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Provisional Peer-Reviewed Toxicity Values for

1,2,4,5-Tetrachlorobenzene
(CASRN 95-94-3)

Superfund Health Risk Technical Support Center
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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
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
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
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PEER-REVIEWED PROVISIONAL TOXICITY VALUES FOR 1,2,4,5-TETRACHLOROBENZENE (CASRN 95-94-3)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVs

Questions regarding the contents and appropriate use of this PPRTV assessment should 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).

INTRODUCTION

1,2,4,5-Tetrachlorobenzene (TCB), CASRN 95-94-3, is a chlorobenzene used in the manufacture of herbicides and defoliants. 1,2,4,5-TCB is also used in insecticides, moisture-resistant impregnates, electrical insulation, and packing material (WHO, 1991). A table of physicochemical properties is provided below (see Table 1).

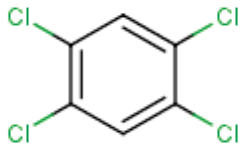

Figure 1. 1,2,4,5-TCB Structure

Table 1. Physicochemical Properties of 1,2,4,5-TCB (CASRN 95-94-3)[a]

Property (unit)	Value
Boiling point (ºC)	244.5
Melting point (ºC)	139.5
Density (g/cm^3)	1.833
Vapor pressure (mmHg at 25ºC)	0.0054
pH (unitless)	No data
Solubility in water (mg/L at 25ºC)	0.595
Relative vapor density (air = 1)	7.4
Molecular weight (g/mol)	215.89

[a]NLM (2011).

Table 2 provides a summary of the available toxicity values for 1,2,4,5-TCB from U.S. EPA and other agencies/organizations.

Table 2. Summary of Available Toxicity Values for 1,2,4,5-TCB (CASRN 95-94-3)[a]

Source/Parameter[a]	Value (Applicability)	Notes	Source	Date Accessed
Noncancer				
ACGIH	NV	NA	ACGIH (2013)	NA
ATSDR	NV	NA	ATSDR (2013)	NA
Cal/EPA	NV	NA	Cal/EPA (2013a,b)[b]	9-26-2013
NIOSH	NV	NA	NIOSH (2010)	NA
OSHA	NV	NA	OSHA (2006, 2011)	NA
IRIS	RfD: 3×10^{-4} mg/kg-d	This RfD is based on the 90-d feeding study performed by Chu et al. (1984a) that identified kidney lesions in male rats and reported a POD as a NOAEL of 0.34 mg/kg-d and a UF_C of 1000 (10 each for intraspecies, interspecies, and extrapolation from subchronic effect level). Confidence in the value was considered to be "low." Note that IRIS did not apply DAF methodology.	U.S. EPA (1991)	NA
Drinking water	NV	NA	U.S. EPA (2012)	NA
HEAST	NV	NA	U.S. EPA (2011a)	NA
CARA HEEP	NV	NA	U.S. EPA (1994)	NA
WHO	TDI: 1×10^{-4} mg/kg-d	Based on the incidence of kidney lesions reported in the study by Chu et al. (1984a), with a NOEL of 0.034 mg/kg-day and a UF of 500.	WHO (1991)	NA
Cancer				
IRIS	NV	NA	U.S. EPA (1991)	NA
HEAST	NV	NA	U.S. EPA (2011a)	NA
IARC	NV	NA	IARC (2013)	NA
NTP	NV	NA	NTP (2011)	NA
Cal/EPA	NV	NA	Cal/EPA (2009, 2013b)	NA

[a]Sources: American Conference of Governmental Industrial Hygienists (ACGIH); Agency for Toxic Substances and Disease Registry (ATSDR); California Environmental Protection Agency (Cal/EPA); National Institute for Occupational Safety and Health (NIOSH); Occupational Safety and Health Administration (OSHA); Chemical Assessments and Related Activities (CARA); Health and Environmental Effects Profile (HEEP); World Health Organization (WHO); Integrated Risk Information System (IRIS); Health Effects Assessment Summary Tables (HEAST); International Agency for Research on Cancer (IARC); National Toxicology Program (NTP).
[b]The Cal/EPA Office of Environmental Health Hazard Assessment (OEHHA) Toxicity Criteria Database (http://oehha.ca.gov/tcdb/index.asp) was also reviewed and found to contain no information on 1,2,4,5-TCB.

