopeptidase activity, hepatic ascorbic acid, and hepatic triglyceride levels (10.5, 22.3, and 125% greater than control levels, respectively) were observed, but changes in body weight, liver weight, hepatic N-demethylation of aminopyrine, and serum ALT activity were not observed. The report includes a general statement that all chemicals tested in this study led to necrosis and fatty degeneration, which suggests that 100 mg/kg was a hepatotoxic dose of 1,1,2,2-tetrachloroethane. However, the significance of the histology results cannot be assessed due to a lack of incidence and severity measures. No other 1,1,2,2-tetrachloroethane-related histological data were reported in this study.

Wolff (1978) exposed 8–10-week-old female Wistar rats in groups of 8–10, to a single gavage dose of 0, 25, or 50 mg/kg 1,1,2,2-tetrachloroethane 30 minutes prior to testing for passive avoidance (shock level of 0.4 milliamperes [mA]). Passive avoidance was measured by allowing the test rats to explore the test apparatus, which consisted of a larger, lit box and a smaller, dark box. After 180 seconds, the darkened box received an electrical shock through the grid floor. During the 180 seconds, the rats remained in the darkened box approximately 80% of the time. The test was repeated 24 hours later. No differences in avoidance were observed between the control and 25 mg/kg groups, but decreased passive avoidance behavior was reported following exposure to 50 mg/kg. In the second test series, the shock level was increased to 0.8 mA and the 1,1,2,2-tetrachloroethane dose was increased to 50 mg/kg. The 1,1,2,2-tetrachloroethane doses were then increased to 80 mg/kg and then to 100 mg/kg. Increasing the shock level to 0.8 mA resulted in no significant differences in avoidance between the controls and the 50 mg/kg dose group (n = 10). Passive avoidance was altered at 80 mg/kg (n = 10), and at 100 mg/kg, the animals (n = 10) were ataxic and did not learn to avoid the shock. The authors stated that the treatment with 1,1,2,2-tetrachloroethane may have affected the threshold of perception of the shock, rather than memory (Wolff, 1978). This conclusion would be consistent with the high-dose anesthetic effects characteristic of volatile organic compounds in general.

4.4.1.2. *Inhalation Studies*

Schmidt et al. (1980a) established a 24-hour median lethal concentration (LC_{50}) of 8,600 mg/m^3 (1,256 ppm) for 1,1,2,2-tetrachloroethane in rats for a single 4-hour exposure. Carpenter et al. (1949) found that a 4-hour exposure to 1,000 ppm 1,1,2,2-tetrachloroethane (6,870 mg/m^3) was lethal in Sherman rats, with mortality in "2/6, 3/6, or 4/6" animals.

Price et al. (1978) exposed rats and guinea pigs to 576, 5,050, and 6,310 ppm 1,1,2,2-tetrachloroethane for 30 minutes. Rats exposed to 576 ppm (3,950 mg/m^3) for 30 minutes showed a slight reduction in activity and alertness, while increasing the concentration

to 5,050 or 6,310 ppm (34,700 or 43,350 mg/m^3) caused lacrimation, ataxia, narcosis, labored respiration, and 30–50% mortality (Price et al., 1978). Eye closure, squinting, lacrimation, and decreased activity were observed in guinea pigs exposed to 576 ppm for 30 minutes; exposure to 5,050 ppm resulted in tremors, narcosis, and labored breathing, and exposure to 6,310 ppm produced 30% mortality (Price et al., 1978). Organ weight measurements and gross pathology and histology evaluations performed 14 days following the 30-minute exposures did not result in chemical-related effects in the lungs, liver, kidneys, heart, brain, adrenals, testes, epididymides, ovaries, or uterus in either species.

Pantelitsch (1933) exposed groups of three mice to 1,1,2,2-tetrachloroethane concentrations of 7,000, 8,000–10,000, 17,000, 29,000, or 34,000 mg/m^3 (1,022, 1,168–1,460, 3,060, 5,220, or 6,120 ppm, respectively) for approximately 1.5–2 hours and examined changes in clinical status of the animals. All concentrations resulted in disturbed equilibrium, prostration, and loss of reflexes, with deaths occurring at ≥8,000–10,000 mg/m^3; increasing the concentration resulted in a more rapid onset of symptoms.

Horvath and Frantik (1973) determined that effective concentrations of 1,1,2,2-tetrachloroethane following a single 6-hour exposure in rats were 360 ppm (2,470 mg/m^3) for a 50% decrease in spontaneous motor activity and 200 ppm (1,370 mg/m^3) for a 50% increase in pentobarbital sleep time. No additional relevant information was reported.

Schmidt et al. (1980a) exposed groups of 10 male Wistar rats to 0, 410, 700, 1,030, 2,100, or 4,200 mg/m^3 (0, 60, 102, 150, 307, or 613 ppm, respectively) 1,1,2,2-tetrachloroethane (mean concentrations) for 4 hours and evaluated the animals immediately (within 15–100 minutes), at 24 hours, or at 120 hours following exposure. The purpose of this study was to determine a threshold concentration for effects on the liver following inhalation exposure. Evaluation of this study is complicated by imprecise and incomplete reporting of results, exposure levels, and observation durations. For example, results for endpoints other than liver histology, ascorbic acid content, and histochemistry were not reported for the lowest concentration (410 mg/m^3), and liver ascorbic acid content and serum and liver triglyceride levels were the only results reported quantitatively. Histological effects included diffuse fine droplet fatty degeneration in the liver at 410 and 700 mg/m^3 (24 hours postexposure), nonspecific inflammation and Councilman bodies (eosinophilic globules derived from necrosis of single hepatocytes) in the liver at 4,200 mg/m^3 (24 hours postexposure), and interstitial nephritis in the kidneys at 700 mg/m^3 (120 hours postexposure). Additional information on these findings, including incidences and results for other exposure concentrations, was not reported.

Hepatic ascorbic acid levels were statistically significantly increased in groups exposed to ≥700 mg/m^3 immediately after exposure (2, 64, 29, 167, and 182% higher than controls at 410, 700, 1,030, 2,100, and 4,200 mg/m^3, respectively), but returned to control levels within 24 hours. Serum triglyceride concentrations were statistically significantly decreased at ≥700 mg/m^3 after 24 hours (35, 23, 29, and 56% at 700, 1,030, 2,100, and 4,200 mg/m^3,

respectively) and at 2,100 and 4,200 mg/m^3 (39 and 42%, respectively) after 120 hours. Hepatic triglyceride levels were significantly increased at 2,100 and 4,200 mg/m^3 (92 and 76%, respectively) at 24 hours postexposure. Hexobarbital sleep time was increased at 2,100 and 4,200 mg/m^3 (not quantified). Assessing the biological significance and adversity of the effects in this study is complicated by factors that include the lack of liver lesion incidence data, the paucity of other quantitative data, and other reporting insufficiencies. The authors concluded that the threshold for effects on the liver was between 410 and 700 mg/m^3 because the fine droplet fatty degeneration was not considered to be biologically significant in the absence of accompanying serum and liver biochemical changes.

Hepatic effects were also reported by Tomokuni (1969), who administered a single 3-hour exposure of 600 ppm (4,120 mg/m^3) 1,1,2,2-tetrachloroethane to female Cb mice. Total hepatic lipids and triglycerides were statistically significantly increased following exposure and continued to increase for 8 hours postexposure. Hepatic triglyceride levels increased more than total lipid levels for 8 hours postexposure. Total hepatic adenosine triphosphate (ATP) levels were decreased immediately following exposure and continued to decrease over the next 8 hours. A later study by the same investigator (Tomokuni, 1970) evaluated female Cb mice (5–8/group) exposed to 800 ppm (5,490 mg/m^3) 1,1,2,2-tetrachloroethane for 3 hours and then followed the time-course of the changes in hepatic lipids and phospholipids over the next 90 hours. Increased triglyceride and decreased phospholipid levels were seen for the first 30–45 hours postexposure, but the effects generally resolved by 90 hours postexposure, demonstrating that hepatic effects resolved after exposure was terminated.

Horiuchi et al. (1962) exposed 10 male mice for a single 3-hour period to an atmosphere containing 5,900 ppm (~40,500 mg/m^3) or 6,600 ppm (~45,300 mg/m^3) 1,1,2,2-tetrachloroethane and then observed the animals for 1 week following exposure. Tissues were obtained for histologic evaluation from animals at sacrifice or when discovered dead. Three mice exposed to 5,900 ppm and four mice exposed to 6,600 ppm died prior to those sacrificed at the end of the study. The histological results reported by Horiuchi et al. (1962) are similar to the repeated vapor exposure study in mice, described in Section 4.4.2.2, where slight to moderate congestion and fatty degeneration of the liver and congestion of the other male tissues were observed.

Deguchi (1972) administered a single 6-hour exposure of 0, 10, 100, or 1,000 ppm (0, 69, 690, or 6,900 mg/m^3, respectively) 1,1,2,2-tetrachloroethane to male rats and evaluated serum AST and ALT activities up to 72 hours postexposure. This study was reported in Japanese and included an English translation of the abstract. Based on information in the English abstract and data graphs in this Japanese study, there was a minimal increase in serum AST at all exposure concentrations 72 hours postexposure.

4.4.2. Short-term Studies (Oral and Inhalation)

4.4.2.1. *Oral Studies*

Dow Chemical Company (1988) exposed groups of male Osborne-Mendel rats (n = 5) to daily gavage doses of 0, 25, 75, 150, or 300 mg/kg-day 1,1,2,2-tetrachloroethane every 24 hours for 4 days, followed by an injection of [^{3}H]-thymidine for DNA incorporation studies 24 hours following the last 1,1,2,2-tetrachloroethane dose. The fourth dose was not administered to the 300 mg/kg-day group due to signs of central nervous system (CNS) depression and debilitation, and one animal in this group died before [^{3}H]-thymidine injection. Terminal body weights of the 300 mg/kg-day animals were statistically significantly decreased, 17% compared to controls. Absolute liver weights at the highest dose were decreased and relative liver weights were statistically significantly increased 14% in the 150 mg/kg-day dose group.

Histological examinations of the livers showed increased numbers of hepatocytes in mitosis in the 75, 150, and 300 mg/kg-day groups, although this response was variable in high-dose rats, possibly due to the increased toxicity observed in this group (Dow Chemical Company, 1988). Increased numbers of reticuloendothelial cells were seen at 300 mg/kg-day. Increased hepatic glycogen content was found in hepatocytes of 75 and 150 mg/kg-day animals, although this could be an outcome of altered feeding patterns resulting from sedative effects of dosing (Dow Chemical Company, 1988).

Hepatic DNA synthesis ([^{3}H]-thymidine incorporation) was increased 2.8-, 4.8-, and 2.5-fold at 75, 150, and 300 mg/kg-day, respectively; the decline at 300 mg/kg-day may have been due to the poor clinical status of the rats in this group (Dow Chemical Company, 1988). Total hepatic DNA content was not increased. Other endpoints were not evaluated. The 300 mg/kg-day dose is a frank effect level (FEL) based on the CNS depression and mortality. The 75 mg/kg-day dose may represent a NOAEL for increased relative liver weight in rats. However, the increase in DNA synthesis and mitosis are not necessarily indicative of hepatotoxicity, and the histological examinations showed no accompanying degeneration or other adverse liver lesions.

Dow Chemical Company (1988) similarly exposed groups of male B6C3F$_1$ mice (n = 5) to daily gavage doses of 0, 25, 75, 150, or 300 mg/kg-day 1,1,2,2-tetrachloroethane for 4 days, followed by [^{3}H]-thymidine injection for the DNA incorporation studies. All animals survived treatment, and changes in body weight were not observed at any dose level. Absolute and relative liver weights were increased 13 and 11%, respectively, at 150 mg/kg-day and 19 and 72%, respectively, at 300 mg/kg-day, although only the increase in relative liver weight at 300 mg/kg-day was statistically significant.

Histopathologic examination of the liver revealed centrilobular swelling with a corresponding decrease in hepatocyte size in the periportal region due to decreased glycogen content in mice at ≥75 mg/kg-day. Increased hepatocyte mitosis was also observed in mice at 300 mg/kg-day. Hepatic DNA synthesis was increased 1.7-fold at 150 mg/kg-day and 4.4-fold at

300 mg/kg-day, although total hepatic DNA content was not increased. Other endpoints were not evaluated.

TSI Mason Laboratories (1993a, unpublished) administered 1,1,2,2-tetrachloroethane in corn oil to groups of male and female (n = 5) F344/N rats at 0, 135, 270, or 540 mg/kg-day for 12 days over a 16-day period. Rats were weighed prior to dosing, after 7 days, and prior to euthanasia, and all surviving rats were euthanized and subject to necropsy. Study endpoints included clinical observations, body weight, necropsy, selected organ weights (liver, kidneys, thymus, lung, heart, and testes), and histology of gross lesions. All of the high-dose rats died by day 5 of the study. Male rats exposed to 270 mg/kg-day displayed an increase in body weight of 37% from day 1 through day 17, compared to an increase of 64% in controls. Female rats exposed to 270 mg/kg-day displayed a decrease in body weight of 3% from day 1 through day 17, compared to an increase of 30% in controls. The automatic watering system for the low- and high-dose males failed prior to the administration of 1,1,2,2-tetrachloroethane, and the low and high doses of the study were repeated in a subsequent study by TSI Mason Laboratories (1993b, unpublished).

Clinical signs were absent in the 135 mg/kg-day animals, but animals exposed to 270 or 540 mg/kg-day were lethargic following treatment. Absolute liver weights were statistically significantly increased (19%) in the 135 mg/kg-day female rats, while relative liver weights were statistically significantly increased at both 135 and 270 mg/kg-day (16 and 34%, respectively). No changes in absolute or relative liver weights were seen in exposed male rats. Absolute right kidney weight was significantly increased 9 and 37% in females at 135 and 270 mg/kg-day, respectively. Absolute thymus weight was statistically significantly decreased in the mid-dose group of male rats (33% at 270 mg/kg-day), while absolute (45%) and relative (32%) thymus weights were statistically significantly decreased in only the mid-dose females. Relative right testis weight was statistically significantly increased (10% at 270 mg/kg-day) in male rats. Absolute but not relative lung weights were statistically significantly decreased in 270 mg/kg-day females (17%), while relative heart weights were statistically significantly increased (14%) in females.

Gross and microscopic lesions were observed in the liver (i.e., hepatodiaphragmatic nodules) of one control, one mid-dose, and one high-dose rat, but these were common spontaneous lesions.

In another study, TSI Mason Laboratories (1993b, unpublished) exposed groups of male F344/N rats (n = 5) to 0, 135, 270, or 540 mg/kg-day 1,1,2,2-tetrachloroethane by gavage in corn oil for 12 days in a 16-day period. Study endpoints included clinical observations, body weight, necropsy, selected organ weights (liver, kidneys, thymus, lung, heart, and testes), and histology of gross lesions. All animals exposed to 540 mg/kg-day died by day 3 of the study. Rats in the 270 and 540 mg/kg-day groups were extremely lethargic following administration of the test article, with recovery observed only in the 270 mg/kg-day rats.

The weight gain observed in the low- and mid-dose rats was 55.2 and 28%, respectively. At 135 mg/kg, statistically significant increases of 17 and 13% in absolute and relative liver weights, respectively, were observed compared to controls. In the mid-dose group, statistically significant decreases in absolute testes weight (7%), absolute kidney weight (9%), absolute and relative heart weight (10 and 6%, respectively), and absolute and relative thymus weight (33 and 21%, respectively) were observed. Statistically significant increases in relative thymus (10%), liver (16%), and kidney weights (7%) were observed at 270 mg/kg-day compared to controls.

Gross and microscopic lesions were observed in the liver of one 270 mg/kg-day male and in the glandular stomach of one 540 mg/kg-day male, but these were diagnosed as spontaneous lesions commonly observed in F344/N rats. The lesion observed in the liver was a dark nodule on the median lobe and corresponded histomorphologically to a hepatodiaphragmatic nodule, and the lesion observed in the glandular stomach was a pale foci.

TSI Mason Laboratories (1993c, unpublished) exposed groups of five male and five female B6C3F_1 mice to 0, 337.5, 675, or 1,350 mg/kg-day 1,1,2,2-tetrachloroethane by gavage in corn oil for 12 days during a 16-day period. Study endpoints included clinical observations, body weight, necropsy, selected organ weights (liver, kidneys, thymus, lung, heart, and testes), and histology of gross lesions. All mice of both genders in the 1,350 mg/kg-day groups were found dead or euthanized by day 3 of the study. Additionally, one 675 mg/kg-day female died and one 337.5 mg/kg-day female was euthanized prior to the end of the study.

No significant changes in body weight were reported in treated groups. Animals in the 675 and 1,350 mg/kg-day groups appeared lethargic within 15 minutes of dosing, and the 1,350 mg/kg-day mice failed to recover after the third treatment. Lethargy also occurred in the 337.5 mg/kg-day female that was sacrificed, but not in other animals in the same exposure group. In male mice, relative liver weight was statistically significantly increased 9% at 337.5 mg/kg, and absolute and relative liver weights were statistically significantly increased 28 and 37%, respectively, at 675 mg/kg-day. In female mice, absolute and relative liver weights were statistically significantly increased by 50 and 42%, respectively, at 675 mg/kg-day.

Gross hepatic changes, described as pale livers, were noted in one male and three females at 337.5 mg/kg-day and in four males and three females at 675 mg/kg-day. Histological examination of the gross lesions showed that they correlated with centrilobular hepatocellular degeneration characterized by hepatocellular swelling, cytoplasmic rarefaction, and hepatocellular necrosis in the 675 and 1,350 mg/kg-day males and the 337.5, 675, and 1,350 mg/kg-day females. Hepatocellular necrosis was the most common lesion observed at 675 mg/kg-day.

In a study examining the potential renal toxicity of orally administered halogenated ethanes, groups of five male F344/N rats received 0, 0.62, or 1.24 mmol/kg-day 1,1,2,2-tetra-chloroethane by gavage in corn oil (0, 104, or 208 mg/kg-day, respectively) for 21 consecutive days (NTP, 1996). All rats in the high-dose group died or were killed moribund on days 13–14

and were not evaluated further. Evaluations of the 0 and 104 mg/kg-day animals included weekly body weights, end-of-study urinalysis (volume, specific gravity, creatinine, glucose, total protein, AST, γ-glutamyl transpeptidase, and N-acetyl-β-D-glucosaminidase), gross necropsy, selected organ weights (right kidney, liver, and right testis), selected histopathology (right kidney, left liver lobe, and gross lesions), and kidney cell proliferation analysis (proliferating cell nuclear antigen [PCNA] labeling index for proximal and distal tubule epithelial cells in S phase). Clinical signs in the high-dose animals included thinness and lethargy (5/5 rats), diarrhea, abnormal breathing, and ruffled fur (3/5 rats). In the low-dose group, no effects on survival, body weight gain, urinalysis parameters, absolute or relative kidney weights, renal or testicular histopathology, or kidney cell PCNA labeling index were observed.

Hepatic effects in the low-dose group included increased absolute and relative liver weights (24 and 29% greater than controls, respectively) and cytoplasmic vacuolization of hepatocytes. The vacuolation occurred in hepatocytes of all low-dose rats and consisted of multifocal areas with clear droplets within the cytoplasm. Changes in the kidneys of the male rats were not observed.

In a range-finding study, the NTP (NTP, 2004; TSI Mason Laboratories, 1993d) exposed male and female F344/N rats (5/sex/group) to 0, 3,325, 6,650, 13,300, 26,600, or 53,200 ppm 1,1,2,2-tetrachloroethane in the diet (microcapsules) for 15 days. Unexposed and vehicle control groups were also evaluated, with the latter being given feed with empty microcapsules. Study endpoints included clinical observations, body weight, food consumption, necropsy, selected organ weights (liver, kidneys, thymus, lung, heart, and testes), and histology of gross lesions; histology was not evaluated in animals without gross lesions. The study authors reported that average daily doses for the three lowest concentrations were 300, 400, or 500 mg/kg-day for both genders. All rats exposed to 26,600 or 53,200 ppm were killed moribund on day 11. The average daily doses for these groups were not reported.

Female rats exposed to 400 mg/kg-day and both genders exposed to 500 mg/kg-day were thin and displayed ruffled fur. Body weight at study termination was statistically significantly lower than controls in both genders of all treated groups. Male rats exposed to 300 mg/kg-day showed decreased weight gain compared to controls and those exposed to higher doses lost weight, with final body weights in male rats 28, 46, and 53% less than vehicle controls at 300, 400, and 500 mg/kg-day, respectively. Females lost weight at doses of ≥300 mg/kg-day, with final body weights in female rats 25, 38, and 47% less than vehicle controls at 300, 400, and 500 mg/kg-day, respectively. Decreased feed consumption likely contributed to the decreased weight gains because consumption was reduced in a dose-related manner in both genders of all treated groups (NTP, 1996).

Absolute thymus weights were decreased 24, 69, and 84% in male rats and 37, 61, and 81% in female rats at doses of ≥300 mg/kg-day, and relative thymus weights were decreased 42 and 65% in male rats and 38 and 65% in female rats at ≥400 mg/kg-day (NTP, 2004; TSI

Mason Laboratories, 1993d). In male rats, absolute liver weights were decreased 22, 49, and 60% compared to controls at 300, 400, and 500 mg/kg-day, respectively. Relative liver weight was increased 7% compared to controls at 300 mg/kg-day and decreased 14% compared to controls at 500 mg/kg-day. In female rats, absolute liver weight was decreased 25 and 34% compared to controls at 400 and 500 mg/kg-day, respectively, and relative liver weight was increased 34 and 23% compared to controls at 300 and 500 mg/kg-day, respectively. Relative kidney weights were increased 14, 26, and 18% in male rats at 300, 400, and 500 mg/kg-day, respectively, and 17 and 36% in female rats at 400 and 500 mg/kg-day, respectively. Absolute kidney weights were decreased 17, 32, and 45% in males and 16, 27, and 27% in females at 300, 400, and 500 mg/kg-day, respectively. Other organ weight decreases were considered a reflection of the decreased body weights.

Focal areas of alopecia occurred on the skin of four female rats in the 500 mg/kg-day group, and these lesions correlated with minimal to moderate acanthosis, which is an abnormal, benign increase in the thickness of the stratum spinosum of the epidermis, a layer of cells that is capable of undergoing mitotic cell division. In the liver, mild or moderate centrilobular degeneration was observed microscopically in the exposed male and female rats.

Groups of five male and five female B6C3F$_1$ mice were exposed to 0, 3,325, 6,650, 13,300, 26,600, or 53,200 ppm of encapsulated 1,1,2,2-tetrachloroethane in the diet for 15 days (NTP, 2004; TSI Mason Laboratories, 1993d). Organ weights, gross necropsy, and histology of gross lesions were evaluated in surviving mice at the termination of the study. Average daily doses were not determined by the study authors because feed consumption could not be measured accurately due to excessive scattering of feed. All male and female mice exposed to 53,200 ppm, all males exposed to 26,600 ppm, and two males exposed to 13,300 ppm were sacrificed in extremis before the end of the study. Final body weights were decreased 16, 24, and 22%, in comparison to vehicle controls, in males at 3,325, 6,650, and 13,300 ppm, respectively. In females, final body weights were decreased 9, 20, 31, and 34% at 3,325, 6,650, 13,300, and 26,600 ppm, respectively.

Clinical findings included hyperactivity in males and females exposed to 3,325, 6,650, or 13,300 ppm and in females in the 26,600 ppm group. Males in the 26,600 and 53,200 ppm groups were lethargic. Males exposed to ≥6,650 ppm and females exposed to 26,600 and 53,200 ppm were thin and had ruffled fur. A statistically significant decrease in absolute (31, 47, 82, and 81%, respectively) and relative (22, 33, 74, and 72%, respectively) thymus weights compared to controls was observed in all exposed female mice. Relative liver weights were statistically significantly increased 22, 31, and 34% in male mice at 3,325, 6,650, and 13,300 ppm, respectively. Absolute liver weights were statistically significantly decreased 11, 9, and 5% in female mice at 6,650, 13,300, and 26,600 ppm, respectively, and relative liver weight increased 30 and 44% at 13,300 and 26,600 ppm, respectively. Other organ weight changes were associated with changes in body weight. Pale or mottled livers were noted in all exposed

groups of male and female mice and correlated microscopically with hepatocellular degeneration, which was characterized by hepatocellular swelling, cytoplasmic rarefaction, single paranuclear vacuoles, hepatocellular necrosis, and infrequent mononuclear infiltrates. The severity of the hepatic changes increased with increasing exposure concentration.

The histological examinations in the surviving mice showed hepatocellular degeneration in 3/3, 4/4, 4/4, 1/1, and 1/1 males, and 4/4, 4/4, 3/3, 3/3, and 3/3 females, at 3,325, 6,650, 13,300, 26,600, and 53,200 ppm, respectively (TSI Mason Laboratories, 1993d). For both genders, the lesions tended to be minimal to mild at 3,325 and 6,650 ppm, with more moderate to marked severity observed at the higher doses.

NCI (1978) conducted a range-finding study in rats and mice in order to estimate the maximum tolerated dose for administration in the chronic bioassay. In this study, Osborne-Mendel rats (5/sex/group) received gavage doses of 0 (vehicle control group), 56, 100, 178, 316, or 562 mg/kg-day 1,1,2,2-tetrachloroethane in corn oil 5 days/week for 6 weeks, followed by a 2-week observation period. $B6C3F_1$ mice (5/sex/group) were similarly exposed to 0, 32, 56, 100, 178, or 316 mg/kg-day 1,1,2,2-tetrachloroethane. It appears that mortality and body weight gain were the only endpoints used to assess toxicity and determine the high-dose levels for the NCI (1978) chronic bioassays in rats and mice. In the rats, one male exposed to 100 mg/kg-day and all five females exposed to 316 mg/kg-day died (mortality rates in the 562 mg/kg-day groups were not reported). Body weight gain was reduced 3, 9, and 38% in male rats and 9, 24, and 41% in female rats at 56, 100, and 178 mg/kg-day, respectively. No deaths or significant alterations in body weight gain were observed in the mice. In male rats, 100 and 178 mg/kg-day, were selected as the NOAEL and LOAEL, respectively, for the observed decrease in body weight, while in female rats the NOAEL and LOAEL were 56 and 100 mg/kg-day, respectively, for the same endpoint. The highest dose in mice, 316 mg/kg-day, was selected as the NOAEL for body weight changes and mortality.

4.4.2.2. *Short-term Inhalation Studies*

Rats (n = 84) were exposed to 0 or 15 mg/m^3 (2.2 ppm) 1,1,2,2-tetrachloroethane 4 hours/day for up to 8 days in a 10-day period (Gohlke and Schmidt, 1972; Schmidt et al., 1972). Following the first, third, and seventh exposures, seven control and exposed rats were given an unknown amount of ethanol. Evaluations were performed on seven males from the control and treated groups, with and without ethanol, following the second, fourth, and eighth exposures.

Statistically significant changes included increased serum total protein and decreased serum α_1- and α_2-globulin fractions compared to controls after the eighth exposure (day 10), although the difference was not quantified (Schmidt et al., 1972). Histological effects included a fine to medium droplet fatty degeneration of the liver that involved increasing numbers of animals with increasing duration of exposure, although the incidences and severity were not

reported (Gohlke and Schmidt, 1972). The results of the serum and histochemical evaluations were illegible in the best copy of the translated reference available. Testicular atrophy in the seminal tubules was observed in five treated animals following the fourth exposure (Gohlke and Schmidt, 1972). This study is limited by imprecise and incomplete reporting of results. Assessment of the adversity of liver and other effects in this study is complicated by the reporting insufficiencies, particularly the paucity of incidence and other quantitative data, as well as effects that were not consistently observed in the three time periods and a lack of information on dose-response due to the use of a single exposure level.

Horiuchi et al. (1962) exposed nine male mice to an average concentration of approximately 7,000 ppm (48,000 mg/m^3) 1,1,2,2-tetrachloroethane for 2 hours once/week for a total of five exposures over 29 days. All animals died during the study with none of the deaths occurring during exposure, and most (5/9) of the mice died within 5 days of the first exposure. The only other reported findings in the exposed animals were slight to moderate congestion and fatty degeneration of the liver and congestion of "other main tissues."

Horiuchi et al. (1962) exposed six male rats to an average concentration of 9,000 ppm (62,000 mg/m^3) 1,1,2,2-tetrachloroethane 2 hours/day, 2–3 times a week for 11 exposures in 29 days. All rats died during the study. No changes in body weight were reported. Exposed animals generally showed hypermotility within the first few minutes of exposure, followed by atactic gait within approximately 20 minutes and eventual near-complete loss of consciousness 1–1.5 hours after the onset of exposure. Hematology was assessed in three rats that survived beyond 2 weeks, and two of these animals showed a decrease in RBC count and Hb content. Exposed animals generally showed moderate congestion and fatty degeneration of the liver and congestion of "other main tissues."

As discussed in Section 4.2.2.1, one monkey was exposed to varying concentrations (2,000–4,000 ppm for the first 20 exposures, 1,000–2,000 ppm for the 20th–160th exposure, and 3,000–4,000 ppm for the remaining exposures) of 1,1,2,2-tetrachloroethane for 2 hours/day, 6 days/week for 9 months (Horiuchi et al., 1962). Effects of short-term exposure included weakness after seven exposures, diarrhea and anorexia between the 12th and 15th exposures, and beginning at the 15th exposure, near-complete unconsciousness for 20–60 minutes after each exposure.

4.4.3. Acute Injection Studies

Paolini et al. (1992) exposed groups of male and female Swiss Albino mice to a single i.p. dose of 0, 300, or 600 mg/kg 1,1,2,2-tetrachloroethane and sacrificed the animals 24 hours after dosing to assess hepatotoxicity. An LD_{50} of 1,476 mg/kg for 1,1,2,2-tetrachloroethane was calculated using six animals/dose and eight dose groups. At 600 mg/kg, absolute and relative liver weights were statistically significantly decreased 16 and 37%, respectively, in female mice. No changes in total microsomal protein were noted. Statistically significant decreases (37–74%)

in hepatic CYP enzymes of numerous classes were reported at both dose levels in male and female mice (see Section 3.3). Other hepatic enzymes with statistically significantly decreased activity included NADPH-cytochrome c-reductase, δ-aminolevulinic acid-synthetase, ethoxyresorufin-O-deethylase, pentoxyresorufin O-dependentylase, GST (600 mg/kg-day only), and epoxide hydrolase. Total hepatic heme was reduced at both doses, and heme oxygenase activity was increased in a dose-related manner, but was statistically significant only in high-dose males and females.

Wolff (1978) exposed groups of female Wistar rats to a single i.p. dose of 0, 20, or 50 mg/kg 30 minutes prior to testing for passive avoidance of a 0.4 mA electric shock. No differences between the control and 25 mg/kg groups were reported, but doses of 50 mg/kg resulted in decreased passive avoidance behavior. Similarly, no differences were seen in the open-field test at any dose level. In male ICR-mice, a single i.p. dose of 20 mg/kg resulted in a significant reduction in spontaneous locomotor activity, and 50–60 mg/kg resulted in a 50% reduction (Wolff, 1978).

In an abstract, Andrews et al. (2002) described the exposure of a rat whole embryo culture system to 1,1,2,2-tetrachloroethane. GD 9 embryos were exposed to concentrations between 0.5 and 2.9 mM 1,1,2,2-tetrachloroethane for 48 hours and then evaluated for morphological changes. At concentrations >1.4 mM, 1,1,2,2-tetrachloroethane resulted in rotational defects and anomalies of the heart and eye. Embryo lethality was observed at ≥2.4 mM.

4.4.4. Immunotoxicological Studies

Shmuter (1977) exposed groups of 12 Chinchilla rabbits to 0, 2, 10, or 100 mg/m^3 (0, 0.3, 1.5, or 14.6 ppm, respectively) 1,1,2,2-tetrachloroethane 3 hours/day, 6 days/week for 8–10 months. Animals were vaccinated with 1 mL of a 1.5×10^9 suspension of heated typhoid vaccine 1.5, 4.5–5, and 7.5–8 months after the initiation of 1,1,2,2-tetrachloroethane exposure. Significant increases and decreases in total antibody levels were observed in the 2 and 100 mg/m^3 groups, respectively. No significant changes in 7S-typhoid antibody levels were observed. Significant alterations in the levels of "normal" hemolysins to the Forsman's antigen of sheep erythrocytes were observed in the 10 and 100 mg/m^3 groups, as levels were increased in the 10 mg/m^3 group after 1.5, 2, and 2.5 months of exposure, decreased after 4 months, and absent at 5 months of exposure. Levels of these hemolysins were decreased in the 100 mg/m^3 group during the first 6 months of exposure. Increases in the electrophoretic mobility of specific antibodies following 1,1,2,2-tetrachloroethane were also reported. Exposure to 100 mg/m^3 1,1,2,2-tetrachloroethane resulted in a decrease in the relative content of antibodies in the γ-globulin fraction and an increase in the T and β□ fractions. This is a poorly reported study that provides inadequate quantitative data. The inconsistent dose-response patterns preclude assessing biological significance and identification of a NOAEL or LOAEL.

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

4.5.1. Genotoxicity

As discussed in Section 3.4, radiolabeled 1,1,2,2-tetrachloroethane may covalently bind to DNA and RNA (Colacci et al., 1987), suggesting the potential for mutagenicity. A summary of the results of genotoxicity studies of 1,1,2,2-tetrachloroethane is presented in Table 4-19.

Table 4-19. Results of in vitro and in vivo genotoxicity studies of 1,1,2,2-tetrachloroethane

In vitro gene mutation assays						
				Results		
Test system	Endpoint	Cells/strain	Concentrations	–S9	+S9	Reference
(a) Bacterial assays						
Salmonella typhimurium (Ames test)	Reverse mutation	TA100, 1535, 1537, 1538, 98	NA	–	–	Nestmann et al., 1980
		TA1530, 1535, 1538	10 µL/plate	NP	+	Rosenkranz, 1977; Brem et al., 1974
		TA1535, 1537, 98	10 µL/plate	–	–	Mitoma et al., 1984
		TA1535	NA	–	–	Ono et al., 1996
		TA97, 98, 100, 1535, 1537	10–3,333 µL/plate	–	–	NTP, 2004
		TA98, 100, 1535, 1537	NA	–	–	Milman et al., 1988
		TA98, 100, 1535, 1537	5–1,000 µL/plate	–	–	Haworth et al., 1983
		TA100	NA	–	–	Warner et al., 1988
	Forward mutation	BA13	0.06–2,979 nmol/	–	–	Roldan-Arjona et al., 1991
Escherichia coli	DNA damage	pol A^+/pol A_1^-	10 µL/plate	NP	+	Rosenkranz, 1977; Brem et al., 1974
		$WP2_S(\lambda)$	15–236 mM	+	–	DeMarini and Brooks, 1992
Saccharomyces cerevisisae	Gene conversion	D7	3.1–7.3 mM	NP	+	Callen et al., 1980
			NA	NP	–	Nestmann and Lee, 1983
	Gene reversion	D7	3.1–7.3 mM	NP	+	Callen et al., 1980
			NA	NP	–	Nestmann and Lee, 1983
	Gene recombination	D7	3.1–7.3 mM	NP	+	Callen et al., 1980
Aspergillus nidulans	Mitotic crossover	P1	0.01–0.04%v:v	NP	+	Crebelli et al., 1988

(b) Mammalian cell assays						
Mouse lymphoma	Gene mutation	L5178Y	25–500 nL/mL	–	–	NTP, 2004
Hepatocytes (primary)	DNA repair	Osborne Mendel rats	NA	NP	–	Milman et al., 1988; Williams, 1983
		$B6C3F_1$ mice	NA	NP	–	
In vitro chromosomal damage assays						
Test system	**Cells/organs**		**Concentrations**	**Results**		**Reference**
Mammalian cells						
Chromosomal aberrations	CHO cells		453–804 µg/mL	–	–	NTP, 2004; Galloway et al., 1987
SCEs	CHO cells		16.8–558 µg/mL	+	+	NTP, 2004; Galloway et al., 1987
	BALB/c-$3T_3$ cells		500–1,000 µg/mL	+	+	Colacci et al., 1992
UDS	Human embryonic intestinal fibroblasts		≤15,869 µg/mL	–	NP	McGregor, 1980
Other in vitro assays						
Cell transformation (initiation)	BALB/c-$3T_3$ cells		1–250 µg/mL	NP	–	Arthur Little, Inc., 1983
			1–250 µg/mL	NP	–	Tu et al., 1985
			125–1,000 µg/mL	+	+	Colacci et al., 1990
			NA	–	–	Milman et al., 1988
Cell transformation (promotion)			0.1–1,000 ng/mL	NP	–	Colacci et al., 1996
In vivo bioassays						
Test system	**Cells/organs**		**Doses**	**Results**		**Reference**
Chromosomal damage: mammalian						
Chromosomal aberrations	Rat bone marrow cells, male		50 ppm	–		McGregor, 1980
	Rat bone marrow cells, female		50 ppm	+		
Micronucleus	Mouse peripheral blood erythrocytes		589–9,100 ppm	+		NTP, 2004
UDS	Mouse hepatocytes		200 mg/kg	+		Miyagawa et al., 1995
	Mouse hepatocytes, male		50–1,000 (mg/kg)	–		Mirsalis et al., 1989
	Mouse hepatocytes, female		50–1,000 mg/kg	–		
DNA alkylation	Mouse hepatocytes		150 mg/kg	+		Dow Chemical Company, 1988
Other in vivo assays						
S-phase DNA synthesis	Mouse hepatocytes, male		200–700 mg/kg	–		Mirsalis et al., 1989
	Mouse hepatocytes, female		200–700 mg/kg	+/–		
Mitotic recombination	*Drosophila melanogaster*		500–1,000 ppm	–		Vogel and Nivard, 1993
Recessive lethal mutation	*D. melanogaster*		800 ppm (injected) 1,500 (feed)	–		Woodruff et al., 1985

+ = positive; – = negative/no change; CHO = Chinese hamster ovary; NA = not available; NP = assay not performed; SCE = sister chromatid exchange; UDS = unscheduled DNA synthesis

1,1,2,2-Tetrachloroethane has been shown to be predominantly inactive in reverse mutation assays in *Salmonella typhimurium* (strains TA97, TA98, TA100, TA1530, TA1535, TA1537, and TA1538), either with or without the addition of S9 metabolic activating mixture, even at concentrations that lead to cytotoxicity (NTP, 2004; Ono et al., 1996; Milman et al., 1988; Warner et al., 1988; Mitoma et al., 1984; Haworth et al., 1983; Nestmann et al., 1980). Two studies reported reverse mutation activity in *S. typhimurium* (Rosenkranz, 1977; Brem et al., 1974). Results of studies employing methods to prevent volatilization were not notably different from those that did not use those methods. 1,1,2,2-Tetrachloroethane also did not induce forward mutations (L-arabinose resistance) in *S. typhimurium* strain BA13 (Roldan-Arjona et al., 1991). Assays with *Escherichia coli* indicated that 1,1,2,2-tetrachloroethane induced DNA damage, as shown by growth inhibition in DNA polymerase deficient *E. coli* (Rosenkranz, 1977; Brem et al., 1974) and induction of prophage lambda (DeMarini and Brooks, 1992). In *Saccharomyces cerevisiae*, 1,1,2,2-tetrachloroethane induced gene conversion, reversion, and recombination in one study (Callen et al., 1980), whereas another study found no conversion or reversion (Nestmann and Lee, 1983). In *Aspergillus nidulans*, 1,1,2,2-tetrachloroethane induced aneuploidy, but no crossing over (Crebelli et al., 1988).

