8-14-2008

Provisional Peer Reviewed Toxicity Values for

1,1,2,2-Tetrachloroethane

(CASRN 79-34-5)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 1,1,2,2-TETRACHLOROETHANE (CASRN 79-34-5)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically

updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Subchronic or chronic RfD or RfC values for 1,1,2,2-tetrachloroethane are not listed in the IRIS (U.S. EPA, 2008) or HEAST (U.S. EPA, 1997) databases. The Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) reports an RfD of 5E-5 mg/kg-day for 1,1,2,2-tetrachloroethane that is based on a route-to-route extrapolation from an inhalation LOAEL of 13.3 mg/m^3 for hepatic changes and decreased body weight in rats exposed for 265 days (Schmidt et al., 1972). The CARA lists (U.S. EPA, 1991, 1994) include a Health Effects Assessment (HEA) for 1,1,2,2-tetrachloroethane (U.S. EPA, 1984) that did not derive an RfD due to lack of data. ATSDR (1996) has derived oral and inhalation MRLs for 1,1,2,2-tetrachloroethane. An intermediate oral MRL of 0.6 mg/kg-day is based on a NOAEL of 56 mg/kg for decreased body weight gain in rats exposed to 1,1,2,2-tetrachloroethane for 6 weeks (NCI, 1978). A chronic, oral MRL of 0.04 mg/kg-day is based on a LOAEL of 43 mg/kg/day for respiratory effects (labored respiration, wheezing, and nasal discharge) in rats exposed for 78 weeks (NCI, 1978). An intermediate-duration inhalation MRL of 0.4 ppm is based on a LOAEL of 130 ppm for liver effects (increased weight and histological changes) in rats exposed for 15 weeks (Truffert et al., 1977).

As listed in IRIS (U.S. EPA, 2008), the U.S. EPA has classified 1,1,2,2-tetrachloroethane in carcinogenicity Group C (possible human carcinogen), based on an oral slope factor of 2.0E-1 per (mg/kg-day) derived from liver tumor data in a 78-week gavage study in mice (NCI, 1978), and an IUR of 5.8E-5 per (µg/m^3) derived by route-to-route extrapolation from the oral data. IARC (2005) classifies 1,1,2,2-tetrachloroethane in carcinogenicity Group 3 (not classifiable as to its carcinogenicity in humans) based on inadequate evidence in humans and

limited evidence in experimental animals. The World Health Organization (WHO) (2005) has not published any health effects documents for 1,1,2,2-tetrachloroethane.

Literature searches through October, 2005 were conducted in MEDLINE, TOXLINE (NTIS subfile), TOXCENTER, TSCATS, CCRIS, DART/ETIC, EMIC, GENETOX, HSDB, RTECS, and Current Contents.

REVIEW OF THE PERTINENT LITERATURE

Human Studies

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) following suicide attempts. Subjects usually lost consciousness within approximately 1 hour and died 3–20 hours postingestion, depending on stomach contents. 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 case are estimated to be approximately 273, 357, 1100, and 9700 mg/kg, respectively. No deaths occurred in 8 patients (6 men and 2 women) who were accidentally given 3 mL of 1,1,2,2-tetrachloroethane (68 mg/kg, using the above assumptions), or in 3 patients (1 young man, 1 young woman, one 12-year-old girl) who were accidentally given 2 or 3 mL (98–118 mg/kg, using the assumed 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 decreased blood pressure.

Inhalation Exposure

The symptoms of high-dose acute inhalation exposure to 1,1,2,2-tetrachloroethane commonly include drowsiness, nausea, headache, constipation, decreased 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 and Schmidt-Kehl, 1936). The results of this study suggest that 3 ppm (6.9 mg/m^3) was the odor detection threshold. Concentrations of 13 ppm (89 mg/m^3) were tolerated without effect for 10 minutes. However, a 10-minute exposure to concentrations of 146 ppm (1003 mg/m^3) and a 30-minute-

exposure concentration of 336 ppm (2308 mg/m^3) were shown to cause irritation of the mucous membranes, pressure in the head, vertigo, and fatigue. Other relevant information was not 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 include increased large mononuclear cells, elevated white 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 on 127 coating workers. Employees were exposed to 75–225 ppm (500–1500 mg/m^3) 1,1,2,2-tetrachloroethane (along with other solvents). The 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-sex group of 380 workers at 23 Indian bangle manufacturing facilities (80% of workers employed at these facilities were examined). In addition to 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 including anemia (33.7%), loss of appetite (22.6%), abdominal pain (23.7%), headaches (26.6%), vertigo (30.5%), and tremors (35%) were reported. The significance of these effects cannot be determined because a control group of unexposed workers was not examined. The study authors noted that the incidence of tremors appeared to be directly related to 1,1,2,2-tetrachloroethane exposure concentrations.

Over a 3-year period, Jeney et al. (1957) examined 34–75 (exact number is unclear from the translation) 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, when in those areas, employees wore gauze masks with organic solvent filters. During the first year of the study, 1,1,2,2-tetrachloroethane levels ranged from 0.016 to 1.7 mg/L (16–1700 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 decreased, ranging 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; 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 a general physical examination, 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 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 gastrointestinal 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 (presumably, this is 66% of the workers with symptoms). Other symptoms included headaches, general weakness and fatigue in 29%, severe weight loss in 4%, and "tormenting itching" in 1% of the examined workers. Enlargement of the liver was observed in 38% of the examined workers. Urobilinogenuria was detected in 50% of the workers; 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% of examined workers). 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% of the examined workers. The investigators noted that the increased urobilinogen levels may have been secondary to excessive alcohol consumption or dietary excess. Enlarged livers were found in 5% of the examined workers. In the first 3 years of the study, no alterations in erythrocyte or hemoglobin levels were found. Leukopenia (defined as leukocyte levels of less than 5800 cells/mL) was found in 20% of the workers, but no relationship between the number of cases and duration of 1,1,2,2-tetrachloroethane exposure was found. A positive relationship between duration of exposure and frequency of abnormal liver function test results was found; statistically significant correlations were found on the thymol and TakatB-Ucko liver function tests, but not on the gold sol reaction test. Abnormal hippuric acid levels were only detected in 1% of the examined workers during the first 2 years of the study 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 the poor reporting of study design and results precludes using this study for quantitative risk assessment.

Norman et al. (1981) examined the mortality of the employees of 39 chemical processing plants formed by the Army during World War II. Ten employees used 1,1,2,2-tetrachloroethane to help treat clothing while the other 29 employees used water in the same process. Estimates of exposure levels were not reported. At the time of evaluation, 2414 deaths were reported from the study cohort. No differences in standard mortality ratios were seen between the 1,1,2,2-tetrachloroethane- and the water-exposed groups in terms of total mortality, cardiovascular disease, liver cirrhosis, or digestive system or respiratory system cancer. The mortality ratio for lymphatic cancers in the 1,1,2,2-tetrachloroethane-exposed group was increased relative to controls and the water group—although the number of deaths was small (4 cases, with an expected 0.85). No other differences were seen between the groups.

Animal Studies

Oral Exposure

The NTP (2004) fed groups of male and female F344 rats (10/sex/group) diets containing 0, 268, 589, 1180, 2300, or 4600 ppm of microencapsulated 1,1,2,2-tetrachloroethane for 14 weeks. The reported average daily doses were 0, 20, 40, 80, 170, or 320 mg/kg-day; vehicle control (feed with empty microcapsules) and untreated control groups were used for both sexes. Endpoints evaluated throughout the study included clinical signs, body weight, and feed consumption. Hematology (12 indices) and clinical chemistry (10 indices) 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 tissue), and uterus. Functional observational batteries (FOBs) (21 parameters) were performed on rats in both control groups and the 20, 40, and 80 mg/kg-day groups during weeks 4 and 13. Sperm evaluations and vaginal cytology evaluations were performed at 0, 40, 80, and 170 mg/kg-day. 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 percentage of time spent in the various estrus stages and estrous cycle length.

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 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 40, 80, 170 and 320 mg/kg-day (3, 9, 29, and 56% lower, respectively); at 320 mg/kg-day, rats of both sexes lost weight. Feed consumption decreased with increasing dose level at 170 and 320 mg/kg-day and may have contributed to the reduced body weight gain and weight loss. Results of the FOBs showed no exposure-related findings of neurotoxicity. The hematology evaluations indicated that 1,1,2,2-tetrachloroethane affected the circulating erythroid mass in both sexes (Table 1). There was evidence of a transient erythrocytosis, as shown by increases in hematocrit values, hemoglobin concentration, and erythrocyte counts on days 5 and 21 at $\geq$170 mg/kg-day. The erythrocytosis was not considered clinically significant and disappeared by week 14, at which time it was replaced by minimal-to-mild, dose-related anemia, as shown by decreases in hematocrit and hemoglobin at $\geq$40 mg/kg-day. For example, although males exposed to 40 mg/kg-day showed a statistically significant decrease in hemoglobin 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.

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Table 1. Body Weight, Liver Weight and Selected Serum Chemistry and Hematology Changes in Rats Exposed to Dietary 1,1,2,2-Tetrachloroethane for 14 Weeks[a,b]