DAF = dosimetric adjustment factor; HED = human equivalent dose; NA = not applicable; NOAEL = no-observed-adverse-effect level; NOEL = no-observed-effect level; NV = not available; POD = point of departure; RfD = reference dose (oral); TDI = tolerable daily intake; UF = uncertainty factor; UF_C = composite uncertainty factor.

Literature searches were conducted on sources published from 1900 through September 2013 for studies relevant to the derivation of provisional toxicity values for 1,2,4,5-TCB, CASRN 95-94-3. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (U.S. EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI; and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following information sources outside of HERO were searched for relevant health information: ACGIH, ATSDR, Cal/EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, U.S. EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 3 provides an overview of the relevant database for 1,2,4,5-TCB and includes all potentially relevant repeated short-term-, subchronic-, and chronic-duration studies. The phrase "statistical significance" used throughout the document indicates a p-value of <0.05.

Table 3. Summary of Potentially Relevant Data for 1,2,4,5-TCB (CASRN 95-94-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Human								
1. Oral (mg/kg-d)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	ND							
Chronic[f]	ND							
2. Inhalation (mg/m^3)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	Workers involved in production of 1,2,4,5-TCB for at least 6 mo, 8 hr/d	No exposure information provided	Increased frequency of chromosomal aberrations	NDr	NDr	NDr	Kiraly et al. (1979)	PR
Chronic[f]	ND							
Animal								
1. Oral (mg/kg-d)[a]								
Short-term	5/5, F344 Rat, diet, 14 d	0, 3.0, 10.5, 30.6, 109, 287 (males) 0, 3.2, 10.5, 29.6, 102, 271 (females) (Adjusted)	Decreased final body weight in males and females (>10% decrement compared to control); liver congestion (no incidence or severity data provided)	NDr	NDr	NDr	NTP (1991a)	PR

Table 3. Summary of Potentially Relevant Data for 1,2,4,5-TCB (CASRN 95-94-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Short-term	5/5, B6C3F$_1$ Mouse, diet, 14 d	0, 6.2, 20.7, 56.1, 213 (males) 0, 8.9, 25.4, 70.8, 273 (females)	Increased absolute and relative liver weights (no raw organ-weight data provided)	NDr	NDr	NDr	NTP (1991b)	PR
Subchronic	**10/10, S-D Rat, diet, 28 d**	**0, 0.041, 0.42, 3.4, 32 (males)** **0, 0.059, 0.61, 6.2, 56 (females)** **(Adjusted)**	**Increased relative liver weight and serum cholesterol; moderate to severe histological changes in the liver, thyroid, kidneys, and lungs**	**NI (males)** **NI (females)**	**0.46 (males; histological changes in liver)**	**0.041 (males; lung and thyroid)** **0.059 (males; thyroid)**	**Chu et al. (1983)**	**PS PR**
	15/15, S-D Rat, diet, 90 d	**0.034−34 (males)** **0.042−41 (females)** **(Adjusted)**	**Increased absolute liver weight; increased serum cholesterol; moderate-to-severe lesions in the liver and kidney in males and females; increased incidence and severity of kidney lesions in male rats at all doses**	**0.34**	**DU**	**3.4**	**Chu et al. (1984a)** **Study authors report average daily doses as ranges only**	**PS PR IRIS (U.S. EPA, 1991)**