1,1,2,2-Tetrachloroethane did not induce trifluorothymidine resistance in L5178Y mouse lymphoma cells, with or without S9, at concentrations up to those producing lethality (NTP, 2004). Primary hepatocytes from rats and mice exposed in vitro to 1,1,2,2-tetrachloroethane did not show altered DNA repair at concentrations that were not cytotoxic (Milman et al., 1988; Williams, 1983). McGregor (1980) reported no increase in unscheduled DNA synthesis (UDS) in human embryonic intestinal fibroblasts exposed to 1,1,2,2-tetrachloroethane. Treatment of Chinese hamster ovary (CHO) cells with up to 653 μg/mL (which was cytotoxic) did not result in increased induction of chromosomal aberrations (NTP, 2004; Galloway et al., 1987) but did produce an increased frequency of sister chromatid exchanges (SCEs) at concentrations of ≥55.8 μg/mL (NTP, 2004; Galloway et al., 1987). SCEs were also induced in BALB/c-3T_3 cells treated in vitro with high concentrations (≥500 μg/mL) of 1,1,2,2-tetrachloroethane, either with or without S9 activating mixture (Colacci et al., 1992).

In BALB/c-3T_3 cells, 1,1,2,2-tetrachloroethane exposure of up to 250 μg/mL in the absence of exogenous metabolic activation did not result in increased numbers of transformed cells (Colacci et al., 1992; Milman et al., 1988; Tu et al., 1985; Arthur Little, Inc., 1983); survival was generally ≥70%. Higher concentrations (≥500 μg/mL) were capable of transforming the cells but also showed higher levels of cytotoxicity (Colacci et al., 1990). However, even relatively low levels (31.25 μg/mL) of 1,1,2,2-tetrachloroethane used as an initiating agent, followed by promotion with 12-O-tetradecanoylphorbol-13-acetate, resulted in increased numbers of transformed cells (Colacci et al., 1992). 1,1,2,2-Tetrachloroethane did not act as a promoter in BALB/c-3T3 cells in vitro without metabolic activation (Colacci et al., 1996).

1,1,2,2-Tetrachloroethane tested negative for sex-linked recessive lethal mutations and mitotic recombination in *Drosophila melanogaster* (NTP, 2004; Vogel and Nivard, 1993; Woodruff et al., 1985; McGregor, 1980). Replicative DNA synthesis was increased in hepatocytes isolated from male $B6C3F_1$ mice exposed to a single gavage dose of 200 mg/kg (24 and 48 hours postexposure) or 400 mg/kg (24, 39, and 48 hours postexposure) relative to hepatocytes from unexposed mice (Miyagawa et al., 1995). Hepatocytes isolated from mice following a single gavage dose of up to 1,000 mg/kg did not show an increase in UDS or S-phase DNA synthesis (Mirsalis et al., 1989). Hepatocytes isolated from $B6C3F_1$ mice 6 hours after a single gavage dose of 150 mg/kg in corn oil demonstrated irreversible alkylation of hepatic DNA (Dow Chemical Company, 1988). Inhalation exposure to 5 or 50 ppm (34.3 or 343 mg/m^3) for 7 hours/day, 5 days/week did not result in increased frequency of chromosomal aberrations in bone marrow cells isolated from male rats (McGregor, 1980); female rats exposed to 50 ppm (343 mg/m^3), but not to 5 ppm (34.3 mg/m^3), showed an increase in bone marrow cell aberrations other than gaps (McGregor, 1980).

In summary, genotoxicity studies provide limited evidence of a mutagenic mode of action. 1,1,2,2-Tetrachloroethane has some genotoxic activity, but in vitro genotoxicity tests generally reported negative results. Similarly, in vivo studies had mostly negative results with the exception of chromosomal aberrations in female rat bone marrow cells and micronucleus formation in mouse bone marrow peripheral erythrocytes. The results of rat liver preneoplastic foci and mouse BALB/c-3T3 cell neoplastic transformation assays suggest that 1,1,2,2-tetrachloroethane may have initiating and promoting activity. Overall, results of genotoxicity studies for 1,1,2,2-tetrachloroethane are mixed and insufficient for establishing a mutagenic mode of action.

4.5.2. Short-Term Tests of Carcinogenicity

Treatment of partially hepatectomized male Osborne-Mendel rats with a single 100 mg/kg gavage dose of 1,1,2,2-tetrachloroethane, followed by 7 weeks of promotion with phenobarbital in the diet, did not result in increased numbers of preneoplastic (gamma-glutamyl transpeptidase [GGT]-positive) foci in the liver (Milman et al., 1988; Story et al., 1986). Exposure of partially hepatectomized male Osborne-Mendel rats to a single i.p. dose of diethylnitrosamine (DEN) as an initiating agent followed by promotion with 100 mg/kg-day of 1,1,2,2-tetrachloroethane by gavage 5 days/week for 7 weeks produced a significantly increased number of GGT-positive foci in the liver (Milman et al., 1988; Story et al., 1986). 1,1,2,2-Tetrachloroethane also significantly increased the number of GGT-positive foci in rats administered the promotion protocol in the absence of the DEN initiator. The study authors concluded that 1,1,2,2-tetrachloroethane induces hepatocarcinogenesis primarily through a promoting mechanism (Story et al., 1986).

Using a mouse strain that had been shown to be susceptible to pulmonary adenomas when exposed to organic chemicals, Theiss et al. (1977) administered i.p. injections of 80, 200, or 400 mg/kg 1,1,2,2-tetrachloroethane in Tricaprylin 5–18 times to groups of 20 male A/St mice for 8 weeks. There was a dose-related increase in number of lung tumors/mouse (Table 4-20), and the dose-response was nearly statistically significant (Theiss et al., 1977).

Table 4-20. Pulmonary adenomas in male A/St mice following repeated i.p. injections of 1,1,2,2-tetrachloroethane

Dose/injection (mg/kg)	0	80	200	400
Number of i.p. injections	24	5	18	16
Total dose (mg/kg)	0	400	3,600	6,400
Number of surviving animals	15/20	10/20	15/20	5/20
Number of lung tumors/mouse	0.27 ± 0.15	0.30 ± 0.21	0.50 ± 0.14	1.00 ± 0.45

Source: Thiess et al. (1977).

Maronpot et al. (1986) tested 65 chemicals at three doses in 6- to 8-week-old male and female strain A/St or A/J mice housed 10/cage. Doses were set based on the highest dose exhibiting a lack of overt toxicity from a preliminary dose-setting study, with the mid and low dose as half the higher dose. Mice were injected i.p. 3 times/week for 8 weeks. Lungs were examined histologically. The data for 1,1,2,2-tetrachloroethane-exposed male and female strain A/St are presented in Table 4-21.

Table 4-21. Pulmonary adenomas in male and female A/St mice following repeated i.p. injections of 1,1,2,2-tetrachloroethane

Compound	Untreated control	Saline vehicle control	Tricaprylin vehicle control	Urethan positive control	1,1,2,2-Tetrachloroethane		
Dose/injection (mg/kg)	–	–	–	1,000	62.5	99	187.5
Vehicle	–	–	–	–	Tricaprylin	Tricaprylin	Tricaprylin
Male A/St mice							
Number of surviving animals[a]	119/120	45/50	54/60	47/50	10/10	8/10	5/10
Percent survivors with tumors	2	9	13	96	10	0	0
Tumors per mouse[b]	0.017	0.089	0.167	11.9	0.1	0	0
Female A/St mice							
Number of surviving animals[a]	79/80	44/50	54/60	47/50	9/10	5/10	3/10
Percent survivors with tumors	8	14	11	96	0	20	0
Tumors per mouse[b]	0.076	0.186	0.11	10.3	0	0.2	0

[a]Numerator is number of mice alive at study termination; denominator is number of mice started on study.
[b]Based on all surviving mice at study termination.

Source: Maronpot et al. (1986).

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

4.6.1.1. *Human Data*

Information on the acute oral toxicity of 1,1,2,2-tetrachloroethane in humans is available from several case reports. Based on amounts of 1,1,2,2-tetrachloroethane recovered from the gastrointestinal tract of deceased subjects following intentional ingestion (Mant, 1953; Sherman, 1953; Lilliman, 1949; Forbes, 1943; Elliot, 1933; Hepple, 1927), estimated lethal doses ranged from 273 to 9,700 mg/kg. Patients who accidentally consumed a known volume of 1,1,2,2-tetrachloroethane, corresponding to single doses ranging from 68 to 117 mg/kg, as medicinal treatment for hookworm experienced loss of consciousness and other clinical signs of narcosis (Ward, 1955; Sherman, 1953). Chronic oral effects of 1,1,2,2-tetrachloroethane in humans have not been reported in the literature.

4.6.1.2. *Animal Data*

Few studies have evaluated acute oral toxicity in animals, and the endpoints assessed consist of data on lethality and neurological and liver effects (Table 4-22). Oral LD_{50} values ranged from 250 to 800 mg/kg in rats (NTP, 2004; Schmidt et al., 1980a; Gohlke et al., 1977; Smyth et al., 1969). Neurological effects of acute, oral 1,1,2,2-tetrachloroethane administration

revealed ataxic effects and decreased passive avoidance behavior (Wolff, 1978). Hepatic changes were noted in two separate acute oral toxicity studies. Male Sprague-Dawley rats administered between 287 and 1,148 mg/kg 1,1,2,2-tetrachloroethane had dose-dependent increases in the serum activity levels of AST and ALT as well as a decrease in hepatic microsomal G6Pase activity (Cottalasso et al., 1998). Male Wistar rats were administered 100 mg/kg 1,1,2,2-tetrachloroethane and had increases in hepatic ascorbic acid levels and serum leucine aminopeptidase activity, but no changes in serum ALT activity (Schmidt et al., 1980a, b). Both studies noted increases in triglyceride levels in the liver.

Table 4-22. Summary of noncancer results of major studies for oral exposure of animals to 1,1,2,2-tetrachloroethane

Species	Sex	Average daily dose (mg/kg-d)	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Response	Comments	Reference
Acute exposure								
Rat (Wistar)	F	0, 25, 50, 80, or 100 (gavage)	Single dose	25	50	Increased electric shock perception threshold.	Results suggestive of a subtle anesthetic effect. Ataxia observed at 100 mg/kg.	Wolff, 1978
Rat (Sprague-Dawley)	M	0, 143.5, 287, 574, or 1,148 (gavage)	Single dose	143.5	287	Increased serum AST activity and ALT activity, increased liver triglyceride levels; decreased liver dolichol levels.	Evaluations performed 1 hr postexposure. Approximately twofold increases in AST and ALT at ≥574 mg/kg. Liver histology and neurotoxicity not assessed.	Cottalasso et al., 1998
Rat (Wistar)	M	0 or 100	Single dose	100	ND	Increased hepatic ascorbic acid levels and serum leucine aminopeptidase activity.	No changes in serum ALT.	Schmidt et al., 1980a, b
Short-term exposure								
Rat (Osborne-Mendel)	M	0, 25, 75, 150, or 300 (gavage)	3–4 d	150	300 (FEL)	CNS depression and mortality. No histopathological changes in liver.	Increased hepatocellular DNA synthesis and mitosis at ≥75 mg/kg-d; increased liver weight at ≥150 mg/kg-d. No nonhepatic endpoints evaluated.	Dow Chemical Company, 1988
Mouse ($B6C3F_1$)	M	0, 25, 75, 150, or 300 (gavage)	4 d	300	ND		Centrilobular swelling at ≥75 mg/kg-d and increased hepatocellular DNA synthesis and mitosis at ≥150 mg/kg-d. No nonhepatic endpoints evaluated.	Dow Chemical Company, 1988
Rat (F344/N)	M, F	0, 135, 270, or 540 (gavage)	12 doses in 16 d	135	270	Decreased body weight in females, plus lethargy and increased organ weights.	The highest dose caused 100% mortality. Limited histology[a].	TSI Mason Laboratories, 1993a, unpubl.
Rat (F344/N)	M	0, 135, 270, or 540 (gavage)	12 doses in 16 d	135	270	Lethargy, decreased body weight gain.	Mortality at 540 mg/kg-d. Limited histology[a].	TSI Mason Laboratories, 1993b, unpubl.
Mouse ($B6C3F_1$)	M, F	0, 337.5, 675, or 1,350 (gavage)	12 doses in 16 d	ND	337.5	Hepatocellular degeneration (females).	Lethargy, increased liver weight, and mortality at higher doses. Limited histology[a].	TSI Mason Laboratories, 1993c, unpubl.
Rat (F344/N)	M	0, 104, or 208 (gavage)	13–21 d	ND	104	Hepatic cytoplasmic vacuolization at low dose, mortality at high dose.	No changes in body weight, kidney weights, kidney histology, or urinalysis.	NTP, 1996;

Table 4-22. Summary of noncancer results of major studies for oral exposure of animals to 1,1,2,2-tetrachloroethane

Species	Sex	Average daily dose (mg/kg-d)	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Response	Comments	Reference
Rat (F344/N)	M, F	0, 300, 400, or 500 (diet)	15 d	ND	300	Decreased body weight gain.	Changes in liver and kidney weights and clinical signs at higher doses. Limited histology[a].	NTP, 2004
Mouse ($B6C3F_1$)	M, F	3,325, 6,650, 13,300, 26,600, or 53,200 ppm	15 d	ND	ND	Decreased body weight, hyperactivity, decreased absolute and relative thymus weight, increased relative liver weight, pale or mottled livers, hepatocellular degeneration.	Feed consumption could not be measured accurately due to feed scattering; thus average daily doses (mg/kg-d) were not estimated.	NTP, 2004; TSI Mason Laboratories, 1993d
Rat (Osborne-Mendel)	M, F	0, 56, 100, 178, 316, or 562	5 d/wk for 6 wks	100 (male) 56 (female)	178 (male) 100 (female)	Decreased body weight gain.	Mortality and body weight gain were the only endpoints used to assess toxicity.	NCI, 1978
Mouse ($B6C3F_1$)	M, F	0, 32, 56, 100, 178, or 316	5 d/wk for 6 wks	316	ND	Body weight changes and mortality.	Mortality and body weight gain were the only endpoints used to assess toxicity.	NCI, 1978
Subchronic exposure								
Rat (F344)	M, F	0, 20, 40, 80, 170, or 320 (diet)	14 wks	20	40	Increased liver weight, as well as decreased sperm motility.	Comprehensive study. More serious hepatic effects, including hepatocyte necrosis and bile duct hyperplasia, as well as effects on other organs, at ≥170 mg/kg-d.	NTP, 2004
				40	80	Increased serum ALT activity, SDH activity, and cholesterol levels, reduced epididymis weight.		
Mouse ($B6C3F_1$)	M, F	0, 100, 200, 370, 700, or 1,360 (male); 0, 80, 160, 300, 600, or 1,400 (female) (diet)	14 wks	80	160	Increased liver weight, increased ALT activity, ALP activity, SDH activity, and bile acid levels.	Comprehensive study. Wide array of endpoints evaluated, including histopathology. More serious hepatic effects, including hepatocyte necrosis and bile duct hyperplasia, as well as effects on other organs, at ≥300 mg/kg-d.	NTP, 2004
Chronic exposure								

Table 4-22. Summary of noncancer results of major studies for oral exposure of animals to 1,1,2,2-tetrachloroethane

Species	Sex	Average daily dose (mg/kg-d)	Exposure duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Response	Comments	Reference
Rat (Osborne-Mendel)	M, F	0, 62, or 108 (male) 0, 43, or 76 (female) (gavage)	78 wks	62 (M) 76 (F)?	108 (M) ND (F)	Fatty changes in liver.	Study is confounded by endemic chronic murine pneumonia, but this is unlikely to have contributed to the liver pathology.	NCI, 1978
Mouse ($B6C3F_1$)	M, F	0, 142, or 284 (gavage)	78 wks	ND 142	142 (M) 284 (F)	Reduced survival. Acute toxic tubular nephrosis, hydronephrosis, and chronic inflammation in the kidneys.		NCI, 1978
Developmental exposure								
Rat (Sprague-Dawley)	F	0, 34, 98, 180, 278, or 330 (diet)	GDs 4–20	34	98	Decreased maternal and fetal body weights.	Effects were more pronounced at higher doses.	Gulati et al., 1991a
Mouse (CD-1)	F	0, 987, 2,120, 2,216, or 4,575 (diet)	GDs 4–17	ND	ND	Maternal mortality and litter resorptions.	High mortality in the exposed mice precluded the identification of a NOAEL or LOAEL.	Gulati et al., 1991b

[a]Histology only evaluated in animals with gross lesions.

F = female; M = male; ND = Not determined

Short-term oral exposure (Table 4-22) to 1,1,2,2-tetrachloroethane produced clinical signs of neurotoxicity and mortality at doses as low as 208–300 mg/kg-day by gavage in rats (NTP, 1996; TSI Mason Laboratories, 1993a, b; Dow Chemical Company, 1988). Body weight gain was decreased at similar dose levels in rats exposed by gavage or diet (NTP, 2004; TSI Mason Laboratories, 1993a, b; Dow Chemical Company, 1988; NCI, 1978). Hepatic effects consisted of increased DNA synthesis and centrilobular swelling in mice exposed to 75 mg/kg-day in the diet (Dow Chemical Company, 1988) and hepatocellular cytoplasmic vacuolation in rats exposed to 104 mg/kg-day (NTP, 1996). At higher doses (337.5 mg/kg-day), hepatocellular degeneration was observed in mice (TSI Mason Laboratories, 1993c).

Subchronic and chronic oral administration studies (Table 4-22) with 1,1,2,2-tetrachloroethane in animals indicated that the liver is the most sensitive organ for toxicity. Oral toxicity studies in F344 and Osborne-Mendel rats and $B6C3F_1$ mice were evaluated (NTP, 2004, NCI, 1978). The 14-week subchronic study by NTP (2004) in both F344 rats and $B6C3F_1$ mice was the most comprehensive evaluation of 1,1,2,2-tetrachloroethane-mediated toxicity through an orally administered route. NCI (1978) conducted a chronic study on Osborne Mendel rats and $B6C3F_1$ mice in which dosing regimens were modified during the course of the study.

In F344 rats, an increased incidence of hepatocellular cytoplasmic vacuolization was observed at 20 mg/kg-day in males and 40 mg/kg-day in females, increased relative liver weights were observed at 40 mg/kg-day, and hepatocellular hypertrophy was observed at 80 mg/kg-day in the subchronic NTP (2004) study. Additional hepatic effects included increases in serum ALT and SDH activity at 80 mg/kg-day, decreases in serum cholesterol levels at 80 mg/kg-day, and increases in serum ALP activity and bile acid levels, hepatocellular necrosis, bile duct hyperplasia, hepatocellular mitotic alterations, foci of cellular alterations, and hepatocyte pigmentation at 170 and 320 mg/kg-day. A NOAEL of 20 mg/kg-day and a LOAEL of 40 mg/kg-day was selected based on the increase in relative liver weight; however, it should be noted that an increased incidence of hepatocellular cytoplasmic vacuolization was observed at 20 and 40 mg/kg-day in male and female rats, respectively. In the Osborne-Mendel rats, significant increases in hepatic fatty metamorphosis were observed in male rats following a chronic exposure to 108 mg/kg-day (TWA, based on changes in dosing regimen) (NCI, 1978). Mortality was significantly increased in female rats dosed at a TWA dose of 43 and 76 mg/kg-day; however, the increased mortality was affected by the deaths of 10 high-dose females, 8 with pneumonia and 2 with no reported lesions, during the first 5 weeks of the study. A NOAEL of 62 mg/kg-day and a LOAEL of 108 mg/kg-day were identified in male rats based on an increased incidence of hepatic fatty metamorphosis (NCI, 1978).

Mice appear to be less sensitive than rats to noncancer effects mediated by orally administered 1,1,2,2-tetrachloroethane. Relative liver weight was statistically significantly increased in female and male $B6C3F_1$ mice at 80 and 200 mg/kg-day, respectively. Effects in the mice also included minimal hepatocellular hypertrophy, increased serum SDH activity, ALT

activity, and bile acid levels, and decreased serum cholesterol levels at 160–200 mg/kg-day, as well as increased serum ALP and 5'-nucleotidase activities, necrosis, pigmentation, and bile duct hyperplasia at 300–370 mg/kg-day. Based on the increase in relative liver weight observed in the NTP (2004) study, a NOAEL of 100 mg/kg-day and a LOAEL of 200 mg/kg-day in male mice and a LOAEL of 80 mg/kg-day in female mice were identified. In addition, male and female $B6C3F_1$ mice were evaluated for chronic oral toxicity by NCI (1978). For this study, a LOAEL of 142 mg/kg-day was selected for chronic inflammation in the kidneys of male mice, while a NOAEL of 142 mg/kg-day and a LOAEL of 284 mg/kg-day were selected for hydronephrosis and chronic inflammation in the kidneys of female mice.

Comprehensive neurobehavioral testing showed no evidence of neurotoxicity in either species at doses equal to or higher than the LOAELs based on liver effects (NTP, 2004), indicating that the liver is more sensitive than the nervous system to subchronic dietary exposure to 1,1,2,2-tetrachloroethane.

Developmental parameters were significantly affected by oral administration of 1,1,2,2-tetrachloroethane in rats and mice. Significant decreases in rat maternal and fetal body weights were noted at doses of ≥98 mg/kg-day (Gulati et al., 1991a). Using statistical significance and a 10% change as the criteria for establishing an adverse effect in maternal body weight, a NOAEL of 34 mg/kg-day and LOAEL of 98 mg/kg-day were selected for developmental toxicity based on the lowest dose that produced a statistically significant decrease in fetal body weight. In mice, the FEL based on maternal toxicity and resorption of litters is 2,120 mg/kg-day (Gulati et al., 1991b). The high mortality in the exposed mice precluded the identification of a NOAEL or LOAEL from this study.

Toxicity to reproductive tissues following 1,1,2,2-tetrachloroethane exposure to adult rats and mice was observed at dose levels as low as 40 mg/kg-day (NTP, 2004). In male rats, sperm motility was decreased at ≥40 mg/kg-day. Higher doses resulted in decreased epididymal absolute weight and increased atrophy of the preputial and prostate gland, seminal vesicle, and testicular germinal epithelium. In female rats, minimal to mild uterine atrophy was increased at ≥170 mg/kg-day, and clitoral gland atrophy and ovarian interstitial cell cytoplasmic alterations were increased at 320 mg/kg-day. Female F344 rats in the 170 mg/kg-day group spent more time in diestrus than did the vehicle controls.

Male $B6C3F_1$ mice had increased incidences of preputial gland atrophy at ≥100 mg/kg-day. Less sensitive effects included decreases in absolute testis weight (≥700 mg/kg-day) and absolute epididymis and cauda epididymis weights (1,360 mg/kg-day) and a decrease in epididymal spermatozoal motility (1,360 mg/kg-day). The only noted reproductive toxicity parameter in female mice affected was a significant increase in the length of the estrous cycle at a dose of 1,400 mg/kg-day (NTP, 2004).

4.6.2. Inhalation

4.6.2.1. *Human Data*

Limited information is available on the acute inhalation toxicity of 1,1,2,2-tetrachloroethane in humans (Table 4-23). The results of an early, poorly reported experimental study with two volunteers suggest that 3 ppm (6.9 mg/m^3) was the odor detection threshold. Irritation of the mucous membranes, pressure in the head, vertigo, and fatigue were observed at 146 ppm (1,003 mg/m^3) for 30 minutes or 336 ppm (2,308 mg/m^3) for 10 minutes. Common reported symptoms of high-level acute inhalation exposure to 1,1,2,2-tetrachloroethane in humans include drowsiness, nausea, headache, and weakness, and at extremely high concentrations, jaundice, unconsciousness, and respiratory failure (Coyer, 1944; Hamilton, 1917).

Table 4-23. Summary of noncancer results of major human studies of inhalation exposure to 1,1,2,2-tetrachloroethane

Study population	Sex	Exposure level (mg/m^3)	Exposure duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Response	Comments	Reference
Acute exposure								
Two volunteers	NS	6.9–2,308	30 min	ND	ND	Irritation, vertigo, head pressure, fatigue.	Effect levels could not be determined due to limited analysis.	Lehmann et al., 1936
Occupational exposure								
127 coating workers	NS	500–1,500	NS	ND	ND	Decreased whole blood specific gravity, decreased RBC count, lymphocytosis, unspecified neurological findings.	Effect levels could not be determined due to limited analysis.	Horiguchi et al., 1964
Workers from 39 chemical processing plants	NS	NS	NS	ND	ND	Increased mortality for lymphatic cancers.	Mortality from cardiovascular disease, cirrhosis of the liver, and digestive or respiratory cancers was not elevated.	Norman et al., 1981
380 workers from 23 factories	M, F	62.5–672	Generally <1 yr	ND	ND	Anemia, loss of appetite, abdominal pain, headache, vertigo, and tremors.	Effect levels could not be determined due to a lack of a control population and possible coexposure.	Lobo-Mendonca, 1963
34–75 workers in penicillin production	NS	10–1,700	Up to 3 yrs	ND	ND	Loss of appetite, epigastric pain, hepatic enlargement, urobilinogenuria, weakness, fatigue, weight loss, and itching.	Effect levels could not be determined due to a lack of a control population, limited reporting, and possible coexposure.	Jeney et al., 1957

F = female; M = male; ND = not determined; NS = not stated

Chronic toxicity of inhaled 1,1,2,2-tetrachloroethane in humans (Table 4-23) resulted in neurological symptoms including headache, weakness, fatigue, and hematological changes such as anemia and elevated WBC count (Norman et al., 1981; Lobo-Mendonca, 1963; Jeney et al., 1957; Minot and Smith, 1921). Most occupational exposure studies failed to evaluate hepatic endpoints, other than an urobilinogen test. Jeney et al. (1957) reported a positive relationship between duration of exposure and frequency of abnormal liver function test results, loss of appetite, bad taste in the mouth, epigastric pain, and a "dull straining pressure feeling in the area of the liver".

4.6.2.2. *Animal Data*

Acute inhalation exposures in animals (Table 4-24) resulted in near-lethal or lethal effects at levels ≥1,000 ppm (Schmidt et al., 1980a; Price et al., 1978; Horiuchi et al., 1962; Carpenter et al., 1949; Pantelitsch, 1933). Death was typically preceded by signs of CNS toxicity (e.g., incoordination, loss of reflexes, labored respiration, prostration, and loss of consciousness) and was often accompanied by congestion and fatty degeneration of the liver. Nonlethal exposures increased lipid and triglyceride levels in the liver in mice following exposure to 600–800 ppm (4,120–5,490 mg/m^3) for 3 hours (Tomokuni, 1970, 1969). Nonlethal exposures also reduced motor activity in rats following exposure to 576 ppm (3,950 mg/m^3) for 30 minutes (Price et al., 1978) and 360 ppm (2,470 mg/m^3) for 6 hours (Horvath and Frantik, 1973) and in guinea pigs following exposure to 576 ppm (3,950 mg/m^3) (Price et al., 1978).

Table 4-24. Summary of noncancer results of major studies for inhalation exposure of animals to 1,1,2,2-tetrachloroethane.

Species	Sex	Exposure level (mg/m^3)	Exposure duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Response	Comments	Reference
Acute exposure								
Rat	NR	NR	4 hrs	NR	8,600	LC_{50}	24-Hr observation.	Schmidt et al., 1980a
Rat (Wistar)	M	0, 410, 700, 1,030, 2,100, or 4,200	4 Hrs	ND	ND	Hepatic effects included histological alterations and increases in serum enzymes and liver triglycerides. Identification of a NOAEL or LOAEL precluded by reporting inadequacies.		Schmidt et al., 1980a
Rat (Sherman)	NR	6,870	4 hrs	ND	ND	Mortality		Carpenter et al., 1949
Rat	NR	3,950, 34,700, or 43,350	30 mins	ND	3,950	Slight reduction in activity and alertness; lacrimation, ataxia, narcosis, labored respiration, and 30–50% mortality when concentration increased.		Price et al., 1978
Guinea pig	NR	3,950, 34,700, or 43,350	30 mins	ND	3,950	Eye closure, squinting, lacrimation, and decreased activity; tremors, narcosis, labored breathing, and mortality when concentration increased		Price et al., 1978
Rat (NR)	NR	1,370 or 2,470	6 hrs	ND	2,470	Effective concentration for a 50% decrease in spontaneous motor activity.	Effective concentration for a 50% increase in pentobarbital sleep time was 1,370 mg/m^3.	Horvath and Frantik, 1973
Mouse (Cb)	F	4,120	3 hrs	ND	4,120	Increased hepatic lipid and triglyceride levels, decreased hepatic ATP.	A limited number of endpoints were evaluated.	Tomokuni, 1969
Mouse (Cb)	F	5,490	3 hrs	ND	ND	Increased triglyceride and decreased phospholipid levels.	Effects generally resolved by 90 hours postexposure.	Tomokuni, 1970
Mouse	NS	7,000, 8,000–10,000, 17,000, 29,000, or 34,000	1.5–2 hrs	ND	7,000	Disturbed equilibrium, prostration, and loss of reflexes.	Limited number of endpoints and poor reporting. Mortality at ≥8,000 mg/m^3.	Pantelitsch, 1933
Mouse	M	40,500 or 45,300	3 hrs	ND	ND	Mortality: 3/10 and 4/10, respectively		Horiuchi et al., 1962
Rat	M	0, 69, 690, or 6,900	6 hrs	ND	69	Minimal increase in serum AST at all exposure concentrations 72 hrs postexposure.		Deguchi, 1970

Table 4-24. Summary of noncancer results of major studies for inhalation exposure of animals to 1,1,2,2-tetrachloroethane.

Species	Sex	Exposure level (mg/m^3)	Exposure duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Response	Comments	Reference
Short-term exposure								
Rat	M	0 or 15	4 hrs/d for up to 8 exposures in 10 d	ND	ND	Increases in serum proteins and histological alterations in the liver. Identification of a NOAEL or LOAEL precluded by reporting inadequacies.		Gohlke and Schmidt, 1972; Schmidt et al., 1972
Rat	M	62,000	2 hrs/d, 2–3 times/wk for 11 exposures in 29 d	ND	ND	All rats died during the study. No changes in body weight were reported. Exposed animals generally showed moderate congestion and fatty degeneration of the liver.		Horiuchi et al., 1962
Mouse	M	48,000	2 hrs/d for 5 exposures in 29 d	ND	ND	Moderate congestion and fatty degeneration of the liver.	Most (5/9) of the mice died within 5 days of the first exposure.	Horiuchi et al., 1962
Subchronic exposure								
Rat (Sprague-Dawley)	F	0 or 3,909	5–6 hrs/d, 5 d/wk for 15 wks	ND	ND	Increased liver weight, transient liver cytoplasmic vacuolization. Identification of a NOAEL or LOAEL precluded by reporting inadequacies.		Truffert et al., 1977
Monkey (Macaca sp.)	M	13,560	2 hrs/d, 6 d/wk for total of 190 exposures in 9 mo	ND	ND	Fatty degeneration and splenic congestion. Identification of a LOAEL or NOAEL is precluded by the use of a single animal and lack of control.		Horiuchi et al., 1962
Rat	M, F	0 or 1,150	7 hrs/d for 6 mo	ND	ND	Pathological effects in the liver, kidney, and lung, precluded by an endemic lung infection.		Mellon Institute of Industrial Research, 1947
Mongrel dog	M	0 or 1,150	7 hrs/d for 6 mo	ND	ND	Increased serum phosphatase and blood urea nitrogen levels, cloudy swelling of the liver and convoluted tubule of the kidney, and light congestion of the lungs. A NOAEL or LOAEL was not identified due to single treated dog.		Mellon Institute of Industrial Research, 1947
Rabbit	NS	0 or 10	3 hrs/d, 6 d/wk for 7–8.5 mo	ND	ND	Altered serum acetylcholine levels. A NOAEL or LOAEL cannot be identified due to incomplete quantitation.		Kulinskaya and Verlinskaya, 1972

Table 4-24. Summary of noncancer results of major studies for inhalation exposure of animals to 1,1,2,2-tetrachloroethane.

Species	Sex	Exposure level (mg/m^3)	Exposure duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Response	Comments	Reference
Rabbit	NS	0, 2, 10, or 100	3 hrs/d, 6 d/wk for 8–10 mo	ND	ND	Increase and decrease in total antibody levels, increase in the mobility of specific antibodies, decrease in the relative content of γ-globulin antibodies and an increase in the T and β□ fractions. Poorly reported study that provides inadequate quantitative data.		Shmuter, 1977
Chronic exposure								
Rat	M	0 or 13.3	4 hrs/d, 110 or 265 d	ND	ND	Increased leukocyte and β_1-globulin levels, increased percentage of segmented nucleated neutrophils, decreased percentage of lymphocytes, increased liver total fat content. Experimental design and results were poorly reported and histological examinations do not appear to have been conducted.		Schmidt et al., 1972

F = female; M = male; ND = not determined; NS = not specified

Acute and short-term inhalation exposure (Table 4-24) to high concentrations (≥7,000 ppm) of 1,1,2,2-tetrachloroethane produced mortality and neurological and liver effects in animals. Mortality occurred in mice exposed to 7,000 ppm (48,000 mg/m^3) for 2 hours, once/week for a total of 4 exposures in 29 days and in rats exposed to 9,000 ppm (62,000 mg/m^3) for 2 hours/day, 2–3 times/week for a total of 11 exposures in 29 days. Congestion and fatty degeneration in the liver (mice and rats), as well as a biphasic change in neurological motor activity (hyperactivity followed by ataxia, rats only), were also reported (Horiuchi et al., 1962). At the lowest inhalation exposure of 2.2 ppm (15 mg/m^3) for 4 hours/day (8–10 days), rats had fine droplet fatty degeneration in the liver and changes in levels of serum proteins, but no neurological changes were reported (Gohlke and Schmidt, 1972; Schmidt et al., 1972).

There are a few subchronic inhalation exposure studies and one chronic exposure study with 1,1,2,2-tetrachloroethane (Table 4-24). Overall these studies either had poor study designs, one exposure concentration, low numbers of animals, or a combination of the above. The available subchronic and chronic inhalation studies indicate that the liver was the most sensitive organ to 1,1,2,2-tetrachloroethane exposure. Increased relative liver weights were reported at exposures of 560 ppm (3,909 mg/m^3) for 15 weeks (Truffert et al., 1977). Other transient hepatic changes (e.g., histological alterations and cytoplasmic vacuolation) were observed, but these effects did not persist (Truffert et al., 1977). In the chronic exposure study, rats exposed to 13.3 mg/m^3 (1.9 ppm) 1,1,2,2-tetrachloroethane 4 hours/day for 265 days exhibited increased liver fat content (Schmidt et al., 1972). In the third rat study (Mellon Institute of Industrial Research, 1947), none of the effects noted from 1,1,2,2-tetrachloroethane exposure could be evaluated since the control animals experienced a high degree of pathological effects in the kidneys, liver, and lung. Hepatic effects from long-term exposure to 1,1,2,2-tetrachloroethane were also reported in a study with one mongrel dog with cloudy swelling of the liver at 167 ppm (1,150 mg/m^3) for 6 months (Mellon Institute of Industrial Research, 1947) and one male monkey with fatty degeneration of the liver at 1,974 ppm (13,560 mg/m^3) for 9 months (Horiuchi et al., 1962).

Other endpoints that were observed following subchronic and chronic inhalation exposure are described below. Hematological alterations including increased leukocyte and β_1-globulin levels, increased percentage of segmented nucleated neutrophils and decreased percentage of lymphocytes, decreased γ-globulin, and decreased adrenal ascorbic acid levels were observed in rats exposed to 1.9 ppm (13.3 mg/m^3) for 265 days (Schmidt et al., 1972), and splenic congestion was noted in a study of a single monkey (Horiuchi et al., 1962). In the mongrel dog study noted above, cloudy swelling of the convoluted tubules of the kidneys and light congestion of the lungs were observed (Mellon Institute of Industrial Research, 1947). Kulinskaya and Verlinskaya (1972) observed alterations in serum acetylcholine levels in rabbits exposed to 10 mg/m^3 (1.5 ppm) 3 hours/day, 6 days/week for 7–8.5 months. Shmuter (1977)

observed immunological alterations (changes in antibody levels) in rabbits exposed to 2–100 mg/m^3 (0.3–14.6 ppm) 3 hours/day, 6 days/week for 8–10 months.

A reproductive toxicity assessment was conducted on seven male rats exposed to 13.3 mg/m^3 1,1,2,2-tetrachloroethane for 258 days. No significant changes in reproductive parameters were observed, indicating that 13.3 mg/m^3 (1.9 ppm) was a NOAEL for male reproductive effects in the rat (Schmidt et al., 1972).

4.6.3. Mode of Action of Noncarcinogenic Effects Information

1,1,2,2-Tetrachloroethane is rapidly and extensively absorbed following both oral and inhalation exposures, with absorption of 70–100% following oral exposure in animals (Dow Chemical Company, 1988; Mitoma et al., 1985) and 40–97% following inhalation exposures in humans (Morgan et al., 1970; Lehmann et al., 1936). Following absorption, the chemical is distributed throughout the body, although the high tissue:air partition coefficient for fat (Gargas et al., 1989) suggests that it may accumulate more in lipid-rich tissues. Metabolism is extensive, with ≥68% of a total administered dose generally found as metabolites (Dow Chemical Company, 1988; Mitoma et al., 1985; Yllner, 1971), and is believed to occur mostly in the liver. Urinary elimination occurs mainly as metabolites, including dichloroacetic acid, glyoxalic acid, formic acid, trichloroethanol, and trichloroacetic acid, while a fraction of an absorbed dose may be eliminated in expired air as parent compound or carbon dioxide.