Dose (mg/kg-day)	0	20	40	80	170	320
Males (10/group)						
Body weight (g)	366 ± 5	354 ± 9	353 ± 6	341 ± 6[c]	259 ± 9[c]	127 ± 5[c]
Liver weight absolute (g) relative (%)	 12.74 ± 0.26 34.79 ± 0.42	 12.99 ± 0.35 36.72 ± 0.44	 14.47 ± 0.44 41.03 ± 0.85[c]	 15.54 ± 0.39 45.61 ± 0.52[c]	 11.60 ± 0.44[c] 44.68 ± 0.45[c]	 6.57 ± 0.18[c] 52.23 ± 1.42[c]
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[c]	6.0 ± 0.1[c]
Serum cholesterol (mg/dL)	73 ± 2	74 ± 3	76 ± 2	67 ± 2	68 ± 2	65 ± 2[c]
ALT (IU/L)	48 ± 2	49 ± 2	53 ± 2	69 ± 3[c]	115 ± 8[c]	292 ± 18[c]
ALP (IU/L)	256 ± 7	260 ± 5	248 ± 5	245 ± 6	353 ± 12[c]	432 ± 24[c]
SDH (IU/L)	23 ± 1	27 ± 1[c]	26 ± 2	31 ± 1[c]	47 ± 2[c]	74 ± 4[c]
Bile acids (µmol/L)	29.2 ± 2.9	27.5 ± 2.7	27.2 ± 2.7	35.9 ± 3.9	92.0 ± 16.6[c]	332.4 ± 47.4[c]
Hematocrit (%)	45.2 ± 0.5	44.9 ± 0.4	44.0 ± 0.9	43.3 ± 0.7	43.1 ± 0.6[c]	39.0 ± 1.1[c]
Hemoglobin (Hb) (g/dL)	15.8 ± 0.1	15.6 ± 0.1	15.2 ± 0.3[c]	14.9 ± 0.1[c]	14.6 ± 0.1[c]	13.6 ± 0.3[c]
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[c]
Mean cell Hb (pg)	17.7 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	17.7 ± 0.2	16.8 ± 0.1[c]	15.5 ± 0.2[c]
Platelets (10^3/µL)	728.4 ± 12.3	707.0 ± 5.8	727.0 ± 25.2	716.3 ± 9.7	692.8 ± 12.6[c]	773.4 ± 23.2[c]
Females (10/group)						
Body weight (g)	195 ± 4	192 ± 4	189 ± 2	177 ± 2[c]	139 ± 4[c]	85 ± 3[c]
Liver weight absolute (g) relative (%)	 6.84 ± 0.17 35.07 ± 0.56	 7.03 ± 0.12 36.69 ± 0.36	 7.14 ± 0.16 37.84 ± 0.51[c]	 7.80 ± 0.08[b] 44.20 ± 0.27[b]	 6.66 ± 0.21 48.03 ± 0.89[c]	 4.94 ± 0.12[c] 58.40 ± 1.42[c]
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[c]	5.6 ± 0.1[c]
Serum cholesterol (mg/dL)	104 ± 4	105 ± 3	98 ± 1	81 ± 2[c]	64 ± 3[c]	55 ± 3[c]
ALT (IU/L)	46 ± 2	42 ± 1	41 ± 2	49 ± 2	112 ± 7[c]	339 ± 18[c]
ALP (IU/L)	227 ± 5	216 ± 4	220 ± 3	225 ± 11	341 ± 7[c]	468 ± 22[c]
SDH (IU/L)	27 ± 1	27 ± 1	28 ± 2	25 ± 1	45 ± 3[c]	82 ± 3[c]
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[c]
Hematocrit (%)	42.8 ± 0.4	43.2 ± 0.4	42.1 ± 0.4	40.1 ± 0.5[c]	42.8 ± 0.7	34.7 ± 0.7[c]
Hb (g/dL)	15.2 ± 0.1	15.3 ± 0.1	14.9 ± 0.1	14.2 ± 0.2[c]	14.5 ± 0.2[c]	12.5 ± 0.2[c]
Mean cell volume (fL)	55.4 ± 0.1	56.1 ± 0.1	55.8 ± 0.1	53.3 ± 0.2[c]	49.0 ± 0.2[c]	44.4 ± 0.4[c]
Mean cell Hb (pg)	19.7 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	18.9 ± 0.1[c]	16.6 ± 0.2[c]	16.0 ± 0.2[c]
Platelets (10^3/µL)	742.1 ± 20.4	725.9 ± 12.7	733.9 ± 8.8	727.4 ± 14.2	639.4 ± 9.9[c]	662.5 ± 19.4[c]

[a] NTP, 2004
[b] Mean ± standard error
[c] These values are statistically significantly different from control value.

The included decreases in mean cell volumes, mean cell hemoglobin values, and mean cell hemoglobin concentration in both males and females, occurred at exposures ≥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; this was supported by bone marrow atrophy observed microscopically. As discussed by NTP (2004), the erythrocytosis suggested a physiological response consistent with the hemoconcentration of dehydration, and a compromised nutritional status, due to the reduced weight gain and food consumption, may have contributed to the development of the anemia.

Statistically significant increases in absolute and relative liver weights were observed in male and female rats exposed to 40 mg/kg-day and higher (Table 1) (NTP, 2004). Significant alterations in absolute and/or relative weights were also observed in several other organs, but these changes likely reflected the decreased body weight gain associated with reduced food intake. Changes in serum clinical chemistry parameters, indicative of liver damage, were observed in both sexes, generally occurring at all time points (day 5, day 21, and week 14) and generally increasing in magnitude with increasing dose and time. At week 14 (Table 1), these effects included statistically significant increases in alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) in males, at ≥80 mg/kg-day, and females, at ≥170 mg/kg-day; increases in alkaline phosphatase (ALP) in both sexes, at ≥170 mg/kg-day; increases in bile acids in male rats, at ≥170 mg/kg-day, and in females, at 320 mg/kg-day; and decreases in serum cholesterol in females, at ≥80 mg/kg-day, and male, at 320 mg/kg-day. There were no exposure-related changes in serum 5'-nucleotidase at week 14, although increases occurred on day 5 in females, at ≥20 mg/kg-day, and on day 21 in both male and female rats, at 80, 170, and/or 320 mg/kg-day. As discussed by NTP (2004), increases in ALT and SDH are specific markers of hepatocellular necrosis or increased cell membrane permeability (leakage) in rodents; increases in bile acids are markers of cholestasis, impaired hepatocellular function, or hepatocellular injury; increases in ALP and 5'-nucleotidase are other markers of cholestasis; and decreases in serum cholesterol could be indicative of liver dysfunction (impaired cholesterol biosynthesis).

Histological evaluation revealed the liver as the primary target of 1,1,2,2-tetrachloroethane toxicity (NTP, 2004); a summary of histopathological alterations is presented in Table 2. Hepatic cytoplasmic vacuolization was noted in male rats exposed to 20 mg/kg-day or more and females exposed to 40 mg/kg-day or more. Although incidence of this alteration was high in the affected groups, severity was only minimal-to-mild and did not increase with dose. Females exposed to 80 mg/kg-day showed an increase in the incidence of hepatocyte hypertrophy, which increased in severity and incidence with increasing exposure level; similar results were seen in males, but they were not statistically significant below 170 mg/kg-day. At 170 mg/kg-day and above, additional effects in the liver in both sexes were hepatocyte necrosis, pigmentation, mitotic alteration, and mixed-cell foci and bile duct hyperplasia. Pigmentation of the spleen was increased in male rats exposed to 80 mg/kg-day or more and in female rats exposed to 170 mg/kg-day or more of 1,1,2,2-tetrachlorethane. Other histological effects included high incidences (70–100%) of atrophy in the spleen (red pulp and lymphoid follicle) of both sexes at 320 mg/kg-day and atrophy of bone (metaphysis) and bone marrow, prostate gland, preputial gland, seminal vesicles, testes (germinal epithelium), uterus, and clitoral gland, as well as ovarian interstitial cell cytoplasmic alterations, in females at ≥170

Table 2. Incidences of Selected Histopathological Lesions in Rats Exposed to Dietary 1,1,2,2-Tetrachlorethane for 14 Weeks[a]

Dose (mg/kg-day)	0	20	40	80	170	320
Males (10/group)[b]						
Hepatocyte cytoplasmic vacuolization	0	7[c] (1.3)	9[c] (2.0)	10[c] (1.9)	8[c] (1.4)	0
Hepatocyte hypertrophy	0	0	0	1 (1.0)	9[c] (1.3)	10[c] (3.2)
Hepatocyte necrosis	0	0	0	0	8[c] (1.0)	10[c] (1.6)
Hepatocyte pigmentation	0	0	0	0	7[c] (1.0)	10[c] (1.9)
Hepatocyte mitotic alteration	0	0	0	0	0	6[c] (2.0)
Mixed cell foci	0	0	0	0	3	5[c]
Bile duct hyperplasia	0	0	0	0	0	10[c] (1.7)
Spleen pigmentation	0	0	1 (1.0)	9[c] (1.0)	9[c] (1.0)	9[c] (1.6)
Spleen red pulp atrophy	0	0	0	0	5[c] (1.0)	9[c] (1.4)
Spleen lymphoid follicle atrophy	0	0	0	0	0	5[c] (1.0)
Females (10/group)[b]						
Hepatocyte cytoplasmic vacuolization	0	0	10[c] (1.7)	10[c] (2.2)	4[c] (1.3)	0
Hepatocyte hypertrophy	0	0	0	4[c] (1.0)	10[c] (1.7)	10[c] (2.8)
Hepatocyte necrosis	0	0	0	1 (1.0)	7[c] (1.0)	10[c] (1.1)
Hepatocyte pigmentation	0	0	0	0	10[c] (1.3)	10[c] (2.0)
Hepatocyte mitotic alteration	0	0	0	0	3 (2.0)	10[c] (1.9)
Mixed cell foci	0	0	0	0	8[c]	1
Bile duct hyperplasia	0	0	0	0	5[c] (1.0)	10[c] (1.9)
Spleen pigmentation	1 (1.0)	0	0	4 (1.0)	8[c] (1.1)	8[c] (1.3)
Spleen, red pulp atrophy	0	0	0	0	0	9[c] (1.6)
Spleen lymphoid follicle atrophy	0	0	0	0	0	3 (1.0)

[a] NTP, 2004
[b] These values represent the number of animals with the lesion, with the severity score in parenthesis; Severity grades are as follows: 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.
[C] These values are statistically, significantly different from vehicle control group.

mg/kg-day and males at 320 mg/kg-day. The reduction in body weight gain at 170 mg/kg-day and body weight loss at 320 mg/kg-day may have contributed to the atrophy of the bone, bone marrow, and reproductive organs. The sperm evaluations showed that motility was 17.1, 14, and 24.0% lower than vehicle control animals at 40, 80, and 170 mg/kg-day, respectively. These reductions in sperm motility are not considered adverse because the decreases are small, not dose-related, and of unclear functional reproductive significance. The vaginal cytology evaluations indicated that the female rats in the 170 mg/kg-day exposure group (320 mg/kg-day not evaluated) spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the vehicle control groups.

In summary, the NTP (2004) 14-week study in rats 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; this minimal effect, which did not increase in severity with dose, was not considered adverse by the NTP. At 40 mg/kg-day, significant increases in relative liver weights were observed. Hepatocellular hypertrophy, spleen pigmentation, and decreases in body weight gain (<10%) were observed at 80 mg/kg-day—although these changes were generally of minimal severity. A decrease in cholesterol and increases in serum ALT and SDH occurred at exposures ≥80 mg/kg-day, and increases in serum ALP and bile acids, hepatocyte necrosis, bile duct hyperplasia, hepatocellular mitotic alterations, foci of cellular alterations, and liver pigmentation occurred at exposure levels of 170 and 320 mg/kg-day. This study identified a NOAEL of 20 mg/kg-day and a LOAEL of 40 mg/kg-day based on a progressive increase in relative liver weight. Liver-related serum chemistry changes began at 80 mg/kg-day, and histological manifestations of hepatocellular damage at higher doses. 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 is less sensitive than the liver for subchronic dietary exposure.