Table 3. Summary of Potentially Relevant Data for 1,2,4,5-TCB (CASRN 95-94-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Subchronic	2/2, Unspecified strain, Rat, diet, 30 d	0, 2.4, 7.0, 23.6, 78.2, 270.6 (males) 0, 2.5, 8.3, 22.4, 90.3, 264.1 (females) (Adjusted)	Gross and microscopic changes in liver (enlarged, light in color, centrilobular necrosis) and kidneys (enlarged, light in color, tubular degeneration, necrosis) in females; enlargement, tubular degeneration, and necrosis in kidneys at all doses in males	NI (males) 8.3 (females)	NDr	2.4 (males) 22.4 (females)	Dow (1984a)	NPR
	10/10, Unspecified strain, Rat, diet, 42 d	26.0, 88.2, 276.0 (males) 30.4, 101.7, 315.3 (females) (Adjusted)	Increased lung, heart, liver, kidney, spleen, and testes weight with increasing dose (no control group animals sacrificed for comparison)	NDr	NDr	NDr	Dow (1984a)	NPR
	10/10, Unspecified strain, Rat, diet, 90 d	0, 2.5, or 8.2 (males) 0, 2.9, or 9.7 (females) (Adjusted)	Centrilobular necrosis in liver and degeneration and necrosis of renal tubular epithelium in males; increased absolute liver weight, centrilobular necrosis, and swelling of hepatocytes in females	NI (males) NI (females)	NDr	2.5 (males) 2.9 (females)	Dow (1984a)	NPR
	10/10, F344 Rat, diet, 13 wk	0, 2.1, 7.1, 22.1, 71.4, 156 (males) 0, 2.1, 7.3, 22.4, 79.1, 151 (females) (Adjusted)	Decreased body weight; increased absolute and relative kidney and liver weights in both sexes; renal lesions in males; thyroid follicular cell hypertrophy in both sexes; decreased free and total thyroxin concentrations in females	NI (females) (decreased free thyroxin)	NDr	2.1 (females) (decreased free thyroxin)	NTP (1991c) Supporting Study for thyroid endpoint	PR

Table 3. Summary of Potentially Relevant Data for 1,2,4,5-TCB (CASRN 95-94-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Subchronic	10/10, B6C3F$_1$ Mouse, diet, 13 wk	0, 4.5, 14.6, 45.2, 150, 278 (males) 0, 6.0, 19.7, 56.6, 143, 302 (females) (Adjusted)	Increased absolute and relative liver weights; increased incidence of liver lesions; increased serum sorbitol dehydrogenase (SDH) and alanine aminotransferase (ALT) activity; increased platelet count	4.5 (males) liver weight	DU	14.6 (males) increased liver weight (>10% compared to control)	NTP (1991d)	PR
	2/2, Beagle Dog, diet, 92 d	0.028, 0.29, 2.63 (males) 0.027, 0.27, 2.86 (females)	No evidence of any compound-related effects	2.63 (males) 2.86 (females)	NDr	None	Dow (1982a) Poor copy: difficult to read	NPR
Chronic	5/5, Unspecified strain, Rat, diet, 101 d	0, 0.01, 0.03, 0.08, 0.25, 0.82 (males) 0, 0.01,0.03, 0.10, 0.29, 0.97 (females) (Adjusted)	Centrilobular necrosis, swelling of parenchymal cells of liver, and degeneration and necrosis of tubular epithelium of kidneys in both sexes	0.08 (males) 0.03 (females)	NDr	0.25 (males) 0.10 (females)	Dow (1984b)	NPR
	Unspecified number and strain, Rat, Unspecified route, 8 mo	0, 0.001, 0.005, 0.05 (Adjusted)	Increased hemoglobin and reticulocytes; disorders of glycogen-forming function of liver	NDr	NDr	NDr	Fomenko (1965) Article in Russian, data obtained from IPCS (1991)	PR

Table 3. Summary of Potentially Relevant Data for 1,2,4,5-TCB (CASRN 95-94-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Chronic	2/2 (4/4 control group), Beagle Dog, diet,144 d	0, 5, 10, 20 (Adjusted)	Increased liver weight in females	NDr	NDr	NDr	Dow (1982b) Poor copy: difficult to read	NPR
	2/2, Beagle Dog, diet, 746 d	5 (Adjusted)	Increased serum alkaline phosphatase (ALP) activity and bilirubin at 24 mo (compared to historical controls); effects not observed at 18 mo and reversed within 3 mo of cessation	NDr	NDr	NDr	Braun et al. (1978a,b) Same data are reported in two different publications in the same year	PR
	2/2 Beagle Dog, diet, 144 d	0, 5, 10, 20	Slight necrosis of hepatocytes	5	NDr	10	Dow (1982b) Poor copy: difficult to read	NPR
Developmental	0/10, S-D Rat, gavage, GDs 6−15	0, 50, 100, 200	Maternal deaths at high dose; no fetal anomalies observed	Maternal: 100 Developmental: 200	NDr	Maternal: 200 (FEL) Developmental: NI	Kacew et al. (1984)	PR
	0/6−8, S-D Rat, gavage, GDs 9−13	0, 30, 100, 300, 1,000	Significantly decreased body-weight gain in dams of highest dose group; no changes observed in any embryonic endpoints examined	Maternal: 300 Developmental: 1,000	NDr	Maternal: 1,000 Developmental: NI	Kitchin and Ebron (1983)	NPR