Metabolism of 1,1,2,2-tetrachloroethane to reactive products is likely to play a key role in the observed noncancer effects. Both nuclear and microsomal CYP enzymes have been implicated in the metabolism of the compound, possibly forming a number of biologically active compounds including aldehydes, alkenes, acids, and free radicals (see Figure 3-1 in Section 3.3), which may react with biological tissues. Evidence for metabolism to reactive compounds comes from studies of radiolabel incorporation following single doses of radiolabeled 1,1,2,2-tetrachloroethane in which incorporated radiolabel was enhanced by pretreatment with phenobarbital, xylene, or ethanol, and the variety of inducers capable of influencing this effect suggest that multiple CYP isozymes may be involved (Casciola and Ivanetich, 1984; Halpert, 1982; Sato et al., 1980), including members of the CYP2A, CYP2B, CYP2E, and CYP3A subfamilies (Omiecinski et al., 1999; Nebert et al., 1987). Additionally, mice are known to metabolize 1,1,2,2-tetrachloroethylene at a 1.1–3.5-fold greater rate than rats and have been demonstrated to have approximately a twofold greater binding of radiolabel to tissues, further implicating metabolic activation as a possible step in the mode of action of noncarcinogenic effects. However, there is uncertainty as to whether the presence of radiolabel in proteins, DNA, and RNA may be radiolabeled carbon that has been incorporated into biomolecules through normal biochemical processes. Studies providing additional mode of action information for the 1,1,2,2-tetrachloroethane-induced noncancer toxicological effects are not available.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) 1,1,2,2-tetrachloroethane is "likely to be carcinogenic to humans" based on data from an oral cancer bioassay in male and female Osborne-Mendel rats and B6C3F_1 mice (NCI, 1978). In B6C3F_1 mice, a statistically significant increase in the incidence of hepatocellular carcinomas in both genders was observed at doses of 142 and 284 mg/kg-day. A decrease in the time to tumor in both genders of mice was also observed. In this same bioassay, male Osborne-Mendel rats exhibited an increased incidence of hepatocellular carcinomas, a rare tumor in this strain (NCI, 1978), at the high dose only, although this increased incidence was not statistically significant. An untreated female control rat also developed a hepatocellular carcinoma. In the high-dose male mice, acute toxic tubular nephrosis was characterized as the cause of death in the mice that died prior to study termination, although hepatocellular carcinomas were observed in most of these mice.

The predominant proposed metabolic pathway for 1,1,2,2-tetrachloroethane involves production of dichloroacetic acid (Casciola and Ivanetich, 1984; Halpert and Neal, 1981; Yllner, 1971). Dichloroacetic acid was identified as the major urinary metabolite in mice treated with 1,1,2,2-tetrachloroethane by i.p. injection (Yllner et al., 1971) and in in vitro systems with rat liver microsomal and nuclear CYP (Casciola and Ivanetich, 1984; Halpert, 1982; Halpert and Neal, 1981). Other pathways may involve the formation of trichloroethylene via dehydrochlorination or tetrachloroethylene via oxidation as initial metabolites (Mitoma et al., 1985; Ikeda and Ohtsuji, 1972; Yllner et al., 1971). 1,1,2,2-Tetrachloroethane may also form free radicals by undergoing reductive dechlorination (ATSDR, 1996).

Dichloroacetic acid induces hepatocellular carcinomas in both genders of F344 rats and B6C3F_1 mice (DeAngelo et al., 1999; DeAngelo et al., 1996; Pereira, 1996; Pereira and Phelps, 1996; Ferreira-Gonzalez et al., 1995; Richmond et al., 1995; Daniel et al., 1992; DeAngelo et al., 1991; U.S. EPA, 1991b; Bull et al., 1990; Herren-Freund et al., 1987). Trichloroethylene, also a metabolite of 1,1,2,2-tetrachloroethane, has been shown to produce hepatocellular carcinomas and hepatocellular adenomas in male and female B6C3F_1 mice, respectively, but did not demonstrate carcinogenicity in Osborne-Mendel or Sprague-Dawley rats (NTP, 1990; NCI, 1976). Tetrachloroethylene, another metabolite of 1,1,2,2-tetrachloroethane, was characterized by NCI (1977) as a liver carcinogen in B6C3F_1 mice, but an evaluation of carcinogenicity in Osborne-Mendel rats was inadequate due to early mortality. In a study by NTP (1986), tetrachloroethylene demonstrated evidence of carcinogenicity in F344 rats, as shown by increased incidences of mononuclear cell leukemia, and in B6C3F_1 mice, as shown by increased incidences of hepatocellular adenomas and carcinomas in males and carcinomas in females.

Additional information on the carcinogenic potential comes from studies on the tumor initiating and promoting activity in mammalian cells (Colacci et al., 1996, 1992). The results of

the in vivo and in vitro genotoxicity studies for 1,1,2,2-tetrachloroethane, which were generally negative, provide limited evidence of a mutagenic mode of action.

No animal cancer bioassay data following inhalation exposure to 1,1,2,2-tetrachloroethane are available. However, U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (2005a) indicates that, for tumors occurring at a site other than the initial point of contact, the cancer descriptor generally applies to all routes of exposure that have not been adequately studied unless there is convincing information to indicate otherwise. No additional information is available for 1,1,2,2-tetrachloroethane (e.g., toxicokinetic data that absorption does not occur by other routes). Thus, based on the observance of systemic tumors following oral exposure, and in the absence of information to indicate otherwise, 1,1,2,2-tetrachloroethane is considered "likely to be carcinogenic to humans" by any route of exposure.

The weight of evidence for the carcinogenicity of 1,1,2,2-tetrachloroethane could be strengthened by additional cancer bioassays demonstrating tumor development. Currently, the NCI (1978) bioassay is the only study available demonstrating 1,1,2,2-tetrachloroethane tumorgenicity. The NCI (1978) study was a 78-week study, compared to a 104-week bioassay, and the limitations of the study included increased mortality in male and female mice, the variable doses given to the mice over the course of the 78-week exposure period, and the acute toxic tubular nephrosis, characterized as the cause of death, in the high-dose male mice that died prior to study termination (although hepatocellular carcinomas were observed in most of these mice).

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

Only one study in humans evaluated the possible carcinogenic effects of 1,1,2,2-tetrachloroethane. Norman et al. (1981) evaluated groups of clothing-treatment workers employed during World War II in which some workers used 1,1,2,2-tetrachloroethane and some used water. Inhalation exposure concentrations and durations were not reported and dermal exposures were likely. In addition, coexposures to dry-cleaning chemicals occurred. No differences in standard mortality ratios were seen between the 1,1,2,2-tetrachloroethane and water groups for total mortality, cardiovascular disease, cirrhosis of the liver, or cancer of the digestive and respiratory systems. The mortality ratio for lymphatic cancers in the 1,1,2,2-tetrachloroethane group was increased relative to controls and the water group, although the number of deaths was small (4 cases observed compared to 0.85 cases expected). No other information was located regarding the carcinogenicity of 1,1,2,2-tetrachloroethane in humans.

The only comprehensive animal study that evaluated the carcinogenicity of 1,1,2,2-tetrachloroethane was performed by the NCI (1978). Male and female Osborne-Mendel rats were exposed to TWA doses of 0, 62, or 108 mg/kg-day (males) or 0, 43, or 76 mg/kg-day (females) 5 days/week for 78 weeks, followed by a 32-week observation period during which the rats were not exposed. No statistically significant increases in tumor incidences were observed in rats.

However, two hepatocellular carcinomas, which were characterized by NCI (1978) as rare in Osborne-Mendel rats, and one neoplastic nodule were observed in the high-dose male rats. A hepatocellular carcinoma was also observed in a female rat in the control group. NCI (1978) characterized the carcinogenic results in male rats as "equivocal." Male and female $B6C3F_1$ mice were exposed to TWA doses of 0, 142, or 284 mg/kg-day 5 days/week for 78 weeks, followed by a 12-week observation period during which the mice were not exposed. Statistically significant, dose-related increases in the incidence of hepatocellular carcinoma were observed in males (3/36, 13/50, and 44/49 in the control, low-, and high-dose groups, respectively) and females (1/40, 30/48, and 43/47, respectively). In addition, a decrease in the time to tumor for the hepatocellular carcinomas was also evident in both genders of mice. Lymphomas were also seen in the male and female mice, but the incidences were not found to be statistically significant. The only other available study observed pulmonary adenomas in female Strain A/St mice given 99 mg/kg-day injections i.p. 3 times/week for 8 weeks (Maronpot et al., 1986).

In vitro studies of the genotoxicity of 1,1,2,2-tetrachloroethane have yielded mixed, though mainly negative, results. Mutagenicity studies in *S. typhimurium* were predominantly negative, with only 2 of 10 available studies reporting activity (NTP, 2004; Ono et al., 1996; Roldan-Arjona et al., 1991; Milman et al., 1988; Warner et al., 1988; Mitoma et al., 1984; Haworth et al., 1983; Nestmann et al., 1980; Rosenkranz, 1977; Brem et al., 1974). Mixed results were reported for gene conversion, reversion, and recombination in *S. cerevisiae* (Nestmann and Lee, 1983; Callen et al., 1980), and aneuploidy but not mitotic cross over was induced in *A. nidulans* (Crebelli et al., 1988). Tests for DNA damage in *E. coli* were positive (DeMarini and Brooks, 1992; Rosenkranz, 1977; Brem et al., 1974). 1,1,2,2-Tetrachloroethane was not mutagenic in mouse L5178Y lymphoma cells (NTP, 2004) and was negative in tests for DNA damage in other mammalian cells, including induction of DNA repair in primary rat or mouse hepatocytes (Milman et al., 1988; Williams, 1983), induction of chromosomal aberrations in CHO cells (NTP, 2004; Galloway et al., 1987), and induction of cell transformation in BALB/c-3T3 cells (Colacci et al., 1992; Milman et al., 1988; Tu et al., 1985; Arthur Little, Inc., 1983). 1,1,2,2-Tetrachloroethane was positive for induction of SCEs in both BALB/c-3T3 (Colacci et al., 1992) and CHO cells (NTP, 2004; Galloway et al., 1987) and for induction of cell transformation in BALB/c-3T3 cells at high (cytotoxic) doses (Colacci et al., 1990).

1,1,2,2-Tetrachloroethane also had mixed results for genotoxicity following in vivo exposure. Tests for sex-linked recessive lethal mutations and mitotic recombination in Drosophila were negative (NTP, 2004; Vogel and Nivard, 1993; Woodruff et al., 1985; McGregor, 1980). Both positive (Miyagawa et al., 1995) and negative results (Mirsalis et al., 1989) have been reported in mouse hepatocytes tested for UDS, and tests for S-phase DNA induction in hepatocytes were negative in male mice and equivocal in female mice (Mirsalis et al., 1989). Rat bone marrow cells were negative for chromosomal aberrations in male rats, but positive in female rats (McGregor, 1980).

1,1,2,2-Tetrachloroethane showed promoting activity but limited initiating activity in rat liver preneoplastic (GGT-positive) foci assays (Milman et al., 1988; Story et al., 1986). 1,1,2,2-Tetrachloroethane initiated but did not promote neoplastic transformation in mouse BALB/c-3t3 cells (Colacci et al., 1996, 1992).

4.7.3. Mode of Action of Carcinogenicity Information

The mode of action of the carcinogenic effects of 1,1,2,2-tetrachloroethane is unknown. Colacci et al. (1987) reported possible covalent binding of radiolabeled 1,1,2,2-tetrachloroethane to DNA, RNA, and protein in the liver, kidneys, lung, and stomach of rats and mice exposed to a single intravenous dose and analyzed 22 hours postexposure. However, the conclusion of covalent binding may be influenced by the presence of radiolabel in the DNA, RNA, and protein that was the result of incorporated radiolabeled carbon into the biomolecules through normal biochemical processes.

The mutagenicity data for 1,1,2,2-tetrachloroethane are inconclusive, with in vitro genotoxicity tests generally reporting negative results, except for assays of SCE and cell transformation, and in vivo tests of genotoxicity showing a similar pattern. Several studies have reported increases in the number of hepatocytes in mitosis, but the possible role these effects may have on the carcinogenicity of 1,1,2,2-tetrachloroethane has not been evaluated. The results of rat liver preneoplastic foci and mouse BALB/c-3T3 cell neoplastic transformation assays suggest that 1,1,2,2-tetrachloroethane may have initiating and promoting activity (Colacci, 1996, 1992; Milman et al., 1988; Story et al., 1986), but tumor initiation and promotion studies have not been conducted.

Tumor formation by 1,1,2,2-tetrachloroethane may involve metabolism to one or more active compounds with the predominant pathway leading to the production of dichloroacetic acid (Casciola and Ivanetich, 1984; Halpert and Neal, 1981; Yllner, 1971). 1,1,2,2-Tetrachloroethane is metabolized extensively following absorption, at least in part, by CYP enzymes from the members of the CYP2A, CYP2B, CYP2E, and CYP3A subfamilies (see Section 3.3). Mice are known to metabolize 1,1,2,2-tetrachloroethane to a greater extent than rats, which may in part account for the fact that liver tumors occurred in mice at statistically significant levels but not in rats following chronic oral exposure.

Dichloroacetic acid, which appears to be the main metabolite of 1,1,2,2-tetrachloroethane, induces hepatocellular carcinomas in both genders of F344 rats and B6C3F_1 mice (DeAngelo et al., 1999; DeAngelo et al., 1996; Pereira, 1996; Pereira and Phelps, 1996; Ferreira-Gonzalez et al., 1995; Richmond et al., 1995; Daniel et al., 1992; DeAngelo et al., 1991; U.S. EPA, 1991b; Bull et al., 1990; Herren-Freund et al., 1987). Dichloroacetic acid is recognized as hepatocarcinogenic in both genders of two rodent species.

1,1,2,2-Tetrachloroethane may be metabolized to form free radicals, which may, in turn, covalently bind to macromolecules including DNA. Formation of free radicals during

1,1,2,2-tetrachloroethane metabolism has been demonstrated in spin-trapping experiments (Tomasi et al., 1984). Both nuclear and microsomal forms of CYP enzymes have been implicated in this process, as increased metabolism and covalent binding of metabolites following pretreatment with phenobarbital (Casciola and Ivanetich, 1984; Halpert, 1982), xylene (Halpert, 1982), or ethanol (Sato et al., 1980) have been reported. The presence of covalently bound label has been reported following inhalation (Dow Chemical Company, 1988), oral (Mitoma et al., 1985), and intravenous (Eriksson and Brittebo, 1991) administration of radiolabeled 1,1,2,2-tetrachloroethane.

In summary, only limited data are available regarding the possible mode(s) of action of 1,1,2,2-tetrachloroethane carcinogenicity. Metabolism to one or more active compounds may play a role in tumor development. Results of genotoxicity studies of 1,1,2,2-tetrachloroethane are mixed and provide inconclusive evidence for establishing a mutagenic mode of action.

There is some evidence to indicate that the mode of carcinogenic action may involve tumor promotion. Milman et al. (1988) and Story et al. (1986) concluded that 1,1,2,2-tetrachloroethane induces hepatocarcinogenesis primarily through a promoting mechanism following treatment of partially hepatectomized male Osborne-Mendel rats with a single 100 mg/kg gavage dose of 1,1,2,2-tetrachloroethane, followed by 7 weeks of promotion with phenobarbital in the diet. This regimen failed to result in increased numbers of preneoplastic (GGT-positive) foci in the liver. However, an exposure of partially hepatectomized male Osborne-Mendel rats to a single i.p. dose of DEN as an initiating agent followed by promotion with 100 mg/kg-day of 1,1,2,2-tetrachloroethane by gavage 5 days/week for 7 weeks produced a significantly increased number of GGT-positive foci in the liver.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

Studies in humans and laboratory animals are not available to determine whether early life stages are particularly susceptible to 1,1,2,2,-tetrachloroethane exposures. However, the Gulati rat study (Gulati et al., 1991b) demonstrated that fetuses exposed in utero can be adversely affected. At scheduled sacrifice, average fetal weights were statistically significantly decreased in all dose groups except the 34 mg/kg-day group. In the Gulati mouse study (Gulati et al., 1991a), complete litter resorption occurred in mice in 1/11, 0/9, 2/8, 1/1, and 1/2 dams in the 0, 987, 2,120, 2,216, and 4,575 mg/kg-day dose groups, respectively. The limited data evaluating the effect of 1,1,2,2-tetrachloroethane on the developing organism have not indicated effects on the offspring at levels that did not also produce maternal effects.

4.8.2. Possible Gender Differences

Studies evaluating the differences in potency of 1,1,2,2-tetrachloroethane in male and female rodents are not available. Some toxicity studies which evaluated both genders in the same study showed close concordance between genders with often no more than one dose distinguishing between response levels for a given effect. Men normally have a smaller volume of body fat than women, even accounting for average size differences, contributing to differential disposition of organic solvents between genders (Sato and Nakajima, 1987). Rats have pronounced sex-specific differences in CYPs, primarily involving the CYP2C family which is not found in humans, but humans have not demonstrated sex-specific isoforms of CYP (Mugford and Kedderis, 1998). Humans have differences in CYP 3A4 activity related to estrogen and progesterone, but these differences are regulated by hormones at the level of gene expression (Harris et al., 1995). Other differences may occur at the Phase 2 level attributable to conjugation. Overall, no consistent differences have been reported between women and men in the handling of xenobiotics such as 1,1,2,2-tetrachloroethane by CYP isoforms (Shimada et al., 1994). These distinctions make it difficult to predict from the animal data gender-relevant differences for human exposure to 1,1,2,2-tetrachloroethane.

4.8.3. Other Susceptible Populations

As metabolism is believed to play an important role in the toxicity of 1,1,2,2-tetrachloroethane, particularly in the liver, individuals with elevated levels of CYP enzymes may have an increased susceptibility to the compound. Halpert (1982) reported an increase in in vitro metabolite formation and in covalently bound metabolites following pretreatment with xylene or phenobarbital, both of which increased CYP activity. Sato et al. (1980) similarly reported an increased metabolism of 1,1,2,2-tetrachloroethane in rats following ethanol pretreatment. Since 1,1,2,2-tetrachloroethane has been demonstrated to inhibit CYP enzymes (Paolini et al., 1992; Halpert, 1982), presumably through a suicide inhibition mechanism, it is also possible that people coexposed to chemicals that are inactivated by CYP enzymes will be more susceptible to those compounds.

In addition, studies of human GST-zeta polymorphic variants show different enzymatic activities toward and inhibition by dichloroacetic acid that could affect the metabolism of 1,1,2,2-tetrachloroethane (Lantum et al., 2002; Blackburn et al., 2001, 2000; Tzeng et al., 2000). Dichloroacetic acid may covalently bind to GST-zeta (Anderson et al., 1999), irreversibly inhibiting one of two stereochemically different conjugates, thus inhibiting its own metabolism and leading to an increase in unmetabolized dichloroacetic acid as the dose and duration of exposure increases (U.S. EPA, 2003). GST zeta is a hepatic enzyme that also functions in the pathway for tyrosine catabolism. Populations or single individuals may be more sensitive to 1,1,2,2-tetrachloroethane toxicity depending on which GST-zeta variant they possess.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Subchronic Oral RfD

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

The data available on subchronic oral exposure to 1,1,2,2-tetrachloroethane are limited to experimental studies in animals. Although a number of case reports provide information on effects of intentional acute oral exposure to lethal oral doses of 1,1,2,2-tetrachloroethane (Mant, 1953; Lilliman, 1949; Forbes, 1943; Elliot, 1933; Hepple, 1927), no subchronic studies of oral exposure to 1,1,2,2-tetrachloroethane in humans exist. A single, well-designed 14-week subchronic study in rats and mice that tested multiple dose levels and examined an array of endpoints and tissues in rats is available (NTP, 2004). Furthermore, a developmental toxicity study in rats and mice exists (Gulati et al., 1991a, b). These studies in laboratory animals provide evidence suggesting that the liver and the developing fetus may be targets of toxicity following subchronic oral exposure to 1,1,2,2-tetrachloroethane.

NTP reported multiple effects on the livers of both male and female rats and mice following subchronic oral exposure to 1,1,2,2-tetrachloroethane. Specifically, NTP (2004) exposed F344 rats (10/sex/group) to 0, 20, 40, 80, 170, or 320 mg/kg-day (both males and females) and B6C3F$_1$ mice (10/sex/group) to 0, 100, 200, 370, 700, or 1,360 mg/kg-day (males) and 0, 80, 160, 300, 600, or 1,400 mg/kg-day (females) in the diet for 14 weeks. A statistically significant decrease in body weight gain (<10%) in both male and female rats at ≥80 mg/kg-day was observed. Low dose effects observed in the liver included statistically significantly increased relative liver weights in both male and female rats at ≥40 mg/kg-day. In addition, hepatocellular vacuolization was observed at ≥20 mg/kg-day in male rats and ≥40 mg/kg-day in female rats. The severity of vacuolization was reported to be minimal to mild. Serum enzyme activity levels of both male and female rats were also affected. For example, increases in serum ALT and SDH activity were observed at ≥80 mg/kg-day in male rats and ≥170 mg/kg-day in female rats. In addition, increased cholesterol levels and ALP activity were observed in female rats at ≥80 and 170 mg/kg-day, respectively. Additional histopathology observed in the liver included a statistically significantly increased incidence of minimal to moderate hepatocellular hypertrophy at ≥170 mg/kg-day in females and ≥200 mg/kg-day in males. Also, increased incidence of necrosis and pigmentation were observed at ≥80 mg/kg-day and hepatocellular mitotic alterations and foci of cellular alterations were observed at ≥80 and ≥170 mg/kg-day, respectively, in male rats. In females, increased incidence of hepatocellular hypertrophy was observed at ≥80 mg/kg-day, and necrosis, pigmentation, and foci of cellular alterations were reported at ≥170 mg/kg-day. Bile duct hyperplasia, increased bile acids, spleen pigmentation, and spleen atrophy were also observed in both male and female rats at the two highest doses.

Evidence of liver effects was also observed in mice by NTP (2004). A statistically significant increase in relative liver weights was observed in both male and female mice at ≥200 and 80 mg/kg-day, respectively. Increases in serum ALT and ALP activity, bile acid levels, and hepatic 5'-nucleotidase activity (males only) were observed in males and females at ≥370 and 160 mg/kg-day, respectively. The study authors also reported an increase in SDH activity at ≥200 and 80 mg/kg-day in male and female mice, respectively. Serum cholesterol levels were statistically significantly increased in female mice at ≥160 mg/kg-day. The incidence of hepatocellular necrosis was statistically significantly increased in male mice at ≥370 mg/kg-day and in female mice at ≥700 mg/kg-day. Hepatocellular hypertrophy was also reported in both genders at ≥160–200 mg/kg-day. A statistically significant increase in incidence of liver pigmentation and bile duct hyperplasia occurred at ≥300 mg/kg-day in females and ≥370 mg/kg-day in males.

In addition to effects on the liver, NTP (2004) also observed effects associated with reproduction in adult rats and mice following subchronic exposure to 1,1,2,2-tetrachloroethane at dose levels as low as 40 mg/kg-day. In male rats, sperm motility was decreased at ≥40 mg/kg-day, and higher doses resulted in decreased epididymis weight and increased atrophy of the preputial and prostate gland, seminal vesicle, and testicular germinal epithelium. In female rats, minimal to mild uterine atrophy was increased at ≥170 mg/kg-day and clitoral gland atrophy and ovarian interstitial cell cytoplasmic alterations were increased at 320 mg/kg-day. Female F344 rats in the 170 mg/kg-day group also spent more time in diestrus compared to controls. Male mice had increased incidences of preputial gland atrophy at ≥100 mg/kg-day. Less sensitive effects included decreases in absolute testes weight (≥700 mg/kg-day), absolute epididymis, and cauda epididymis weights (1,360 mg/kg-day), and a decrease in epididymal spermatozoal motility (1,360 mg/kg-day). The only noted reproductive toxicity parameter in female mice affected was a significant increase in the length of the estrous cycle at a dose of 1,400 mg/kg-day.

A developmental toxicity study by Gulati et al. (1991a) demonstrated that the developing fetus may be sensitive to 1,1,2,2-tetrachloroethane exposure. Gulati et al. (1991a) exposed pregnant CD Sprague-Dawley rats to 0, 34, 98, 180, 278, or 330 mg/kg-day 1,1,2,2-tetrachloroethane from GDs 4 through 20. Small but statistically significant decreases were observed in maternal body weight and average fetal weight at ≥98 mg/kg-day. No other maternal or fetal effects were reported by the study authors. In a second study, Gulati et al. (1991b) exposed pregnant Swiss CD-1 mice to 0, 987, 2,120, 2,216, or 4,575 mg/kg-day 1,1,2,2-tetrachloroethane from GDs 4 through 17. All animals (9/9) in the high-dose group died prior to the end of the study, precluding calculation of the average dose in this exposure group. Maternal body weights were statistically significantly decreased compared to controls at ≥2,120 mg/kg-day beginning on study day 9. Gross hepatic effects such as pale or grey and/or enlarged livers and a prominent lobulated pattern were also reported in dams from all groups except at the low dose. Complete

litter resorption occurred in 1/11, 0/9, 2/8, 1/1, and 1/2 dams in the 0, 987, 2,120, 2,216, and 4,575 mg/kg-day groups, respectively. No other developmental effects were reported. Gulati et al. (1991a, b) suggested that the developing fetus may be a target of 1,1,2,2-tetrachloroethane-induced toxicity. However, these developmental studies were conducted at doses higher than the subchronic NTP (2004) study, which demonstrated liver effects at lower doses. Therefore, Gulati et al. (1991a, b) was not selected as the principal study, and the observed reproductive effects were not selected as the critical effect following subchronic exposure to 1,1,2,2-tetrachloroethane. Nevertheless, potential PODs based on the observed developmental effects from Gulati et al. (1991a) were provided for comparison (see Section 5.1.2 and Appendix B).

In consideration of the available studies reporting effects of subchronic oral exposure to 1,1,2,2-tetrachloroethane in animals, NTP (2004) was chosen as the principal study for the derivation of the subchronic RfD. This study was conducted in both genders of two species, used five dose levels and a concurrent control group, measured a wide-range of endpoints and tissues, and provides data that were transparently and completely reported. NTP (2004) identified the liver as the most sensitive target organ of 1,1,2,2-tetrachloroethane-induced toxicity. Specifically, NTP (2004) identified effects on the liver including increased liver weight and increased incidence of hepatocellular vacuolization at low dose levels. Other liver effects observed in rats and mice at higher doses included increased liver weight, increased ALT, ALP, and SDH serum activity levels, increased bile acid levels, and an increased incidence of hepatocellular vacuolization and necrosis.

Based on the available data from the NTP (2004) study, the liver appears to be the most sensitive target organ for 1,1,2,2-tetrachloroethane-induced toxicity. Thus, the observed effects in the liver were considered in the selection of the critical effect for the derivation of the subchronic RfD. Specifically, liver effects including increased liver weight, increased ALT, ALP, and SDH serum levels, increased bile acid levels, and an increased incidence of hepatocellular vacuolization were modeled and considered for the determination of the critical effect and POD (Section 5.1.1.2 and Appendix B). EPA selected increased liver weight as the critical effect because this effect may represent a sensitive endpoint that occurs early in the process leading to hepatocellular necrosis associated with subchronic oral exposure to 1,1,2,2-tetrachloroethane; however, chemical-specific data demonstrating the relationship between increased liver weight and hepatocellular necrosis are not available. The increase in relative liver weight, as opposed to an increase in absolute liver weight, was selected because the calculation of relative liver weight takes into account the substantive, dose-dependent decreases in body weight that were observed in both genders of rats. Rats were selected as the representative species because they appeared to be more sensitive than mice to the hepatotoxic effects of 1,1,2,2-tetrachloroethane.

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

BMD modeling was conducted using the EPA's benchmark dose software (BMDS, version 2.1.1.) to analyze the hepatotoxic effects associated with subchronic exposure to 1,1,2,2-tetrachloroethane (see Appendix B for modeling details). The software was used to calculate potential PODs for deriving the subchronic RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$). For all continuous endpoints, a benchmark response (BMR) of 1SD of the control mean was considered appropriate for derivation of the RfD under the assumption that it represents a minimally biologically significant response level. A BMR of 1 standard deviation (SD) of the control mean was also included for comparative purposes. For the dichotomous data (i.e., the incidence of hepatocellular cytoplasmic vacuolization), a BMR of 10% extra risk was considered appropriate for derivation of the RfD under the assumption that it represents a minimally biologically significant response level. The effects modeled include liver weight changes, serum ALT and SDH, bile acids, hepatocellular cytoplasmic vacuolization, and rat fetal body weights. Table 5-1 summarizes the BMD modeling results for the selected toxicological endpoints.

Table 5-1. Summary of BMD model results for rats exposed to 1,1,2,2-tetra-chloroethane

Endpoint	Model	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)
Males				
Cytoplasmic vacuolization	Polynomial	10% extra risk	1.7	1.1
Relative liver weight	None	NA	NA	NA
Absolute live weight	Polynomial	1 SD	30	23
ALT	Polynomial	1 SD	41	26
SDH	None	NA	NA	NA
Bile acids	Power	1 SD	72	57
Females				
Cytoplasmic vacuolization	Weibull	10% extra risk	31	19
Relative liver weight	Polynomial	1 SD	22	15
Absolute liver weight	Polynomial	1 SD	36	26
ALT	Hill	1 SD	82	69
SDH	Power	1 SD	157	113
Bile acids	Polynomial	1 SD	188	170
Developmental				
Rat fetal weight	Linear	1 SD	83	60

Changes in hepatocellular cytoplasmic vacuolization, ALT, SDH, ALP, and bile acid serum levels from NTP (2004), as well as mean rat fetal weights from Gulati et al. (1991a), were modeled for comparison in identifying a POD. For serum ALT levels in female rats, a BMD of 82 mg/kg-day and a BMDL of 69 mg/kg-day was derived from the Hill model. For serum SDH

in female rats, a BMD of 157 mg/kg-day and a BMDL of 113 mg/kg-day was derived from the power model. The serum ALP data were not amenable to BMD modeling; a LOAEL of 160 mg/kg-day was identified. For bile acid levels in female rats, a BMD of 188 mg/kg-day and a BMDL of 170 mg/kg-day were derived from the polynomial model. BMD modeling derived a BMD of 83 mg/kg-day and a BMDL of 60 mg/kg-day from a linear model for decreased rat fetal weight.

A BMD of 31 mg/kg-day and BMDL of 19 mg/kg-day were derived from the multistage model for the increased incidence of hepatocellular cytoplasmic vacuolization in female rats. The POD for the increased incidence of hepatocellular vacuolization is approximately an order of magnitude lower than the POD for increased relative liver weight, and would result in a lower RfD than that derived for increased relative liver weight (See Sections 5.1.1.2 and 5.1.3 for more information). However, the biological significance of this effect following 1,1,2,2-tetrachloro-ethane exposure is unclear based on the following considerations. Vacuoles are defined as cavities bound by a single membrane that serve several functions, usually providing storage areas for fat, glycogen, secretion precursors, liquid, or debris (Osol, 1972). Vacuolization is defined as the process of accumulating vacuoles in a cell or the state of accumulated vacuoles (Grasso, 2002). This process can be classified as either a normal physiological response or may reflect an early toxicological process. As a normal physiological response, vacuolization is associated with the sequestration of materials and fluids taken up by cells, and also with secretion and digestion of cellular products (Henics and Wheatley, 1999). In addition, Robbins et al. (1976) characterized vacuolization (i.e., intracellular autophagy) as a normal cellular functional, homeostatic, and adaptive response.

Vacuolization is not only a normal physiological response. Vacuolization has been identified as one of four principal types of chemical-induced injury (the other three being cloudy swelling, hydropic change, and fatty change) (Grasso, 2002). It is one of the most common responses of the liver following a chemical exposure, typically in the accumulation of fat in parenchymal cells, most often in the periportal zone (Plaa and Hewitt, 1998). The ability to detect subtle ultrastructural defects, such as vacuolization, early in the course of toxicity often permits identification of the initial site of the lesion and thus can provide clues to possible biochemical mechanisms involved in the pathogenesis of liver injury (Hayes, 2001).

The hepatocellular vacuolization reported by NTP (2004) was not observed consistently across species (i.e., reported only in male and female rats); whereas the other observed liver effects were reported in both sexes of both species. In addition, NTP (2004) did not characterize the vacuole content following exposure to 1,1,2,2-tetrachloroethane. The study authors indicated that the severity of the hepatocellular vacuolization was minimal to mild and was concentration independent, but NTP (2004) did not report the localization of the vacuolization in the liver. The observed vacuolization in the liver at low doses appeared to diminish as dose increased. Specifically, hepatocellular vacuolization increased in a dose dependant manner from 20 to

80 mg/kg-day in male rats. At 80 mg/kg-day, 100% of male rats were affected, and at doses of ≥80 mg/kg-day, the incidence of vacuolization began to decrease. Concurrent with this decrease in incidence of vacuolization, an increased incidence of hepatocellular hypertrophy, necrosis, and pigmentation were observed. In female rats, the incidence of vacuolization was 100% at 40 and 80 mg/kg-day, followed by a diminished response at the two highest doses. Necrosis and pigmentation were observed in the females at the two high doses. Thus, the qualitative and quantitative biological relationship between the observed hepatocellular toxicity (i.e., hepatocellular necrosis) and the increased incidence of hepatocellular cytoplasmic vacuolization in NTP (2004) is unknown.

The BMD_{1SD} of 22 mg/kg-day and $BMDL_{1SD}$ of 15 mg/kg-day based on increased relative liver weight in the female rat was selected as the POD for the subchronic RfD. The observed changes in liver weights, serum liver enzyme levels, and hepatocellular necrosis combine to support hepatotoxicity as the major toxic effect following 1,1,2,2-tetrachloroethane exposure.

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

To derive the subchronic RfD, the $BMDL_{1SD}$ of 15 mg/kg-day for increased relative liver weight in female rats is divided by a total UF of 300. The UF of 300 comprises component factors of 10 for interspecies extrapolation, 10 for interhuman variability, and 3 for database deficiencies.

A default UF of 10 was selected to account for the interspecies variability in extrapolating from laboratory animals (rats) to humans because information was not available to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans for 1,1,2,2-tetrachloroethane.

A default UF of 10 was selected to account for intraspecies variability (UF_H) in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 1,1,2,2-tetrachloroethane in humans. However, studies of human GST-zeta polymorphic variants demonstrate different enzymatic activities toward and inhibition by dichloroacetic acid that could affect the metabolism of 1,1,2,2-tetrachloroethane (Lantum et al., 2002; Blackburn et al., 2001, 2000; Tzeng et al., 2000). Populations or single individuals may be more sensitive to 1,1,2,2-tetrachloroethane toxicity depending on which GST-zeta variant they possess. Animal toxicity studies did not show consistent sex-related differences.

An UF of 3 was selected to account for deficiencies in the database. The NTP (2004) 14-week study provides comprehensive evaluations of systemic toxicity and neurotoxicity in two species. The NTP (2004) study provides information on effects on sperm, estrous cycle, and male and female reproductive tissues in rats and mice, but the database lacks a two-generation reproductive toxicity study. Available developmental toxicity studies provide information on

embryo or fetotoxicity in orally exposed rats and mice (Gulati et al., 1991a, b), but the studies did not include skeletal and visceral examinations.

An UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR associated with a change of 1 SD from the control mean was selected under an assumption that it represents a minimally biologically significant change.

The subchronic RfD for 1,1,2,2-tetrachloroethane is calculated as follows:

Subchronic RfD	=	$BMDL_{1SD} \div UF$
	=	15 mg/kg-day ÷ 300
	=	0.05 mg/kg-day (or 5×10^{-2} mg/kg-day)

5.1.2. Chronic Oral RfD

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

Information on the chronic oral toxicity of 1,1,2,2-tetrachloroethane is limited to a 78-week cancer bioassay in rats and mice that were exposed by gavage (NCI, 1978). Interpretation of the rat study may be confounded by high incidences of endemic chronic murine pneumonia, although it is unlikely that this contributed to effects observed in the liver. Based on an increased incidence of hepatic fatty changes, the NOAEL and LOAEL for liver effects were 62 and 108 mg/kg-day, respectively. In the mouse study, a LOAEL of 142 mg/kg-day was selected for chronic inflammation in the kidneys of males, and a NOAEL of 142 mg/kg-day and a LOAEL of 284 mg/kg-day were selected for hydronephrosis and chronic inflammation in the kidneys of females, respectively.

The 14-week dietary study in rats and mice (NTP, 2004) used to derive the subchronic RfD was also considered for the derivation of the chronic RfD. The subchronic NTP (2004) study appears to be a more sensitive assay than the chronic NCI (1978) bioassay. The NTP (2004) study also uses lower dose levels and a wider dose range than the NCI (1978) study, and thereby provides a better characterization of the dose-response curve in the low-dose region. Additionally, dietary exposure is a more relevant route of exposure for the general population exposed to 1,1,2,2-tetrachloroethane in the environment than is gavage exposure. For these reasons, the NTP (2004) subchronic study was selected as the principal study.

EPA selected increased liver weight as the critical effect because this effect may represent a potential sensitive endpoint that may occur early in the process leading to hepatocellular necrosis associated with subchronic oral exposure to 1,1,2,2-tetrachloroethane. The increase in relative liver weight, as opposed to an increase in absolute liver weight, was selected because the calculation of relative liver weight takes into account the substantive, dose-dependent decreases in body weight that were observed in both sexes of rats. Additional liver effects observed included increased liver weight, increased ALT, ALP, and SDH serum levels,

increased serum bile acid levels, and increased incidence of hepatocellular vacuolization and necrosis.

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

The subchronic $BMDL_{1SD}$ of 15 mg/kg-day based on the increased relative liver weight in female rats was used as the POD for the chronic RfD. The observed increases in liver weights, serum liver enzyme levels, and incidence of hepatocellular necrosis combine to support hepatotoxicity as the critical effect of toxicity of 1,1,2,2-tetrachloroethane.

5.1.2.3. *RfD Derivation—Including Application of UFs*

To derive the chronic RfD, the subchronic $BMDL_{1SD}$ of 15 mg/kg-day, based on increased relative liver weights in female rats, was divided by a UF of 1,000. The UF of 1,000 comprises component factors of 10 for interspecies extrapolation, 10 for interhuman variability, 3 for subchronic to chronic duration extrapolation, and 3 for database deficiencies, as explained below.

A default UF of 10 was selected to account for the interspecies variability in extrapolating from laboratory animals (rats) to humans because information was not available to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans for 1,1,2,2-tetrachloroethane.

A default UF of 10 was selected to account for interindividual variability (UF_H) in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 1,1,2,2-tetrachloroethane in humans. However, studies of human GST-zeta polymorphic variants demonstrate different enzymatic activities toward and inhibition by dichloroacetic acid that could affect the metabolism of 1,1,2,2-tetrachloroethane (Lantum et al., 2002; Blackburn et al., 2001, 2000; Tzeng et al., 2000). Populations or single individuals may be more sensitive to 1,1,2,2-tetrachloroethane toxicity depending on which GST-zeta variant they possess. Animal toxicity studies which evaluated both sexes in the same study did not show consistent sex-related differences. Developmental toxicity studies in animals are limited in scope, but have not indicated effects on the offspring at levels that did not also cause maternal effects.