NTP (2004) also exposed groups of male and female B6C3F1 mice (10/sex/group) to diets containing 0, 589, 1120, 2300, 4550, or 9100 ppm of microencapsulated 1,1,2,2-tetrachloroethane for 14 weeks. The reported average daily doses were 0, 100, 200, 370, 700, or 1360 mg/kg-day for males and 80, 160, 300, 600, or 1400 mg/kg-day for females; vehicle and untreated control groups were used for each sex. Endpoints evaluated throughout the study included clinical signs, body weight, and feed consumption. Clinical chemistry (10 indices) was assessed at the end of the study, and hematology 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, and thymus in both sexes, and the preputial gland in the male mice and lungs in the female mice. FOBs (21 parameters) were performed on both control and the 160/200, 300/370, and 600/700 mg/kg-day exposure 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 1360/1400 mg/kg-day exposure groups.

All mice survived to the end of the study (NTP, 2004). A clinical sign of thinness was observed at exposures of 300/370 mg/kg-day (3/10 males, 1/10 females), 600/700 mg/kg-day (9/10 males, 2/10 females), and 1360/1400 mg/kg-day (10/10 males, 10/10 females). Final body weights were significantly lower than vehicle controls in male mice at 370, 700, and 1360 mg/kg-day (12, 16, and 33% reduced, respectively) and female mice at 300, 600, and 1400 mg/kg-day (4, 10, and 11% reduced, respectively) (Table 3). Feed consumption was slightly less than the controls in males, at ≥700 mg/kg-day, but it was similar to controls in the females. Significant increases in absolute and relative liver weights were observed in the male mice exposed to 200 mg/kg-day or higher and in female mice exposed to 80 mg/kg-day or higher (Table 3). Other organ weight changes (increased kidney weights in males, at ≥370 mg/kg-day,

Table 3. Body Weight, Liver Weight and Selected Clinical Chemistry Changes in Mice Exposed to Dietary 1,1,2,2-Tetrachloroethane for 14 Weeks[a,b]

Dose (mg/kg-day)	0	100	200	370	700	1360
Males (10/group)						
Body weight (g)	30.1 ± 0.6	30.6 ± 0.6	30.0 ± 0.3	26.5 ± 0.4[c]	25.2 ± 0.2[c]	23.1 ± 0.5[c]
Liver weight absolute (g) relative (%)	 1.47 ± 0.02 48.84 ± 1.17	 1.56 ± 0.04 50.94 ± 0.93	 1.70 ± 0.02[c] 56.82 ± 0.63[c]	 1.61 ± 0.04[c] 60.63 ± 1.20[c]	 1.53 ± 0.05 60.71 ± 1.76[c]	 1.56 ± 0.04 67.43 ± 1.83[c]
Serum total protein (g/dL)	5.4 ± 0.1	5.2 ± 0.1	5.1 ± 0.1[c]	5.1 ± 0.1[c]	5.1 ± 0.1[c]	5.1 ± 0.1[c]
Serum cholesterol (mg/dL)	131 ± 7	125 ± 4	94 ± 3[c]	110 ± 5	112 ± 4	126 ± 5
ALT (IU/L)	66 ± 8	62 ± 19	74 ± 8	207 ± 18[c]	172 ± 18[c]	296 ± 24[c]
ALP (IU/L)	85 ± 2	78 ± 2	89 ± 2	130 ± 3[c]	143 ± 7[c]	184 ± 11[c]
SDH (IU/L)	55 ± 3	53 ± 2	76 ± 3[c]	288 ± 20[c]	288 ± 29[c]	448 ± 25[c]
5'-Nucleotidase (IU/L)	18 ± 1	16 ± 1	18 ± 0	30 ± 2[c]	37 ± 3[c]	62 ± 7[c]
Bile acids (µmol/L)	25.3 ± 1.2	22.8 ± 1.5	24.8 ± 0.6	56.5 ± 5.1[c]	63.3 ± 7.5[c]	108.7 ± 8.1[c]
Females (10/group)						
Dose (mg/kg-day)	**0**	**80**	**160**	**300**	**600**	**1400**
Body weight (g)	24.3 ± 0.5	24.2 ± 0.2	24.3 ± 0.6	23.3 ± 0.4	21.7 ± 0.2[c]	21.5 ± 0.6[c]
Liver weight absolute (g) relative (%)	 1.05 ± 0.03 43.26 ± 1.05	 1.16 ± 0.02[c] 47.90 ± 0.85[c]	 1.36 ± 0.06[c] 55.54 ± 1.17[c]	 1.34 ± 0.04[c] 57.39 ± 0.84[c]	 1.28 ± 0.03[c] 58.73 ± 1.23[c]	 1.39 ± 0.05[c] 64.42 ± 1.14[c]
Serum total protein (g/dL)	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.0	5.4 ± 0.1[c]	5.4 ± 0.0[c]	5.1 ± 0.1[c]
Serum cholesterol (mg/dL)	109 ± 2	109 ± 3	85 ± 3[c]	68 ± 2[c]	64 ± 3[c]	92 ± 4[c]
ALT (IU/L)	34 ± 5	50 ± 15	65 ± 5[c]	189 ± 33[c]	197 ± 21[c]	351 ± 35[c]
ALP (IU/L)	131 ± 5	126 ± 2	139 ± 5	150 ± 3[c]	161 ± 7[c]	195 ± 6[c]
SDH (IU/L)	36 ± 1	44 ± 3[c]	76 ± 4[c]	197 ± 15[c]	243 ± 23[c]	461 ± 59[c]
5'-Nucleotidase (IU/L)	59 ± 3	71 ± 2	84 ± 5[c]	62 ± 2	62 ± 3	83 ± 4[c]
Bile acids (µmol/L)	27.2 ± 1.2	26.1 ± 1.9	30.9 ± 1.1[c]	44.2 ± 3.9[c]	51.5 ± 3.6[c]	101.7 ± 12.0[c]

[a] NTP, 2004.
[b] Mean ± standard error
[c] Statistically significantly different from control value.

and increased thymus weights in males and females, at 1360/1400 mg/kg-day, respectively) were considered to be secondary to the body weight changes. Results of the FOBs showed no exposure-related neurotoxicity.

Clinical chemistry findings in the mice are summarized in Table 3 and include statistically significant decreases in serum total protein in male mice at ≥200 mg/kg-day, serum total protein in the female mice exposed to ≥300 mg/kg-day, and serum albumin in the females exposed to 1,1,2,2-tetrachlorethane concentrations of 1400 mg/kg-day (NTP, 2004). Decreased serum albumin could not fully account for the decreased total protein concentrations, suggesting that other factors (e.g., changes in other protein fractions, hydration status, and/or hepatic function) contributed to the hypoproteinemia (NTP, 2004). Other serum chemistry changes were indicative of dose-related liver effects beginning at exposure concentrations of 160 mg/kg-day; these included statistically significant increased SDH in both sexes at ≥160/200 mg/kg-day, decreased serum cholesterol in females at ≥160 mg/kg-day, increased ALT and total bile acids in females at ≥160 and in males at ≥370 mg/kg-day, increased ALP in both sexes at 300/370 mg/kg-day, and increased 5'-nucleotidase in males at ≥370 mg/kg-day exposures. As previously discussed for the rat study, these serum indices are markers of hepatocellular damage, cholestasis, and/or impaired hepatic function (NTP, 2004).

Histopathological findings are consistent with the serum chemistry data in indicating that the liver is the most sensitive target of 1,1,2,2-tetrachloroethane toxicity in the mice (NTP, 2004). As summarized in Table 4, minimal hepatocyte hypertrophy was observed at ≥160 mg/kg-day in females and ≥200 mg/kg-day in males. Although hypertrophy can reflect an adaptive nonadverse hepatic response, serum chemistry changes more clearly indicative of hepatotoxicity (increased SDH, ALT, and 5'nucleotidase and decreased cholesterol) also occurred at ≥160 mg/kg-day in females and ≥200 mg/kg-day in males. Degenerative and other adverse liver lesions, including necrosis, pigmentation, and bile duct hyperplasia, occurred at ≥300 mg/kg-day in females and ≥370 mg/kg-day in males. Other histological findings included increased incidences of preputial gland atrophy in the 100, 700, and 1360 mg/kg-day male groups (Table 4), but this effect was not clearly dose-related and is possibly associated with decreased body weight gain. Additional effects included decreased absolute testis weight at ≥700 mg/kg-day and absolute epididymis and cauda epididymis weights at 1360 mg/kg-day (relative weights not reported), decreased epididymal spermatozoal motility at 1360 mg/kg-day (3.1% less than vehicle controls), and increased estrous cycle length at 1400 mg/kg-day (8.7% longer than vehicle controls). Based on the increases in liver weight in female mice at 80 mg/kg-day, serum chemistry changes at ≥160 mg/kg-day, and clear evidence of histopathology at higher doses, this study identified a minimal LOAEL of 80 mg/kg-day for liver effects; a NOAEL was not identified.

The National Cancer Institute (NCI) conducted a subchronic dose range-finding study in rats and mice (NCI, 1978). In this study, groups of five male and five female Osborne Mendel rats received gavage doses of 0 (vehicle control group), 56, 100, 178, 316, or 562 mg/kg of 1,1,2,2-tetrachloroethane in corn oil 5 days/week for 6 weeks followed by a 2-week observation period. Groups of five male and five female B6C3F1 mice were similarly exposed to 0, 32, 56, 100, 178, or 316 mg/kg of 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 and all five females exposed to 316 mg/kg died (mortality rates for the 562 mg/kg groups were not reported). Body weight gain was reduced in the rats at 56, 100, and 178 mg/kg-day exposure

Table 4. Incidences of Selected Histopathological Lesions in Mice Exposed to Dietary 1,1,2,2-Tetrachloroethane for 14 Weeks[a]

Dose (mg/kg-day)	0	100	200	370	700	1360
Males (10/group)[b]						
Hepatocyte hypertrophy	0	0	7[c] (1.0)	10[c] (2.2)	10[c] (2.8)	10[c] (3.1)
Hepatocyte necrosis	0	0	1 (2.0)	8[c] (1.1)	8[c](1.0)	9[c] (1.0)
Liver focal pigmentation	0	0	0	10[c] (1.2)	10[c] (1.4)	8[c] (1.3)
Bile duct hyperplasia	0	0	0	7[c] (1.4)	9[c] (1.3)	10[c] (2.0)
Preputial gland atrophy	0	4[c] (2.0)	2 (1.0)	0	4[c] (2.5)	5[c] (2.2)
Females (10/group)[b]						
Oral Dose (mg/kg-day)	**0**	**80**	**160**	**300**	**600**	**1400**
Hepatocyte hypertrophy	0	2 (1.5)	9[c] (1.0)	10[c] (1.9)	10[c] (2.5)	10[c] (3.0)
Hepatocyte necrosis	0	0	0	3 (1.0)	7[c] (1.0)	4[c] (1.0)
Liver focal pigmentation	0	0	2 (1.0)	9[c] (1.0)	8[c] (1.0)	7[c] (1.1)
Bile duct hyperplasia	0	0	0	8[c] (1.0)	10[c] (1.4)	10[c] (2.0)

[a] NTP, 2004.
[b] Values represent number of animals with the lesion, with the severity score in parenthesis; Severity grades are as follows: 1=minimal, 2=mild, 3=moderate, 4=severe.
[c] Statistically significantly different from vehicle control group.

groups; corresponding reductions were 3, 9, and 38% in males and 9, 24, and 41% in females. No deaths or significant alterations in body weight gain were observed in the mice. The limited number of endpoints examined in this study precludes identifying NOAELs and/or LOAELs for sensitive effects in either species.