Table 3. Summary of Potentially Relevant Data for 1,2,4,5-TCB (CASRN 95-94-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Developmental	0/25, F344 Rat, gavage, GDs 6−15	0, 25, 75, 125	Reduced weight gain and food consumption in dams; urine stains, audible respiration, nasal and ocular discharge in dams; reduced ossification in fetuses	NDr	NDr	NDr	Fisher et al. (1990a) Abstract only	NPR
	0/15; New Zealand White Rabbit, gavage, GDs 6−18	0, 5, 15, 25	Mortality, abortions, and reduced body-weight gain in dams; one visceral and one cranial variation observed in two fetuses, respectively. FEL at lowest dose tested (maternal mortality and fetal loss).	NDr	NDr	NDr	Fisher et al. (1990b) Abstract only	NPR
Reproductive	F0: 28/28, S-D Rat, diet, 10 wk; F1: 28/28, S-D Rat, diet, 11 wk	0, 2.2, 21.1, 70.3 (F0 males) 0, 2.6, 25.5, 82.5 (F0 females) 0, 2.0, 21.3 (F1 males) 0, 2.5, 25.4 (F1 females)	Histologic changes in kidneys of F0 and F1 males; stillborn F1 pups and perinatal deaths	Parental: NI (males) Developmental: 2.6	NDr	Parental: 2.2 (males) Developmental: 25.5	Tyl and Neeper-Bradley (1989) Also cited as Union Carbide Corporation (1992) Bushy Run Research Center (1988)	NPR

Table 3. Summary of Potentially Relevant Data for 1,2,4,5-TCB (CASRN 95-94-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Reproductive	20/20 (40 pairs included in the control group), Swiss CD-1 Mouse, diet, 105 d	0, 42, 109, 246 (F0 males) 0, 43, 108, 253 (F0 females) 0, 108 (F1 males) 0, 127 (F1 females)	Mortality in high-dose F0 females (19/20); decreased number of total live pups/litter only in litters born to F0 but not F1 mice	Parental and Reproductive: 42 (males) 43 (females)	NDr	Parental and Reproductive: 109 (males) 108 (females)	NTP (1991e) Also cited as Chapin (1997)	PR
Carcinogenicity	ND							
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	ND							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects. All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (daily) exposure. Values from animal developmental studies are not adjusted to a continuous exposure.
[b]Notes: IRIS = Utilized by IRIS, date of last update; PS = principal study, PR = peer reviewed, NPR = not peer reviewed.
[c]Acute = Exposure for 24 hr or less (U.S. EPA, 2002).
[d]Short-term = Repeated exposure for >24 hr ≤30 d (U.S. EPA, 2002).
[e]Long-term = Repeated exposure for >30 d ≤10% lifespan (based on 70-yr typical lifespan) (U.S. EPA, 2002).
[f]Chronic = Repeated exposure for ≥ 10% lifespan (U.S. EPA, 2002).

DU = data unsuitable, FEL = frank effect level, GD = gestation day, NA = not applicable, ND = no data, NDr = not determined, NI = not identified, NP = not provided, NR = not reported, NR/Dr = not reported but determined from data, NS = not selected, NV = not available, S-D = Sprague-Dawley.

HUMAN STUDIES

Oral Exposures

No studies were identified.

Inhalation Exposures

The effects of inhalation exposure of humans to 1,2,4,5-TCB have been evaluated in one long-term-duration study (Kiraly et al., 1979), which was limited to considering chromosomal aberrations in peripheral blood lymphocytes. No acute-, short-term-, or chronic-duration studies on inhalation exposure of humans to 1,2,4,5-tetrachlorobenzene were identified in the literature.