An UF of 3 was selected to account for extrapolation from a subchronic exposure duration study to a chronic RfD. The study selected as the principal study was a 14-week study by NTP (2004), a study duration that is minimally past the standard subchronic (90-day) study and falls well short of a standard lifetime study. In addition, some data are available to inform the nature and extent of effects that would be observed with a longer duration of exposure to 1,1,2,2-tetrachloroethane. Specifically, the available chronic cancer bioassay data (NCI, 1978) suggest that liver damage observed in F344 rats following subchronic exposure to 1,1,2,2-tetrachloroethane (NTP, 2004) (e.g., increased liver weight and incidence of necrosis, and altered

serum enzyme and bile levels) may not progress to more severe effects following chronic exposures. The chronic cancer bioassay was conducted in Osborne-Mendel rats and did not measure liver enzyme levels. However, NCI (1978) observed minimal alterations in liver pathology including inflammation, fatty metamorphosis, focal cellular change, and angiectasis in rats, and organized thrombus and nodular hyperplasia in mice. NCI (1978) reported that the study authors performed complete histological analysis on the liver, but specific endpoints assessed were not included. The available database does not abrogate all concern associated with using a subchronic study as the basis of the RfD. For these reasons, a threefold UF was used to account for the extrapolation from subchronic to chronic exposure duration for the derivation of the chronic RfD.

An UF of 3 was selected to account for deficiencies in the database. The NTP (2004) 14-week study provides comprehensive evaluations of systemic toxicity and neurotoxicity in both rats and mice. However, the database is limited by the lack of a two-generation reproductive toxicity study. The NTP (2004) study provides information on effects on sperm, estrous cycle, and male and female reproductive tissues in rats and mice, but the database lacks a two-generation reproductive toxicity study. Available developmental toxicity studies provide information on embryo or fetotoxicity in orally exposed rats and mice (Gulati et al., 1991a, b), but the studies did not include skeletal and visceral examinations.

An UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR associated with a change of 1 SD from the control mean was selected under an assumption that it represents a minimally biologically significant change.

The chronic RfD for 1,1,2,2-tetrachloroethane is calculated as follows:

$$\begin{aligned} \text{Chronic RfD} &= BMDL_{1SD} \div UF \\ &= 15 \text{ mg/kg-day} \div 1{,}000 \\ &= 0.02 \text{ mg/kg-day (or } 2 \times 10^{-2} \text{ mg/kg-day)} \end{aligned}$$

5.1.3. RfD Comparison Information

Figure 5-1 is an exposure-response array that presents NOAELs, LOAELs, and the dose range tested corresponding to selected health effects. The effects observed in the subchronic and chronic studies were considered candidates for the derivation of the sample subchronic and chronic RfDs.

In addition to the increase in relative liver weight and the increased incidence of hepatocellular cytoplasmic vacuolization, changes in absolute liver weight and serum levels of ALT and SDH, bile acid levels, and serum cholesterol levels were considered for comparison. Mean rat fetal weights observed following subchronic or chronic exposure to 1,1,2,2-tetrachloro-ethane were also considered for comparison. Table 5-2 provides a tabular summary of sample

PODs and resulting subchronic sample RfDs for these endpoints in female rats. Additionally, Figure 5-2 provides a graphical representation of this information. This figure should be interpreted with caution since the PODs across studies are not necessarily comparable, nor is the confidence the same in the data sets from which the PODs were derived. Figure 5-3 provides a graphical representation of the derivation of sample chronic RfDs for sample PODs from the subchronic data.

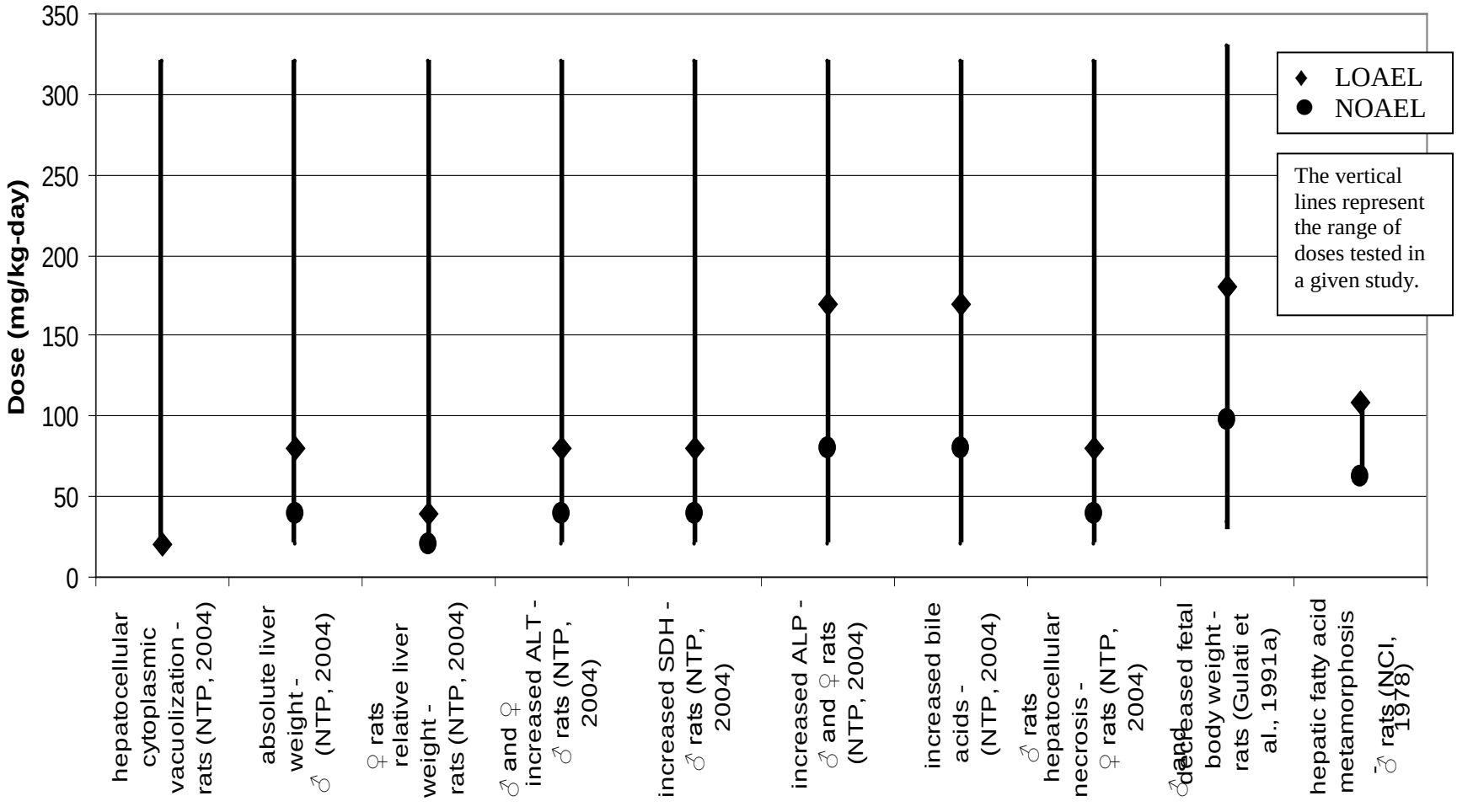


Figure 5-1. Exposure response array for subchronic and chronic oral exposure to 1,1,2,2-tetrachloroethane.

Table 5-2. Potential PODs with applied UFs and resulting subchronic RfDs

Effect	POD (mg/kg-d)	Gender and species	UFs[a]						Subchronic RfD
			A	H	L	S	D	Total	
Hepatocellular cytoplasmic vacuolization	1.1[b]	Male Rat	10	10	–	–	3	300	4×10^{-3}
Relative liver weight	15[c]	Female Rat	10	10	–	–	3	300	5×10^{-2}
Absolute liver weight	23[c]	Male Rat	10	10	–	–	3	300	8×10^{-2}
ALT	26[c]	Male Rat	10	10	–	–	3	300	9×10^{-2}
SDH	113[c]	Female Rat	10	10	–	–	3	300	0.38
Bile acids	57[c]	Male Rat	10	10	–	–	3	300	0.20
Fetal body weight	60[d]	Rat	10	10	–	–	3	300	0.20

[a]UFs: A = animal to human (interspecies); H = interindividual (intraspecies); L = LOAEL to NOAEL; S = subchronic-to-chronic duration; D = database deficiency.
[b]POD based on BMDL determined through BMD modeling of a 10% response; source: NTP (2004).
[c]POD based on BMDL determined through BMD modeling of a 1 SD response; source: NTP (2004).
[d]POD based on BMDL determined through BMD modeling of a 5% response; source: Gulati et al. (1991a).

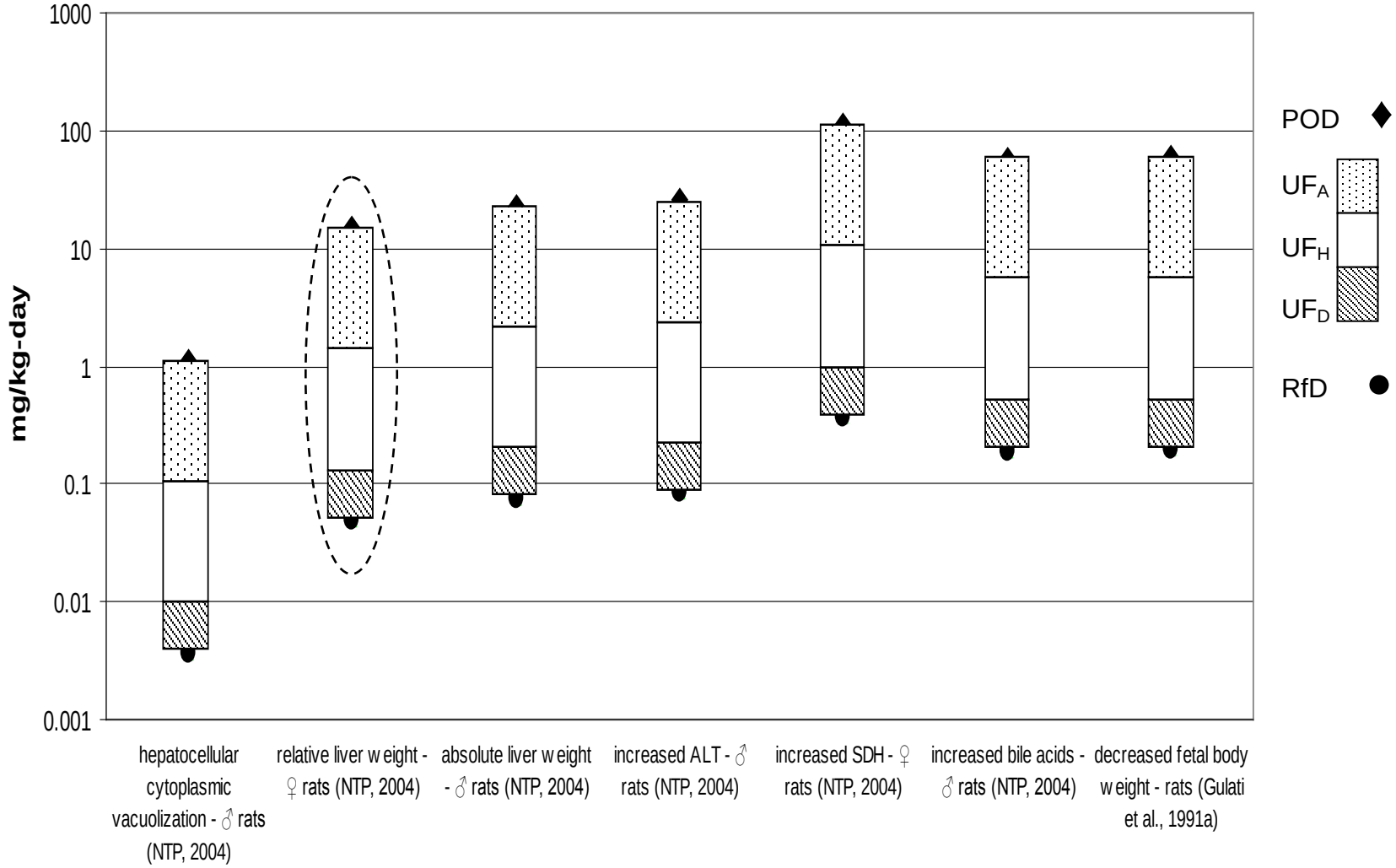


Figure 5-2. PODs for selected endpoints (with critical effect circled) from Table 5-2 with corresponding applied UFs and derived sample subchronic oral reference values (RfVs).

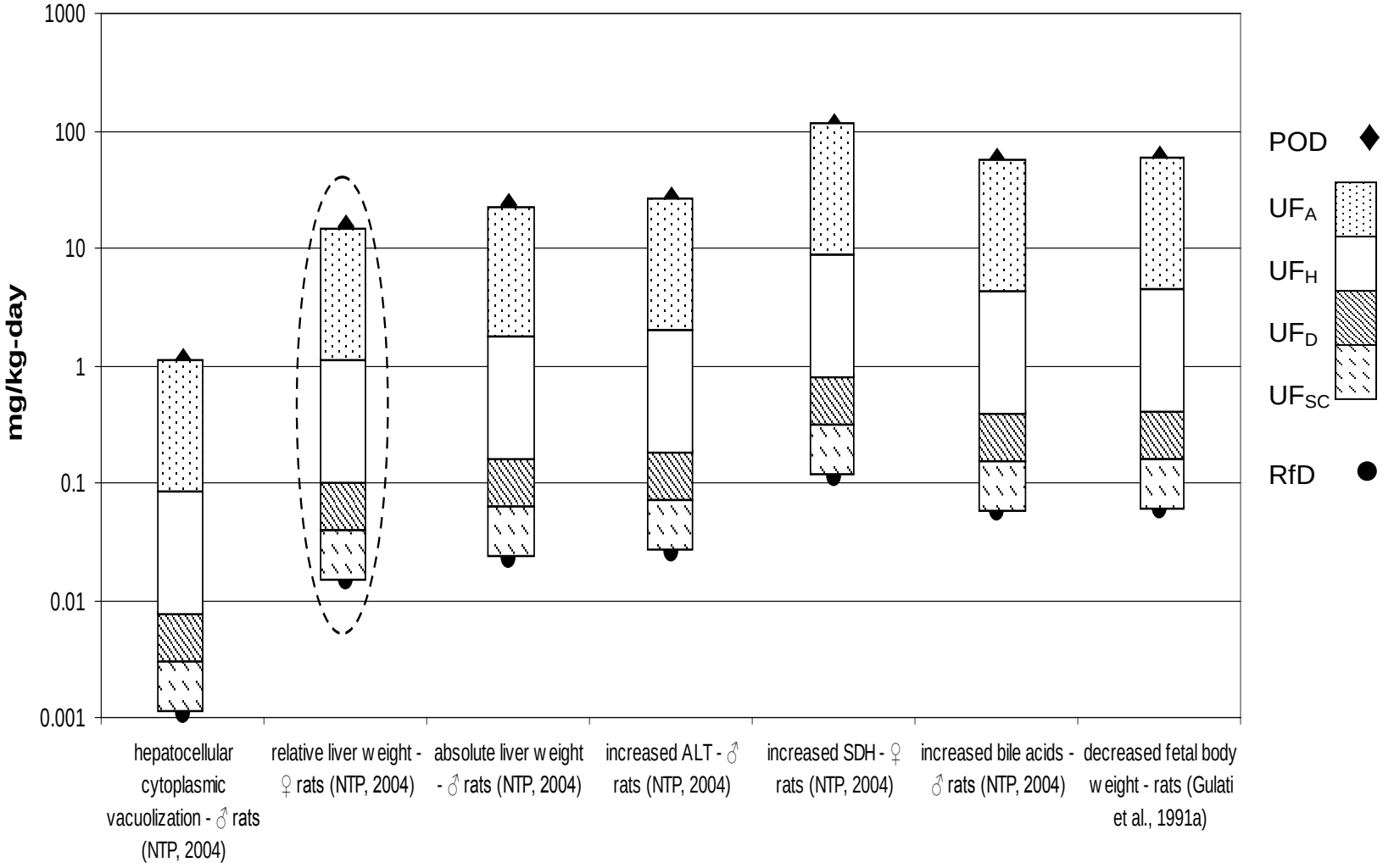


Figure 5-3. PODs for selected endpoints (with critical effect circled) from Table 5-2 with corresponding applied UFs and derived sample chronic oral reference values (RfVs).

5.1.4. Previous RfD Assessment

An oral assessment for 1,1,2,2-tetrachloroethane was not previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

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

Information on the inhalation toxicity of 1,1,2,2-tetrachloroethane is limited. In the Truffert et al. (1977) study, rats were exposed to a presumed concentration of 560 ppm (3,909 mg/m^3) for a TWA duration of 5.1 hours/day, 5 days/week for 15 weeks. Findings included transient histological alterations in the liver including granular appearance and cytoplasmic vacuolation, which were observed after 9 exposures and were no longer evident after 39 exposures. Because of the uncertainty regarding the actual exposure concentration for the single dose, and a lack of incidence and severity data, this report cannot be used to identify a NOAEL or LOAEL or for possible derivation of an RfC.

Horiuchi et al. (1962) observed fatty degeneration of the liver and splenic congestion in a single monkey exposed to a TWA of 1,974 ppm (15,560 mg/m^3) 1,1,2,2-tetrachloroethane 2 hours/day, 6 days/week for 9 months. The monkey was weak after approximately seven exposures and had diarrhea and anorexia between the 12th and 15th exposures. Beginning at the 15th exposure, the monkey was "almost completely unconscious falling upon his side" for 20–60 minutes after each exposure. Also, hematological parameters demonstrated sporadic changes in hematocrit and RBC and WBC counts, but the significance of these findings cannot be determined. This study cannot be utilized to identify a NOAEL or LOAEL due to the use of a single test animal with no control group.

Mellon Institute of Industrial Research (1947) observed an increased incidence of lung lesions and an increase in kidney weight in rats following a 6-month exposure to 200 ppm 1,1,2,2-tetrachloroethane, but these results were not evaluated because the control animals experienced a high degree of pathological effects in the kidney, liver, and lung, and because of the presence of an endemic lung infection in both controls and treated groups. Mellon Institute of Industrial Research (1947) also observed increased serum phosphatase levels and blood urea nitrogen levels in a dog exposed to 200 ppm 1,1,2,2-tetrachloroethane, compared to control values, along with cloudy swelling of the liver and the convoluted tubules of the kidney, and light congestion of the lungs. However, identification of a LOAEL or NOAEL is precluded by poor study reporting, high mortality and lung infection in the rats, and the use of a single treated animal in the dog study.

Kulinskaya and Verlinskaya (1972) observed inconsistent changes in acetylcholine levels in Chinchilla rabbits exposed to 10 mg/m^3 (1.5 ppm) 1,1,2,2-tetrachloroethane 3 hours/day, 6 days/week for 7–8.5 months. A NOAEL or LOAEL was not identified because the changes in acetylcholine were not consistent across time and incompletely quantified, and the biological significance of the change is unclear.

Shmuter (1977) observed increases in antibody levels in Chinchilla rabbits at 2 mg/m^3 1,1,2,2-tetrachloroethane and decreases in antibody levels at 100 mg/m^3. Exposure to 100 mg/m^3 1,1,2,2-tetrachloroethane also resulted in a decrease in the relative content of antibodies in the γ-globulin fraction and an increase in the T and β fractions. This is a poorly reported study that provides inadequate data, including reporting limitations, toxicological uncertainty in the endpoints, and inconsistent patterns of response, which preclude the identification of a NOAEL or LOAEL.

Effects following the chronic inhalation toxicity of 1,1,2,2-tetrachloroethane included hematological alterations and increased liver fat content in rats exposed to 1.9 ppm (13.3 mg/m^3) 4 hours/day for 265 days (Schmidt et al., 1972). Statistically significant changes included increased leukocyte (89%) and β_1-globulin (12%) levels compared to controls after 110 days, and an increased percentage of segmented nucleated neutrophils (36%), decreased percentage of lymphocytes (17%), and increased liver total fat content (34%) after 265 days. A statistically significant decrease in γ-globulin levels (32%) at 60 days postexposure and a decrease in adrenal ascorbic acid content (a measure of pituitary ACTH activity) were observed at all three time periods (64, 21, and 13%, respectively). This study is insufficient for identification of a NOAEL or LOAEL for systemic toxicity because most of the observed effects occurred at a single dose or time point, or there was a reversal of the effect at the next dose or time point. A reproductive assessment in the Schmidt et al. (1972) study was sufficient for identification of a NOAEL for the single dose tested, 1.9 ppm (13.3 mg/m^3), for reproductive effects in male rats, including percentage of mated females having offspring, littering interval, time to 50% littered, total number of pups, pups per litter, average birth weight, postnatal survival on days 1, 2, 7, 14, 21, and 84, sex ratio, and average body weight on postnatal day 84. However, macroscopic malformations or significant group differences in the other indices were not observed at 13.3 mg/m^3. The lack of information on the reproductive toxicity precludes utilizing the selected NOAEL in the derivation of the RfC.

In addition, effects of chronic exposure to 1,1,2,2-tetrachloroethane included alterations in serum acetylcholinesterase activity in rabbits exposed to 1.5 ppm (10 mg/m^3) 1,1,2,2-tetrachloroethane 3 hours/day, 6 days/week for 7–8.5 months (Kulinskaya and Verlinskaya, 1972) and immunological alterations in rabbits exposed to 0.3–14.6 ppm (2–100 mg/m^3) 3 hours/day, 6 days/week, for 8–10 months (Shmuter, 1977). These studies are inadequate for identification of NOAELs or LOAELs for systemic toxicity due to inadequate study reporting.

The inhalation toxicity database lacks a well-conducted study that demonstrates a dose-related toxicological effect following subchronic and/or chronic exposure to 1,1,2,2-tetrachloroethane. Therefore, an inhalation RfC was not derived.

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

A route-to-route extrapolation using the computational technique of Chiu and White (2006), as described in Section 3.5, was considered. However, U.S. EPA (1994b) recommends not conducting a route-to-route extrapolation from oral data when a first-pass effect by the liver or respiratory tract is expected, or a potential for a portal-of-entry effect in the respiratory tract is indicated following an analysis of the available short-term inhalation, dermal irritation, and in vitro studies, or after evaluation of the physical/chemical properties. In the case of 1,1,2,2-tetrachloroethane, a first-pass effect by the liver is expected. In addition, the presence of tissue-bound metabolites in the epithelial linings in the upper respiratory tract may demonstrate a first-pass effect by the respiratory tract (Eriksson and Brittebo, 1991). Lehmann et al. (1936) observed irritation of the mucous membranes of two humans following inhalation of 146 ppm (1,003 mg/m^3) for 30 minutes or 336 ppm (2,308 mg/m^3) for 10 minutes, indicating the potential for portal-of-entry effects in the respiratory system.

5.2.3. Previous RfC Assessment

An inhalation assessment for 1,1,2,2-tetrachloroethane was not previously available on IRIS.

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

The following discussion identifies some uncertainties associated with the RfD for 1,1,2,2-tetrachloroethane. As presented earlier (Sections 5.1.2 and 5.1.3; 5.2.2 and 5.2.3), EPA standard practices and RfC and RfD guidance (U.S. EPA, 1994b) were followed in applying a UF approach to a POD, a $BMDL_{1SD}$ for the subchronic and chronic RfDs. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal bioassay to human exposure, a diverse human population of varying susceptibilities, and database deficiencies. These extrapolations are carried out with standard approaches given the lack of extensive experimental and human data on 1,1,2,2-tetrachloroethane to inform individual steps.

An adequate range of animal toxicology data is available for the hazard assessment of 1,1,2,2-tetrachloroethane, as described in Section 4. Included in these studies are short-term and long-term bioassays and a developmental toxicity bioassay in rats and mice, as well as numerous supporting genotoxicity and metabolism studies. Toxicity associated with oral exposure to 1,1,2,2-tetrachloroethane is observed in the liver, kidney, and developing organism, including decreased fetal body weight and increased number of litter resorptions.

Consideration of the available dose-response data to determine an estimate of oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 14-week oral dietary study in rats (NTP, 2004) and increased relative

liver weight in females as the principal study and critical effect, respectively, for deriving the subchronic and chronic RfDs for 1,1,2,2-tetrachloroethane. The NTP (2004) data demonstrate hepatocellular damage including increased liver weight, increased serum liver enzyme levels, and increased incidence of hepatic necrosis. Increased liver weight was chosen as the critical effect because it may represent a sensitive indicator of 1,1,2,2,-tetrachloroethane-induced hepatoxicity and occurs at a dose lower than the observed overt liver necrosis. However, chemical-specific data demonstrating a relationship between increased liver weight and hepatocellular necrosis is not available. The increase in relative liver weight was selected as the basis for the selection of the POD because this analysis takes into account the substantive, dose-dependent decreases in body weight that were observed in both sexes of rats. The dose-response relationships between oral exposure to 1,1,2,2-tetrachloroethane and fetal body weight in rats and mice are also suitable for deriving an RfD, but are associated with BMDLs that are less sensitive than the selected critical effect and corresponding BMDL.

For comparison purposes, Figure 5-2 presents potential PODs, applied UFs, and derived potential RfDs for the additional endpoints that were modeled using the EPA's BMDS, version 2.1.1. The additional endpoints included increased absolute liver weight, changes in serum ALT and SDH, increased bile acids, and increased incidence of hepatocellular necrosis, all of which support the liver as the target of 1,1,2,2-tetrachloroethane-induced toxicity following oral exposure. A decrease in rat fetal weight was also modeled. The change in serum ALP was modeled, but a model with adequate fit was not available.

The selection of the BMD model for the quantitation of the RfD does not lead to significant uncertainty in estimating the POD, since benchmark effect levels were within the range of experimental data. However, the selected model, the polynomial model, does not represent all possible models one might fit, and other models could be selected to yield more extreme results, both higher and lower than those included in this assessment.

Extrapolating from animals to humans embodies further issues and uncertainties. An effect and its magnitude associated with the concentration at the POD in rodents are extrapolated to human response. Pharmacokinetic models are useful in examining species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not possible for the toxicity observed following oral and inhalation exposure to 1,1,2,2-tetrachloroethane. Additional interspecies uncertainty may arise from the rate of metabolism across species, as it has been demonstrated that mice have greater metabolic capacity following exposure to tetrachloroethylene than rats and humans (Reitz et al., 1996). Reitz et al. (1996) demonstrated that mice possessed a greater relative ability to metabolize tetrachloroethylene than rats and humans, and, although data are not available, a similar situation may exist for 1,1,2,2-tetrachloroethane.

Heterogeneity among humans is another uncertainty associated with extrapolating from animals to humans. Uncertainty related to human variation needs to be considered; also,

uncertainties in extrapolating from a subpopulation, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population need to be addressed. In the absence of 1,1,2,2-tetrachloroethane-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation in the derivation of the RfD. Human variation may be larger or smaller; however, 1,1,2,2-tetrachloroethane-specific data to examine the potential magnitude of over- or underestimation are unavailable.

Extrapolating from subchronic PODs to derive chronic reference values (RfVs) is also an uncertainty encountered in this assessment. A threefold UF was selected to account for extrapolation from a subchronic exposure duration study to a chronic RfD. Based on the available data for 1,1,2,2-tetrachloroethane, the toxicity observed in the liver does not appear to increase over time. The use of data from a subchronic study to derive a chronic RfD becomes a concern when the damage, in this case hepatoxicity, has the potential to accumulate; however, if the progression of the effect is not apparent, a reduced UF may be considered (U.S. EPA, 1994b). Specifically, liver damage observed in F344 rats following subchronic exposure to 1,1,2,2-tetrachloroethane (NTP, 2004) (e.g., increased incidence of necrosis or altered serum enzyme and bile levels) did not progress to more severe effects such as cirrhosis or major liver disease following chronic exposures (NCI, 1978). NCI (1978) observed minimal alterations in liver pathology including inflammation, fatty metamorphosis, focal cellular change, and angiectasis in rats, and organized thrombus and nodular hyperplasia in mice. Therefore, the available database does not abrogate all concern associated with using a subchronic study as the basis of the RfD, but supports the utilization of a database UF of 3.

Data gaps have been identified that are associated with uncertainties in database deficiencies specific to the developmental and reproductive toxicity of 1,1,2,2-tetrachloroethane following oral exposure. The developing fetus may be a target of toxicity, and the absence of a study specifically evaluating the full range of developmental toxicity endpoints represents an area of uncertainty or gap in the database. The database of inhalation studies is of particular concern due to the paucity of studies, especially subchronic and chronic studies, a multi-generational reproductive study, and a developmental toxicity study.

5.4. CANCER ASSESSMENT

As discussed in Section 4.7, under U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,1,2,2-tetrachloroethane is "likely to be carcinogenic to humans" based on data from an oral cancer bioassay in male and female Osborne-Mendel rats and $B6C3F_1$ mice (NCI, 1978) demonstrating an increase in the incidence of hepatocellular carcinomas in both sexes of mice. In this study, the incidence of hepatocellular carcinomas was statistically significantly increased in both sexes of $B6C3F_1$ mice at 142 (13/50 males; 30/48 females) and 284 mg/kg-day (44/49 males; 43/47 females), with incidences in the male

and female controls of 3/36 and 1/40, respectively. NCI (1978) also demonstrated a decrease in the time to tumor in both sexes of mice. Male rats exhibited an increased incidence in hepatocellular carcinomas, characterized as rare tumors, but the increased incidence was not statistically significantly different from controls. NCI (1978) has characterized the carcinogenic results in male rats as "equivocal."

The epidemiological human data available are inadequate for evaluation for cancer risk (IARC, 1999). There are a limited number of positive results from genotoxicity studies which suggest that 1,1,2,2-tetrachloroethane treatment in animals can result in UDS (Miyagawa et al., 1995), chromosomal aberrations (McGregor, 1980), SCE (NTP, 2004; Colacci et al., 1992), and micronucleus formation (NTP, 2004). The ability of 1,1,2,2,-tetrachloroethane to alkylate enzymatically purified hepatic DNA was observed following a single oral dose of 150 mg/kg of 1,1,2,2-tetrachloroethane in B6C3F_1 mice (Dow Chemical Company, 1988). 1,1,2,2-Tetrachloroethane may have tumor initiating and promoting activity in mammalian cells (Colacci et al., 1996, 1992; Milman et al., 1988; Story et al., 1986).

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

The only carcinogenicity bioassay for 1,1,2,2-tetrachloroethane is a chronic gavage study in Osborne-Mendel rats and B6C3F_1 mice performed by NCI (1978). This study was conducted in both sexes in two species with an adequate number of animals per dose group, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Selection of doses was aided by range-finding toxicity tests. The rat study did not identify statistically significant increases in tumor incidences in males or females. Three rare liver tumors in high-dose male rats were noted. Limitations in the study included increased mortality in male and female mice, the variable doses given to the mice over the course of the 78-week exposure period, and the exposure duration of the study (78 weeks) was less than the standard 104 week chronic exposure duration. In the high-dose male mice, acute toxic tubular nephrosis was characterized as the cause of death in the mice that died prior to study termination, although hepatocellular carcinomas were observed in most of these mice.

The mouse study identified statistically significant, dose-related increases in the incidences of hepatocellular carcinomas in both sexes. Based on these increases in hepatocellular carcinomas, NCI (1978) concluded that orally administered 1,1,2,2-tetrachloroethane is a liver carcinogen in male and female B6C3F_1 mice. NCI (1978) stated that there was no evidence for carcinogenicity of 1,1,2,2-tetrachloroethane in Osborne-Mendel rats (NCI, 1978). The tumor data in mice from the NCI study was used for dose-response analysis for oral exposure.

5.4.2. Dose-response Data

Data on the incidences of hepatocellular carcinomas in male and female mice from the NCI (1978) study were used for cancer dose-response assessment. These data are shown in Table 5-3. The control data were pooled from vehicle control groups. The cancer bioassay for 1,1,2,2-tetrachloroethane demonstrated evidence of increased incidence of tumors in both sexes of one species.

Table 5-3. Incidences of hepatocellular carcinomas in B6C3F$_1$ mice used for dose-response assessment of 1,1,2,2-tetrachloroethane

Sex	Dose (mg/kg-d)[a]		
	0	142	284
Male	3/36[b]	13/50	44/49
Female	1/40[b]	30/48	43/47

[a]TWA dose administered by gavage 5 d/wk for 78 wks.
[b]Pooled vehicle (corn oil) control groups from this and another concurrent bioassay. Pooling based on identical housing and care, similar spontaneous tumor rates, placed on test at about the same time, and examined by the same pathologists.

Source: NCI (1978).

5.4.3. Dose Adjustments and Extrapolation Method(s)

Conversion of the doses in the NCI (1978) mouse study to human equivalent doses (HEDs) to be used for dose-response modeling was accomplished in three steps. The mice were treated with 1,1,2,2-tetrachloroethane by gavage 5 days/week for 78 weeks and then observed untreated for 12 weeks for a total study duration of 90 weeks. Because the reported TWA doses were for a 5 day/week, 78 week exposure, they were duration-adjusted to account for the partial week exposure (by multiplying by 5 days/7 days) and untreated observation period (by multiplying by 78 weeks/90 weeks). These duration-adjusted animal doses were then converted to HEDs by adjusting for differences in body weight and lifespan between humans and mice. In accordance with the U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, a factor of $BW^{3/4}$ was used for cross-species scaling. Because the study duration (90 weeks) was less than the animal lifespan (104 weeks), the scaled dose was then multiplied by the cubed ratio of experimental duration to animal lifespan to complete the extrapolation to a lifetime exposure in humans. The equation and data used to calculate the HEDs are presented below, and the calculated HEDs are presented in Table 5-4.

$$HED = Dose^{*} \times (W/70\ kg)^{1/4} \times (Le/L)^{3}$$

Where:

Dose = average daily animal dose (* TWA converted for 5/7 days, 78/90 weeks)
W = average animal body weight (0.030 kg for male and female B6C3F$_1$ mice [U.S. EPA, 1988]).
70 kg = reference human body weight (U.S. EPA, 1988)
Le = duration of experiment (90 weeks)
L = reference mouse lifespan (104 weeks) (U.S. EPA, 1988)

Table 5-4. HEDs corresponding to duration-adjusted TWA doses in mice

	Dose (mg/kg-d)		
Duration-adjusted dose in male and female mice (mg/kg-d)	0	87.9	175.8
HED for use with both male and female mouse incidence data (mg/kg-d)	0	8.22	16.5

The mode of action of 1,1,2,2-tetrachloroethane carcinogenicity is unknown. It appears that metabolism to one or more active compounds is likely to play a role in the development of the observed liver tumors, but insufficient data preclude proposing a specific mode of action. Dichloroacetic acid, a metabolite of 1,1,2,2-tetrachloroethane, induces hepatocellular carcinomas in male and female B6C3F$_1$ mice and F344 rats. Trichloroethylene (NTP, 1990; NCI, 1976) and tetrachloroethylene (NTP, 1996; NCI, 1977), also metabolites of 1,1,2,2-tetrachloroethane, have also been shown to be hepatocarcinogens in rodents.

Results of genotoxicity and mutagenicity studies of 1,1,2,2-tetrachloroethane are mixed and insufficient for informing whether 1,1,2,2-tetrachloroethane carcinogenicity is associated with a mutagenic mode of action. Given that the mechanistic and other information available on cancer risk from exposure to 1,1,2,2-tetrachloroethane is sparse and that the existing data do not inform the mode of action of carcinogenicity, a linear low-dose extrapolation was conducted as a default option for the derivation of the oral slope factor.

Dose-response modeling was performed to obtain a POD for quantitative assessment of cancer risk. The data sets for hepatocellular carcinoma in both sexes of mice were modeled for determination of the POD. In accordance with the U.S. EPA (2005a) cancer guidelines, the BMDL$_{10}$ (lower bound on dose estimated to produce a 10% increase in tumor incidence over background) was estimated by applying the multistage cancer model in the EPA's BMDS (version 2.1.1.) for the dichotomous incidence data and selecting the results of the model that best characterized the cancer incidences. The BMD modeling of the male mouse data did not achieve adequate model fit for any of the dichotomous models; thus, a cancer slope factor was not derived from the male data. The 1° multistage model was selected for the derivation of the cancer slope factor from the female data because this model provided adequate model fit and the lowest Akaike's Information Criterion (AIC) when compared to the results of the 2° multistage model. In addition, the 2° multistage model had insufficient degrees of freedom (DF) to test the

goodness-of-fit. The BMDL of 0.65 mg/kg-day from the modeling of the tumor incidence data in female mice was selected as the POD for use in calculation of an oral slope factor (Table 5-5). Details of the BMD modeling are presented in Appendix C.

Table 5-5. Summary of human equivalent BMDs and BMDLs based on hepatocellular carcinoma incidence data in female B6C3F$_1$ mice

	BMR (% extra risk)	BMD (mg/kg-d)[a]	BMDL$_{10}$ (mg/kg-d)[a]
Female mice	10	0.81	0.65

[a]HED.

5.4.4. Oral Slope Factor and Inhalation Unit Risk

The oral slope factor was derived from the BMDL$_{10}$ (the lower bound on the exposure associated with a 10% extra cancer risk) by dividing the BMR by the BMDL$_{10}$, and represents an upper bound on cancer risk associated with a continuous lifetime exposure to 1,1,2,2-tetrachloroethane. In accordance with the U.S. EPA (2005a) guidelines, an oral slope factor (mg/kg-day)$^{-1}$ was calculated by dividing the human equivalent BMDL$_{10}$ into 0.1 (10%) (Appendix C). The BMDL$_{10}$ is 0.65 mg/kg-day, and the cancer slope factor (the slope of the linear extrapolation from the BMDL$_{10}$ to 0) is 0.10/0.65 = 0.2 per mg/kg-day. The slope of the linear extrapolation from the central estimate (i.e., BMD) is 0.1/0.81 mg/kg-day or 0.1 per mg/kg-day.

In the absence of any suitable data on the carcinogenicity of 1,1,2,2-tetrachloroethane via the inhalation route, an inhalation unit risk has not been derived.

5.4.5. Uncertainties in Cancer Risk Values

Extrapolation of data from animals to estimate potential cancer risks to human populations from exposure to 1,1,2,2-tetrachloroethane yields uncertainty. Several types of uncertainties may be considered quantitatively, but other important uncertainties cannot be considered quantitatively. Thus, an overall integrated quantitative uncertainty analysis is not presented. This section and Table 5-6 summarize the principal uncertainties.