Information on the chronic oral toxicity of 1,1,2,2-tetrachloroethane is available from an NCI (1978) bioassay in rats and mice. Groups of 50 male and 50 female Osborne-Mendel rats were administered 1,1,2,2-tetrachloroethane in corn oil by gavage on a 5 days/week regimen for 78 weeks. Vehicle and untreated control groups (20 animals/sex/species/group) were also used. The initial low and high doses used for rats of both sexes were 50 and 100 mg/kg-day. In week 15, the doses were raised to 65 mg/kg-day for low-dose males and 130 mg/kg-day for high-dose males. In 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 in week 33, intubation of all high-dose rats was suspended for 1 week, followed by 4 weeks of dosing; 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 for male rats and 43 and 76 mg/kg for female rats. The exposure period was followed by a 32-week 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 female rats, survival in the vehicle control, and both the 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, it was mainly due to the deaths of 10 high-dose females during the first 5 weeks of the study. NCI (1978) stated that the high-dose females died from apparent compound toxicity, but this conclusion is questionable because 8 of the 10 early deaths had pneumonia, and there was a high prevalence of endemic chronic murine pneumonia in both sexes. 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 sexes, 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; the differences between body weights of the vehicle control rats and the low- and high-dose rats were less than 10% for both sexes in the low-dose group and approximately 20 and 15% for the high-dose males and females, respectively. The body weight curves for dosed and control rats converged during the post-treatment observation period. Causes of the respiratory, ocular, and body weight effects were not discussed by the authors, but they are likely related to the chronic murine pneumonia. Other effects included significantly increased incidences of hepatic fatty metamorphosis in high-dose males (0/20, 2/50, and 9/49 in the vehicle control, low- and high-dose groups). No significant increases in tumor incidence were observed in the rats; however, two hepatocellular carcinomas and one neoplastic nodule, which are rare tumors in male Osborne-Mendel rats, were observed in the high-dose males. Although interpretation of this study is confounded by the chronic murine pneumonia, it is unlikely to have contributed to the fatty changes in the liver, indicating that a NOAEL of 62 mg/kg-day and a LOAEL of 108 mg/kg-day were identified for liver pathology.

NCI (1978) also exposed groups of 50 male and 50 female B6C3F1 mice to 1,1,2,2-tetrachloroethane in corn oil via gavage on 5 days/week for 78 weeks. Initial dose levels were 100 and 200 mg/kg-day in both sexes. In week 19, the doses were raised to 150 and 300 mg/kg-day, respectively. Three weeks later, the doses were raised again, to 200 and 400 mg/kg-day, respectively. In week 27, the doses were lowered to 150 and 300 mg/kg-day, respectively. The reported TWA doses were 142 and 284 mg/kg for both male and female mice; when adjusted for continuous (7 days/week) exposure, the TWA doses were equivalent to 101 and 203 mg/kg-day in both sexes. The exposure period was followed by a 12-week period in which the mice were not exposed to 1,1,2,2-tetrachloroethane. Vehicle and untreated control groups (20 animals/sex/group) were also used. 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; survival markedly decreased after 45 weeks of exposure 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 and both the 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. A very slight decrease in body weight gain (less than 10%) occurred in the high-dose male mice; no other effects on body weight gain were observed. A

high incidence (95%) of pronounced abdominal distension, probably resulting from liver tumors, was observed in the high-dose females beginning in week 60 and continuing throughout the recovery period. Statistically significant ($p < 0.001$) increases in incidences of hepatocellular carcinoma occurred in both sexes and dose levels; incidences in the vehicle control, 101, and 203 mg/kg-day groups were 3/36, 13/50, and 44/49 in males and 1/40, 30/48, and 43/47 in females. There were no increases in nonneoplastic liver lesions, but detection of such lesions could have been obscured by the liver tumors. Information on the progression from preneoplastic pathology to hepatocellular carcinoma is not available due to the lack of interim sacrifices. Nonneoplastic lesions that were significantly increased were limited to hydronephrosis (0/20, 0/46, and 16/46) and chronic inflammation in the kidneys (0/20, 0/46, and 5/46) in high-dose females, and acute toxic tubular nephrosis in high-dose males (33/49; incidences in other groups were not reported).

In a developmental toxicity study, groups of 8–9 timed-pregnant CD Sprague-Dawley rats were exposed 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 (Gulati et al., 1991a). Based on body weight and food consumption data, reported corresponding estimated doses of 1,1,2,2-tetrachloroethane were 0, 34, 98, 180, 278, and 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 and dead fetuses). Necropsies were performed on the maternal animals, but the fetuses were not examined for malformations. All dams survived to study termination. Maternal body weight and weight gain decreased significantly in all dose groups except the 34 mg/kg-day group (9.3, 11.6, 13.8, and 24% decrease in body weight in the 98, 180, 278, or 330 mg/kg-day groups, respectively). Daily food consumption was significantly lower in all dose groups; this may have contributed to the decreased body weights seen in the study. Four animals in the 278 mg/kg-day group had rough fur beginning on GD 10, while rough fur was present in all animals in the 330 mg/kg-day group. No changes were seen in the numbers of live fetuses per litter, dead fetuses per litter, resorptions per litter, or implants per litter. One dam in the 98 mg/kg-day exposure group and 4 dams in the 330 mg/kg-day group completely resorbed their litters. At scheduled sacrifice, average fetal weights were statistically significantly decreased in all dose groups except the 34 mg/kg-day group (4.9, 4, 12.8, 10.6, and 20.7% decrease in the 34, 98, 180, 278, and 330 mg/kg-day groups, respectively). The apparently contradictory results in the 34 and 98 mg/kg-day groups with regard to statistical significance were due to greater variability in the 34 mg/kg-day group. Gravid uterine weight was reduced only in the 330 mg/kg-day animals. No statistically significant changes were seen in the 34 mg/kg-day animals, while small—but statistically significant—decreases were seen in maternal body weight and average fetal weight at 1,1,2,2-tetrachloroethane exposures of 98 mg/kg-day. Using a 10% change as the criterion for an adverse change in maternal body weight, this study identified a maternal NOAEL of 98 mg/kg-day and LOAEL of 180 mg/kg-day. A NOAEL of 98 mg/kg-day and LOAEL of 180 mg/kg-day are identified for developmental toxicity based on the lowest dose that caused a clear decrease in fetal rat body weight.

Gulati et al. (1991b) similarly exposed groups of 9–11 timed-pregnant Swiss CD-1 mice 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, reported corresponding estimated doses of 1,1,2,2-tetrachloroethane were 0, 987, 2120, 2216, and 4575 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 and feed consumption, as well as 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 mice (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, 2120, 2216, or 4575 mg/kg-day exposure groups, respectively; the mortality in the higher dose groups affected the statistical power of the study for those groups. Maternal body weights were generally decreased at ≥2120 mg/kg-day, beginning on study day 9; although the data from study day 17 were not statistically significantly different from controls for any treatment group. Average daily feed consumption was decreased in all treated groups except the 987 mg/kg-day exposure group. Gross hepatic effects were reported in dams from all groups except the 987 mg/kg-day group. The noted effects include pale, enlarged and/or grey 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, 2120, 2216, and 4575 mg/kg-day groups, respectively. No changes in developmental endpoints were noted in the 987 or 2120 mg/kg-day exposure groups; other groups had too few litters to permit statistical analysis of the findings. This study identified a NOAEL of 987 mg/kg-day for maternal and developmental toxicity in mice; the next highest dose, 2120 mg/kg-day, is a FEL for maternal mortality and litter resorptions.

Inhalation Exposure

Truffert et al. (1977) exposed groups of 55 female Sprague-Dawley rats to 1,1,2,2-tetrachloroethane vapor in reported calculated atmospheric concentrations of 0 or 560 mL/m^3 on 5 days/week for 15 weeks (78 exposures in total). There is uncertainty regarding the actual concentration due to the unusual mL/m^3 unit of exposure. If it is assumed that mL/m^3 is a volume/volume vapor concentration, the reported concentration is equivalent to 560 ppm (3909 mg/m^3). If it is assumed that 560 mL is the volume of liquid volatilized in 1 m^3 of air, the reported concentration is equivalent to 892,640 mg/m^3 (130,325 ppm), an unlikely level because it is approximately 100 times higher than concentrations reported to be acutely lethal in rats given a single 4-hour exposure (Schmidt et al., 1980; Carpenter et al., 1949). The daily exposure duration was 6 hours for the first 8 exposures and 5 hours for the remaining 70 exposures; the TWA daily exposure duration was 5.1 hours/day. Interim sacrifices were conducted after 2, 4, 9, 19, 39, and 63 exposures (number of animals killed at each time period was not reported). Clinical signs and body weight were evaluated during the entire study, and hematology (RBC and WBC counts, hematocrit), histology (lungs, liver, kidneys, adrenals, ovaries, and uterus), relative liver weight and uptake of injected ^{3}H-thymidine in hepatic DNA were evaluated at each sacrifice. 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" (additional information not reported). Though body weight gain was decreased towards the end of the study, the magnitude of the change was not reported. Unquantified increases in relative liver weights were observed beginning 15 days after exposure initiation. Hematological alterations consisted of an unquantified, slight decrease in hematocrit

"confirmed by the joint RBC and WBC counts" at the end of the study. A marked increase (313%) in thymidine uptake in hepatic DNA was observed after four exposures; by the ninth exposure, thymidine uptake had decreased to levels similar to controls. After the ninth exposure, histological alterations began to manifest. These included granular appearance, cytoplasmic vacuolization, and evidence of hyperplasia (increase in number of binucleated cells and 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, the lack of information on dose-response due to the use of a single exposure level, and the uncertainty regarding the exposure concentration, a reliable NOAEL or LOAEL cannot be determined 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 2000–4000 ppm (13,700–27,500 mg/m^3) for the first 20 exposures, 1000–2000 ppm (6870–13,700 mg/m^3) for the next 140 exposures, and 3000-4000 ppm (20,600–27,500 mg/m^3) for the last 30 exposures; the TWA concentration was 1974 ppm (13,560 mg/m^3). The authors noted that the monkey was weak after 7 exposures and had diarrhea and anorexia between exposures 12 and 15. Beginning at the exposure 15, the monkey was "almost completely unconscious falling upon his side" for 20–60 minutes after each exposure. The authors noted some alterations in body weight gain and hematological parameters (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; no effects were observed in the heart, lung, kidney, pancreas, or testis. This study cannot be used to identify a NOAEL or LOAEL for subchronic exposure due to the frank neurotoxicity and the use of a single animal.