Acute Studies

No studies were identified.

Short-Term Studies

No studies were identified.

Long-Term Study

Kiraly et al. (1979)

In a peer-reviewed occupational study, Kiraly et al. (1979) investigated chromosomal aberrations in peripheral blood lymphocytes of factory workers involved in the production of insecticides for at least 6 months and for 8 hours per day. The study included a control group, a factory workers control group, and an exposure group. The normal control group consisted of 43 males and 6 females whose ages ranged from 26−52 years. The factory workers control group consisted of engineers, technicians, and office staff working with the Budapest Chemical Works for 10−30 years but never directly exposed to pesticides. This group included 11 males and 3 females, whose ages ranged from 28−47 years. The exposure group consisted of 24 male and 1 female workers, whose ages ranged from 31−59 years. Individuals in the exposure group were involved in the production of 1,2,4,5-TCB for at least 6 months and for 8 hours per day. The concentration of 1,2,4,5-TCB in the air of workshops was not reported by the study authors. Blood samples were taken from each group, and lymphocytes were examined for chromosome aberrations (see Table B.1). The overall frequency of chromatid aberrations was higher in workers exposed to 1,2,4,5-TCB than in either of the control groups. However, due to lack of exposure information, identification of a NOAEL or LOAEL is precluded and the study is unsuitable for dose-response evaluation.

Chronic Studies

No studies were identified.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to 1,2,4,5-TCB have been evaluated in two short-term-duration studies (NTP, 1991a,b), eight subchronic-duration studies (Chu et al., 1984a, 1983; Dow, 1984a [3 studies], 1982a; NTP 1991c,d), four chronic-duration studies (Dow, 1984b, 1982b; Braun et al., 1978a,b; Fomenko, 1965), four developmental toxicity studies (Kacew et al., 1984; Kitchin and Ebron, 1983; Fisher et al., 1990a,b), and two reproductive toxicity studies (Tyl and Neeper-Bradley, 1989; NTP, 1991e).

As described in Table 3, the studies by NTP (1991) are identified as NTP (1991a) for the 14-day feeding study in rats, NTP (1991b) for the 14-day feeding study in mice, NTP (1991c) for the 13-week feeding study in rats, NTP (1991d) for the 13-week feeding study in mice, and NTP (1991e) for the reproductive toxicity study in mice.

Short-Term Studies

NTP (1991a)

The NTP conducted a dietary feeding study on F344 rats (5/sex/group) where animals were administered diets containing 1,2,4,5-TCB (>99% pure) at concentrations of 0; 30; 100; 300; 1,000; or 3,000 ppm for 14 days. The control and treatment diets each contained 1% corn oil. Based on food consumption and body-weight data, the study authors calculated average daily doses of 0, 3.0, 10.5, 30.6, 109, and 287 mg/kg-day for males and 0, 3.2, 10.5, 29.6, 102, and 271 mg/kg-day for females. Histopathological examinations of animals in the control group and in the two highest dose groups (1,000 and 3,000 ppm) were conducted on the following tissues: adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal passage and turbinates, pancreas, parathyroid glands, pituitary gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Livers from male rats at the 300-ppm dose level were also examined histopathologically.

No mortality was observed in the control or treatment groups. Body weights were significantly lower in males and females at the high dose compared to concurrent controls (18% decrement in males, 15% decrement in females). Feed consumption for high-dose animals was decreased by about 20%. Treatment-related clinical signs included tremors, lethargy, thin appearance, rough hair coats, ataxia, and chromodacryorrhea (bloody tears) in both sexes of rats in the high-dose group. Rapid breathing was observed in all females in the high-dose group. At dietary concentrations ≥300 ppm (30.6 mg/kg-day in males, 29.6 mg/kg-day in females), absolute and relative liver and kidney weights were increased; however, the study authors provide only a qualitative narrative for this 14-day study with no numerical data. Liver congestion was also observed in males (≥109 mg/kg-day) and females (271 mg/kg-day), although NTP (1991a) did not provide incidence or severity data. NTP (1991a) observed large hyaline droplets in cortical tubular epithelial cytoplasm in kidneys of all treated male rats but did not provide severity data. Immunostaining to verify the presence of alpha-2u protein was not done. Due to deficiencies in reporting of organ weights and histopathology, identification of a NOAEL or LOAEL is precluded.