Table 5-6. Summary of uncertainty in the 1,1,2,2-tetrachloroethane cancer risk assessment

Consideration/ approach	Impact on oral slope factor	Decision	Justification
Low-dose extrapolation procedure	Departure from U.S. EPA's *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ slope factor an unknown extent	Multistage cancer model to determine POD, linear low-dose extrapolation from POD	Available mode of action data do not inform selection of dose-response model; linear approach used in absence of an alternative as per U.S. EPA's *Guidelines for Carcinogen Risk Assessment*.
Dose metric	Alternatives could ↑ or ↓ slope factor by an unknown extent	Used administered exposure	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not clearly identified.
Cross-species scaling	Alternatives could ↓ or ↑ slope factor (e.g., 3.5-fold ↓ [scaling by BW] or twofold ↑ [scaling by $BW^{2/3}$])	$BW^{3/4}$	There are no data to support alternatives. Because the dose metric was not an AUC, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks.
Statistical uncertainty at POD	↓ slope factor if MLE of the POD is used rather than lower bound on POD (i.e., LEC)	LEC (method for calculating reasonable upper bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on administered exposure.
Bioassay	Alternatives could ↑ or ↓ slope factor by an unknown extent	NCI study	Alternative bioassays were unavailable.
Species/gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Female mice liver cancer	There are no mode of action data to guide extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across species. Generally, direct site concordance is not assumed; consistent with this view, some human tumor types are not found in rodents, and rat and mouse tumor types also differ.
Human relevance of mouse tumor data	Human relevance of mouse tumor data could ↓ slope factor	Liver tumors in mice are relevant to human exposure	1,1,2,2-Tetrachloroethane is carcinogenic through an unknown mode of action.
Human population variability in metabolism and response/sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity, including whether children are more sensitive. Metabolic activation mode of action (if fully established) could indicate ↑ or ↓ early-life susceptibility.

LEC = lower confidence limit on a concentration producing a given effect; MLE = maximum likelihood estimate

Choice of low-dose extrapolation approach. The mode of action is a key consideration in clarifying how risks at low-dose exposures should be estimated. A linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,1,2,2-tetrachloroethane

exposure due to the unavailability of data that supports any specific mode of carcinogenic action for 1,1,2,2-tetrachloroethane.

The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the mode of action for 1,1,2,2-tetrachloroethane were known is of interest, but data on the mode of action of 1,1,2,2-tetrachloroethane are not available.

Dose metric. 1,1,2,2-Tetrachloroethane is metabolized to intermediates with carcinogenic potential. Dichloroacetic acid is recognized as hepatocarcinogenic in male $B6C3F_1$ mice and F344 rats (U.S. EPA, 2003). However, it is unknown whether a metabolite or some combination of parent compound and metabolites is responsible for the observed toxicity. If the actual carcinogenic moiety is proportional to administered exposure, then use of administered exposure as the dose metric is the least biased choice. On the other hand, if this is not the correct dose metric, then the impact on the slope factor is unknown.

Cross-species scaling. An adjustment for cross-species scaling ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between the rodent species and humans, consistent with the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). It is assumed that equal risks result from equivalent constant lifetime exposures.

Statistical uncertainty at the POD. Parameter, or probabilistic, uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the multistage cancer model applied to the female mice data, there is a reasonably small degree of uncertainty at a 10% increase in tumor incidence (the POD for linear low-dose extrapolation).

Bioassay selection. The study by NCI (1978) was used for development of an oral slope factor. This study was conducted in both sexes in two species with an adequate number of animals per dose group, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Alternative bioassays were unavailable. Both genders of mice exhibited liver tumors. Uncertainties associated with the use of this study in the derivation of the oral slope factor arise, primarily, from the study design. The dose levels used in the study were poorly selected and were modified over the exposure duration, and the exposure duration of the study (78 weeks) was less than the standard 104-week chronic exposure duration. In addition, the bolus nature of the 1,1,2,2-tetrachloroethane gavage exposures in NCI (1978), as well as the use of corn oil as the gavage vehicle, may lead to more pronounced irritation, inflammation, cell death, and an eventual increase in tumor incidence at portals of entry; however, chemical-specific data demonstrating this progression are not available. There was also an increased incidence of endemic chronic murine pneumonia in male and female rats and mice, and while interpretation of this study is complicated by the chronic murine pneumonia, it is unlikely to have contributed to the carcinogenicity results observed in male and female rats.

Choice of species/gender. The oral slope factor for 1,1,2,2-tetrachloroethane was quantified using the tumor incidence data for female mice. The hepatocelluar carcinoma data in

male mice demonstrated tumorigenicity, but the data in male mice did not achieve adequate model fit for any of the dichotomous models when BMD modeled. The male and female rat tumor incidence data were not suitable for deriving low-dose quantitative risk estimates, and NCI described the rat strain as relatively insensitive to the carcinogenic effects of chlorinated organic compounds.

Relevance to humans. The oral slope factor is derived from the incidence of hepatocellular carcinomas in female $B6C3F_1$ mice. Using liver tumors in $B6C3F_1$ mice as the model for human carcinogenesis is a concern because of the prevalence of and susceptibility to developing liver tumors in this strain of mice, which may result in the derivation of an oral slope factor that is overly health protective in relation to human risk assessment. Hasemen et al. (1998) reported an increased liver carcinoma rate of 17.9 and 8.4% for male and female $B6C3F_1$ mice, respectively, from NTP carcinogenicity feeding bioassays, and a combined adenoma and carcinoma rate of 42 and 24% for male and female $B6C3F_1$ mice, respectively. However, the incidence in the control $B6C3F_1$ mice in NCI (1978) was 1/18 in the male vehicle controls and 0/20 in the female vehicle controls, and 3/36 and 1/40 in male and female pooled-vehicle controls, respectively, and comparison of an experimental group with its concurrent controls has been considered to be the most appropriate comparison (Haseman et al., 1992; Tarone et al., 1981; Gart et al., 1979 as cited in Haseman et al., 1998; Goodman et al., 1980).

Additional interspecies uncertainty may arise from the rate of metabolism across species. Reitz et al. (1996) demonstrated that mice possessed a greater relative ability to metabolize tetrachloroethylene than rats and humans, and, although data are not available, a similar situation may exist for 1,1,2,2-tetrachloroethane.

In addition, the genotoxicity and mutagenicity studies provide limited evidence of a mutagenic mode of action, with 1,1,2,2-tetrachloroethane displaying equivocal results of mutagenic activity. There are inadequate data to support any mode of action hypothesis.

Human population variability. The extent of interindividual variability in animals for 1,1,2,2-tetrachloroethane metabolism has not been characterized. A separate issue is that the human variability in response to 1,1,2,2-tetrachloroethane is also unknown. This lack of understanding about potential differences in metabolism and susceptibility across exposed animal and human populations, thus, represents a source of uncertainty.

5.4.6. Previous Cancer Assessment

In the previous IRIS assessment, posted to the IRIS database in 1987, 1,1,2,2-tetrachloroethane was characterized as "Classification — C; possible human carcinogen" based on the increased incidence of hepatocellular carcinomas in mice observed in the NCI (1978) bioassay. An oral slope factor of 0.2 $(mg/kg\text{-}day)^{-1}$ was derived using the increased incidence of hepatocellular carcinomas in female mice (NCI, 1978) and a linearized multistage approach.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

1,1,2,2-Tetrachloroethane (CAS No. 79-34-5) has been used as an insecticide, fumigant, and weed killer (Hawley, 1981), although it presently is not registered for any of these purposes. It was once used as an ingredient in an insect repellent, but registration was canceled in the late 1970s. In the past, the major use for 1,1,2,2-tetrachloroethane was in the production of trichloroethylene, tetrachloroethylene, and 1,2-dichloroethylene (Archer, 1979). It was also used as a solvent, in cleaning and degreasing metals, in paint removers, varnishes, and lacquers, in photographic films, and as an extractant for oils and fats (Hawley, 1981). With the development of new processes for manufacturing chlorinated ethylenes, the production of 1,1,2,2-tetrachloroethane as a commercial end-product in the United States and Canada steadily declined since the late 1960s and had ceased by the early 1990s (NLM, 2009; Environment Canada and Health Canada, 1993). 1,1,2,2-Tetrachloroethane may still appear as a chemical intermediate in the production of a variety of other common chemicals.

1,1,2,2-Tetrachloroethane is well absorbed from the respiratory and gastrointestinal tracts, is rapidly and extensively metabolized, and is eliminated mainly as metabolites in the urine and breath. Both reductive and oxidative metabolisms occur, producing reactive radical and organochlorine intermediates, respectively. Trichloroethanol, trichloroacetic acid, and dichloroacetic acid are initial metabolites that subsequently yield glyoxalic acid, oxalic acid, and carbon dioxide.

A limited amount of information is available addressing the toxicity of 1,1,2,2-tetrachloroethane in humans. CNS depression was the predominant effect of high-dose acute oral and inhalation exposures, although acute inhalation also caused irritation of the mucous membranes. Occupational studies suggest that repeated exposure to 1,1,2,2-tetrachloroethane can affect the liver and the nervous system.

Animal studies have established that the CNS and liver are the main targets of toxicity at high levels of oral and inhalation exposures. Death in laboratory animals typically was preceded by signs of CNS depression (e.g., lethargy, incoordination, loss of reflexes, depressed respiration, prostration, and loss of consciousness), and postmortem examinations mainly showed fatty degeneration in the liver. The most sensitive target of sublethal ingestion and inhalation appears to be the liver, and short-term and subchronic exposures caused hepatic effects that included serum chemistry changes, hepatocellular degeneration, and other histopathological alterations. Comprehensive neurobehavioral testing in 14-week feeding studies showed no effects in rats or mice, indicating that the liver was more sensitive than the nervous system for subchronic oral exposure (Chan, 2004). A limited amount of information is available

on other effects of 1,1,2,2-tetrachloroethane. Reduced body weight gain and weight loss were effects of repeated oral exposures in rats and mice that generally occurred at high doses and may have contributed to mild anemia and atrophy in the spleen, bone, bone marrow, and reproductive tissues in these animals. Kidney lesions (acute toxic tubular necrosis and chronic inflammation) occurred in mice that were chronically exposed to oral doses that also caused reduced survival. Adequate immunological testing of 1,1,2,2-tetrachloroethane has not been performed.

The reproductive and developmental toxicity of 1,1,2,2-tetrachloroethane has not been adequately evaluated. Significant decreases in maternal and fetal body weights were observed in rats. In mice, litter resorption was observed along with high maternal mortality. Toxicity to reproductive tissues following 1,1,2,2-tetrachloroethane exposure to adult rats and mice was observed in F344 rats and $B6C3F_1$ mice. Effects observed in rats and/or mice include: decreased sperm and spermatozoal motility; decreased testis and epididymal weight; increased atrophy of the preputial and prostate gland, seminal vesicle, testicular germinal epithelium, uterus, and clitoral gland; ovarian interstitial cell cytoplasmic alterations; and lengthened estrus cycle. Chronic low-level inhalation caused no effects on reproductive function in male mice, but multigeneration or other tests of reproductive function in females have not been conducted for any route of exposure. Developmental toxicity was assessed in rats and mice that were gestationally exposed to 1,1,2,2-tetrachloroethane in the diet. These studies did not include examinations for skeletal or visceral abnormalities, although effects that included reduced fetal body weight gain in rats and litter resorptions in mice occurred at doses that were maternally toxic.

The carcinogenicity of 1,1,2,2-tetrachloroethane was evaluated in a chronic gavage study in rats and mice conducted by NCI (1978). Hepatocellular carcinomas were induced in male and female mice, but there were no statistically significant increases in tumor incidences in the rats. Three rare tumors in high dose male rats were noted. Thus, 1,1,2,2-tetrachloroethane is "likely to be carcinogenic to humans" by any route of exposure, according to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

The NTP (2004) study was selected as the principal study because it was a well-designed, subchronic dietary study, conducted in both sexes in two rodent species with a sufficient number of animals per dose group. The number of test animals allocated among three dose levels and an untreated control group was acceptable, with examination of appropriate toxicological endpoints in both sexes of rats and mice. The liver was the most sensitive target in both species, and the rats were more sensitive than the mice. In addition to the observed liver weight increases, there is evidence of hepatocellular effects including increased serum liver enzyme levels and an increased incidence of both hepatocellular cytoplasmic vacuolization and necrosis from the

subchronic NTP (2004) study. EPA selected increased liver weight as the critical effect because this effect may represent an indicator of liver toxicity that occurs early in the process leading to hepatocellular necrosis associated with subchronic oral exposure to 1,1,2,2-tetrachloroethane; however, chemical-specific data demonstrating the relationship between increased liver weight and hepatocellular necrosis is not available.

Potential PODs for a subchronic RfD were derived by BMD modeling of dose-response data for increases in liver weight, increases in serum levels of ALT, SDH, and ALP, increased levels of bile acids, and increased incidence of hepatocellular cytoplasmic vacuolization in rats. All available dichotomous models in the EPA's BMDS (version 2.1.1) were fit to the incidence data for hepatocellular cytoplasmic vacuolization, and all available continuous models in the software were applied to the data for liver weight and serum enzyme levels, as well as the data for rat fetal body weight. A BMR of 10% (10% extra risk above control) was selected for derivation of the BMDL for hepatocellular cytoplasmic vacuolization in female rats, and a BMR of 1 SD (a change in the mean equal to 1 SD from the control mean) was selected for the derivation of the BMDL for the continuous female rat liver weight and rat fetal body weight data.

The BMD_{1SD} of 22 mg/kg-day and $BMDL_{1SD}$ of 15 mg/kg-day based on the relative liver weight effects seen in the female rat was selected as the POD for the derivation of the RfD. To derive the subchronic RfD, the 15 mg/kg-day $BMDL_{1SD}$ based on female rat relative liver weight was divided by a total UF of 300, yielding a subchronic RfD of 0.05 mg/kg-day. The UF of 300 comprises component factors of 10 for interspecies extrapolation, 10 for interhuman variability, and 3 for database deficiencies.

The choice of BMD model is not expected to introduce a considerable amount of uncertainty in the risk assessment since the chosen response rate of 1 SD is within the observable range of the data. Additional BMD modeling for other amenable data sets, including serum liver enzyme levels, liver lesions, and fetal body weight, were also conducted to provide other PODs for comparison purposes (see Appendix B). A graphical representation of these potential PODs and resulting subchronic RfVs is shown below in Figure 6-1.

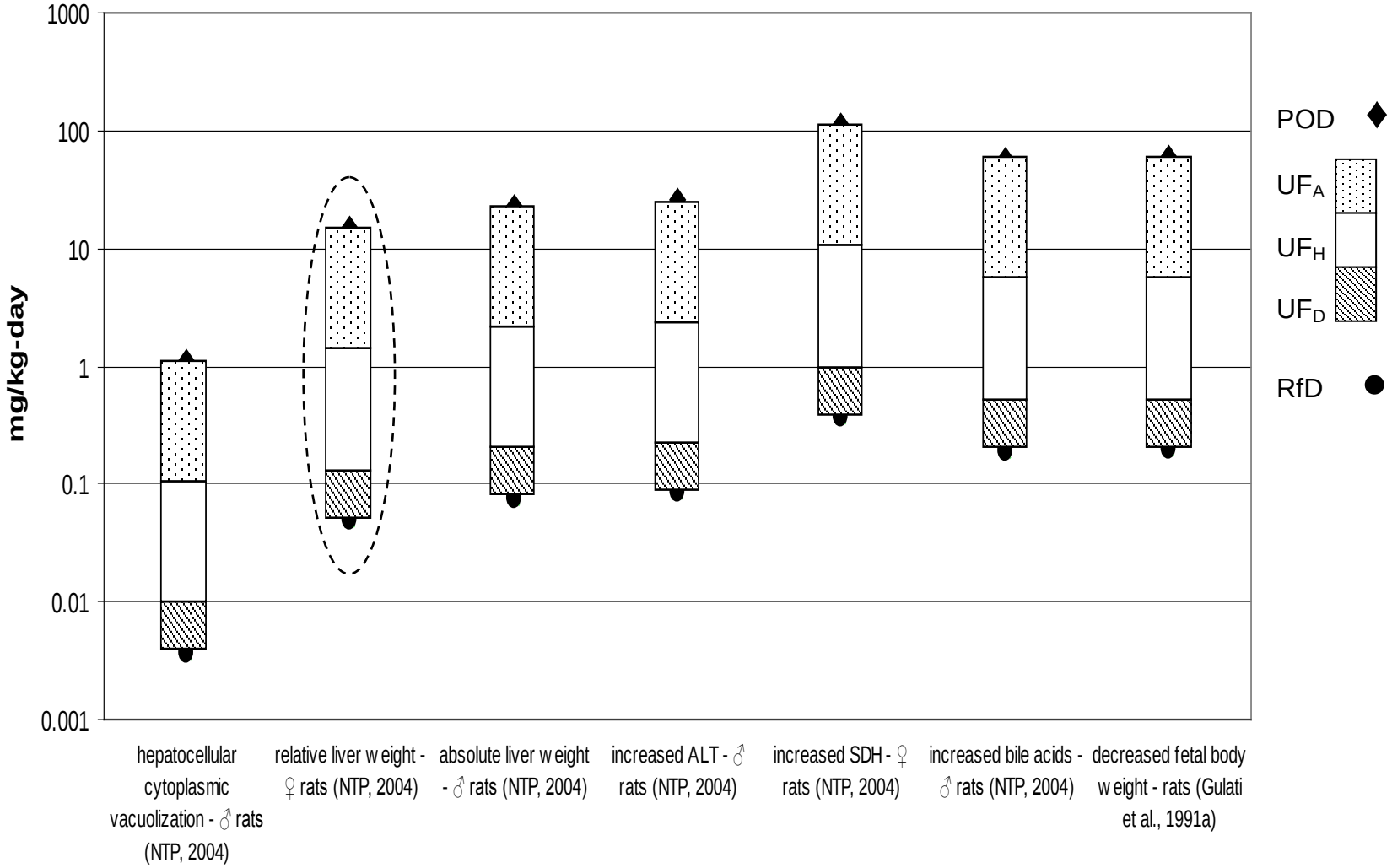


Figure 6-1. PODs for selected endpoints (with critical effect circled) with corresponding applied UFs and derived sample subchronic oral RfVs.

The default UF of 10 for the extrapolation from animals to humans is a composite of uncertainty to account for toxicokinetic differences and toxicodynamic differences between the animal species in which the POD was derived and humans.

PBPK models can be useful for the evaluation of interspecies toxicokinetics; however, information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans and the potential variability in human susceptibility; thus, the interspecies and intraspecies UFs of 10 were applied for a total UF of 100. Human variation may be larger or smaller; however, 1,1,2,2-tetrachloroethane-specific data to examine the potential magnitude of human variability of response are unknown.

In addition, a threefold database UF was applied due to the lack of information addressing the potential reproductive toxicity associated with 1,1,2,2-tetrachloroethane. Uncertainties associated with data gaps in the 1,1,2,2-tetrachloroethane database have been identified, specifically, uncertainties associated with database deficiencies characterizing reproductive toxicity associated with oral exposure to 1,1,2,2-tetrachloroethane. The developing fetus may be a target of toxicity (Gulati et al., 1991a), and the absence of a study specifically evaluating the full range of developmental toxicity represents an additional area of uncertainty or gap in the database.

The overall confidence in this subchronic RfD assessment is medium. Confidence in the principal study (NTP, 2004) is high. Confidence in the database is medium. Reflecting high confidence in the principal study and medium confidence in the database, confidence in the subchronic RfD is medium.

Information on the chronic oral toxicity of 1,1,2,2-tetrachloroethane consists of a limited 78-week gavage study in rats and mice (NCI, 1978). The high incidences of hepatocellular tumors in all treated groups of mice precluded evaluation of noncancer effects in the liver and identification of a NOAEL or LOAEL. Additionally, the NCI (1978) study performed histological examinations on the animals when they died or at the termination of the study, which was beyond the point at which more sensitive hepatotoxic effects, including nonneoplastic effects, would be expected. The 14-week dietary study (NTP, 2004) was used to derive the subchronic oral RfD. The NTP (2004) study also utilized a more relevant type of exposure (i.e., oral feeding) for the general population exposed to 1,1,2,2-tetrachloroethane in the environment.

The chronic RfD of 0.02 mg/kg-day was calculated by dividing the subchronic $BMDL_{1SD}$ of 15 mg/kg-day for increased relative liver weight by a total UF of 1,000: 10 for interspecies extrapolation, 10 for interhuman variability, 3 for subchronic to chronic duration extrapolation, and 3 for database deficiencies.

The choice of BMD model is not expected to introduce a considerable amount of uncertainty in the risk assessment since the chosen BMR of 1 SD from the control mean is within the observable range of the data. Additional BMD modeling for other amenable data sets, including serum liver enzyme levels, liver lesions, and fetal body weight, were also conducted to

provide other PODs for comparison purposes (see Appendix B). A graphical representation of these potential PODs and resulting chronic RfVs is shown below in Figure 6-2.

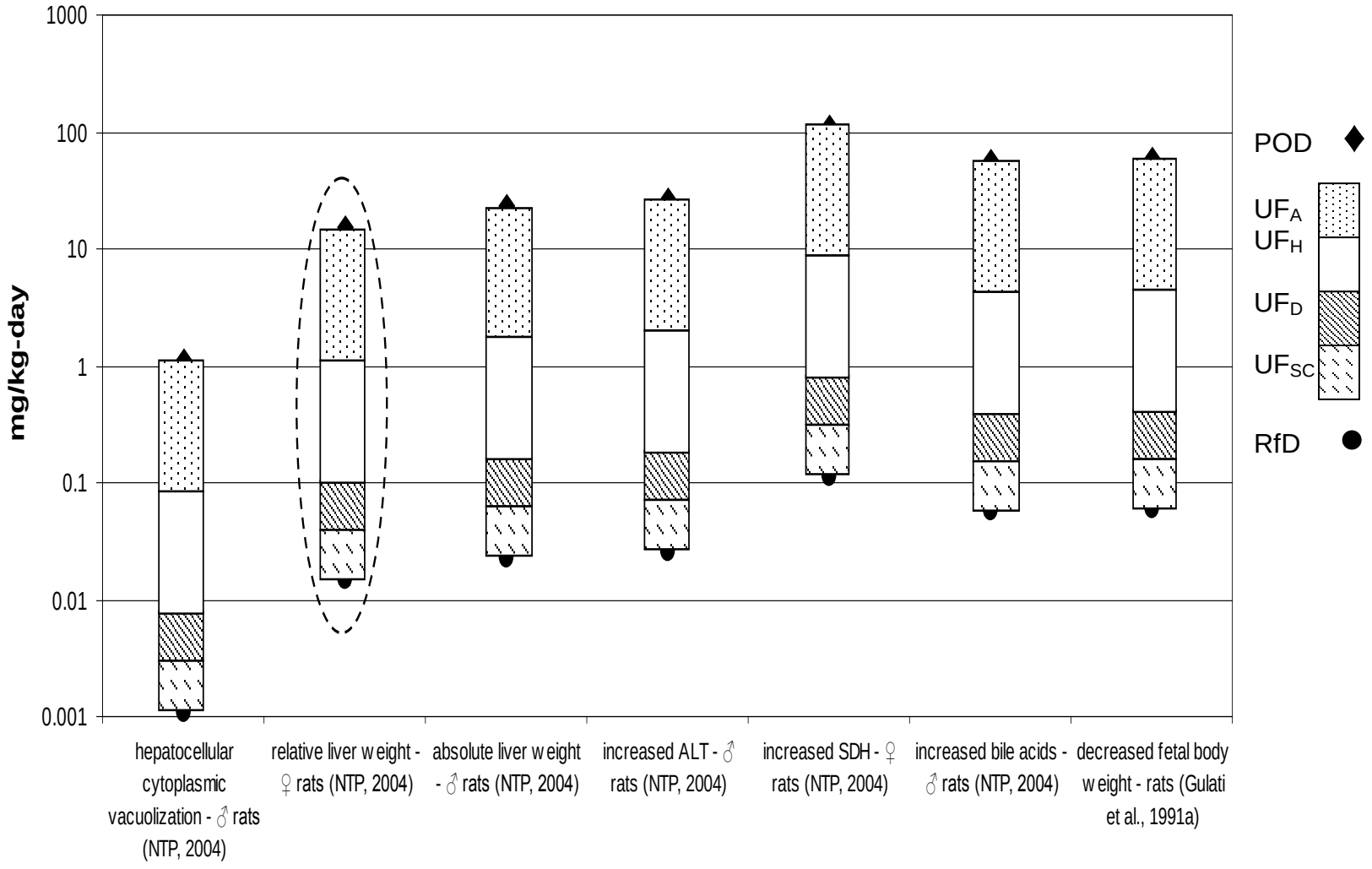


Figure 6-2. PODs for selected endpoints (with critical effect circled) from Table 5-2 with corresponding applied UFs and derived sample subchronic oral RfVs.

The default UF of 10 for the extrapolation from animals to humans is a composite of uncertainty to account for toxicokinetic differences and toxicodynamic differences between the animal species in which the POD was derived and humans.

PBPK models can be useful for the evaluation of interspecies toxicokinetics; however, information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans and the potential variability in human susceptibility; thus, the interspecies and intraspecies UFs of 10 were applied for a total UF of 100. Human variation may be larger or smaller; however, 1,1,2,2-tetrachloroethane-specific data to examine the potential magnitude of human variability of response are unknown.

A threefold UF was applied for extrapolation from a subchronic exposure duration study to a chronic RfD. Based on the available data for 1,1,2,2-tetrachloroethane, the toxicity observed in the liver does not appear to increase over time. Specifically, liver damage observed in F344 rats following subchronic exposure to 1,1,2,2-tetrachloroethane (NTP, 2004) (e.g., increased incidence of necrosis or altered serum enzyme and bile levels) did not progress to more severe effects such as cirrhosis or major liver disease following chronic exposures (NCI, 1978). Therefore, the available database does not abrogate all concern associated with using a subchronic study as the basis of the RfD but supports the utilization of a database UF of 3.

In addition, a threefold database UF was applied due to the lack of information addressing the potential reproductive toxicity associated with 1,1,2,2-tetrachloroethane. Uncertainties associated with data gaps in the 1,1,2,2-tetrachloroethane database have been identified, specifically, uncertainties associated with database deficiencies characterizing reproductive toxicity associated with oral exposure to 1,1,2,2-tetrachloroethane. The developing fetus may be a target of toxicity (Gulati et al., 1991a), and the absence of a study specifically evaluating the full range of developmental toxicity represents an additional area of uncertainty or gap in the database.

The overall confidence in this chronic RfD assessment is medium. Confidence in the principal study (NTP, 2004) is high. Confidence in the database is medium. Reflecting high confidence in the principal study and medium confidence in the database, confidence in the chronic RfD is medium.

6.2.2. Noncancer/Inhalation

An RfC was not calculated due to insufficient data. Information on the subchronic and chronic inhalation toxicity of 1,1,2,2-tetrachloroethane is limited to the results of one study in rats that found transient liver effects (Truffert et al., 1977). Reporting inadequacies preclude identification of a NOAEL or LOAEL and derivation of an RfC in the usual manner.

A route-to-route extrapolation using the computational technique of Chiu and White (2006), as described in Section 3.5, was considered. However, U.S. EPA (1994b) recommends not conducting a route-to-route extrapolation from oral data when a first-pass effect by the liver

or respiratory tract is expected, or a potential for portal-of-entry effects in the respiratory tract is indicated following analysis of short-term inhalation, dermal irritation, in vitro studies, or evaluation of the physical properties of the chemical. In the case of 1,1,2,2-tetrachloroethane, a first-pass effect by the liver is expected. In addition, the presence of tissue-bound metabolites in the epithelial linings in the upper respiratory tract may demonstrate a first-pass effect by the respiratory tract (Eriksson and Brittebo, 1991). Lehmann et al. (1936) observed irritation of the mucous membranes of two humans following exposure to 1,1,2,2-tetrachloroethane air concentrations of 146 ppm (1,003 mg/m^3) for 30 minutes or 336 ppm (2,308 mg/m^3) for 10 minutes, indicating the potential for portal-of-entry effects in the respiratory system.

Information regarding the chronic inhalation toxicity of 1,1,2,2-tetrachloroethane is available from four animal studies that include limited data on liver effects and serum acetylcholinesterase, or hematological and immunological alterations (Shmuter, 1977; Kulinskaya and Verlinskaya, 1972; Schmidt et al., 1972; Mellon Institute of Industrial Research, 1947). However, the reporting of results from these chronic bioassays is inadequate for identification of NOAELs or LOAELs for systemic toxicity. A chronic NOAEL was identified for reproductive effects in male rats (Schmidt et al., 1972); however, macroscopic malformations or significant group differences in the other indices were not observed at 13.3 mg/m^3. This lack of information on reproductive toxicity precludes utilizing this selected NOAEL in the derivation of an RfC.

6.2.3. Cancer/Oral and Inhalation

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,1,2,2-tetrachloroethane is characterized as "likely to be carcinogenic to humans", based on the existence of evidence of the compound's tumorigenicity in a single study in two animal species (NCI, 1978). The epidemiological human data available are inadequate for evaluation of cancer risk (IARC, 1999). The NCI (1978) provided evidence that 1,1,2,2-tetrachloroethane causes hepatocellular tumors in male and female mice, and rare tumors (not statistically significant) were seen in high-dose male rats.

The only carcinogenicity bioassay for 1,1,2,2-tetrachloroethane was a chronic gavage study in Osborne-Mendel rats and B6C3F_1 mice performed by NCI (1978). This was a well-designed study, conducted in both sexes in two rodent species with an adequate number of animals per dose group and with examination of appropriate toxicological endpoints in both sexes of rats and mice. Although limitations in the study included increased mortality in male and female mice, the variable doses given to the mice over the course of the 78-week exposure period, and the exposure duration of the study (78 weeks) was less than the standard 104 week chronic exposure duration.

The rat study found no statistically significant increases in tumor incidences in males or females. Three rare hepatocellular tumors in high-dose male rats were noted, and NCI (1978)

characterized the carcinogenic results in male rats as "equivocal." The mouse study found significant, dose-related increases in the incidences of hepatocellular carcinomas in both sexes. Based on the increased incidences of hepatocellular carcinomas, NCI (1978) concluded that orally administered 1,1,2,2-tetrachloroethane is a liver carcinogen in male and female $B6C3F_1$ mice. This NCI study was used for dose-response analysis for oral exposure.

Data on the incidences of hepatocellular carcinomas in male and female mice from the NCI (1978) study were used for cancer dose-response assessment. Conversion of the doses in the NCI (1978) mouse study to HEDs to be used for dose-response modeling was accomplished in two steps. The mice were treated with 1,1,2,2-tetrachloroethane by gavage 5 days/week for 78 weeks, and then observed untreated for 12 weeks for a total study duration of 90 weeks. Because the reported TWA doses were doses for 5 days/week for 78 weeks, they were duration-adjusted to account for the partial week exposure (by multiplying by 5 days/7 days) and untreated observation period (by multiplying by 78 weeks/90 weeks). The duration-adjusted animal doses were converted to HEDs by adjusting for differences in body weight and lifespan between humans and mice. In accordance with U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, a factor of $BW^{3/4}$ was used for cross-species scaling. Because the study duration (90 weeks) was less than the animal lifespan (104 weeks), the scaled dose was then multiplied by the cubed ratio of experimental duration to animal lifespan to complete the extrapolation to a lifetime exposure in humans.

The mode of action of 1,1,2,2-tetrachloroethane carcinogenicity is unknown. It appears that metabolism to one or more active compounds is likely to play a role in the development of the observed liver tumors, but insufficient data preclude proposing this as a mode of action. Results of genotoxicity and mutagenicity studies of 1,1,2,2-tetrachloroethane are mixed and insufficient for informing the mode of action. Given that the mechanistic and other information available on cancer risk from exposure to 1,1,2,2-tetrachloroethane is sparse and that the data that do exist are equivocal, there is inadequate information to inform the low dose extrapolation.

Dose-response modeling was performed to obtain a POD for quantitative assessment of cancer risk. The incidences of hepatocellular carcinomas in both sexes of mice were modeled for determination of the POD. In accordance with the U.S. EPA (2005a) cancer guidelines, the $BMDL_{10}$ (lower bound on dose estimated to produce a 10% increase in tumor incidence over background) was estimated by applying the multistage cancer model in the EPA's BMDS (version 2.1.1) for the dichotomous incidence data and selecting the results for the model that best fit the data. The BMD modeling of the male mouse data did not achieve adequate fit for any of the dichotomous models; thus, a cancer slope factor was not derived from the male data. The 1° multistage model was selected for the derivation of the cancer slope factor from the female data because this model provided adequate model fit and the lowest AIC when compared to the results of the 2° multistage model. In addition, the 2° multistage model had insufficient DF to test the goodness-of-fit. The $BMDL_{10}$ of 0.65 mg/kg-day from the modeling of the tumor

incidence data in female mice is selected as the POD for use in calculation of an oral slope factor. In accordance with the U.S. EPA (2005a) guidelines, an oral slope factor of 0.2 (mg/kg-day)$^{-1}$ is calculated by dividing the human equivalent $BMDL_{10}$ of 0.65 mg/kg-day into 0.1 (10%) (Appendix C).

In the absence of any data on the carcinogenicity of 1,1,2,2-tetrachloroethane via the inhalation route, an inhalation unit risk has not been derived in this evaluation.

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

The Toxicological Review of 1,1,2,2-tetrachloroethane (dated August, 2009) has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). An external peer-review workshop was held January 27, 2010. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases, the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA did not receive any scientific comments from the public.

EXTERNAL PEER REVIEW PANEL COMMENTS

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

In addition, the reviewers provided comments specific to particular decisions and analyses presented in the Toxicological Review under multiple charge questions. These comments were organized and responded to under the most appropriate charge question.

A. General Comments

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

Comments: The reviewers generally commented that the Toxicological Review was logically written. One reviewer recommended an improvement to the clarity of the document by reducing the text describing the available studies and presenting the individual study data in a bulleted format, and this was echoed by another reviewer who recommended condensing the study summaries and discussions.

Response: The content of the Toxicological Review is consistent with the current outline for IRIS Toxicological Reviews, although an effort has been made to streamline the document and reduce the redundancy. The general structure of a Toxicological Review is to present a factual summary of toxicity studies and a qualitative synthesis of these studies in Section 4 and a quantitative critical interpretation in Section 5.

2. **Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of 1,1,2,2-tetrachloroethane.**

Comments: Several reviewers did not provide additional studies. One reviewer identified the following studies:

Ashley, DL; Bonin, MA; Cardinali, FL; et al. (1994) Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposure. Clin Chem 40(7 Pt 2):1401–4.

Matsuoka, A; Yamakage, K; Kusakabe, H; et al. (1996) Re-evaluation of chromosomal aberration induction on nine mouse lymphoma assay "unique positive' NTP carcinogens. Mutat Res 12;369(3–4):243–52.

Sofuni, T; Honma, M; Hayashi, M; et al. (1996) Detection of in vitro clastogens and spindle poisons by the mouse lymphoma assay using the microwell method: interim report of an international collaborative study. Mutagenesis 11(4):349–55.

Response: The references (Matsuoka et al. [1996]; Sofuni et al. [1996]; Ashley et al. [1994]) were examined but have not been added to the Toxicological Review, as these references do not contribute significant information to the discussion and analysis in the document.

B. Oral Reference Dose (RfD) for 1,1,2,2-tetrachloroethane

1. **Subchronic and chronic RfDs for 1,1,2,2-tetrachloroethane have been derived from a 13-week oral gavage study (NTP, 2004) in rats and mice. Please comment on whether the selection of this study as the principal study has been scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.**

Comment: The reviewers generally agreed that the selection of the NTP (2004) report as the principal study was scientifically justified.

Response: Comment acknowledged.

Comment: One reviewer commented that the Gulati et al. (1991a, b) study is the only other study that could be a candidate principal study and provides what may be a more significant

endpoint for human health protection; but also states that EPA has made a reasonable selection in the NTP study.

Response: The Gulati et al. (1991a, b) developmental studies were conducted at doses higher than the subchronic NTP (2004) study, which demonstrated liver effects at lower doses. Therefore, the Gulati et al. (1991a, b) studies were not selected as the principal studies. However, potential PODs based on the observed developmental effects from Gulati et al. (1991a) were provided in the document for comparison purposes.

Comment: One reviewer requested additional explanation regarding the statement that high incidences of hepatocellular tumors in all mouse groups of the NCI (1978) study precluded evaluation of noncancer effects in the liver.

Response: The statement in Section 5.1.2.1., *Choice of Principal Study and Critical Effect - with Rationale and Justification*, regarding the high incidence of hepatocellular tumors in all mouse dose groups precluding the evaluation of noncancer effects in the liver was deleted. The effects observed in the NCI (1978) study were considered in the identification of the principal study and critical effect, and a LOAEL of 142 mg/kg-day was identified for chronic inflammation in the kidneys of male mice, while a NOAEL of 142 mg/kg-day and a LOAEL of 284 mg/kg-day were identified for hydronephrosis and chronic inflammation in the kidneys of female mice. This information is included in Section 5.1.2.1.

2. **Increased relative liver weight was selected as the critical effect for the derivation of the subchronic and chronic RfDs. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Please provide a detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.**

Comment: The reviewers generally agreed that the selection of increased relative liver weight as the critical effect for the derivation of the subchronic and chronic RfDs was justified. However, one reviewer did not concur with the selection of the critical effect and stated that there is no scientific evidence to support the conclusion that the increase in liver weight represents a sensitive endpoint early in the process leading to hepatocellular necrosis. A second reviewer questioned whether increases in liver weight reflect other, earlier changes that have been going on long enough to cause the cell proliferation, inflammation, or other effects responsible for the observed weight gain.

One reviewer commented that increased relative liver weight is a less toxicologically significant index of liver change than increased absolute liver weight, due to the treatment-

induced loss of body weight; whereas another reviewer believed the change in relative liver weight is more appropriate than absolute liver weight where body weights in general are being affected. Another reviewer commented that increased serum enzyme activity is an alternative critical effect and a true measure of hepatocellular damage, and the most toxicologically-significant endpoint should be selected as the critical effect. A reviewer commented that the only other endpoint that is a candidate critical effect is reduced fetal body weight in the Gulati et al. (1991a, b) studies, but also states that EPA's selection of the relative liver weight as the critical effect is reasonable.