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 (1150 mg/m^3) of 1,1,2,2-tetrachloroethane for 7 hours/day on alternate days for the 6-month study period. Endpoints included survival, body weight, blood cell counts and hemoglobin determinations, liver and kidney organ weights, and chemical indices (bromsulphalein retention, serum phosphatase, thymol-barbital test, blood urea nitrogen), and gross pathology and histology (adrenal, heart, kidney, liver, lung, pancreas, thyroid, parathyroid, spleen, testis). The reported effects include apparent increases in liver and lung pathology in the exposed animals. An endemic lung infection, which predisposed the exposed animals to numerous early deaths (57 and 69% mortality in the control and exposed groups, respectively), precludes meaningful interpretation of these and other results and identification of a LOAEL or NOAEL. This study also included one mongrel dog that had the same exposure and evaluations as the rats. The dog survived the 6-month exposure with effects that included light cloudy swelling of the convoluted tubules of the kidney, marked cloudy swelling of the liver, and light congestion of the lungs. Identification of a reliable LOAEL or NOAEL is precluded by the use of a single animal.

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) for 3 hours/day, 6 days/week, for 7–8.5 months. The exposed group contained six animals, and the size of the control group was not specified. In comparison with both starting and control levels, serum acetylcholine levels were significantly decreased after 1.5 months, significantly increased after 4.5 months, and significantly decreased at the end of the study. No changes in serum acetylcholinesterase activity were reported, although serum butyrylcholinesterase activity was significantly reduced after 5–6 months of exposure. This study is poorly reported and does not examine any other relevant endpoints. Because the changes in acetylcholine were not consistent across time and incompletely quantified, and the biological significance is unclear, no NOAEL or LOAEL can be identified.

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) of 1,1,2,2-tetrachloroethane for 3 hours/day, 6 days/week, for 8–10 months. Animals were challenged with 1 mL of a 1.5×10^9 suspension of heated typhoid vaccine (no further details given), 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 Forssman antigen of sheep erythrocytes were observed in the 10 and 100 mg/m^3 groups; levels were increased in the 10 mg/m^3 group after 1.5, 2, and 2.5 months of exposure; and levels decreased after 4 months of exposure and decreased in the 100 mg/m^3 group during the first 6 months of exposure. Increases in the electrophoretic mobility of specific antibodies were also reported. Exposure to 100 mg/m^3 resulted in a decrease in the relative γ globulin fraction (protein fraction that includes the antibodies) and an increase in the α and β globulin fractions.

The apparent lack of treatment-associated change in typhoid-specific antibody following challenge with typhoid suggests a lack of immunotoxicity (Shmuter, 1977). In contrast, the decrease in total antibody at 100 mg/m^3 and decrease in "normal" hemolysin to Forssman antigen at 10 mg/m^3 after 4 months and decrease at 100 mg/m^3 "during the first 6 months," suggests a general suppression of the antibody response at higher 1,1,2,2-tetrachloroethane exposure levels and with increasing exposure duration. This potential general suppression of antibody response is supported by the decrease in the relative gamma globulin fraction of serum proteins. Therefore, the results are equivocal, in part because total antibody levels, gamma globulin fraction, and "normal" hemolysin-to-Forssman antigen are not standard assays for immunotoxicity and do not have strong predictive value for general immunosuppression. This study is poorly reported and provides inadequate quantitative data. The reporting limitations, endpoints of uncertain toxicological significance, and inconsistent patterns of response preclude assessing biological significance and identification of a NOAEL or LOAEL.

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; 1.9 ppm) 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; the remaining animals were observed until natural death.

The scope of the study and the results of systemic toxicity evaluations were incompletely reported. There were no significant alterations in survival. Exposed rats weighed 2.1, 11.6, and 12.2% less than controls on study days 110, 260, and 324, although the only statistically significant decreases in body weight gain occurred between days 90 and 170. Other statistically significant changes included increased leukocyte and β_1-globulin levels after 110 days; increased percentage of segmented nucleated neutrophils, decreased percentage of lymphocytes, and increased liver total fat content after 265 days; decreased γ-globulin levels at 60 days post-exposure, and decreased adrenal ascorbic acid content (a measure of ACTH activity in hypophysectomized rats) at all three time periods. This study is insufficient for identification of a NOAEL or LOAEL for systemic toxicity because the experimental design and results were poorly reported and the histological examinations do not appear to have been conducted.

The Schmidt et al. (1972) chronic study summarized above included a limited reproductive function/developmental toxicity assessment. One week before the end of the 265-day exposure period, each of 7 control and 7 exposed male rats was mated with 5 unexposed virgin females, yielding corresponding groups of 35 mated females. The offspring were observed for 84 days and were examined macroscopically for malformations. Other reported study endpoints were 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, and sex ratio and average body weight on postnatal day 84. No macroscopic malformations or significant group differences in the other indices were found, indicating that 13.3 mg/m^3 (1.9 ppm) is a NOAEL for reproductive toxicity in male rats.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC p-RfDs FOR 1,1,2,2-TETRACHLOROETHANE

Subchronic p-RfD

Information on the subchronic oral toxicity of 1,1,2,2-tetrachloroethane is available from 6-week gavage and 14-week dietary studies in rats and mice. The 6-week gavage studies (NCI, 1978) were limited in scope and insufficient for identification of a NOAEL or LOAEL in either species, although 316 mg/kg-day was a FEL for mortality in rats. No mortality occurred in rats exposed to 320 mg/kg-day (through diet) for 14 weeks (NTP, 2004), indicating that subchronic dietary exposure is less toxic than exposure by gavage.

The 14-week dietary studies (NTP, 2004) are comprehensive and found that the liver was the most sensitive target organ in both rats and mice. Hepatic effects in the rats include increased hepatic cytoplasmic vacuolization at 20 mg/kg-day (the lowest tested dose), increased liver weight at 40 mg/kg-day, and hepatocellular hypertrophy at 80 mg/kg-day. The hepatic cytoplasmic vacuolation is not considered adverse because the severity was minimal-to-mild and did not increase with dose. Increases in serum ALT, SDH, and cholesterol also occurred at 80 mg/kg-day, and increases in serum ALP and bile acids, hepatocyte necrosis, bile duct hyperplasia, hepatocellular mitotic alterations, foci of cellular alterations, and liver pigmentation occurred at 170 and 320 mg/kg-day. The rat-targeted portion of the study identified a NOAEL of 20 mg/kg-day and a LOAEL of 40 mg/kg-day, based on increased relative liver weight

beginning at this dose, liver-related serum chemistry changes beginning at 80 mg/kg-day, and histological manifestations of hepatocellular damage at higher doses. Hepatic effects in the mice included increased liver weight at 80 mg/kg-day; minimal hepatocellular hypertrophy, increased serum SDH, ALT and bile acid levels, and decreased serum cholesterol at 160–200 mg/kg-day; and increases in serum ALP and 5'-nucleotidase, necrosis, pigmentation, and bile duct hyperplasia at 300–370 mg/kg-day. Based on the liver weight changes at 80 mg/kg-day and serum chemistry and histopathology at higher doses, the mouse study identified a minimal LOAEL of 80 mg/kg-day.

Adverse effects on body weight, histology of nonhepatic tissues, and reproductive endpoints were observed in both species (NTP, 2004)—but only at doses higher than the hepatic LOAELs and in the 170–320 mg/kg-day range, which also caused necrotic and other histopathological lesions in the liver. Comprehensive neurobehavioral testing at doses as high as 80 mg/kg-day in the rats and 600–700 mg/kg-day in the mice showed no evidence of neurotoxicity in either species (NTP, 2004), further indicating that the liver was the most sensitive target for subchronic dietary exposure. Gestational exposure to 1,1,2,2-tetrachoroethane was fetotoxic in rats (decreased fetal body weight) and mice (litter resorptions) at dietary doses that were both higher than the 14-week hepatic LOAELs and maternally toxic (180 mg/kg-day in rats and 987 mg/kg-day in mice) doses, although it is not known if lower doses can cause other developmental effects because the fetuses were not examined for malformations (Gulati et al., 1991a,b). Although both mouse and rat data were modeled, the rats are more sensitive than the mice. Because the liver is the most sensitive target of 1,1,2,2-tetrachloroethane toxicity, hepatic data in rats are used as the basis of the p-RfD.

Potential points of departure (PODs) for the subchronic p-RfD were derived by BMD analysis (U.S. EPA, 2000) of the NTP (2004) rat liver data shown in Table 5. Data for relative liver weight, serum ALT, SDH, bile acids, and cholesterol were selected for modeling because these endpoints showed statistically significant changes and best reflected the progression and spectrum of hepatotoxic effects. Hepatocyte necrosis was not modeled because there is no clear dose-response relationship throughout the dose range. The continuous-variable models in the BMD software (version 1.4.1) were applied to the data for changes in relative liver weight and serum ALT, SDH, bile acids, and cholesterol levels.

Appropriate model fits were obtained for the relative liver weight data in females, serum ALT data in males, and serum bile acids data in both sexes. A summary of the predicted BMDs and 95% lower confidence limits (BMDLs) using the best fitting models for these endpoints is presented in Table 6. For the continuous data, the calculated BMDs and BMDLs are estimates of the doses associated with a change in the mean equal to 1 standard deviation (SD) from the control mean. Predicted doses associated with an increase of 100% (i.e., 2-fold) were also calculated for the best fitting model for the changes in liver enzymes (serum ALT), as an increase of this magnitude is sometimes considered to be an indicator of clinical significance for these effects. Details of the BMD modeling are presented in Appendix A.