NTP (1991b)

NTP also conducted a 14-day feeding study in $B6C3F_1$ mice (5/sex/group) using the same protocol as described above for F344 rats; in addition, the gallbladders of the mice were also removed for examination at necropsy. Only qualitative summaries were provided for this 14-day study with no numerical information. The study authors calculated average daily doses for the 30-; 100-; 300-; or 1,000-ppm groups as 6.2, 20.7, 56.1, and 213 mg/kg-day, respectively, for males and 8.9, 25.4, 70.8, and 273 mg/kg-day for females. No animals in the high-dose group (3,000 ppm) survived until the end of study. Because none of the animals in the 3,000-ppm group survived, the study authors were unable to calculate an average daily dose for

this group. At moribund sacrifice or at premature death, males and females in the highest dose group were observed to have effects typical of moribund animals including depletion and necrosis of the lymphoid tissue in the spleen, thymus, and lymph nodes. Tremors, rapid breathing, lethargy, hunched posture, rough hair coats, dyspnea, and prostration were observed in males and females in the highest dose group. In the remaining treatment groups at terminal sacrifice, final body weights in control and treated animals were comparable. Absolute and relative liver weights were statistically significantly increased in males receiving 213 mg/kg-day and females receiving 70.8 or 273 mg/kg-day. Due to deficiencies in reporting of organ weight data, a NOAEL or LOAEL cannot be identified from this study.

Subchronic Studies

Chu et al. (1983)

The peer-reviewed, 28-day dietary feeding study in Sprague-Dawley rats (Chu et al., 1983) is selected as the principal study for derivation of the subchronic provisional reference dose (p-RfD). The 28-day study is suitable to consider as subchronic in duration. No statement on good laboratory practice (GLP) compliance was provided. The study authors administered diets containing 1,2,4,5-TCB (>99.5% pure) at concentrations of 0, 0.5, 5.0, 50, or 500 ppm to Sprague-Dawley rats (10/sex/dose) for 28 days. The control and treatment diets each contained 4% corn oil. Based on body weight and food consumption data, the study authors calculated average daily doses as 0, 0.041, 0.42, 3.4, and 32 mg/kg-day for males and 0, 0.059, 0.61, 6.2, and 56 mg/kg-day for females. Body weight and food consumption were measured weekly, and clinical observations were made daily. At necropsy, serum biochemical parameters (sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase [ALP], aspartate aminotransferase [AST], total protein, calcium, cholesterol, glucose, uric acid, lactic dehydrogenase [LDH], and sorbitol dehydrogenase [SDH]) were measured. The brain, heart, liver, spleen, and kidneys were excised and weighed. The brain, pituitary, liver, adrenals, thyroid, parathyroid, thymus, lungs, trachea, bronchi, thoracic aorta, esophagus, gastric cardia, fundus and pylorus, duodenum, pancreas, colon, kidneys, spleen, bone marrow, mesenteric and mediastinal lymph nodes, testes, epididymis, skeletal muscle, and heart were fixed in buffered formalin and examined microscopically. The study authors also examined hemoglobin concentration (Hgb), packed cell volume (PCV), red blood cell (RBC) count, total and differential white blood cell (WBC) count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Liver samples were measured for microsomal aniline hydroxylase (AH), aminopyrine demethylase (APDM), and ethoxyresorufin (ER) deethylase activities and liver porphyrin concentrations. The study authors performed one-way statistical analysis of variance (ANOVA), and when significant differences were indicated, the data were subjected to the Student-Newman-Keuls post hoc multiple comparison test to identify groups that significantly differed.

Chu et al. (1983) stated that there were no treatment-related effects in weight gain or food consumption in rats of either sex. Liver porphyrin concentrations were not affected by treatment. No treatment-related hematological aberrations were observed in rats of either sex at any dose.