Response: EPA considered that, given the available data, increased liver weight represents the most sensitive effect observed in the liver. In addition to increased liver weight following subchronic exposure, the evidence of hepatocellular damage includes increased serum concentrations of hepatocellular enzymes (ALT and SDH), decreased serum cholesterol, and increased incidences of hepatocellular necrosis, bile duct hyperplasia, hepatocellular mitotic alterations, and hepatic pigmentation. Evidence of the 'earlier changes' reflected by the increase in liver weight as suggested by one reviewer is unavailable. Thus, EPA retained increased liver weight as a critical effect for the subchronic and chronic RfDs. Clarification text has been added to Section 5.1.1.1, *Choice of Principal Study and Critical Effect – with Rationale and Justification* addressing the lack of chemical-specific data demonstrating the relationship between increased liver weight and hepatocellular necrosis.

The increase in relative liver weight was selected as the basis for the POD because the relative liver weight analysis takes into account the substantive, dose-dependent decreases in body weight that were observed in both sexes of rats.

The increase in serum enzyme activity was included as a comparison to the increased liver weight in Section 5.1.3., *RfD Comparison Information*; however, it was determined that the observed increase in liver weight may represent the most sensitive effect that occurs early in the process of 1,1,2,2-tetrachloroethane-induced hepatotoxicity.

The reduction in fetal body weight was observed at doses higher than the demonstrated liver effects from the subchronic NTP (2004) study. Therefore, the decrease in fetal body weight was not selected as the critical effect. However, PODs based on the observed developmental effects from Gulati et al. (1991a) were provided in the document for comparison purposes.

3. **Hepatocellular vacuolization was observed at the lowest dose in the principal study (NTP, 2004). This effect was not selected as the critical effect for the determination of the POD for derivation of the subchronic and chronic RfDs. Please comment on the rationale and justification for not selecting this endpoint as the critical effect.**

Comment: The reviewers generally considered the rationale and justification for not selecting hepatocellular vacuolization as the critical effect as reasonable, justified, logical, and comprehensive. One reviewer recommended slight refinements to the justification, and questioned whether the comments that vacuolization was not observed across species and the severity was not dose-dependent supported the conclusion. Another reviewer asked if NTP (2004) specified the lobular distribution of the vacuoles.

Response: The decision to not select hepatocellular vacuolization as the critical effect involved more than a consideration of cross species observations and severity (see Section 5.1.1.1., *Choice of Principal Study and Critical Effect – with Rationale and Justification*). The biological significance of the hepatocellular vacuolization observed following 1,1,2,2-tetrachloroethane exposure was unclear based on the paucity of available information.

NTP did not specify the lobular distribution of the observed vacuoles.

4. **The subchronic and chronic RfDs have been derived utilizing benchmark dose (BMD) modeling to define the point of departure (POD). All available models were fit to the data in both rats and mice for increased absolute and relative liver weight, increased incidence of hepatocellular cytoplasmic vacuolization (rats only), increased levels of ALT, SDH, and bile acids, and decreased fetal body weight. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., one standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.**

Comment: Three reviewers stated that the BMD modeling was appropriate. One reviewer disagreed with the reasoning provided in the document for eliminating the two highest dose groups from the BMD modeling analysis for all of the endpoints, and stated that dropping doses is typically only done when the issues of model fit are encountered. A second reviewer commented that EPA should at least show earlier BMD modeling results with the highest doses included and show the lack of model fit that led to the elimination of the two highest doses.

Response: In agreement with the reviewers' comments, the reasoning, provided in Section 5.1.1.2 of the document, *Methods of Analysis—Including Models (PBPK, BMD, etc.)*, for dropping the two highest dose groups (exceeding the MTD) was removed. In its place, a rationale for dropping dose groups based on adequacy of model fit was employed. In addition, the endpoints in Table 5-1 were remodeled using all of the dose groups within the

dataset. The BMD modeling results (generated using version 2.1.1 of BMDS) with the highest dose groups included are presented in Appendix B. This analysis demonstrated that lack of model fit led to the elimination of one or more of these dose groups in order to obtain adequate fit. As a result of this remodeling, the critical effect, increased relative liver weight, was based on the data in female rats, where before, relative liver weight in male rats had been selected as the most sensitive species/sex.

5. **Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfDs. For instance, are they scientifically justified? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factor:**
    - **A database uncertainty factor of 3 was used to account for the lack of oral reproductive and developmental toxicity data for 1,1,2,2-tetrachloroethane. Please comment on whether the application of this uncertainty factor has been scientifically justified.**

Comment: The reviewers generally considered the applications of the UFs to be adequate, acceptable, reasonable, and appropriate.

Response: Comment acknowledged.

Comment: One reviewer requested a comparison between the RfD derived from the subchronic NTP study and an approximate RfD derived from the chronic NCI study.

Response: The RfD from the subchronic NTP study was based on a study that used lower dose levels and a wider dose range than the NCI (1978) study, and thereby provided a better characterization of the dose-response curve in the low-dose region. Additionally, the route of exposure used in the NTP study (dietary exposure) is a more relevant route of exposure for the general population exposed to 1,1,2,2-tetrachloroethane in the environment than the gavage exposure used in the NCI study. However, if one were to use the observance of chronic inflammation in the kidneys of male mice in the NCI study as a LOAEL, for purposes of comparison, the POD of 142 mg/kg-day could be divided by a total UF of 300 to yield a potential RfD of 0.5 mg/kg-day. This information is shown in Figure 5-1.

Comment: A reviewer recommended the addition of text addressing the major metabolites of 1,1,2,2-tetrachloroethane (dichloroacetic acid, trichloroethylene, perchloroethylene) and how the results of these assessments compare to those derived for 1,1,2,2-tetrachloroethane.

Response: The RfDs derived for the major metabolites are not an appropriate comparison because the principal studies, critical effects, PODs, and UFs are chemical-specific, and are not directly comparable, nor is the confidence the same in the data sets from which the PODs were derived. While the datasets may have similarities, the overall assessment development will be quite different.

Comment: One reviewer commented that there is a considerable amount of information about the toxicokinetics of related halocarbons (e.g., trichloroethylene, perchloroethylene, chloroform, 1,1,1-trichloroethane) in rodents and humans, and that the rank of metabolic activation of the compounds is: mice >> rats > humans. Therefore, the toxicokinetic component of the interspecies UF of 10 could be reduced, resulting in an interspecies uncertainty factor of 3.

Response: The potential for difference between animal and human toxicokinetics following 1,1,2,2-tetrachloroethane exposure based on information from related halocarbons was added to Section 5.3, *Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration*. Upon further evaluation, this information was not considered sufficient to reduce the UF for 1,1,2,2-tetrachloroethane and the UF of 10 was retained.

Comment: A reviewer commented that Section 5.3 is a restatement of the features that contributed to the valuation of the standard uncertainty factors, and recommended a consideration of what additional uncertainties are present that might impact the results.

Response: Additional text was added to this section in response to the reviewer's comments.

C. Inhalation Reference Concentration (RfC) for 1,1,2,2-tetrachloroethane

1. An RfC for 1,1,2,2-tetrachloroethane has not been derived. Has the scientific justification for not deriving an RfC been described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study. Please identify and provide the rationale for any endpoints that should be considered in the selection of the critical effect.

Comment: The reviewers agreed with the decision not to derive an RfC. One reviewer commented that a comparison to metabolically-related compounds is useful and recommended including this information in the discussion of the uncertainties associated with not deriving an RfC.

Response: Additional text related to uncertainties was added to Section 5.3.

D. Carcinogenicity of 1,1,2,2-tetrachloroethane

1. Under EPA's 2005 *Guidelines for carcinogen risk assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that 1,1,2,2-tetrachloroethane is *likely to be carcinogenic to humans* by all routes of exposure. Please comment on the cancer weight of the evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

Comment: One reviewer did not concur with the conclusion that 1,1,2,2-tetrachloroethane is *likely to be carcinogenic to humans*, and thought it would be more accurate to characterize 1,1,2,2-tetrachloroethane as a possible human carcinogen. This reviewer also commented that the WOE characterization for 1,1,2,2-tetrachloroethane may not be applicable to all routes of exposure based on general toxicokinetic differences between oral and inhalation exposure. A second reviewer commented that the conclusion that 1,1,2,2-tetrachloroethane is *likely to be carcinogenic to humans* is one of the weakest *likely to be carcinogenic to humans* characterizations demonstrated when the data is singularly considered; in addition, given the prevalence of and susceptibility to developing liver tumors in $B6C3F_1$ mice, the reviewer questioned whether a slope factor should be derived from this study. This reviewer also advocated incorporating carcinogenicity information from dichloroacetic acid, trichloroethylene, and perchloroethylene into the document to strengthen the WOE characterization. A third reviewer agreed with the *likely to be carcinogenic to humans* determination..

Response: The cancer weight of evidence descriptor for 1,1,2,2-tetrachloroethane is based on the statistically significant increase in the incidence of hepatocellular carcinomas in both male and female $B6C3F_1$ mice, and the rare hepatocellular tumors observed in the male Osborne-Mendel rats were considered a rare tumor by NCI (1978). According to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the *likely to be carcinogenic to humans* descriptor is supported when an agent has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route with or without evidence of carcinogenicity in humans, and in the case of 1,1,2,2-tetrachloroethane, a positive tumor response was observed in both male and female mice. This descriptor is also supported when a rare animal tumor is observed in a single experiment that is assumed to be relevant to humans, and in the case of 1,1,2,2-tetrachloroethane, NCI (1978) considered the liver tumors observed in male rats to be a rare tumor response. Goodman et al. (1980) observed hepatocellular carcinomas in 2 male (n=975) and 4 female (n=970) Osborne-Mendel rats that

were untreated, corn oil fed, or corn oil gavage controls, and stated that historical data indicates the general rarity or prevalence of tumors and may be useful in assessing biological significance.

According to the 2005 Cancer Guidelines, the cancer WOE characterization applies to all exposure routes that have not been adequately tested at sufficient doses when tumors are observed at a site other than the initial point of contact. In the case of 1,1,2,2-tetrachoroethane, tumors were observed in the liver of both sexes of mice and in rats following oral exposure, and the database for 1,1,2,2-tetrachloroethane lacks inhalation studies that would be useful in determining a WOE characterization for the inhalation route.

Additional text was added to the discussion of the potential susceptibility of $B6C3F_1$ mice to developing hepatocellular carcinomas following 1,1,2,2-tetrachloroethane exposure in Section 5.4.5, *Uncertainties in Cancer Risk Values.*

Section 4.7.1, *Summary of Overall Weight of Evidence*, presents the carcinogenicity data available for 1,1,2,2-tetrachloroethane and describes the weight of the evidence cancer descriptor. This section also includes a discussion of the carcinogenicity data available for dichloroacetic acid, trichloroethylene, and perchloroethylene.

2. A two-year oral gavage cancer bioassay (NCI, 1978) was selected as the principal study for the derivation of an oral slope factor. Please comment on the appropriateness of the selection of the principal study.

Comment: The reviewers generally agreed with the selection of the NCI (1978) study as the principal study for the development of an oral slope factor, although the reviewers highlighted that this was the only study available for this purpose.

Response: Comment acknowledged.

Comment: One reviewer commented that the NCI study used poorly selected dose levels that were adjusted during the course of the study, the exposure duration was 78 weeks as opposed to the more standard 104 weeks, that there was also a concurrent disease (pneumonia) observed, and that these deficiencies and resulting uncertainties need to be stated in the document.

Response: Text was added to Sections 5.4.1., *Choice of Study/Data—with Rationale and Justification*, and 5.4.5, *Uncertainties in Cancer Risk Values*, to address the concern associated with the dose selection and modification, the exposure duration, and the increased incidence of chronic murine pneumonia in the rats.

Comment: A reviewer expressed concerns that gavage dosing may deliver the chemical in a short term bolus dose and may not provide the same results as a dietary or other oral dosing method that delivers the chemical more gradually over time.

Response: The potential effect of the corn oil vehicle, as well as the bolus nature of the gavage dose, on the effects observed in the liver following 1,1,2,2-tetrachloroethane exposure has been added to Section 5.4.5, *Uncertainties in Cancer Risk Values*.

3. An increased incidence of hepatocellular carcinomas in B6C3F$_1$ mice was used to estimate the oral cancer slope factor. Please comment on the scientific justification of this analysis. Has the BMD modeling been appropriately conducted?

Comment: Several reviewers considered the modeling of the increased incidence of hepatocellular tumors in B6C3F$_1$ mice to be justified and appropriate. One reviewer commented that maybe an oral slope factor should not be derived given the prevalence of and susceptibility to developing liver tumors in this strain of mice. A reviewer commented that both sexes of B6C3F$_1$ mice have a high spontaneous cancer incidence and referenced a study by Haseman et al. (1998) which reported that male B6C3F$_1$ control mice have a 42% liver cancer incidence. This reviewer recommended including a discussion addressing this in the uncertainty section.

Response: The U.S. EPA considers liver tumors in mice to be relevant to humans unless chemical-specific information is available to indicate otherwise. Text addressing this issue is included in Section 5.4.5, *Uncertainties in Cancer Risk Values*.

Text was also added to Section 5.4.5, *Uncertainties in Cancer Risk Values*, addressing the high spontaneous cancer incidence of liver cancer in male B6C3F$_1$ mice. The 42% liver cancer rate for male B6C3F$_1$ mice was for liver adenomas and carcinomas combined, but the NCI (1978) study reported only hepatocellular carcinomas. Haseman et al. (1998) reported a lower spontaneous cancer incidence for hepatocellular carcinomas; 17.9 and 8.4% for male and female B6C3F$_1$ mice, respectively.

Even though the B6C3F$_1$ strain is frequently associated with a high spontaneous cancer incidence, the incidence in the control mice in NCI (1978) was rather low; 1/18 in the male vehicle controls and 0/20 in the female vehicle controls, and 3/36 and 1/40 in male and female pooled-vehicle controls, respectively. Comparison of an experimental group with its concurrent controls was considered to be the most appropriate comparison (Haseman et al., 1992; Tarone et al., 1981; Gart et al., 1979 as cited in Haseman et al., 1998; Goodman et al., 1980) and, in this case, the control values were considered low.

Comment: One reviewer requested additional model output information in Appendix C describing how the multi-stage model fit the data points, even if the reported goodness-of-fit *p*-value was provided as "NA" because of too many model parameters.

Response: In response to this comment, the incidence of hepatocellular carcinomas in male and female mice was remodeled using the most recent version of BMDS (version 2.1.1). The relevant information describing the fit of both the one- and two-stage multistage models to these incidence data have now been included in Appendix C.

Comment: A reviewer requested additional analysis of the mode of action of carcinogenesis, as the preponderance of genotoxicity data suggest that 1,1,2,2-tetrachloroethane is not genotoxic and the data available indicate promotion potential. This reviewer recommended an uncertainty factor approach for the cancer assessment. A second reviewer also commented that it is more likely that 1,1,2,2-tetrachloroethane may act as a tumor promoter, and that regenerative hyperplasia is a more likely mode of action, provided that the majority of the in vitro and in vivo genotoxicity and mutagenicity studies yielded non-positive results.

Response: The two studies (Milman et al. [1988] and Story et al. [1986]) providing some evidence to support the promotion potential of 1,1,2,2-tetrachloroethane were added to Section 4.7.3, *Mode of Action of Carcinogenicity Information*. However, the key events associated with any hypothesized mode of action of carcinogenesis of 1,1,2,2-tetrachloroethane, whether mutagenic or promotional, cannot be determined with the information available.

Comment: A reviewer commented that mice and other rodents metabolize a considerably larger portion of high doses of halocarbons than humans, and, therefore, experience more severe hepatocellular injury, greater formation of covalent adducts, and higher cancer incidences.

Response: Text was added to Section 5.4.5, *Uncertainties in Cancer Risk Values*, addressing the potential difference between animal and human toxicokinetics following 1,1,2,2-tetrachloroethane exposure, based on information from related halocarbons demonstrating increased metabolic activation in mice compared with humans.

Comment: A reviewer recommended that the document highlight that dichloroacetic acid and trichloroacetic acid are metabolites of both trichloroethylene and tetrachloroethylene, so the hepatocarcinogenic effects of these metabolites should be additive; however, it is important to point out that quantitative data on the quantities of these metabolites formed by

rodents or humans are unavailable. The reviewer recommended including a discussion addressing this in the uncertainty section.

Response: The document was not modified because the hepatocarcinogenic effects of dichloroacetic acid and trichloroacetic acid, whether they are additive or not, have no bearing on the carcinogenicity observed for 1,1,2,2-tetrachloroethane.

Comment: A reviewer commented that the document should recognize that administration of large quantities of corn oil promotes lipid accumulation and lipoperoxidative damage of hepatocytes, and that corn oil is believed to be tumorigenic in rats and humans through increased expression of protooncogenes, decreased apotosis, mitogenesis, etc. The reviewer recommended including a discussion addressing this in the uncertainty section.

Response: EPA has included text in Section 5.4.5, *Uncertainties in Cancer Risk Values*, that addresses the bolus administration of 1,1,2,2-tetrachloroethane was corn oil.

APPENDIX B. BENCHMARK DOSE MODELING RESULTS FOR THE DERIVATION OF THE RfD

Dichotomous Endpoints

Incidence of hepatocellular cytoplasmic vacuolization in male and female rats (NTP, 2004)

Table B-1. Incidences of hepatocellular cytoplasmic vacuolization in rats exposed to dietary 1,1,2,2-tetrachlorethane for 14 weeks

Nonneoplastic lesion	Dose (mg/kg-d)					
	Vehicle control	20	40	80	170	320
Males[a]						
Hepatocellular cytoplasmic vacuolization	0/10	7/10[b] (1.3)	9/10[b] (2.0)	10/10[b] (1.9)	8/10[b] (1.4)	0/10
Females[a]						
Hepatocellular cytoplasmic vacuolization	0/10	0/10	10/10[b] (1.7)	10/10[b] (2.2)	4/10[b] (1.3)	0/10

[a]Values represent proportion of animals with the lesion; for those dose groups in which lesions were found, the average severity score is in parentheses; severity grades were as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[b]Statistically significantly different from vehicle control group.

Source: NTP (2004).

All available dichotomous models (except the "quantal-linear" and "quantal-quadratic") in the EPA's BMDS (version 2.1.1) were fit to the incidence of hepatocellular cytoplasmic vacuolization in male and female rats administered 1,1,2,2-tetrachloroethane in the diet for 14 weeks. Table B-1 displays the incidence data for this endpoint for both males and females. BMDs and their associated 95% lower confidence limits (i.e., BMDLs) at an extra risk of 10% were estimated by each model. The results of this BMD modeling for male and female rats are summarized in Tables B-2 and B-3, respectively, and the BMDS output from the selected model is displayed following each table.

Table B-2. Summary of BMD modeling results for the incidence of hepatocellular cytoplasmic vacuolization in male rats

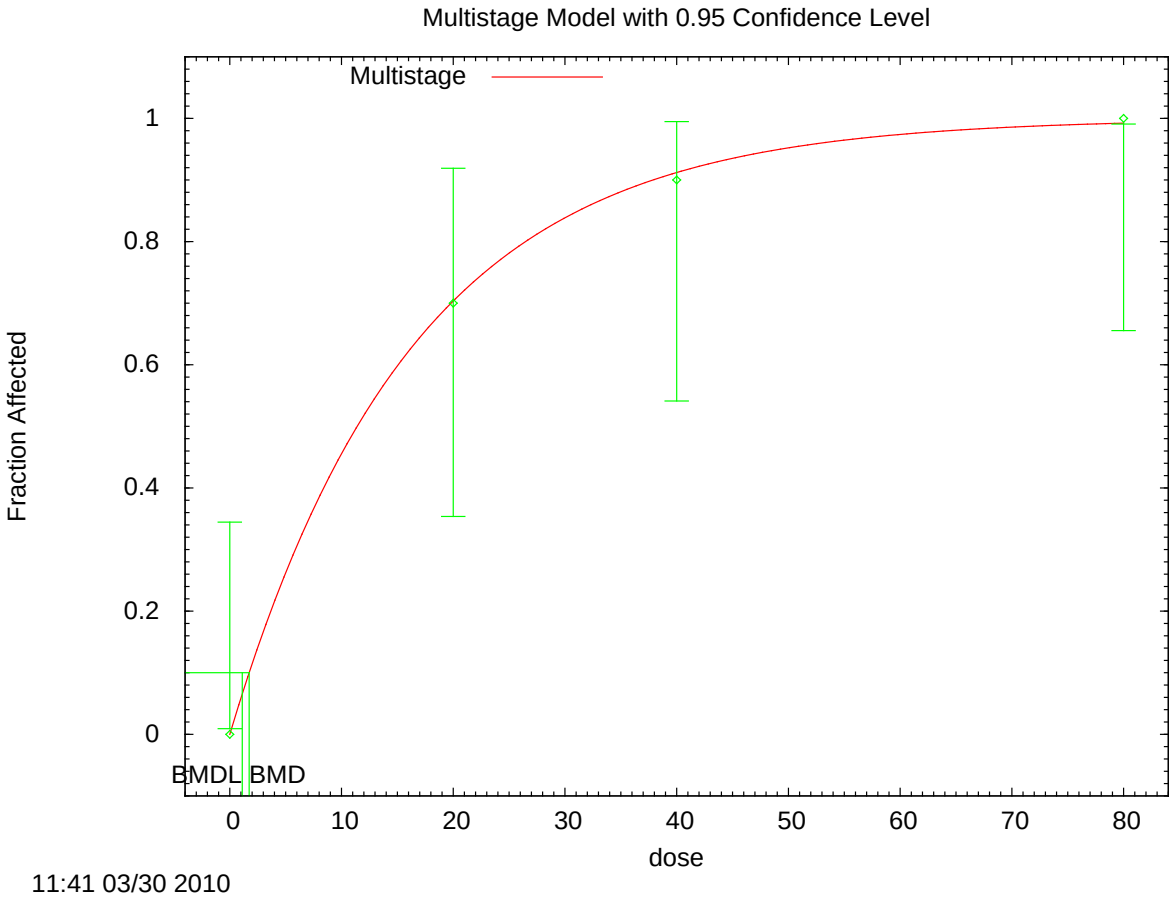
Model	DF	χ^2	χ^2 Goodness of fit *p*-value[a]	Scaled residuals of interest[b]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
All dose groups included							
BMDS was unable to generate model outputs							
Highest dose group dropped							
Gamma[c]	4	57.61	<0.001	0.00/1.66	47.97	3.64	2.60
Logistic	3	22.78	<0.001	-2.77/1.01	57.05	10.59	6.70
Log-logistic[d,e]	4	6.78	0.15	0.00/-0.06	36.14	0.91	0.40
Log-probit[d]	4	36.46	<0.001	0.00/0.85	41.77	4.70	3.03
Multistage (1-degree)[f]	4	57.61	<0.001	0.00/1.66	47.97	3.64	2.60
Probit	3	20.45	<0.001	3.00/0.94	58.24	13.29	8.99
Weibull[c]	4	57.61	<0.001	0.00/1.66	47.97	3.64	2.60
Two highest dose groups dropped							
Gamma[c]	2	0.10	0.95	0.00/0.08	22.87	2.47	1.12
Logistic	2	2.50	0.29	-0.82/0.81	25.51	6.78	3.67
Log-logistic[d]	2	0.25	0.88	0.00/0.09	23.09	6.16	0.31
Log-probit[d]	2	0.18	0.92	0.00/0.10	22.98	5.49	1.80
Multistage (1-degree)[f,g]	**3**	**0.10**	**0.99**	**0.00/-0.02**	**20.89**	**1.73**	**1.12**
Multistage (2-degree)[f]	2	0.08	0.96	0.00/0.12	22.83	1.99	1.12
Multistage (3-degree)[f]	2	0.06	0.97	0.00/0.13	22.80	1.89	1.13
Probit	2	2.56	0.28	-0.81/1.03	25.71	6.45	3.73
Weibull[c]	2	0.10	0.95	0.00/0.10	22.86	2.32	1.12

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; DF = degrees of freedom

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and immediately above the BMD.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Although the overall goodness of fit p-value suggested adequate fit of this model to the data, the model was rejected because the very high residual at the high dose (-2.32) suggested that fit of the model to the data would be improved by dropping that dose.
[g]Selected model is displayed in boldface type. BMDLs for models with adequate fit differed by greater than threefold. However, the results from the log-logistic model were rejected as unreliable due to the large spread between BMD and BMDL (20-fold) and because the BMDL from this model was an outlier in relation to the results of the other models. After dropping this model, the results of the other models were within approximately threefold. Among the remaining models, the 1-degree polynomial had the lowest AIC and also produced the lowest BMDL and was therefore selected as the most suitable model for this dataset.

As shown in Table B-2, in attempting to model the incidence of hepatocellular cytoplasmic vacuolization in male rats with all six dose groups included, the BMDS failed to generate any output because response was not a monotonically increasing function of dose (i.e., the response in the penultimate dose group was 80%, while the response in the highest dose group was 0). A key underlying assumption for the fitting of the dichotomous models in BMDS is that response must be a monotonically non-decreasing function of dose. Therefore, the highest dose group was dropped and the models were fit to the data again. In this instance, the χ^2 goodness-of-fit test found that all models exhibited inadequate fit (i.e., $p < 0.1$). Finally, in an attempt to find a model that fit, the two highest dose groups were dropped and the models were refit to these data. In this case, all of the models exhibited adequate fit ($p \geq 0.10$).

Of these models exhibiting adequate fit, a "best-fit" model was selected consistent with the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b) as follows. If the BMDL estimates from the models exhibiting adequate fit are "sufficiently close," then the model with the lowest AIC is to be used to estimate the BMDL from which the POD will be derived. In this particular case, as explained in the footnote in Table B-2, BMDLs for models with adequate fit differed by greater than threefold. However, the results from the log-logistic model were rejected as unreliable due to the large spread between BMD and BMDL (20-fold) and because the BMDL from this model was an outlier in relation to the results from the other models. After dropping the log-logistic model, the BMDLs from the other models were within approximately threefold. Among the remaining models, the one-stage multistage model had the lowest AIC and also produced the lowest BMDL, and was, therefore, selected as the most suitable model for this dataset. The $BMDL_{10}$ from this model (i.e., 1.12 mg/kg-day) was then selected as a possible POD. The standard BMDS output from the one-stage multistage model is displayed below.




```
 ======================================================================
        Multistage Model. (Version: 3.0;  Date: 05/16/2008)
        Input Data File:
C:\USEPA\IRIS\TCE\NTP\hepcytvac\male\mst_hepcytvacM2HDD_MS_1.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\hepcytvac\male\mst_hepcytvacM2HDD_MS_1.plt
                                          Tue Mar 30 12:41:48 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 4
 Total number of records with missing values = 0
```

Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 1.28571e+018

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.0607678	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-9.35947	4			
Fitted model	-9.44611	1	0.173273	3	0.9818
Reduced model	-25.8979	1	33.0768	3	<.0001

AIC: 20.8922

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
20.0000	0.7034	7.034	7.000	10	-0.024
40.0000	0.9120	9.120	9.000	10	-0.134
80.0000	0.9923	9.923	10.000	10	0.279

Chi^2 = 0.10 d.f. = 3 P-value = 0.9922

Benchmark Dose Computation

```
Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.73382

            BMDL =        1.11682

            BMDU =        2.71595

Taken together, (1.11682, 2.71595) is a 90     % two-sided confidence
interval for the BMD
```

Table B-3. Summary of BMD model results for the incidence of hepatocellular cytoplasmic vacuolization in female rats

Model	DF	χ^2	χ^2 Goodness of fit *p*-value[a]	Scaled residuals of interest[b]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
All dose groups included							
BMDS was unable to generate model outputs							
Highest dose group dropped							
Gamma[c]	4	45.13	<0.001	0.00/-1.66	61.33	8.65	6.18
Logistic	3	38.70	<0.001	-2.52/3.63	69.75	30.61	18.21
Log-logistic[d]	4	31.61	<0.001	0.00/-2.36	53.57	3.99	2.24
Log-probit[d]	4	49.11	<0.001	0.00/-1.61	58.57	12.62	8.86
Multistage (1-degree polynomial)[e]	4	45.13	<0.001	0.00/-1.66	61.33	8.65	6.18
Probit	3	38.70	<0.001	-2.50/3.65	69.79	31.28	19.39
Weibull[c]	4	45.13	<0.001	0.00/-1.66	61.33	8.65	6.18
Two highest dose groups dropped							
Gamma[c]	3	1.56	0.67	-0.95/0.82	5.00	20.59	17.05
Logistic	2	0.00	1.00	0.00/0.00	4.00	29.46	19.38
Log-logistic[d]	3	0.04	1.00	-0.14/0.14	2.08	25.03	19.51
Log-probit[d]	**3**	**0.00**	**1.00**	**0.00/0.00**	**2.00**	**26.36**	**19.56**
Multistage (1-degree polynomial)[e]	3	13.83	0.003	0.00/-3.09	22.89	3.14	2.05
Multistage (2-degree polynomial)[e]	3	7.48	0.06	0.00/-2.24	14.54	10.17	5.95
Multistage (3-degree polynomial)[e]	3	4.41	0.22	0.00/-1.78	9.85	14.53	9.15
Probit	2	0.00	1.00	0.00/0.00	4.00	28.77	19.85
Weibull[c,f]	**3**	**0.00**	**1.00**	**-0.02/0.01**	**2.00**	**30.68**	**19.16**

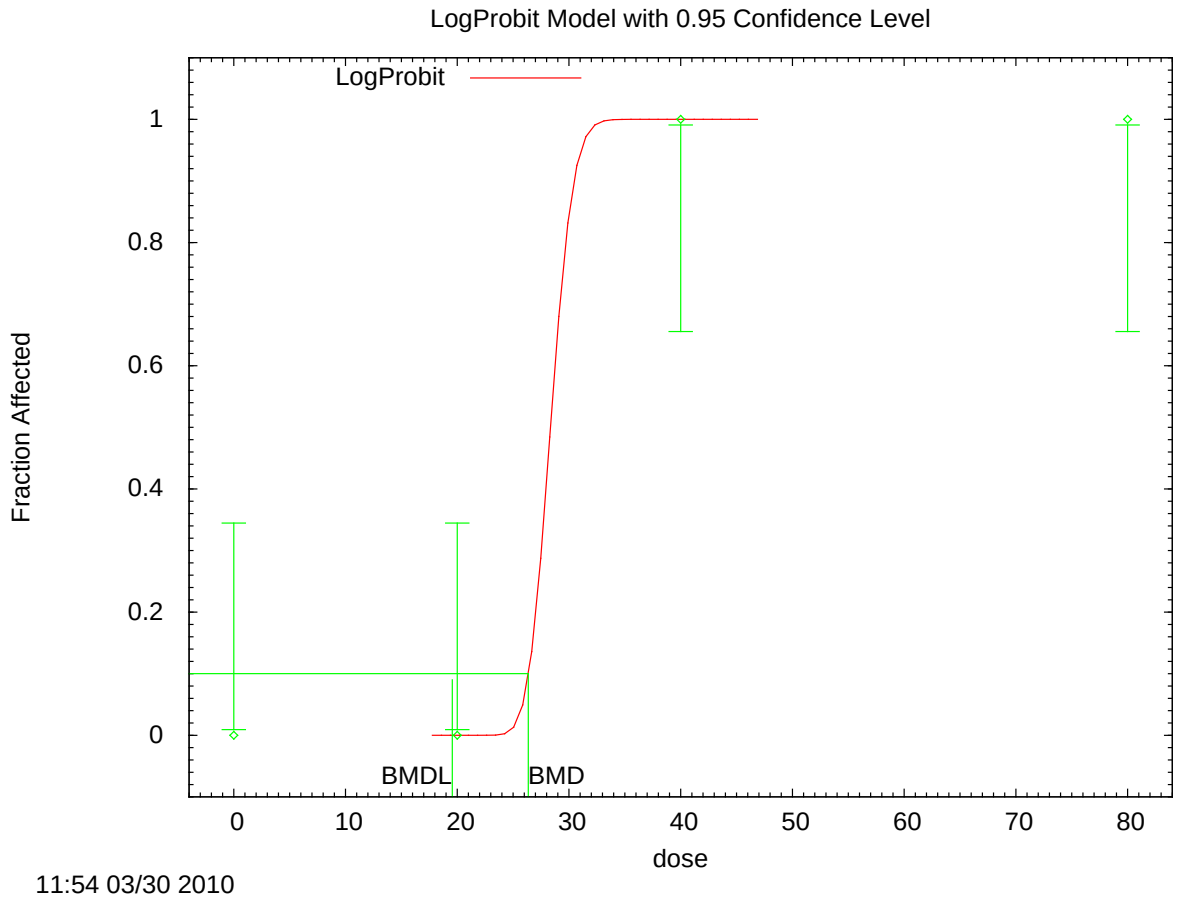
AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; DF = degrees of freedom

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and immediately above the BMD.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model is displayed in boldface type. BMDLs for models with adequate fit differed by less than threefold, so the models with the lowest AIC (Log-probit and Weibull models) were initially selected as the best fitting. The Weibull model had a slightly lower BMDL between the two models; thus, the Weibull was selected.

As shown in Table B-3, in attempting to model the incidence of hepatocellular cytoplasmic vacuolization in female rats with all six dose groups included, the BMDS failed to generate any output because response was not a monotonically increasing function of dose (i.e., the response in the penultimate dose group was 40%, while the response in the highest dose group was 0). A key underlying assumption for the fitting of the dichotomous models in BMDS is that response must be a monotonically non-decreasing function of dose. Therefore, the highest dose group was dropped, and the models were fit to the data again. In this instance, the χ^2 goodness-of-fit test showed that all models exhibited inadequate fit (i.e., $p < 0.1$). Finally, in an

attempt to find a model that fit, the two highest dose groups were dropped and the models were refit to these data. In this case, all of the models exhibited adequate fit, except for the one- and two-stage multistage models ($p \geq 0.10$).

Of the models exhibiting adequate fit, a "best-fit" model was selected consistent with the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b) as follows. If the BMDL estimates from the models exhibiting adequate fit are "sufficiently close," then the model with the lowest AIC is to be used to estimate the BMDL from which the POD will be derived. In this particular case, as explained in the footnote in Table B-3, BMDLs for models with adequate fit differed by less than threefold. Among these models, the log-probit and Weibull models shared the lowest AIC, and, thus, the average $BMDL_{10}$ from these two models (i.e., 19.36 mg/kg-day) was used to derive a possible POD. The standard BMDS outputs from the log-probit and Weibull models are displayed below.




```
 ================================================================
        Probit Model. (Version: 3.1;  Date: 05/16/2008)
        Input Data File:
C:\USEPA\IRIS\TCE\NTP\hepcytvac\female\lnp_hepcytvacF2HDD_logprobit.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\hepcytvac\female\lnp_hepcytvacF2HDD_logprobit.plt
                                          Tue Mar 30 12:54:34 2010
 ================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = incidence
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
```

Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0
intercept = -8.43383
slope = 2.43905

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-60.1746	2420.13	-4803.54	4683.19
slope	18	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	0	4			
Fitted model	-4.43789e-009	1	8.87578e-009	3	1
Reduced model	-27.7259	1	55.4518	3	<.0001

AIC: 2

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
20.0000	0.0000	0.000	0.000	10	-0.000
40.0000	1.0000	10.000	10.000	10	0.000
80.0000	1.0000	10.000	10.000	10	0.000

Chi^2 = 0.00 d.f. = 3 P-value = 1.0000

Benchmark Dose Computation

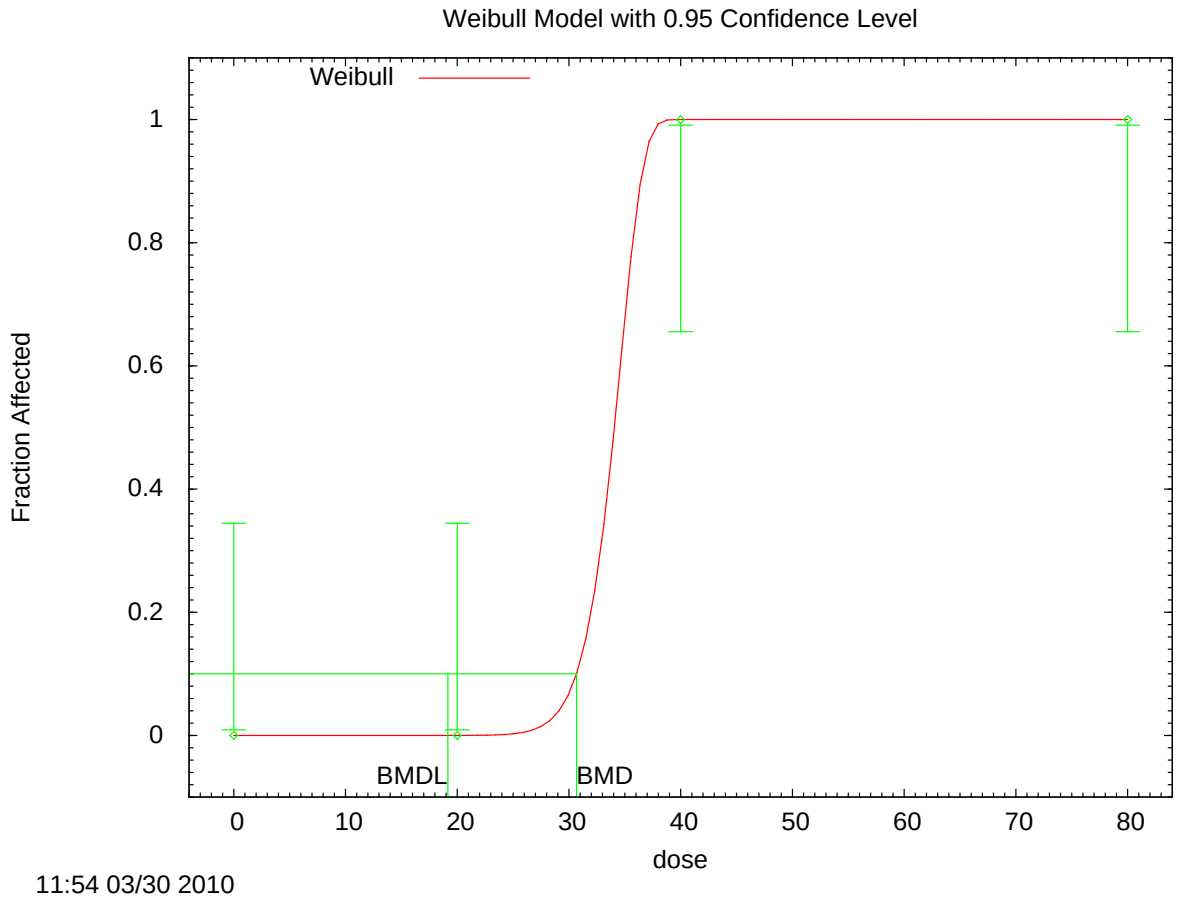
Specified effect = 0.1

```
Risk Type        =      Extra risk

Confidence level =            0.95

             BMD =         26.3597

            BMDL =          19.557
```




```
=============================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File:
C:\USEPA\IRIS\TCE\NTP\hepcytvac\female\wei_hepcytvacF2HDD_weibull.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\hepcytvac\female\wei_hepcytvacF2HDD_weibull.plt
                                          Tue Mar 30 12:54:37 2010
 ============================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = incidence
   Independent variable = dose
   Power parameter is restricted as power >=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
```

Default Initial (and Specified) Parameter Values
Background = 0.0454545
Slope = 0.00369372
Power = 1.53227

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Slope
Slope	-1.$

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	1.81559e-028	1.#QNAN	1.#QNAN	1.#QNAN
Power	18	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	0	4			
Fitted model	-0.000514093	1	0.00102819	3	1
Reduced model	-27.7259	1	55.4518	3	<.0001

AIC: 2.00103

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
20.0000	0.0000	0.000	0.000	10	-0.022
40.0000	1.0000	10.000	10.000	10	0.006
80.0000	1.0000	10.000	10.000	10	0.000

Chi^2 = 0.00 d.f. = 3 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 30.681

BMDL = 19.1631

Continuous Endpoints

Organ weight and serum chemistry changes in male and female rats (NTP, 2004)

Table B-4. Selected organ weight and serum chemistry changes in male and female F344 rats administered 1,1,2,2-tetrachlroethane in the diet for 14 weeks

Endpoint	Sex	Dose (mg/kg-d)					
		0	20	40	80	170	320
Absolute liver wt (g)	Male	12.74 ± 0.26a	12.99 ± 0.35	14.47 ± 0.44	15.54 ± 0.40	11.60 ± 0.44	6.57 ± 0.18
	Female	6.84 ± 0.17	7.03 ± 0.13	7.14 ± 0.16	7.80 ± 0.08	6.66 ± 0.22	4.94 ± 0.12
Relative liver wt (mg organ wt/g body wt)	Male	34.79 ± 0.42	36.72 ± 0.44	41.03 ± 0.85	45.61 ± 0.52	44.68 ± 0.45	52.23 ± 1.42
	Female	35.07 ± 0.56	36.69 ± 0.36	37.84 ± 0.51	44.20 ± 0.27	48.03 ± 0.89	58.40 ± 1.42
Serum ALT activity (IU/L)	Male	48 ± 2	49 ± 2	53 ± 2	69 ± 3	115 ± 8	292 ± 18
	Female	46 ± 2	42 ± 1	41 ± 2	49 ± 2	112 ± 7	339 ± 18
Serum SDH activity (IU/L)	Male	23 ± 1	27 ± 1	26 ± 2	31 ± 1	47 ± 2	74 ± 4
	Female	27 ± 1	27 ± 1	28 ± 2	25 ± 1	45 ± 3	82 ± 3
Serum bile acid levels (µmol/L)	Male	29.2 ± 2.9	27.5 ± 2.7	27.2 ± 2.7	35.9 ± 3.9	92.0 ± 16.6	332.4 ± 47.4
	Female	37.0 ± 7.1	46.6 ± 6.5	39.1 ± 5.6	36.3 ± 3.9	39.3 ± 7.9	321.5 ± 50.6

[a]Values are means ± SE for 10 animals.