Table 5. Selected Liver Effects in Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks (from Table 1)[a]

Endpoint[b]	Dose (mg/kg-day)					
	0	20	40	80	170	320
Males (10/group)						
Liver weight, relative (%)	34.79 ± 1.33	36.72 ± 1.39	41.03 ± 2.69[c]	45.61 ± 1.64[c]	44.68 ± 1.42[c]	52.23 ± 4.49[c]
Serum ALT (IU/L)	48 ± 6.32	49 ± 6.32	53 ± 6.32	69 ± 9.49[c]	115 ± 25.30[c]	292 ± 56.92[c]
Serum SDH (IU/L)	23 ± 3.16	27 ± 3.16[c]	26 ± 6.32	31 ± 3.16[c]	47 ± 6.32[c]	74 ± 12.65[c]
Bile acids (µmol/L)	29.2 ± 9.17	27.5 ± 8.54	27.2 ± 8.54	35.9 ± 12.33	92.0 ± 52.49[c]	332.4 ± 149.89[c]
Females (10/group)						
Liver weight, relative (%)	35.07 ± 1.77	36.69 ± 1.14	37.84 ± 1.61[c]	44.20 ± 0.85[c]	48.03 ± 2.81[c]	58.40 ± 4.49[c]
Serum ALT (IU/L)	46 ± 6.32	42 ± 3.16	41 ± 6.32	49 ± 6.32	112 ± 22.14[c]	339 ± 56.92[c]
Serum SDH (IU/L)	27 ± 3.16	27 ± 3.16	28 ± 6.32	25 ± 3.16	45 ± 9.49[c]	82 ± 9.49[c]
Bile acids (µmol/L)	37.0 ± 22.45	46.6 ± 20.55	39.1 ± 17.71	36.3 ± 12.33	39.3 ±24.98	321.5 ± 160.01[c]
Serum cholesterol (mg/dL)	104 ± 12.65	105 ± 9.49	98 ± 3.16	81 ± 6.32[c]	64 ± 9.49[c]	55 ± 9.49[c]

[a]NTP, 2004.
[b]Mean ± standard deviation (calculated from the standard error)
[c]Statistically significantly different from control value.

Table 6. Summary of BMD Model Predictions for Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks (Best Fitting Models)[a]

Endpoint	BMR	BMD (mg/kg-day)	BMDL (mg/kg-day)
Males			
Relative liver weight	1 control SD	13.1	10.8
Serum ALT	1 control SD	38.23	26.56
	100% relative deviation	134.06	121.35
Serum SDH	1 control SD	38	25
	100% relative deviation	No adequate fit to the data	
Bile acids	1 control SD	72.45	57.17
Females			
Relative liver weight	1 control SD	23.57	15.68
Serum ALT	1 control SD	64	56
Serum SDH	1 control SD	No adequate fit to the data	
Bile acids	1 control SD	216.74	177.00
Serum cholesterol	1 control SD	No adequate fit to the data	

[a]NTP, 2004.

As shown in Table 6, the lowest BMD and BMDL were calculated for the male rats' relative liver weight data using 1 SD from the control mean as the benchmark response (BMR). The full BMDS output for this endpoint is found in Appendix B. The BMDL of 10.8 mg/kg-day based on the relative liver weight data in males was selected as a conservative POD for the subchronic p-RfD.

To derive the subchronic p-RfD, the 10.8 mg/kg-day BMDL for increased relative liver weight is divided by a total Composite UF of 1000. The Composite UF of 1000 comprises component factors of 10 for interspecies extrapolation, 10 for interhuman variability, and 10 for database deficiencies, as explained below.

A 10-fold UF is used to account for the interspecies variability in extrapolating from laboratory animals (rats) to humans. No relevant information is available on the toxicity of 1,1,2,2-tetrachloroethane in humans, and data on toxicokinetic differences between animals and humans in the disposition of ingested 1,1,2,2-tetrachloroethane are not available.

A 10-fold UF is also used to account for variations in sensitivity within human populations because there is insufficient information on the degree to which humans of varying gender, age, health status, or genetic makeup might vary in the disposition of, or response to, ingested 1,1,2,2-tetrachloroethane. Animal toxicity studies that evaluated both sexes in the same study did not show consistent sex-related differences. Developmental toxicity studies in animals are limited in scope, but they have not indicated effects on the offspring at levels that did not also cause maternal effects.

Lastly, a 10-fold UF is used to account for deficiencies in the database. The NTP (2004) 14-week study provides comprehensive evaluations of systemic toxicity and neurotoxicity in two species (rats and mice). Although hepatotoxicity data in this study provide a suitable basis for subchronic RfD derivation, the database is limited by a lack of adequate reproductive and developmental toxicity studies. The NTP (2004) study provides information on effects on sperm, estrus cycle, and male and female reproductive tissues in rats and mice, and a one-generation inhalation study provides limited data on reproductive effects in male rats (Schmidt et al., 1972), but the database lacks a multigeneration reproduction study and a state-of-the-art immunotoxicology 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 fetal skeletal and visceral examinations.

A UF to account for extrapolation from a LOAEL to a NOAEL was not applied because a NOAEL was identified and a BMDL was used as the POD for the p-RfD.

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

$$\begin{aligned} \text{subchronic p-RfD} &= \text{BMDL} \div \text{Composite UF} \\ &= 10.8 \text{ mg/kg-day} \div 1000 \\ &= \mathbf{0.01 \text{ mg/kg-day } (1 \times 10^{-2} \text{ mg/kg-day})} \end{aligned}$$

For the comparative purpose, using the NOAEL/LOAEL approach and the NOAEL of 20 mg/kg-day for liver effects in rats, and a Composite UF of 1000, the subchronic oral p-RfD would be 0.02 mg/kg-day, which is similar to the value calculated by the BMD approach.

Chronic RfD

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-target portion of the study is confounded by high incidences of endemic chronic murine pneumonia, but it is unclear whether 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. The mouse-targeted portion of the study identified a FEL of 284 mg/kg-day for reduced survival and lethal kidney lesions, but high incidences of hepatocellular tumors in all treated groups precluded evaluation of noncancer effects in the liver and identification of a NOAEL or LOAEL. The 14-week diet study in rats and mice (NTP, 2004) used to derive the subchronic RfD is the most appropriate basis for a chronic RfD because it is more comprehensive and sensitive than the chronic study, and it identified lower LOAELs than the chronic study. Additionally, dietary exposure is a more relevant type of exposure for the general population exposed to 1,1,2,2-tetrachloroethane in the environment.

The subchronic BMDL of 10.8 mg/kg-day for increased relative liver weight in male rats is used as the POD for the chronic p-RfD. To derive the chronic p-RfD, the subchronic BMDL is divided by a Composite UF of 3000. The Composite UF of 3000 comprises component factors of 10 for interspecies extrapolation, 10 for interhuman variability, 3 for subchronic to chronic duration extrapolation, and 10 for database deficiencies, as explained below.

A 10-fold UF is used to account for the interspecies variability in extrapolating from laboratory animals (rats) to humans. No relevant information is available on the toxicity of 1,1,2,2-tetrachloroethane in humans, and data on the toxicokinetic differences between animals and humans in the disposition of ingested 1,1,2,2-tetrachloroethane are not available.

A 10-fold UF is also used to account for variations in sensitivity within human populations because there is insufficient information on the degree to which humans of varying gender, age, health status, or genetic makeup might vary in the disposition of, or response to, ingested 1,1,2,2-tetrachloroethane. Animal toxicity studies that evaluated both sexes in the same study did not show consistent sex-related differences. Developmental toxicity studies in animals are limited in scope, but they have not indicated effects on the offspring at levels that did not also cause maternal effects.

A 3-fold UF is used to account for extrapolation from subchronic to chronic duration because pharmacokinetic data suggest that the biological half-life of 1,1,2,2-tetrachloroethane is short, and that a 14-week study should be sufficient to achieve steady-state. Available studies indicated that 60–80% of a single oral or intraperitoneal dose of 1,1,2,2-tetrachloroethane was metabolized and excreted within 48–72 hours in rats and mice (Dow Chemical Company, 1988;

Mitoma et al., 1985; Yllner, 1971). Furthermore, a 78-week exposure study in male and female Osborne Mendel rats and B6C3F1 mice (NCI, 1978) did not report evidence of the progression of hepatotoxicity to cirrhosis or other major liver effects.

A 10-fold UF is used to account for deficiencies in the database. Although the NTP (2004) 14-week study provides comprehensive evaluations of systemic toxicity and neurotoxicity in two species (rats and mice), the database is limited by the lack of an adequate chronic study. The database also lacks adequate reproductive and developmental toxicity studies, as discussed in the subchronic p-RfD derivation.

A UF to account for extrapolation from a LOAEL to a NOAEL was not applied because a NOAEL was identified and a BMDL was used as the POD for the p-RfD.

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

$$\begin{aligned} \text{chronic p-RfD} &= \text{BMDL} \div \text{CompositeUF} \\ &= 10.8 \text{ mg/kg-day} \div 3000 \\ &= \mathbf{0.004 \text{ mg/kg-day } (4 \times 10^{-3} \text{ mg/kg-day})} \end{aligned}$$

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR 1,1,2,2-TETRACHLOROETHANE

Subchronic p-RfC

Information on the subchronic inhalation toxicity of 1,1,2,2-tetrachloroethane consists of an English translation of a poorly reported French study in which rats were exposed to a presumed concentration of 3909 mg/m^3 (560 ppm) for a TWA duration of 5.1 hours/day, 5 days/week, for 15 weeks (Truffert et al., 1977). Findings included transient histological alterations in the liver: granular appearance and cytoplasmic vacuolation occurred after 9 exposures, regressed after 19 exposures, and were no longer evident after 39 exposures. Due to uncertainty regarding the actual exposure concentration, a lack of incidence and severity data, the lack of information on dose-response due to the use of a single exposure level, and other inadequately reported results, this study cannot be used to identify a reliable NOAEL or LOAEL or for p-RfC derivation.

Chronic p-RfC

Information pertaining to the chronic toxicity of inhaled 1,1,2,2-tetrachloroethane in humans is available from several occupational studies (Norman et al., 1981; Lobo-Mendonca, 1963; Jeney et al., 1957; Minot and Smith, 1921) that are inadequate for identification of effect levels due to limitations that include insufficient exposure monitoring, lack of control data, dermal exposure, and/or coexposure to other chemicals. Though neurological symptoms (including headache, weakness, and fatigue) were reported in these studies, hepatic effects were generally not evaluated.