Chu et al. (1983) reported a statistically significant increase in relative liver weights in males and females of the highest dose group compared to controls (see Table B.2). A statistically significant increase in serum cholesterol in both male and female rats occurred at the highest dose (500 ppm). However, the study authors did not report data on relative liver weights or serum cholesterol data for the intermediate dose groups (only control and high-dose animals).

Treatment-related increases in hepatic mixed-function oxidase activity also were observed (see Table B.3). Statistically significant increases in aniline hydroxylase (AH) were observed in males and females in the highest dose group, and statistically significant increases in APDM were observed in females in the highest dose group and in males at the two highest doses. Table B.4 indicates histopathological lesions in liver, thyroid, and lung beginning at the low dose for males, as well as liver, thyroid, and kidney beginning at the low dose for females. The study authors reported dose-dependent morphological changes in the livers of both male and female rats that consisted of parenchymal cytoplasmic vacuolation and anisokaryosis, and a reduction in aggregated basophilia in the perivenous area, beginning at the lowest dose. The histological changes were considered moderate-to-severe in the high-dose animals; however, the study authors did not provide mean severity grades. In the thyroid, the study authors observed increased epithelial height, angular collapse of thyroid follicles, and reduction in colloid density at all doses. Although the study authors did not provide mean severity grades for each dose group, changes in the thyroid were reported as mild, even within the highest dose group. In the kidney, the study authors noted the presence of eosinophilic inclusions in the proximal convoluted tubule of the renal cortex, which bulged more prominently into the tubular lumina with increasing dose. The study authors considered the changes in the kidney to be statistically significant only in males in the highest and second-highest dose groups (3.4 and 32 mg/kg-day). Immunostaining to verify the presence of alpha-2u protein was not done. The study authors concluded that 1,2,4,5-TCB caused hepatomegaly, hepatic microsomal enzyme induction, and serum biochemical changes. The study authors further concluded that 1,2,4,5-TCB is a P450-type microsomal enzyme inducer. Based on increased incidence of histological changes in the thyroid in males, a LOAEL of 0.041 mg/kg-day (the lowest dose tested) is identified, with no corresponding NOAEL.

Chu et al. (1984a)

Chu et al. (1984a) is the principal study identified by IRIS RfD. Chu et al. (1984) fed groups of weanling Sprague-Dawley rats (15/sex/dose) diets containing 0, 0.5, 5.0, 50, and 500 ppm of 1,2,4,5-TCB for 13 weeks. The corresponding dose range in mg/kg-day was given as 0.034−34. Dose-related increases in the frequency and severity of kidney lesions for male rats were observed at 1,2,4,5-TCB doses of 5.0 ppm (0.34 mg/kg-day) and greater. The severity of effects was considered statistically significant only at the 50- and 500-ppm doses (3.4 and 34 mg/kg-day) because of a high incidence of mild kidney lesions in the controls. Liver lesions were observed in female rats at 500 ppm.

Dow (1984a)

Dow Chemical Company conducted subchronic and chronic toxicity studies in rats with varying doses and durations. The subchronic study with 30, 42, and 90-day sacrifices is designated as Dow (1984a) while the 101-day study is designated as Dow (1984b).

In a proprietary, non-peer-reviewed study, Dow (1984a) fed groups of 12 male and 12 female rats (strain not specified) diets containing 0; 30; 100; 300; 1,000; or 3,000 ppm of 1,2,4,5-TCB (99.5% purity, recrystallized), and sacrifices were conducted at 30, 42, or 90 days. At 30 days, 2 rats/sex/dose were sacrificed to obtain preliminary histopathological data. At 42 days, all rats in the three-highest dose groups (300; 1,000; and 3,000 ppm) were necropsied; however, no control animals were sacrificed at this timepoint. The remaining dose groups (0, 30, and 100 ppm) were maintained on the diet for 90 days and sacrificed. Adjusted daily doses are calculated using average body weights and average food consumption data provided by the study