Source: NTP (2004).

All available continuous models in the EPA's BMDS (version 2.1.1) were fit to each of the endpoints listed in Table B-4 for both male and female rats administered 1,1,2,2-tetrachloroethane in the diet for 14 weeks. BMDs and their 95% lower confidence limits (i.e., BMDLs) associated with a change in the response of 1 SD from the control were estimated by each model. The results of this BMD modeling for male and female rats are summarized in Tables B-5 through B-14. Following each table is the BMDS output for the selected model.

The model fitting procedure for continuous data was as follows. The simplest model (linear) is first applied to the data while assuming constant variance. If the data are consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means is evaluated and the polynomial, power, and Hill models are fit to the data while assuming constant variance. In accordance with U.S. EPA (2000b) guidance, BMDs and BMDLs are estimated employing a BMR that represents a change of 1 SD from the control. Adequate model fit is judged primarily by the goodness-of-fit *p*-value ($p > 0.1$), but visual inspection of the dose-response curve and the examination of scaled residual at the data point (except the control) closest to the predefined BMR also play a role. If the test for constant variance is negative, the linear model is run again while applying the power model integrated into BMDS to account for nonhomogeneous variance. If the nonhomogeneous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means is evaluated and the

polynomial, power, and Hill models are fit to the data and evaluated while the variance model is applied. If no model provides adequate fit to the data based on these criteria, then the highest dose is dropped, if appropriate, and the continuous modeling procedure is repeated.

Absolute liver weights in male and female rats (Tables B-5 and B-6)

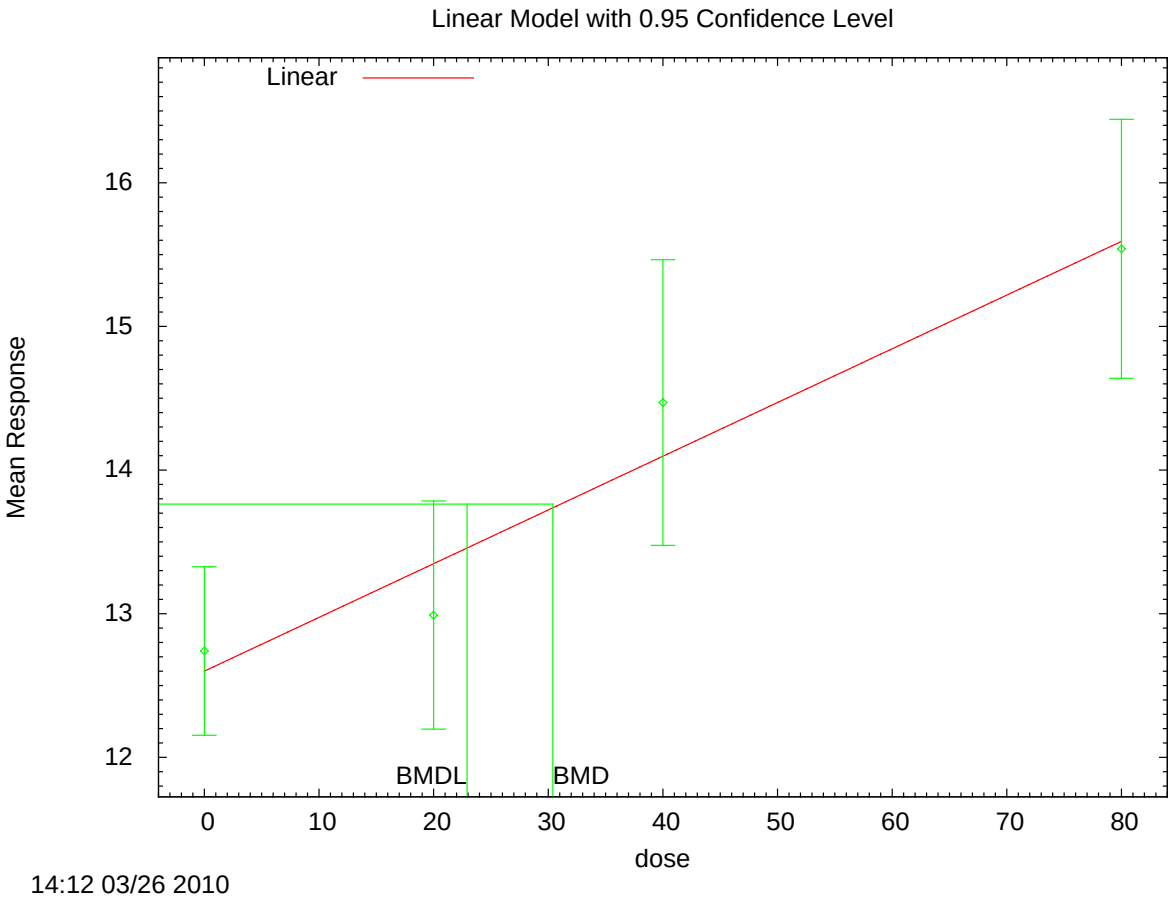
No adequate fit to the data for absolute liver weight in males or females was achieved until the two highest doses were dropped. After dropping the two highest doses, the assumption of constant variance was met, and all models provided adequate fit (except the Hill model, which has too many parameters for the number of remaining data points). BMDL estimates across the models with adequate fit differed by less than threefold. In accordance with U.S. EPA (2000b), the model with the lowest AIC (linear, for both males and females) was selected as the basis for the BMD_{1SD} and $BMDL_{1SD}$ estimates for these endpoints (respectively, 30 and 23 mg/kg-day for males, and 36 and 26 mg/kg-day for females).

Table B-5. Summary of BMD modeling results for absolute liver weight in male rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	0.07	<0.0001	NA	198.13	NA	3,925.92
Non-constant variance							
Hill[e]	<0.0001	0.39	<0.0001	-0.7/1.81	160.48	36.49	NA
Linear[d]	<0.0001	0.39	<0.0001	NA	200.13	NA	10.43
Polynomial (2-degree)[d]	<0.0001	0.39	<0.0001	NA	200.13	NA	10.45
Polynomial (3-degree)[d]	<0.0001	0.39	<0.0001	NA	200.13	NA	733.03
Polynomial (4-degree)[d]	<0.0001	0.39	<0.0001	NA	200.13	NA	595.06
Polynomial (5-degree)[d]	<0.0001	0.39	<0.0001	NA	200.13	NA	533.37
Power[e]	<0.0001	0.39	<0.0001	-1.43/0.08	106.77	173.92	141.52
Highest dose group dropped							
Constant variance							
Hill[e]	<0.0001	0.49	<0.0001	3.3/0.00	100.95	165.58	94.36
Linear[d]	<0.0001	0.49	<0.0001	NA	112.67	NA	606.09
Polynomial (2-degree)[d]	<0.0001	0.49	<0.0001	NA	112.67	NA	416.42
Polynomial (3-degree)[d]	<0.0001	0.49	<0.0001	NA	112.67	NA	326.66
Polynomial (4-degree)[d]	<0.0001	0.49	<0.0001	NA	112.67	NA	282.11
Power[e]	<0.0001	0.49	<0.0001	3.3/0.00	98.95	166.09	145.65
Two highest dose groups dropped							
Constant variance							
Hill[e]	<0.0001	0.41	NA	0.00/0.00	57.97	32.10	20.62
Linear[d,f]	**<0.0001**	**0.41**	**0.32**	**-1.07/0.97**	**56.26**	**30.40**	**22.92**
Power[e]	<0.0001	0.41	0.13	-1.03/1.01	58.25	31.30	22.93

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; ; NA = not applicable (BMD/BMDL computation failed or insufficient DF)

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Best-fitting model is displayed in boldface type. BMDLs for models providing adequate fit differed by less than threefold, so the model with the lowest AIC was selected.


Linear Model with 0.95 Confidence Level
Linear
Mean Response
16
15
14
13
12
BMDL
BMD
0
10
20
30
40
50
60
70
80
dose
14:12 03/26 2010

```
==========================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File:
C:\USEPA\IRIS\TCE\NTP\abslivwt\male\lin_abslivwtM2HDD_linear.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\abslivwt\male\lin_abslivwtM2HDD_linear.plt
                                          Fri Mar 26 15:12:39 2010
 ==========================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   rho is set to 0
   The polynomial coefficients are restricted to be positive
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =      1.35605
                            rho =            0   Specified
                         beta_0 =       12.626
                         beta_1 =       0.0374


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been
specified by the user,
                 and do not appear in the correlation matrix )

                   alpha       beta_0       beta_1

     alpha             1    -6.9e-010    -4.8e-011

    beta_0    -6.9e-010            1        -0.76

    beta_1    -4.8e-011        -0.76            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable        Estimate       Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         alpha         1.29235        0.288979           0.725966             1.85874
       beta_0          12.626        0.278462            12.0802             13.1718
```

beta_1	0.0374	0.00607655	0.0254902	0.0493098

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	12.7	12.6	0.82	1.14	0.317
20	10	13	13.4	1.11	1.14	-1.07
40	10	14.5	14.1	1.39	1.14	0.968
80	10	15.5	15.6	1.26	1.14	-0.217

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-23.984311	5	57.968622
A2	-22.556035	8	61.112070
A3	-23.984311	5	57.968622
fitted	-25.129323	3	56.258645
R	-38.455553	2	80.911106

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	31.799	6	<.0001
Test 2	2.85655	3	0.4143
Test 3	2.85655	3	0.4143
Test 4	2.29002	2	0.3182

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

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

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 30.3962

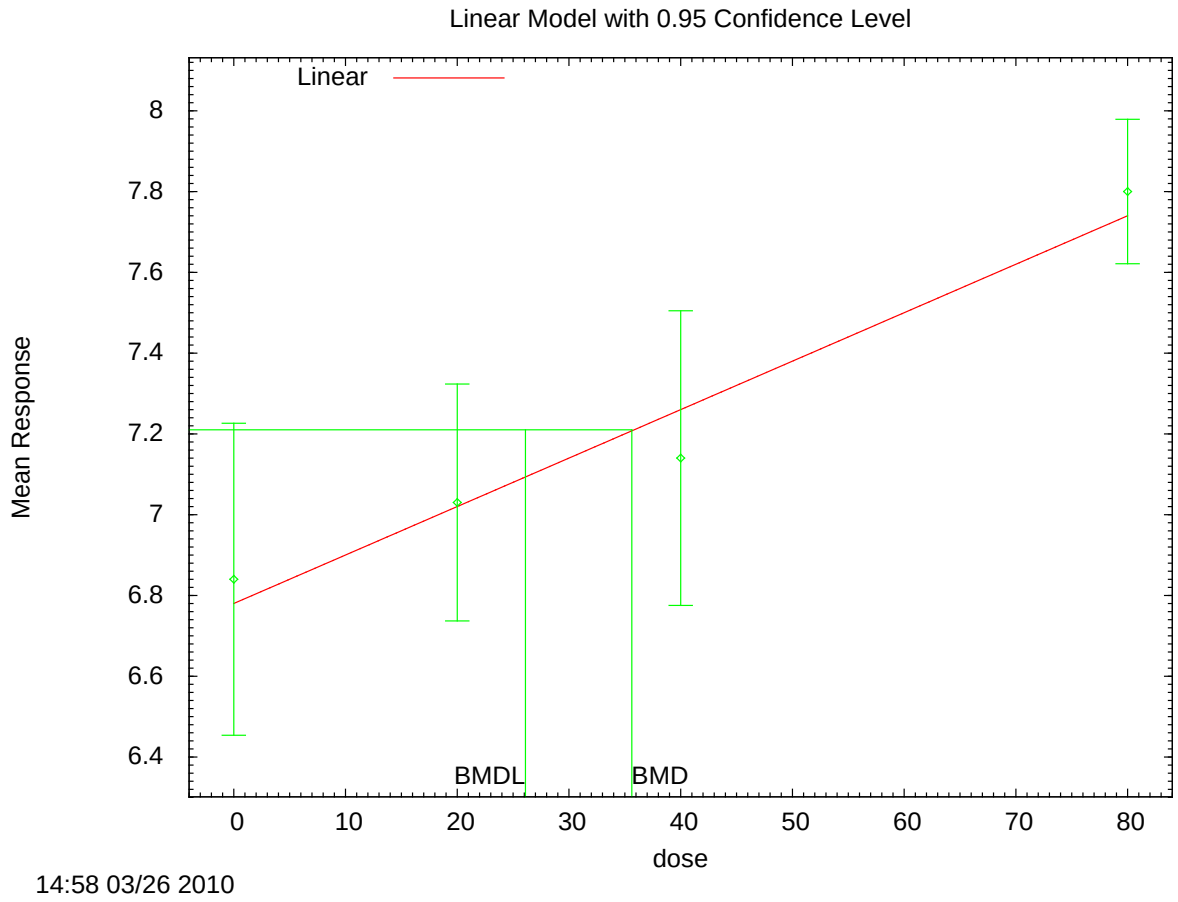
BMDL = 22.9198

Table B-6. Summary of BMD modeling results for absolute liver weight in female rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	0.05	<0.0001	NA	62.98	NA	3,632.46
Non-constant variance							
Linear[d]	<0.0001	0.02	<0.0001	NA	64.98	NA	24.07
Highest dose group dropped							
Constant variance							
Linear[d]	<0.0001	0.04	<0.0001	NA	5.69	NA	377.10
Non-constant variance							
Hill[e]	<0.0001	0.84	<0.0001	0.00[f]	4.52	170.20	NA
Linear[d]	<0.0001	0.84	<0.0001	NA	7.69	NA	397.23
Polynomial (2-degree)[d]	<0.0001	0.84	<0.0001	NA	7.69	NA	343.87
Polynomial (3-degree)[d]	<0.0001	0.84	<0.0001	NA	7.69	NA	290.54
Polynomial (4-degree)[d]	<0.0001	0.84	<0.0001	NA	7.69	NA	67.91
Power[e]	<0.0001	0.84	<0.0001	0.00[f]	2.52	170.19	153.95
Two highest dose groups dropped							
Constant variance							
Hill[e]	<0.0001	0.11	NA	-0.30/0.05	-19.17	48.28	25.37
Linear[d,g]	**<0.0001**	**0.11**	**0.55**	**0.05/-0.91**	**-22.27**	**35.62**	**26.10**
Polynomial (2-degree)[d]	<0.0001	0.11	0.63	-0.28/0.05	-21.25	48.21	27.58
Polynomial (3-degree)[d]	<0.0001	0.11	0.71	-0.19/0.02	-21.35	49.83	27.77
Power[e]	<0.0001	0.11	0.57	-0.30/0.05	-21.17	48.28	27.44

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; NA = not applicable (BMD/BMDL computation failed or insufficient DF to fit model)

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Residual at highest dose tested.
[g]Best-fitting model displayed in boldface type. BMDLs for models providing adequate fit differed by less than threefold, so the model with the lowest AIC was selected.




```
 ======================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File:
C:\USEPA\IRIS\TCE\NTP\abslivwt\female\lin_abslivwtF2HDD_linear.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\abslivwt\female\lin_abslivwtF2HDD_linear.plt
                                          Fri Mar 26 15:58:54 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   rho is set to 0
   The polynomial coefficients are restricted to be positive
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	0.195575	
rho =	0	Specified
beta_0 =	6.784	
beta_1 =	0.0119571	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	-8e-009	8.2e-009
beta_0	-8e-009	1	-0.76
beta_1	8.2e-009	-0.76	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.181435	0.04057	0.101919	0.26095
beta_0	6.784	0.104336	6.5795	6.9885
beta_1	0.0119571	0.00227681	0.00749468	0.0164196

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	6.84	6.78	0.54	0.426	0.416
20	10	7.03	7.02	0.41	0.426	0.0509
40	10	7.14	7.26	0.51	0.426	-0.908
80	10	7.8	7.74	0.25	0.426	0.441

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	14.743437	5	-19.486874
A2	17.781442	8	-19.562884
A3	14.743437	5	-19.486874
fitted	14.137196	3	-22.274391
R	3.648385	2	-3.296770

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	28.2661	6	<.0001
Test 2	6.07601	3	0.108
Test 3	6.07601	3	0.108
Test 4	1.21248	2	0.5454

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

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

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 35.6232

BMDL = 26.1046

Relative liver weights in male and female rats (Tables B-7 and B-8)

No model provided an adequate fit to the relative liver weight data in male rats even after dropping the two highest dose groups. Therefore, these data are considered unsuitable for BMD modeling. For the relative liver weight data in females, the assumption of constant variance was satisfied and the power and 2- and 3-degree polynomial models provided adequate fit to the data after the highest two dose groups were dropped. BMDL estimates across these models differed by less than threefold. In accordance with U.S. EPA (2000b), the model with the lowest AIC (3-degree polynomial) was selected as the basis for the BMD_{1SD} and $BMDL_{1SD}$ estimates of 22 and 15 mg/kg-day, respectively, for this endpoint.

Table B-7. Summary of BMD modeling results for relative liver weight in male rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	<0.0001	1.6/4.15	208.74	68.02	56.64
Non-constant variance							
Linear[d]	<0.0001	0.03	<0.0001	1.93/4.36	208.89	55.05	37.77
Highest dose group dropped							
Constant variance							
Linear[d]	<0.0001	0.09	<0.0001	1.84/4.25	165.27	51.62	40.95
Non-constant variance							
Linear[d]	<0.0001	0.06	<0.0001	-0.79/-0.95	157.11	12.93	8.10
Two highest dose groups dropped							
Constant variance							
Linear[d]	<0.0001	0.07	0.15	0.25/-1.24	94.60	13.14	10.76
Non-constant variance							
Linear[d]	<0.0001	0.08	0.09	0.35/-1.32	95.74	10.97	7.77
Three highest doses dropped							
Constant variance							
Linear[d]	<0.0001	0.03	0.10	0.66/-1.32	74.39	12.16	9.27
Non-constant variance							
Hill[e]	NA						
Linear[d]	<0.0001	0.52	0.05	0.45/-1.32	71.18	8.47	6.05
Polynomial (2-degree)[d]	<0.0001	0.52	NA	-0.07/0.12	69.32	15.27	8.46
Power[e]	<0.0001	0.52	NA	-0.07/0.12	69.32	15.50	9.02

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; NA = not applicable (insufficient DF to fit the model)

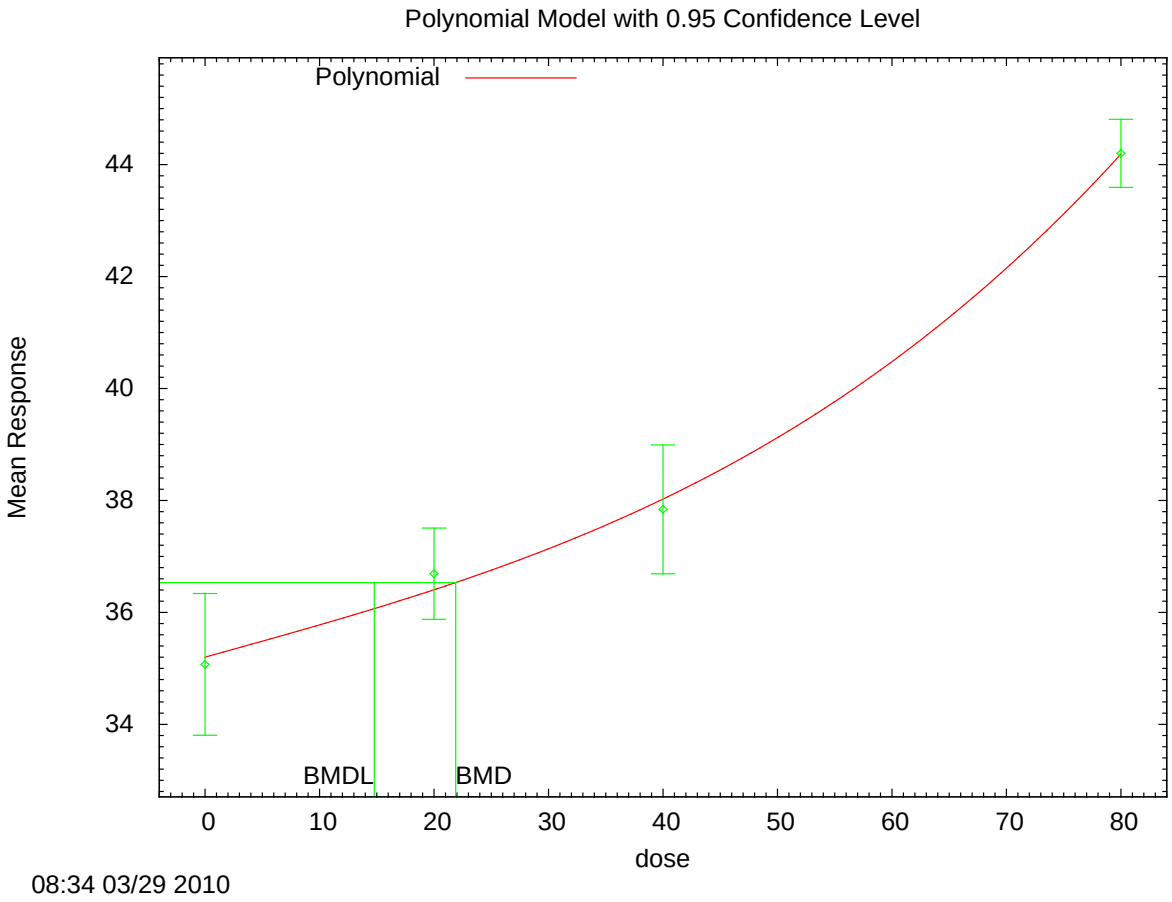
[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.

Table B-8. Summary of BMD modeling results for relative liver weight in female rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	0.01	-0.66/-1.01	181.20	36.16	30.95
Non-constant variance							
Linear[d]	<0.0001	0.01	<0.0001	<-10/<-10	6.00	0.003	NA
Highest dose group dropped							
Constant variance							
Linear[d]	<0.0001	0.002	<0.0001	-0.52/-1.19	129.06	26.16	21.87
Non-constant variance							
Linear[d]	<0.0001	0.01	0.001	-0.12/-0.30	123.73	16.52	12.39
Two highest dose groups dropped							
Constant variance							
Hill[e]	<0.0001	0.11	NA	1.12/-0.72	74.32	25.33	17.12
Linear[d]	<0.0001	0.11	0.005	1.31/-0.09	78.98	13.20	10.81
Polynomial (2-degree)[d]	<0.0001	0.11	0.22	0.94/-0.70	71.76	23.57	15.68
Polynomial (3-degree)[d,f]	**<0.0001**	**0.11**	**0.38**	**0.69/-0.43**	**70.98**	**21.90**	**14.78**
Power[e]	<0.0001	0.11	0.15	1.12/-0.72	72.32	25.31	17.12

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; NA= not applicable (BMD/BMDL computation failed or insufficient DF to fit model)

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Best-fitting model is displayed in boldface type. BMDLs for models providing adequate fit differed by less than threefold, so the model with the lowest AIC was selected.




```
 ======================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File:
C:\USEPA\IRIS\TCE\NTP\rellivwt\female\ply_rellivwtF2HDD_Poly_3.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\rellivwt\female\ply_rellivwtF2HDD_Poly_3.plt
                                          Mon Mar 29 09:34:20 2010
 ======================================================================

 BMDS Model Run
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   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   rho is set to 0
   The polynomial coefficients are restricted to be positive
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	1.93677	
rho =	0	Specified
beta_0 =	35.07	
beta_1 =	0.115542	
beta_2 =	0	
beta_3 =	2.84896e-005	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho -beta_2
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1	beta_3
alpha	1	-6e-009	3.2e-009	-1.7e-009
beta_0	-6e-009	1	-0.76	0.56
beta_1	3.2e-009	-0.76	1	-0.92
beta_3	-1.7e-009	0.56	-0.92	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	1.77636	0.397207	0.997852	2.55487
beta_0	35.1967	0.395218	34.4221	35.9713
beta_1	0.0567055	0.0185417	0.0203645	0.0930465
beta_2	1.59898e-026	NA		
beta_3	8.68894e-006	2.57808e-006	3.636e-006	1.37419e-005

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	35.1	35.2	1.77	1.33	-0.301
20	10	36.7	36.4	1.14	1.33	0.687
40	10	37.8	38	1.61	1.33	-0.43
80	10	44.2	44.2	0.85	1.33	0.043

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

```
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                     Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1          -31.113274            5      72.226548
            A2          -28.050020            8      72.100041
            A3          -31.113274            5      72.226548
         fitted         -31.491356            4      70.982711
             R          -72.394938            2     148.789876


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              88.6898          6          <.0001
   Test 2              6.12651          3          0.1056
   Test 3              6.12651          3          0.1056
   Test 4             0.756163          1          0.3845

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

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


              Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean
```

```
Confidence level =          0.95

             BMD =        21.8955

            BMDL =        14.7785
```

Serum ALT activity in male and female rats (Tables B-9 and B-10)

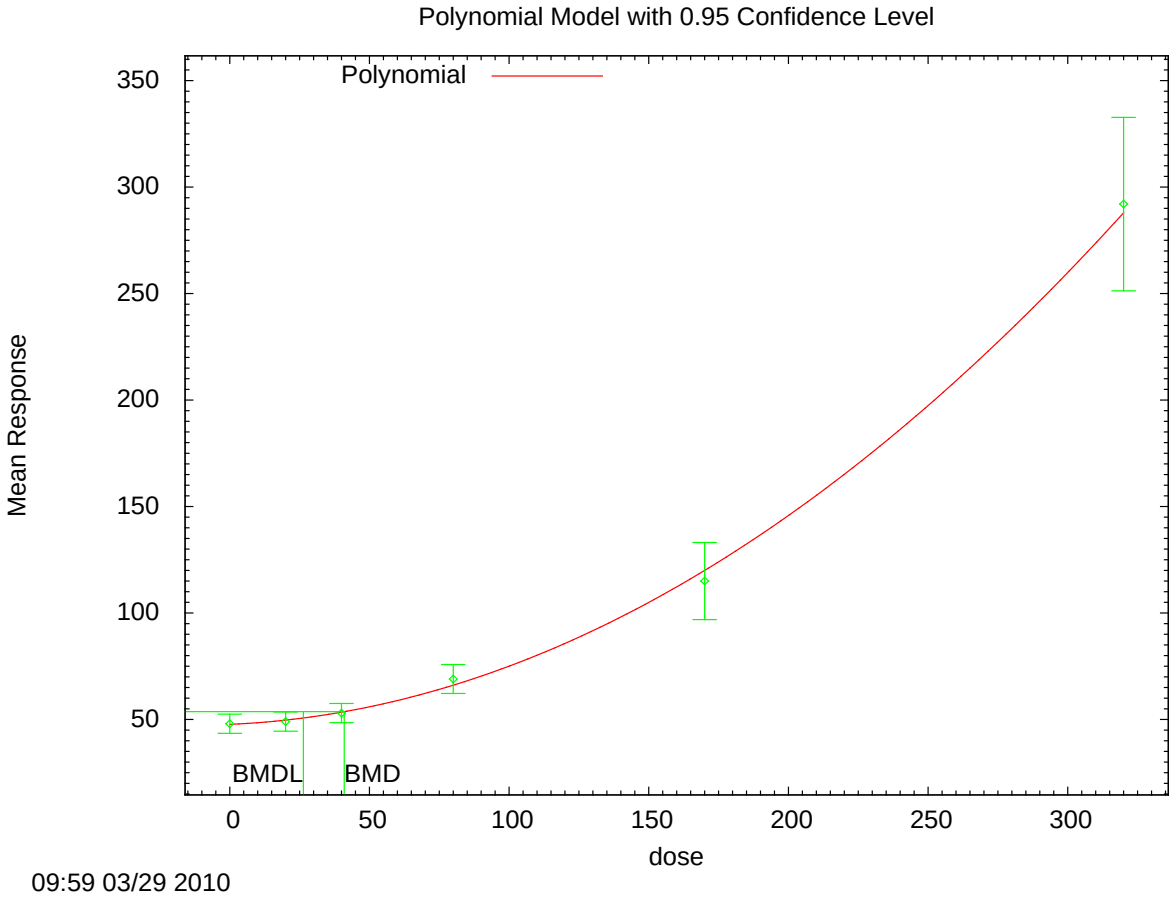
All doses were retained in the BMD modeling of serum ALT in males and females. The assumption of constant variance was not upheld for either dataset, but in each case, the power model for variance built into the BMDS provided adequate fit to the variance data. With the variance model applied, adequate fit to the means was provided by the Hill, power, and 2- and 5-degree polynomial models for the males, and by the Hill model alone for the females. For the males, estimated BMDLs from the adequately fitting models differed by less than threefold. In accordance with U.S. EPA (2000b), the model with the lowest AIC (i.e., 2-degree polynomial) was selected as the basis for the BMD_{1SD} and $BMDL_{1SD}$ estimates of 41 and 26 mg/kg-day. For the females, BMD_{1SD} and $BMDL_{1SD}$ estimates of 82 and 69 mg/kg-day were based on the Hill model.

Table B-9. Summary of BMD modeling results for serum ALT activity in male rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	<0.0001	-0.19/-1.55	486.88	43.91	37.37
Non-constant variance							
Hill[e]	<0.0001	0.72	0.51	0.10/0.77	370.02	42.19	34.33
Linear[d]	<0.0001	0.72	<0.0001	>10	6.00	0.00	NA
Polynomial (2-degree)[d,f]	**<0.0001**	**0.72**	**0.84**	**-0.21/1.00**	**366.08**	**40.98**	**26.35**
Polynomial (3-degree)[d]	<0.0001	0.72	<0.0001	>10	10.00	0.00	NA
Polynomial (4-degree)[d]	<0.0001	0.72	<0.0001	NA	606.63	NA	28.22
Polynomial (5-degree)[d]	<0.0001	0.72	0.47	-0.14/1.06	370.17	40.62	26.19
Power[e]	<0.0001	0.72	0.73	-0.11/0.76	367.96	41.97	32.24

Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; NA= not applicable (BMD/BMDL computation failed)

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Best-fitting model is displayed in boldface type. BMDLs for models providing adequate fit differed by less than threefold, so the model with the lowest AIC was selected.




```
================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\IRIS\TCE\NTP\ALT\male\ply_ALTM_poly_2.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\ALT\male\ply_ALTM_poly_2.plt
                                          Mon Mar 29 10:59:45 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   The polynomial coefficients are restricted to be positive
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

lalpha =	6.52437
rho =	0
beta_0 =	48.8991
beta_1 =	0.00912505
beta_2 =	0.00233971

!!! Warning: optimum may not have been found. !!!
!!! You may want to try choosing different initial values. !!!

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	beta_0	beta_1	beta_2
lalpha	1	-0.0021	-0.015	0.027
beta_0	-0.0021	1	-0.71	0.49
beta_1	-0.015	-0.71	1	-0.86
beta_2	0.027	0.49	-0.86	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-6.58334	0.182468	-6.94097	-6.22571
rho	2.62555	NA		
beta_0	47.7312	1.57297	44.6483	50.8142
beta_1	0.05625	0.0541054	-0.0497946	0.162295
beta_2	0.00216953	0.000281829	0.00161716	0.0027219

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	48	47.7	6.3	5.95	0.143
20	10	49	49.7	6.3	6.28	-0.365
40	10	53	53.5	6.3	6.9	-0.207
80	10	69	66.1	9.5	9.12	1
170	10	115	120	25.3	19.9	-0.792
320	10	292	288	56.9	62.9	0.206

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
             A1         -222.570247            7     459.140493
             A2         -177.293103           12     378.586206
             A3         -178.329731            8     372.659462
         fitted         -179.039110            4     366.078220
              R         -300.315008            2     604.630016


                   Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              246.044         10          <.0001
   Test 2              90.5543          5          <.0001
   Test 3              2.07326          4          0.7223
   Test 4              1.41876          4          0.8409

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

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


              Benchmark Dose Computation

Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean
```

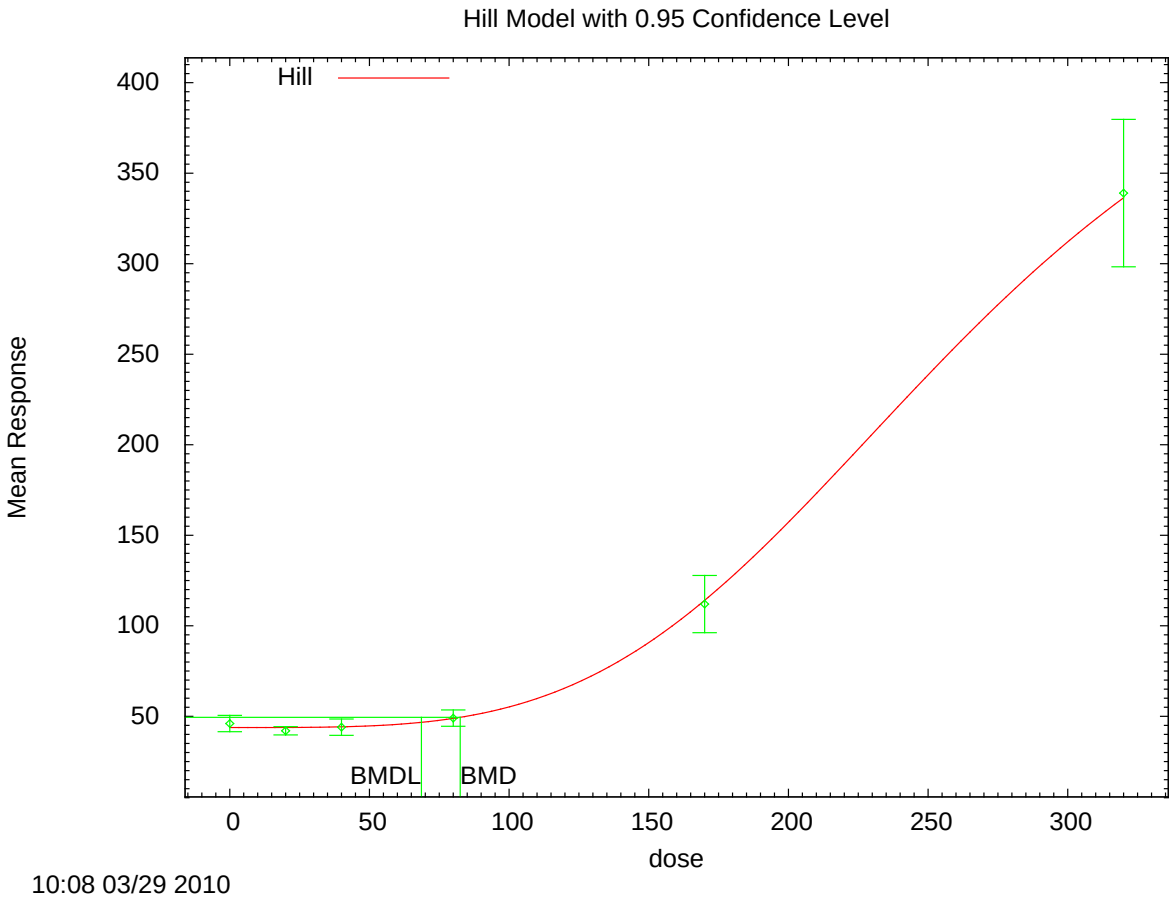
```
Confidence level =          0.95

             BMD =        40.9754

            BMDL =        26.3459
```