Information regarding the chronic inhalation toxicity of 1,1,2,2-tetrachloroethane in animals comes from several poorly reported studies in rats and rabbits (Shmuter, 1977; Schmidt et al., 1972; Kulinskaya and Verlinskaya, 1972; Mellon Institute of Industrial Research, 1947). The effects reported in these studies included hematological alterations and increased liver fat content in rats exposed to 13.3 mg/m^3 (1.9 ppm), for 4 hours/day, for 265 days (Schmidt et al., 1972); alterations in serum acetylcholinesterase activity in rabbits exposed to 10 mg/m^3 (1.5 ppm) for 3 hours/day, 6 days/week, for 7–8.5 months (Kulinskaya and Verlinskaya, 1972); and immunological alterations in rabbits exposed to 2–100 mg/m^3 (0.3–14.6 ppm) for 3 hours/day, 6 days/week, for 8–10 months (Shmuter, 1977). These studies are inadequate for the identification of reliable NOAELs or LOAELs for systemic toxicity. Among other reporting limitations, the studies provide insufficient data on incidence, magnitude, and/or effect severity. A reproductive assessment in the Schmidt et al. (1972) chronic study is, however, sufficient for identification of a NOAEL of 13.3 mg/m^3 (1.9 ppm) for male reproductive effects in rats—but the lack of reliable information on systemic toxicity precludes derivation of a p-RfC.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 1,1,2,2-TETRACHLOROETHANE

Cancer potency is not addressed in this PPRTV document because an OSF and an IUR have been previously determined and are available on-line (U.S. EPA, 2008).

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APPENDIX A. DETAILS OF BMD ANALYSIS FOR 1,1,2,2-TETRACHLOROETHANE

Continuous Data

The model-fitting procedure for continuous data is as follows. The BMD modeling was conducted with the U.S. EPA's BMD software (BMDS version 1.4.1). For all the continuous data (relative liver weight increase in rats; clinical enzyme values), the original data were modeled with all the continuous models available within the software. An adequate fit was judged based on the goodness-of-fit P value (P > 0.1), scaled residue at the range of BMR, and visual inspection of the model fit. In addition to the three criteria for judging the adequate model fit, whether the variance needed to be modeled, and if so, how it was modeled also determined final use of the model results. If a homogenous variance model was recommended based on statistics provided from the BMD model runs (Test 2), the final BMD results would be estimated from a homogenous variance model. If the test for constant variance (Test 2) was negative (P < 0.1), the model is run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model did not provide an adequate fit to the variance data (Test 3; P < 0.1), then the data set was considered unsuitable for modeling. Among all the models that provided an adequate data fit, the lowest BMDL is selected if the BMDLs estimated from different models varied >3-fold, otherwise, the BMDL from the model with the lowest AIC is selected as the POD.

The data on rat liver weight changes observed in NTP (2004) were modeled with continuous models available in BMDS version 1.4.1. All the models available in the BMDS were used to fit the data with a constant or non-constant variance setting. The BMD modeling results are summarized in the following table and Appendix. Results from the best fitting models of female rat data are presented for comparison.

Table A-1. Benchmark Dose Modeling for Rat Liver Weight Changes

Model Type	Variance Model	Goodness-of-Fit *p*-value[a]	AIC	Scaled Residual	BMD (mg/kg-day)	BMDL (mg/kg-day)
			Males (10)			
Linear[b]	constant	**0.153**	94.57	-1.26	13.1	10.8
Linear	non-constant	0.08887	95.73	-1.34	11.0	7.8
Polynomial	constant	0.05931	96.37	-1.41	12.0	8.6
Polynomial	non-constant	0.0304	97.58	-1.49	10.3	6.8
Power[b]	constant	**0.153**	94.57	-1.26	13.1	10.8
Power	non-constant	0.02779	97.73	-1.33	11.1	7.8
Hill	constant	N/A	94.82	-2.22 e-7	19.2	12.2
Hill	non-constant	N/A	94.89	0.0552	18.3	failed
			Females (10)			
Polynomial	constant	0.216	69.76	0.94	23.6	15.7
Power	constant	0.1481	72.32	0.35	25.3	17.1

[a] Values < 0.10 fail to meet conventional goodness-of-fit criteria
[b] Models with best fit
N/A = not applicable

For all the continuous models, the test of constant variance model (Test 2) indicated a P value of 0.07016 ($P < 0.1$), and BMDS suggested to run the model with a non-homogeneous variance model. However, non-homogeneous variance model did not provide satisfactory fit to the variance data as indicated in Test 3, where the P value is less than 0.1 ($P = 0.07016$), suggesting a different variance model might be appropriate. Due to a lack of additional variance model available in the current BMDS, BMD results from the models providing the best fit to the data were selected for the further analysis.

Among all the available continuous models in BMDS (version 1.4.1), only Linear model and Power models with constant variance setting provide acceptable goodness-of-fit P values ($p = 0.153$). Therefore, these models were considered the best models available, and the BMDL of 10.8 mg/kg-day estimated from these models is the appropriate estimate for liver weight increase after the treatment with 1,1,2,2-tetrachloroethane. Details of the modeling results are presented in Appendix B.

Serum ALT

For the serum ALT data, the assumption of constant variance did not hold for either the male or female rat data. The nonhomogeneous variance model was applied and provided adequate fit to the variance for both the male and female data. With the nonhomogeneous variance model applied, the linear model did not provide an adequate fit to the means for either the male or female data. For the males, both the polynomial and power models provided adequate fits to the means while the variance model was applied. The AIC was slightly lower for the polynomial model, which was selected as the best fitting model. Doses associated with a 100% change from the control (2-fold) in the polynomial model were also calculated (Table A-2; Figure A-1). For the females, none of the models were able to provide an adequate fit to the means when the variance model was applied.

Serum SDH

For the serum SDH data, the assumption of constant variance did not hold for either the male or female rat data. The nonhomogeneous variance model was applied and provided inadequate fit to the variance for both the male and female data (Table A-3).

Table A-2. Model Predictions for Changes in Serum ALT in Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks by NTP (2004)

Model	BMR	Variance *p*-value[a]	Means *p*-value[a]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Male						
Linear[b] (constant variance)	1 SD	<0.0001	<0.0001	484.88	43.91	37.37
Linear[b] (modeled variance)	1 SD	0.7223	<0.0001	412.89	12.72	10.07
Polynomial[b,c,d] (modeled variance)	1 SD	0.7223	0.7302	367.955	38.23	26.56
	100%	0.7223	0.7302	367.955	134.06	121.35
Power[e] (modeled variance)	1 SD	0.6731	0.7945	367.956	41.97	32.24
Hill[e] (modeled variance)	Model failed					
Female						
Linear[b] (constant variance)	1 SD	<0.0001	<0.0001	511.01	44.94	38.22
Linear[b] (modeled variance)	1 SD	0.1849	<0.0001	447.29	17.59	13.50
Polynomial[f] (modeled variance)	1 SD	0.1849	<0.0001	370.32	49.47	45.00
Power[e] (modeled variance)	1 SD	0.1782	0.0074	358.41	64.68	56.13
Hill[e] (modeled variance)	Model failed					

[a] Values < 0.10 fail to meet conventional goodness-of-fit criteria
[b] Coefficients restricted to be positive
[c] 2-degree polynomial (lowest degree with significant fit)
[d] Best fitting model
[e] Power restricted to ≥1
[f] 2-degree polynomial; no adequate fit with any polydegree.

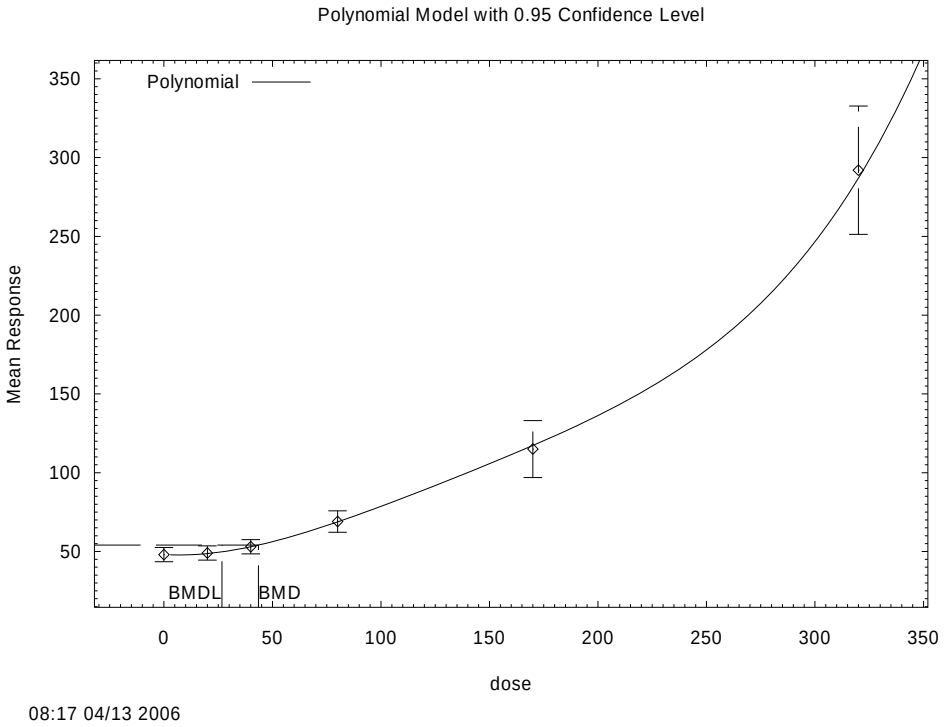


Figure A-1. Changes in Serum ALT in Male Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks by NTP (2004)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control, and are in units of mg/kg-day.

Table A-3. Model Predictions for Changes in Serum SDH in Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks by NTP (2004)

Model	BMR	Variance *p*-value[a]	Means *p*-value[a]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Male						
Linear[b] (constant variance)	1 SD	<0.0001	0.1889	291.96	41.70	35.55
Linear[b] (modeled variance)	1 SD	0.0499	0.0471	274.89	23.86	19.11
Female						
Linear[b] (constant variance)	1 SD	<0.0001	<0.0001	319.64	47.70	40.47
Linear[b] (modeled variance)	1 SD	0.0429	<0.0001	310.32	34.45	26.54

[a]Values < 0.10 fail to meet conventional goodness-of-fit criteria
[b] Coefficients restricted to be positive

Bile Acids

For the serum bile acids data, the assumption of constant variance did not hold for either the male or female rat data. The nonhomogeneous variance model was applied and provided an adequate fit to the variance for both the male and female data. With the variance model applied, the linear and Hill models did not provide adequate fits to the means for either the male or female data, and the polynomial model did not provide an adequate fit to the mean for the female data. For the males, both the polynomial and power models provided adequate fits to the means when the variance model was applied. The power model was selected as the best fitting model for the male data because it had a slightly lower AIC than the polynomial model (Table A-4; Figure A-2). For the female, the power model was the only model that provided adequate fit to the means while the variance model was applied (Table A-4; Figure A-3).