Table B-10. Summary of BMD modeling results for serum ALT activity in female rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	<0.0001	-0.12/2.54	512.92	45.04	38.30
Non-constant variance							
Hill[e,f]	**<0.0001**	**0.23**	**0.16**	**0.09/-0.29**	**351.50**	**82.49**	**68.61**
Linear[d]	<0.0001	0.23	<0.0001	0.79/3.84	444.14	142.23	12.12
Polynomial (2-degree)[d]	<0.0001	0.23	<0.0001	-0.91/-0.16	413.32	65.95	19.55
Polynomial (3-degree)[d]	<0.0001	0.23	<0.0001	-0.95/-0.20	415.39	71.30	15.90
Polynomial (4-degree)[d]	<0.0001	0.23	<0.0001	-0.77/-0.40	392.73	71.75	22.50
Polynomial (5-degree)[d]	<0.0001	0.23	<0.0001	-0.85/-0.14	432.77	79.16	13.16
Power[e]	<0.0001	0.23	0.02	-0.26/-1.58	355.84	64.07	55.45

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Best-fitting model is displayed in boldface type. In this case, Hill model was the only model that provided an adequate fit to the data.




```
================================================================
        Hill Model. (Version: 2.14;  Date: 06/26/2008)
        Input Data File: C:\USEPA\IRIS\TCE\NTP\ALT\female\hil_ALTF_Hill.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\ALT\female\hil_ALTF_Hill.plt
                                          Mon Mar 29 11:08:43 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = mean
   Independent variable = dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

lalpha =	6.46604
rho =	0
intercept =	46
v =	293
n =	2.07344
k =	416.806

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	intercept	v	n	k
lalpha	1	-0.99	-0.12	0.1	-0.0074	0.051
rho	-0.99	1	0.098	-0.11	0.0073	-0.052
intercept	-0.12	0.098	1	-0.41	0.49	-0.42
v	0.1	-0.11	-0.41	1	-0.9	0.98
n	-0.0074	0.0073	0.49	-0.9	1	-0.95
k	0.051	-0.052	-0.42	0.98	-0.95	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-5.48513	1.18231	-7.80242	-3.16783
rho	2.36002	0.272384	1.82615	2.89388
intercept	43.8372	1.06856	41.7428	45.9315
v	440.049	121.144	202.612	677.486
n	3.71466	0.661842	2.41747	5.01185
k	266.476	45.4588	177.378	355.573

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	46	43.8	6.3	5.58	1.23
20	10	42	43.9	3.2	5.58	-1.06
40	10	44	44.2	6.3	5.63	-0.124
80	10	49	48.8	6.3	6.33	0.0904
170	10	112	114	22.1	17.1	-0.29
320	10	339	336	56.9	61.6	0.159

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that

were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-220.820465	7	455.640931
A2	-165.059425	12	354.118851
A3	-167.889045	8	351.778089
fitted	-169.749216	6	351.498431
R	-312.021870	2	628.043741

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	293.925	10	<.0001
Test 2	111.522	5	<.0001
Test 3	5.65924	4	0.2261
Test 4	3.72034	2	0.1556

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

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

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 82.493

BMDL = 68.6138

Serum SDH activity in male and female rats (Tables B-11 and B-12)

No model provided an adequate fit to the data for changes in serum SDH activity in male rats. This was due to the difficulty in modeling the reported variances. As a result, these data are considered unsuitable for BMD modeling. For females, only the power model provided an adequate fit to the serum SDH activity data after the highest dose was dropped and the variance was modeled using the non-constant variance model included in BMDS. This model served as the basis for the BMD_{1SD} and $BMDL_{1SD}$ estimates of 157 and 113 mg/kg-day for this endpoint.

Table B-11. Summary of BMD modeling results for serum SDH activity in male rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	0.19	-0.75/-1.42	293.96	41.70	35.55
Non-constant variance							
Linear[d]	<0.0001	0.05	<0.0001	-0.92/0.60	307.18	62.52	11.14
Highest dose group dropped							
Constant variance							
Linear[d]	<0.0001	0.02	0.08	1.33/-1.16	212.18	34.45	28.37
Non-constant variance							
Linear[d]	<0.0001	0.03	0.05	1.09/-1.28	212.07	32.47	19.12
Two Highest dose groups dropped							
Constant variance							
Linear[d]	0.0004	0.04	0.26	-0.92/0.15	159.19	45.73	31.69
Non-constant variance							
Linear[d]	0.0004	0.03	0.17	-0.91/0.13	161.04	42.28	25.15
Three highest dose groups dropped							
Constant variance							
Linear[d]	0.03	0.04	0.14	-0.60[e]	125.02	58.79	27.97
Non-constant variance							
Linear[d]	0.03	0.05	0.64	1.20/-0.82	122.10	27.88	13.75

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD

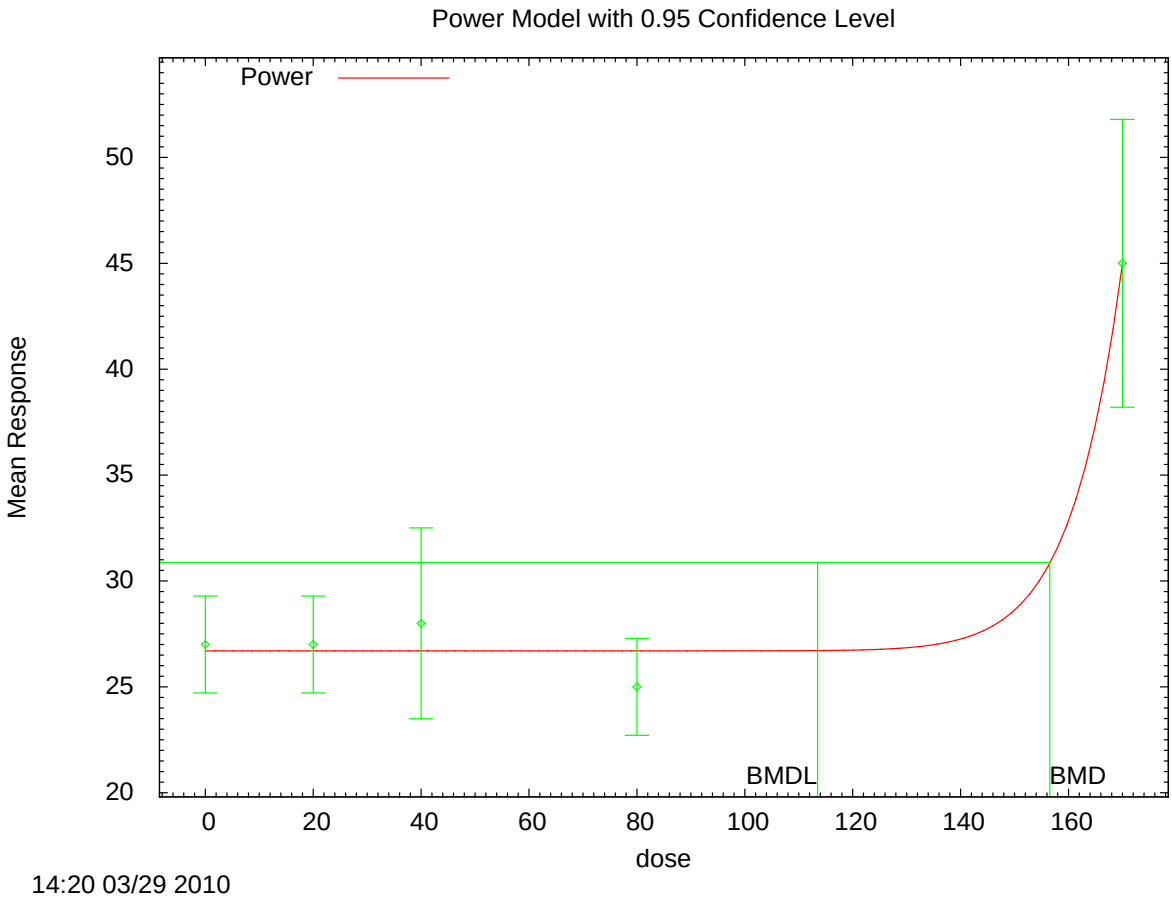
[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Residual reported for highest dose tested.

Table B-12. Summary of BMD modeling results for serum SDH activity in female rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	<0.0001	0.18/-3.60	321.64	47.70	40.47
Non-constant variance							
Linear[d]	<0.0001	0.04	<0.0001	NA	432.91	NA	24.11
Highest dose group dropped							
Constant variance							
Linear[d]	<0.0001	0.0002	0.0001	-0.05/-3.48	244.99	63.45	48.93
Non-constant variance							
Hill[e]	<0.0001	0.18	0.05	-1.34/0.00	217.37	153.80	NA
Linear[d]	<0.0001	0.18	0.00	-0.09/-2.36	229.76	67.45	38.00
Polynomial (2-degree)[d]	<0.0001	0.18	0.00	-2.77/1.04	224.39	87.97	66.87
Polynomial (3-degree)[d]	<0.0001	0.18	0.01	-2.19/0.42	219.90	106.18	87.33
Polynomial (4-degree)[d]	<0.0001	0.18	0.04	-1.78/0.17	217.52	118.22	102.34
Power[e,f]	**<0.0001**	**0.18**	**0.10**	**-1.34/0.00**	**215.37**	**156.52**	**113.49**

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; NA = not applicable (BMD/BMDL computation failed)

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Best-fitting model is displayed in boldface type. Power model was the only model that provided an adequate fit to the data.




```
 ======================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File:
C:\USEPA\IRIS\TCE\NTP\SDH\female\pow_SDHFHDD_power.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\SDH\female\pow_SDHFHDD_power.plt
                                        Mon Mar 29 15:20:23 2010
 ======================================================================

 BMDS Model Run
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = mean
   Independent variable = dose
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

lalpha =	3.46985
rho =	0
control =	25
slope =	0.0617409
power =	1.1118

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	control	slope
lalpha	1	-1	-0.15	0.37
rho	-1	1	0.14	-0.37
control	-0.15	0.14	1	-0.22
slope	0.37	-0.37	-0.22	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-7.0365	3.52075	-13.937	-0.135945
rho	3.00361	1.03813	0.968917	5.0383
control	26.75	0.652491	25.4711	28.0289
slope	1.29772e-039	2.07902e-040	8.90244e-040	1.7052e-039
power	18	NA		

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	27	26.7	3.2	4.13	0.192
20	10	27	26.7	3.2	4.13	0.192
40	10	28	26.7	6.3	4.13	0.958
80	10	25	26.8	3.2	4.13	-1.34
170	10	45	45	9.5	9.01	3.88e-006

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

```
 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1         -109.112298            6     230.224595
              A2          -98.178926           10     216.357851
              A3         -100.610596            7     215.221192
          fitted         -103.685379            4     215.370759
               R         -135.518801            2     275.037602

                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              74.6798          8          <.0001
   Test 2              21.8667          4        0.000213
   Test 3              4.86334          3          0.1821
   Test 4              6.14957          3          0.1046

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

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


                Benchmark Dose Computation

Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95
```

BMD = 156.523

BMDL = 113.491

Serum bile acids in male and female rats (Tables B-13 and B-14)

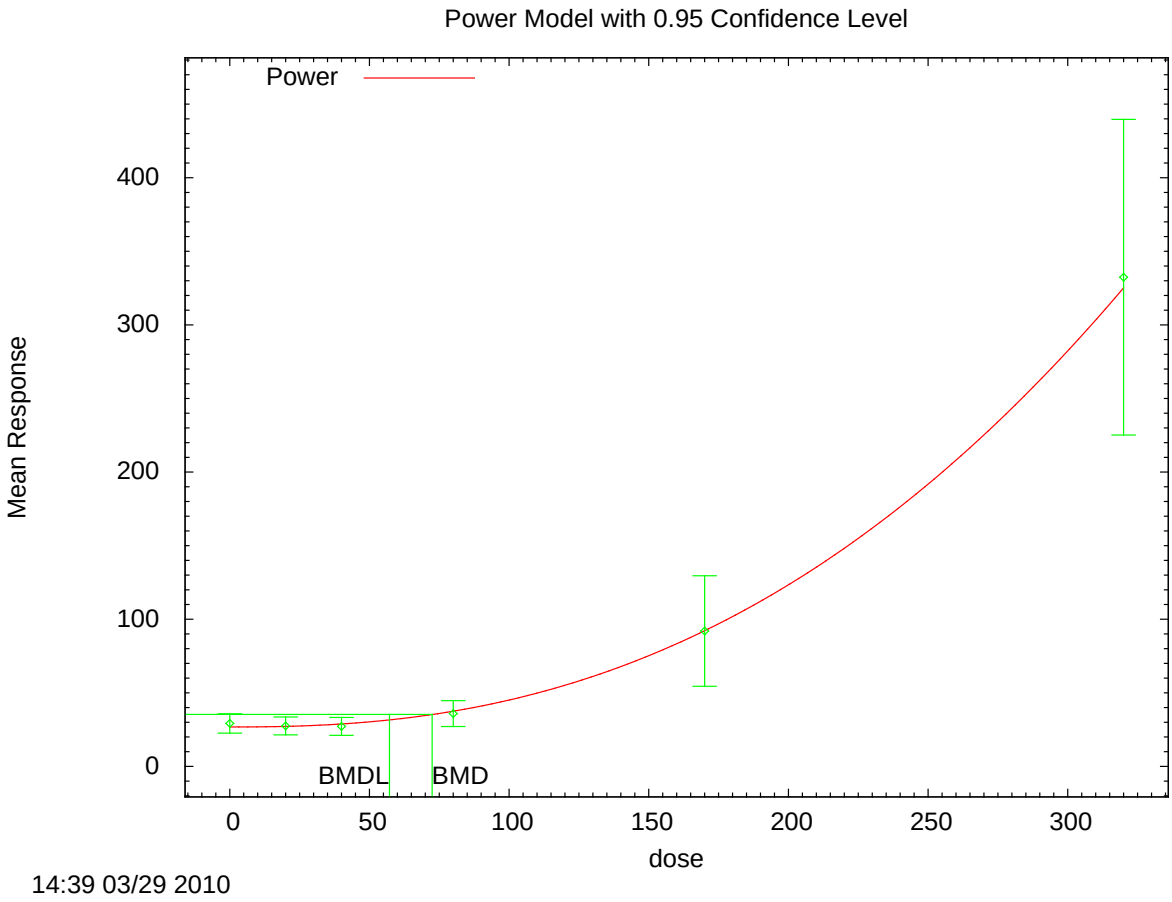
All doses were retained in the modeling of serum bile acids in males and females. The assumption of constant variance was not upheld for either dataset, but in each case, the power model for variance included in BMDS provided adequate fit to the variance data. With the variance model applied, adequate fit to the mean data was provided by several models for each sex, and for both datasets, BMDL estimates across models with adequate fit differed by less than threefold. In accordance with U.S. EPA (2000b), the models with the lowest AIC (power model for males and 5-degree polynomial model for females) were selected as the basis for the BMD_{1SD} and $BMDL_{1SD}$ estimates for these endpoints (respectively, 72 and 57 mg/kg-day for males and 188 and 170 mg/kg-day for females).

Table B-13. Summary of BMD results for serum bile acid levels in male rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	0.002	-0.10/-1.38	578.68	76.00	62.75
Non-constant variance							
Hill[e]	<0.0001	0.77	0.69	0.17/-0.74	427.84	82.84	66.69
Linear[d]	<0.0001	0.77	<0.0001	0.48/2.69	454.67	115.63	36.05
Polynomial (2-degree)[d]	<0.0001	0.77	0.21	-0.88/-1.16	428.95	58.37	50.80
Polynomial (3-degree)[d]	<0.0001	0.77	0.32	-0.65/-0.56	428.58	69.21	54.31
Polynomial (4-degree)[d]	<0.0001	0.77	0.32	-0.65/-0.56	428.58	69.21	54.31
Polynomial (5-degree)[d]	<0.0001	0.77	<0.0001	-1.08/0.17	449.32	76.72	25.65
Power[e,f]	**<0.0001**	**0.77**	**0.46**	**-0.56/-0.43**	**427.70**	**72.45**	**57.17**

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Best-fitting model is displayed in boldface type. BMDLs for models providing adequate fit differed by less than threefold, so the model with the lowest AIC was selected.




```
 ======================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\USEPA\IRIS\TCE\NTP\bile\male\pow_BileM_power.(d)
        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\bile\male\pow_BileM_power.plt
                                          Mon Mar 29 15:39:39 2010
 ======================================================================

 BMDS Model Run
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = mean
   Independent variable = dose
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

lalpha =	8.35885
rho =	0
control =	27.2
slope =	0.000160062
power =	2.50584

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	control	slope	power
lalpha	1	-0.98	-0.31	-0.17	0.22
rho	-0.98	1	0.25	0.18	-0.23
control	-0.31	0.25	1	-0.3	0.28
slope	-0.17	0.18	-0.3	1	-1
power	0.22	-0.23	0.28	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-3.601	1.08576	-5.72905	-1.47295
rho	2.39924	0.272426	1.86529	2.93318
control	26.8064	1.58205	23.7056	29.9071
slope	0.000289806	0.000360688	-0.00041713	0.000996743
power	2.40282	0.233505	1.94515	2.86048

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	29.2	26.8	9.2	8.54	0.886
20	10	27.5	27.2	8.5	8.69	0.111
40	10	27.2	28.9	8.5	9.33	-0.561
80	10	35.9	37.6	12.3	12.8	-0.429
170	10	92	93.1	52.5	38	-0.0914
320	10	332	330	150	173	0.0463

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that

were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-277.604668	7	569.209336
A2	-206.636351	12	437.272702
A3	-207.553828	8	431.107657
fitted	-208.851786	5	427.703572
R	-320.497188	2	644.994376

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	227.722	10	<.0001
Test 2	141.937	5	<.0001
Test 3	1.83495	4	0.7661
Test 4	2.59591	3	0.4582

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

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

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 72.4471

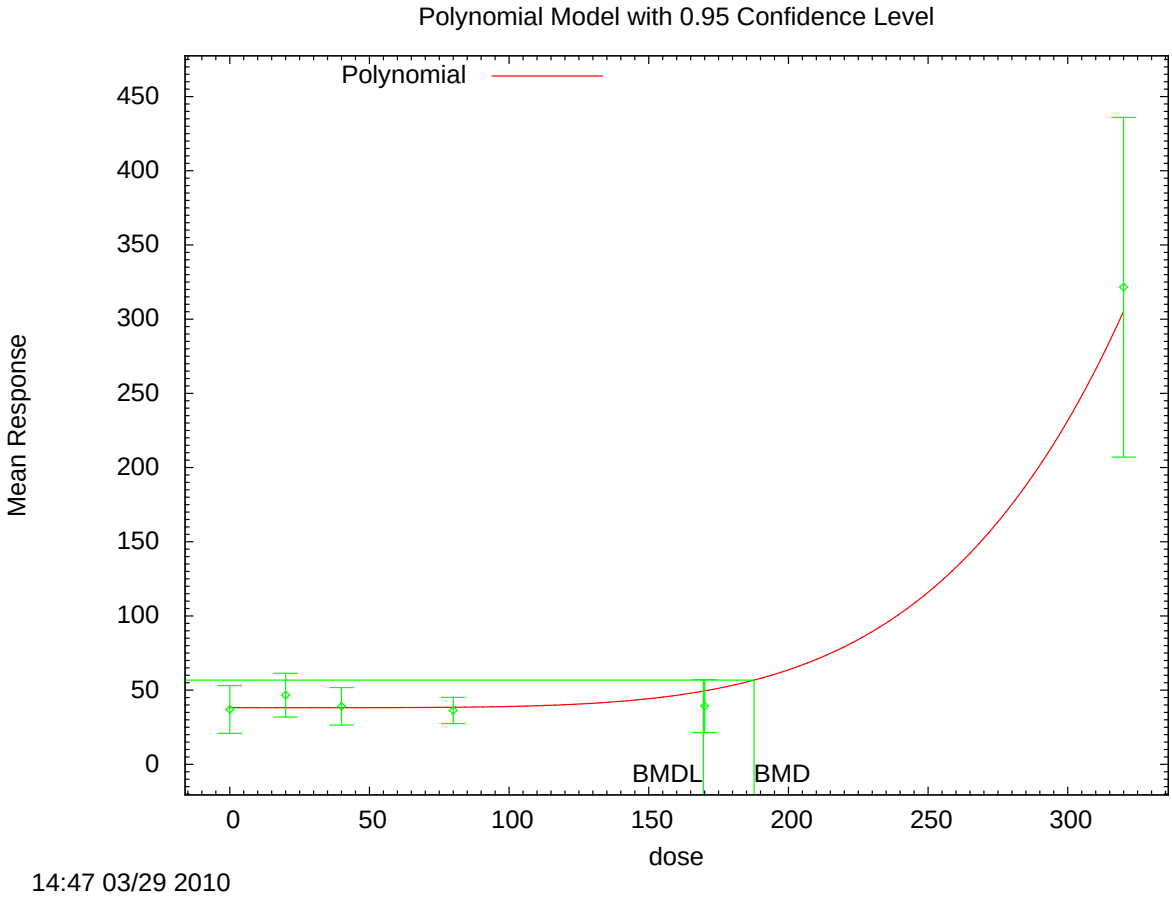
BMDL = 57.1682

Table B-14. Summary of BMD modeling results for serum bile acid levels in female rats

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	<0.0001	-1.13/-3.83	596.57	101.36	81.28
Non-constant variance							
Hill[e]	<0.0001	0.47	0.38	-0.51/0.02	466.68	186.94	177.64
Linear[d]	<0.0001	0.47	<0.0001	3.70[f]	505.52	343.48	139.12
Polynomial (2-degree)[d]	<0.0001	0.47	<0.0001	3.09[f]	485.36	344.76	145.95
Polynomial (3-degree)[d]	<0.0001	0.47	0.003	-0.71/-2.18	477.39	149.70	129.07
Polynomial (4-degree)[d]	<0.0001	0.47	0.08	-0.42/-1.95	469.90	168.35	152.78
Polynomial (5-degree)[d,g]	**<0.0001**	**0.47**	**0.33**	**-1.34/0.34**	**466.14**	**187.71**	**169.55**
Power[e]	<0.0001	0.47	0.38	-0.50/0.02	466.68	216.74	177.00

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1.
[f]Residual at highest dose tested.
[g]Best-fitting model is displayed in boldface type. BMDLs for models providing adequate fit differed by less than threefold, so the model with the lowest AIC was selected.




```
================================================================
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        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\NTP\bile\female\ply_BileF_Poly_5.plt
                                          Mon Mar 29 15:47:49 2010
 ================================================================

 BMDS Model Run
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   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   The polynomial coefficients are restricted to be positive
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

lalpha =	8.43454
rho =	0
beta_0 =	37
beta_1 =	0
beta_2 =	0
beta_3 =	0
beta_4 =	0
beta_5 =	0

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -beta_1 -beta_2 -beta_3 -beta_4
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	beta_0	beta_5
lalpha	1	-0.98	-0.049	0.16
rho	-0.98	1	0.049	-0.16
beta_0	-0.049	0.049	1	-0.15
beta_5	0.16	-0.16	-0.15	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-1.58198	1.00675	-3.55517	0.391218
rho	2.03725	0.245366	1.55634	2.51816
beta_0	38.2101	2.76802	32.7849	43.6353
beta_1	1.25128e-026	NA		
beta_2	0	NA		
beta_3	0	NA		
beta_4	0	NA		
beta_5	7.95519e-011	1.43294e-011	5.14667e-011	1.07637e-010

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	37	38.2	22.5	18.5	-0.206
20	10	46.6	38.2	20.6	18.5	1.43
40	10	39.1	38.2	17.7	18.5	0.15
80	10	36.3	38.5	12.3	18.7	-0.368
170	10	39.3	49.5	25	24.1	-1.34
320	10	322	305	160	154	0.336

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-279.875470	7	573.750939
A2	-224.999384	12	473.998768
A3	-226.787639	8	469.575277
fitted	-229.071113	4	466.142225
R	-318.845182	2	641.690364

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	187.692	10	<.0001
Test 2	109.752	5	<.0001
Test 3	3.57651	4	0.4663
Test 4	4.56695	4	0.3347

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels

It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

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

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 187.713

BMDL = 169.553

Fetal body weights in Sprague-Dawley rats (Tables B-15 and B-16)

Fetal body weight data from Gulati et al. (1991a) in Sprague-Dawley rats administered 1,1,2,2-tetrachloroethane in the diet on GD 4–20 are shown in Table B-15. BMD modeling results based on these data are shown in Table B-16. Adequate model fit was achieved for the fetal body weight data only after the highest two dose groups were dropped. This was due to difficulty in modeling the reported variances. After dropping the two highest dose groups, the remaining dose groups satisfied the assumption of constant variance. Assuming constant variance, the linear model provided adequate fit to the mean fetal body weight data. The higher order models either did not fit ($p < 0.1$: higher order polynomial, power) or failed due to too many parameters for the available data points (Hill). The linear model is the basis for the BMD_{1SD} and $BMDL_{1SD}$ estimates of 83 and 60 mg/kg-day, respectively, for this endpoint shown in Table B-16.

Table B-15. Fetal body weight in Sprague-Dawley rats administered 1,1,2,2-tetrachloroethane in the diet on GDs 4–20

Dose (mg/kg-d)	Number of animals	Mean (g)	Standard error
0	9	2.28	0.04
34	8	2.17	0.04
98	8	2.19	0.03
180	9	1.99	0.05
278	9	2.04	0.14
330	5	1.81	0.12

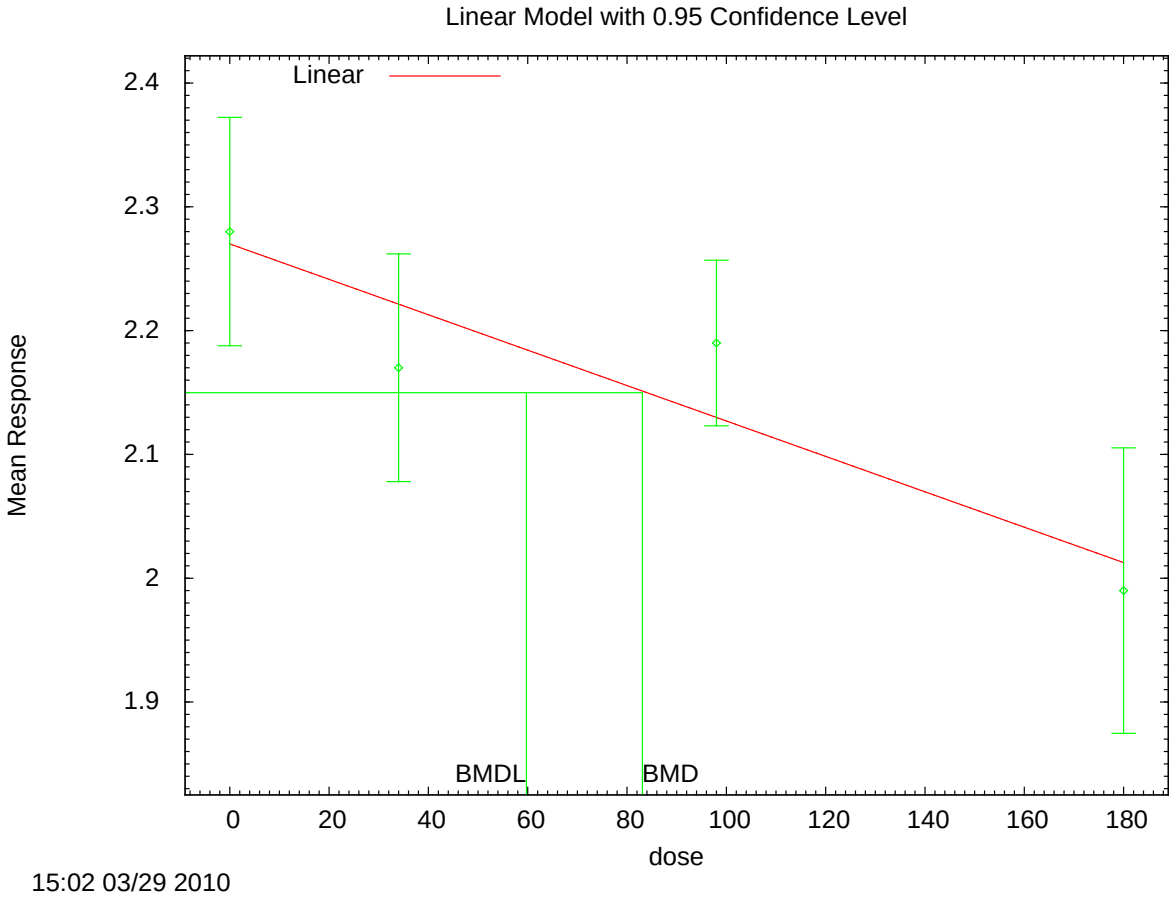
Source: Gulati et al. (1991a).

Table B-16. Summary of BMD modeling results for fetal body weight following exposure of pregnant Sprague-Dawley rats on GDs 4–20

Model	Test for significant difference *p*-value[a]	Variance *p*-value[b]	Mean *p*-value[b]	Scaled residuals of interest[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
All dose groups included							
Constant variance							
Linear[d]	<0.0001	<0.0001	0.40	-0.92/1.23	-91.54	201.09	139.17
Non constant variance							
Linear[d]	<0.0001	0.07	0.20	-1.25/0.88	-112.47	84.64	56.25
Highest dose group dropped							
Constant variance							
Linear[d]	<0.0001	<0.0001	0.40	-1.24/0.70	-83.65	238.24	147.87
Non constant variance							
Linear[d]	<0.0001	0.05	0.18	-1.27/0.83	-105.40	84.31	53.36
Two highest dose groups dropped							
Constant variance							
Hill[e]	0.0002	0.35	NA	0.38/-0.06	-101.33	129.74	61.35
Linear[d,f]	**0.0002**	**0.35**	**0.12**	**-1.19/1.46**	**-104.84**	**83.10**	**59.73**
Polynomial (2-degree)[d]	0.0002	0.35	0.06	0.87/-0.20	-103.53	110.21	62.16
Polynomial (3-degree)[d]	0.0002	0.35	0.08	0.65/-0.09	-103.98	118.06	64.06
Power[e]	0.0002	0.35	0.06	0.38/-0.06	-103.33	129.71	61.40

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and immediately above the BMD.
[d]Coefficients restricted to be negative.
[e]Power restricted to ≥1.
[f]Best-fitting model is displayed in boldface type. The linear model is the only model providing an adequate fit to the data.




```
==========================================================================
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        Gnuplot Plotting File:
C:\USEPA\IRIS\TCE\gulati\fetalbdwt\lin_fetalbdwt2HDD_linear.plt
                                          Mon Mar 29 16:02:57 2010
 ==========================================================================

 BMDS Model Run
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   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	0.0141567	
rho =	0	Specified
beta_0 =	2.26747	
beta_1 =	-0.0014099	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	-1.3e-010	2e-010
beta_0	-1.3e-010	1	-0.75
beta_1	2e-010	-0.75	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.0141234	0.00342543	0.00740968	0.0208371
beta_0	2.26874	0.0306445	2.20868	2.3288
beta_1	-0.00143017	0.000290756	-0.00200004	-0.000860296

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	9	2.28	2.27	0.12	0.119	0.284
34	8	2.17	2.22	0.11	0.119	-1.19
98	8	2.19	2.13	0.08	0.119	1.46
180	9	1.99	2.01	0.15	0.119	-0.538

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	57.506457	5	-105.012914
A2	59.148779	8	-102.297557
A3	57.506457	5	-105.012914
fitted	55.418685	3	-104.837369
R	46.282389	2	-88.564779

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.7328	6	0.0002497
Test 2	3.28464	3	0.3498
Test 3	3.28464	3	0.3498
Test 4	4.17554	2	0.124

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

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

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 83.0965

BMDL = 59.7345

APPENDIX C. BENCHMARK DOSE MODELING RESULTS FOR THE DERIVATION OF THE ORAL SLOPE FACTOR

Hepatocellular carcinomas in male and female B6C3F$_1$ mice (Tables C-1 and C-2)

The incidence data for hepatocellular carcinomas in male and female B6C3F$_1$ mice exposed via gavage to 1,1,2,2-tetrachloroethane 5 days/week for 78 weeks are shown in Table C-1 (NCI, 1978).

Table C-1. Incidence of hepatocellular carcinomas in B6C3F$_1$ mice administered 1,1,2,2-tetrachloroethane by gavage for 78 weeks

Endpoint	Sex	Dose (mg/kg-d)[a]		
		0[b]	8.22	16.5
Hepatocellular carcinomas	Male	3/36	13/50	44/49
	Female	1/40	30/48	43/47

[a]HED as calculated in Section 5.4.3 and shown in Table 5-5.
[b]Pooled vehicle controls.

Source: NCI (1978).

The BMD modeling results from the data in Table C-1 are summarized in Tables C-2 (for males) and C-3 (for females) followed by the standard BMDS output for the selected models from version 2.1.1 of the software. The multistage cancer model did not provide an adequate fit to the incidence data for hepatocellular carcinomas in male mice; these data are considered unsuitable for BMD modeling. The one-stage multistage model provided the best fit to the incidence data for hepatocellular carcinomas in females, and this model was used as the basis for the BMD$_{10}$ and BMDL$_{10}$ estimates (0.81 and 0.65 mg/kg-day, respectively, as HEDs) for this endpoint.

Table C-2. Summary of BMD modeling results for the incidence of hepatocellular carcinomas in male mice

Model	DF	χ^2	χ^2 Goodness of fit *p*-value[a]	Scaled residuals of interest[b]	AIC	$BMD_{10[HED]}$ (mg/kg-d)	$BMDL_{10[HED]}$ (mg/kg-d)
Multistage (1-degree polynomial)[c]	1	18.30	<0.001	0.51/-3.27	134.58	1.42	1.11
Multistage (2-degree polynomial)[c]	1	5.24	0.02	0.53/-1.83	119.87	4.10	3.08

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; DF = degrees of freedom

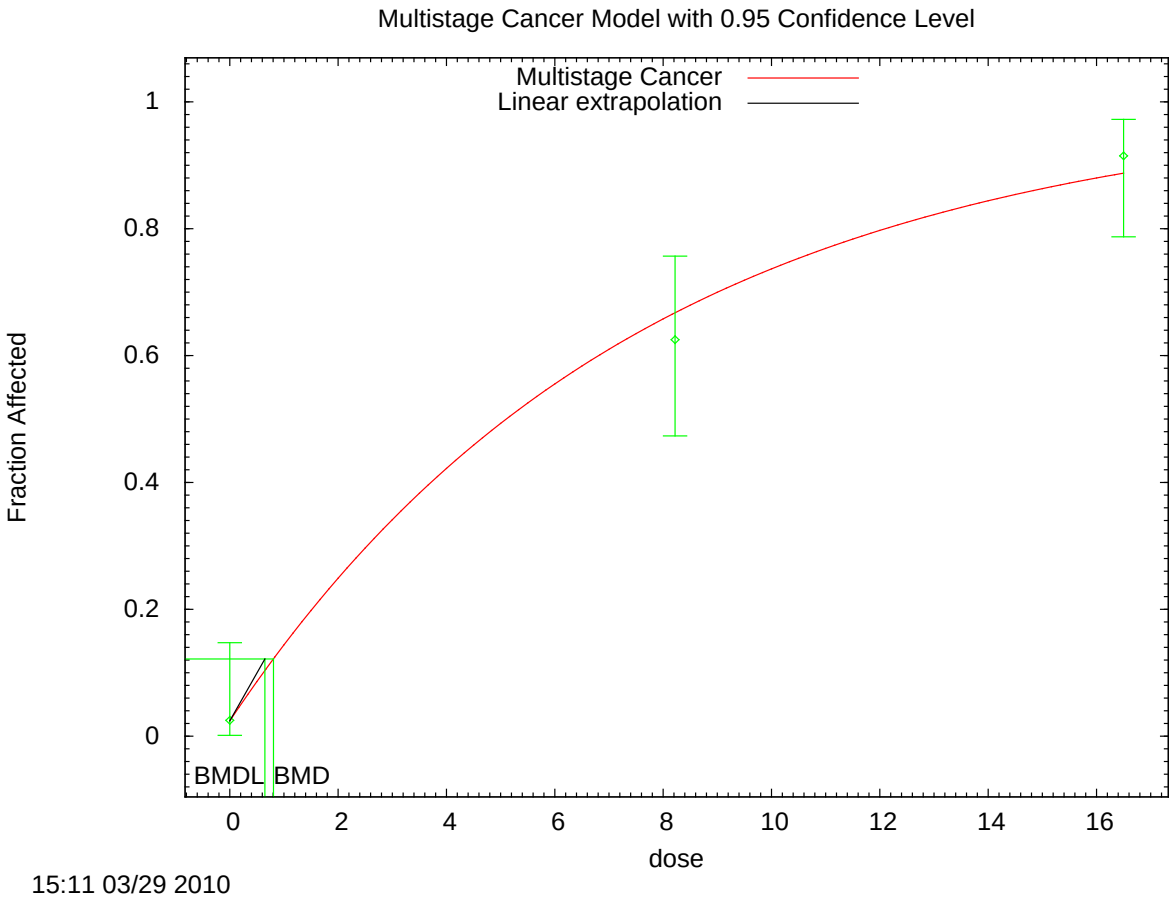
[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and immediately above the BMD.
[c]Betas restricted to ≥0.

Table C-3. Summary of BMD modeling results for the incidence of hepatocellular carcinomas in female mice

Model	DF	χ^2	χ^2 Goodness of fit *p*-value[a]	Scaled residual of interest[b]	AIC	$BMD_{10[HED]}$ (mg/kg-d)	$BMDL_{10[HED]}$ (mg/kg-d)
Multistage (1-degree polynomial)[c,d]	**1**	**0.74**	**0.39**	**0.04/-0.61**	**104.99**	**0.81**	**0.65**
Multistage (2-degree polynomial)[c]	0	0.00	NA	0.00/0.00	106.22	1.18	0.67

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD; DF = degrees of freedom; NA= not applicable (*p*-value was not generated due to insufficient DF)

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and immediately above the BMD.
[c]Betas restricted to ≥0.
[d]Selected model is displayed in boldface type.




```
 ======================================================================
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C:\USEPA\IRIS\TCE\NCI\hepcarc\female\msc_hepcarcF_MS_1.plt
                                          Mon Mar 29 16:11:43 2010
 ======================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = incidence
   Independent variable = dose

 Total number of observations = 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
Beta(1) = 0.147828

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.54
Beta(1)	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0240983	*	*	*
Beta(1)	0.130589	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-50.1115	3			
Fitted model	-50.4931	2	0.763231	1	0.3823
Reduced model	-92.948	1	85.673	2	<.0001
AIC:	104.986				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0241	0.964	1.000	40	0.037
8.2200	0.6664	31.988	30.000	48	-0.608
16.5000	0.8869	41.682	43.000	47	0.607

Chi^2 = 0.74 d.f. = 1 P-value = 0.3897

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

```
              BMD =       0.806812

             BMDL =       0.648049

             BMDU =        1.01577

Taken together, (0.648049, 1.01577) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.154309
```