Table A-4. Model Predictions for Changes in Bile Acids in Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks by NTP (2004)

Model	BMR	Variance *p*-value[a]	Means *p*-value[a]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Male						
Linear[b] (constant variance)	1 SD	<0.0001	0.0013	577.11	79.44	61.93
Linear[b] (modeled variance)	1 SD	0.7661	<0.0001	464.43	24.81	20.06
Polynomial[b,c] (modeled variance)	1 SD	0.7661	0.1194	428.95	58.37	49.57
Power[d,e] (modeled variance)	1 SD	0.7661	0.4582	427.70	72.45	57.17
Hill[e] (modeled variance)	Model failed					
Female						
Linear[b] (constant variance)	1 SD	<0.0001	<0.0001	594.57	101.36	81.28
Linear[b] (modeled variance)	1 SD	0.4663	<0.0001	576.14	NA[g]	54.83
Polynomial[f] (modeled variance)	1 SD	0.4663	<0.0001	487.96	149.50	106.40
Power[d,e] (modeled variance)	1 SD	0.4663	0.3751	466.68	216.74	177.00
Hill[e] (modeled variance)	Model failed					

[a] Values < 0.10 fail to meet conventional goodness-of-fit criteria
[b] Coefficients restricted to be positive
[c] 2-degree polynomial (lowest degree with significant fit)
[d] Best fitting model
[e] Power restricted to ≥1
[f] 2-degree polynomial; no adequate fit with any polydegree.
[g] NA = not available (BMD software could not generate a model output)

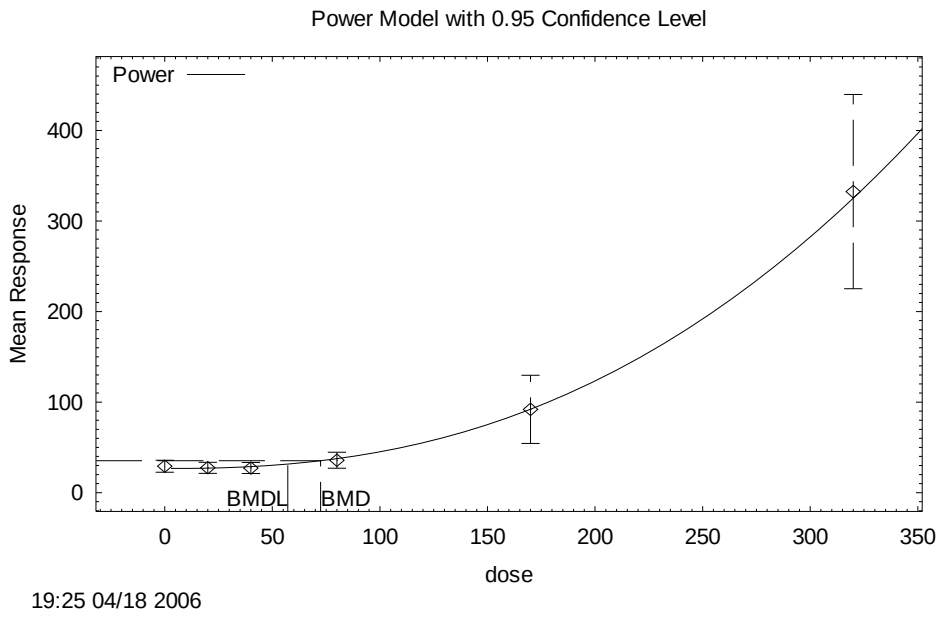


Figure A-2. Changes in Bile Acids in Male Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks by NTP (2004)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units of mg/kg-day.

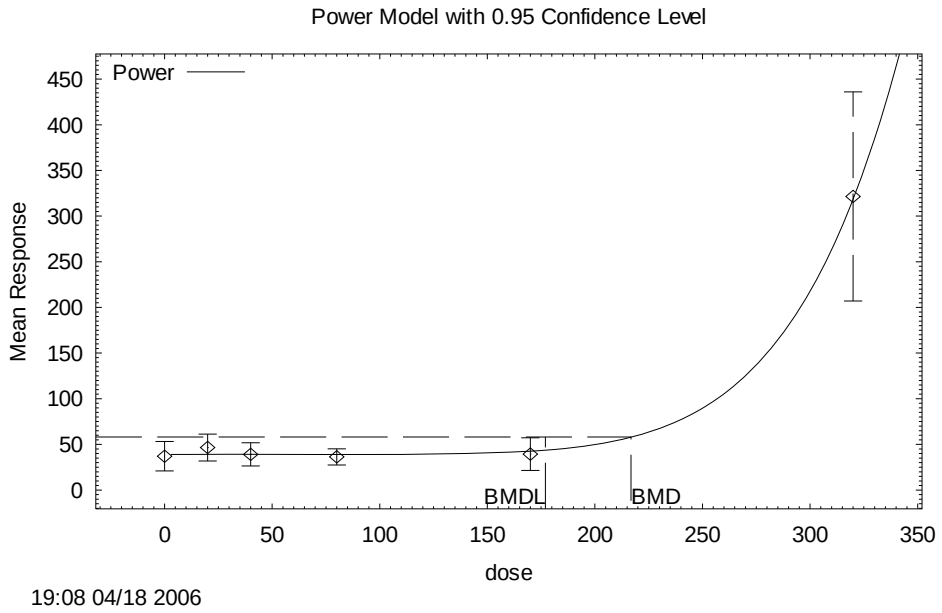


Figure A-3. Changes in Bile Acids in Female Rats Exposed to 1,1,2,2-Tetrachloroethane in the diet for 14 weeks by NTP (2004)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units of mg/kg-day.

Serum Cholesterol (Females Rats Only)

Statistical tests indicated that variances were not constant across exposure groups (this is reflected in the standard errors listed in Table 5). The nonhomogeneous variance model did not adequately fit the variance data; therefore, there is no good fit to the data for change in serum cholesterol in female rats (Table A-5).

Table A-5. Model Predictions for Changes in Serum Cholesterol in Female Rats Exposed to 1,1,2,2-Tetrachloroethane in the Diet for 14 Weeks by NTP (2004)

Model	Variance *p*-value[a]	Means *p*-value[a]	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear[b] (constant variance)	0.0044	<0.0001	63.66	53.24
Linear[b] (modeled variance)	0.0019	<0.0001	56.37	39.96

[a]Values < 0.10 fail to meet conventional goodness-of-fit criteria.
[b] Coefficients restricted to be positive

APPENDIX B. DETAILS OF BMD ANALYSIS FOR THE SUBCHRONIC ORAL RfD FOR 1,1,2,2-TETRACHLOROETHANE—FEMALE RAT RELATIVE LIVER WEIGHT

```
POLYNOMIAL MODEL CONSTANT VARIANCE

2-degree


=====================================================================
         Polynomial Model. Revision: 2.2  Date: 9/12/2002
         Input Data File: C:\BMDS\DATA\1122-TCE_RAT_REL_LIVER_WT.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\1122-TCE_RAT_REL_LIVER_WT.plt
                                                Fri Sep 28 22:28:06 2007
 ====================================================================

 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 = 6
   Total number of records with missing values = 2
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                   Default Initial Parameter Values
                           alpha =      1.93677
                             rho =            0   Specified
                          beta_0 =      35.2192
                          beta_1 =    0.0343205
                          beta_2 =  0.000966477



                                  Parameter Estimates

                                                                 95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha           1.8111         0.404974             1.01736
2.60484
         beta_0          35.2192         0.407789             34.4199
36.0184
         beta_1        0.0343205        0.0258825          -0.0164082
0.0850491
         beta_2      0.000966477      0.000300067         0.000378356
0.0015546


           Asymptotic Correlation Matrix of Parameter Estimates
```

	alpha	beta_0	beta_1	beta_2
alpha	1	1.5e-009	-2.9e-010	-1.8e-009
beta_0	1.5e-009	1	-0.74	0.59
beta_1	-2.9e-010	-0.74	1	-0.96
beta_2	-1.8e-009	0.59	-0.96	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	10	35.1	1.77	35.2	1.35	-0.351
20	10	36.7	1.14	36.3	1.35	0.935
40	10	37.8	1.61	38.1	1.35	-0.701
80	10	44.2	0.85	44.2	1.35	0.117

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

Likelihoods of Interest

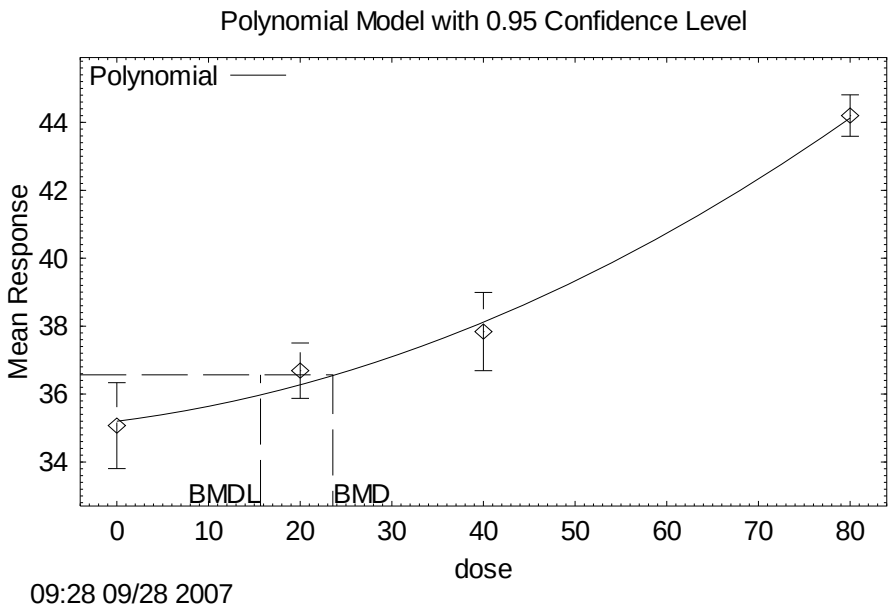
Model	Log(likelihood)	DF	AIC
A1	-31.113274	5	72.226548
A2	-28.050020	8	72.100041
fitted	-31.878683	3	69.757366
R	-72.901294	2	149.802588

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	89.7025	6	<.0001
Test 2	6.12651	3	0.1056
Test 3	1.53082	1	0.216

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 .05. A homogeneous variance
model appears to be appropriate here

```
The p-value for Test 3 is greater than .05.  The model
chosen appears
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 =        23.569


            BMDL =       15.6757
```