authors whenever possible; if food consumption data were not provided, doses are calculated using allometric equations for food consumption according to provided body-weight data. For rats administered 0; 30; 100; 300; 1,000; or 3,000 ppm of 1,2,4,5-TCB in the diet for 30 days, the adjusted daily doses are calculated as 0, 2.4, 7.0, 23.6, 78.2, and 270.6 mg/kg-day, respectively, for males and 0, 2.5, 8.3, 22.4, 90.3, and 264.1 mg/kg-day, respectively, for females. For rats administered 300; 1,000; or 3,000 ppm of 1,2,4,5-TCB in the diet for 42 days, adjusted daily doses are calculated as 26.0, 88.2, and 276.0 mg/kg-day, respectively, for males and 30.4, 101.7, and 315.3 mg/kg-day, respectively, for females. For rats administered 0, 30, or 100 ppm of 1,2,4,5-TCB in the diet for 90 days, adjusted daily doses are calculated as 0, 2.5, and 8.2 mg/kg-day, respectively, for males and 0, 2.9, and 9.7 mg/kg-day, respectively, for females.

Animals were weighed twice weekly for the first month and once weekly in the subsequent weeks. The lungs, heart, liver, spleen, and testes were removed and weighed after necropsy, and portions of these organs were removed and prepared for histological examination.

Among the 2 rats/sex/dose that were sacrificed after 30 days, males exposed to the highest dose exhibited a body weight depression and slightly decreased average food consumption and food utilization. Male rats at the lowest dose level (30 ppm) also exhibited kidney histopathology. An increase in the average weight of the lungs, liver, and kidneys was noted at the three highest doses in male rats, and pathological changes in these organs were also observed in all but the low-dose group. Male rats also had evidence of pneumonia in the lungs and enlargement and lightening of the kidneys. The study authors also observed tubular degeneration and necrosis of the kidneys. In female rats, no effects were noted in the two lowest dose groups as judged by gross appearance and behavior, food consumption, mortality, final organ and body weights, and gross and microscopic examination of tissues. Females in the highest dose group displayed a retardation of growth and decreased average food consumption and food utilization. Increased average weight of lungs, liver, and kidneys were observed in females of the two highest dose groups. Gross and microscopic changes in lungs, liver, and kidneys of female rats in the three highest dose groups were similar to those observed in male rats. Based on kidney effects in the male rat kidney, a LOAEL of 2.4 mg/kg-day (the lowest dose tested) is identified for the 30-day study, with no corresponding NOAEL.

After the 30-day sacrifice, Dow (1984a) sacrificed all rats remaining in the three highest dose groups on Day 42. No control rats were sacrificed at this timepoint, and no histopathological examinations were conducted. The study authors only reported average organ weights and did not provide analysis of variance for the organ-weight values. The study authors noted that the average organ weights (lungs, heart, liver, kidneys, spleen, testes) increased with increasing dose. No additional data from the 42-day sacrifice were reported. Due to lack of control animals sacrificed at this timepoint, identification of a NOAEL or LOAEL is precluded for the 42-day segment of this study.

Dow (1984a) sacrificed the remaining animals in the 0-, 30-, and 100-ppm exposure groups (adjusted daily doses of 0, 2.5, and 8.2 mg/kg-day for males and 0, 2.9, and 9.7 mg/kg-day for females) after 90 days. Male and female rats both showed gross and microscopic changes in the liver and kidneys at all doses tested (incidence and severity not reported). In males fed 8.2 mg/kg-day, livers were enlarged and light in color. In all females in the three lowest dose groups, centrilobular necrosis and slight generalized cloudy swelling of the remaining parenchymal cells were observed. Statistically significantly increased average relative

liver weights (g/100 g body weight) of female rats in the highest dose group also were reported (see Table B.5). Degeneration and necrosis of renal tubular epithelium also were observed in all treated males and females. Based on gross and microscopic effects seen in liver and kidney of males, a 90-day LOAEL of 2.5 mg/kg-day (the lowest dose tested) is identified, with no corresponding NOAEL.

NTP (1991c)

In a peer-reviewed, 13-week oral toxicity study, NTP (1991c) administered diets containing 1,2,4,5-TCB (98% pure) at concentrations of 0; 30; 100; 300; 1,000; or 2,000 ppm of to F344/N rats (20/sex/dose). The control and treatment diets each contained 1% corn oil. The study was performed in compliance with GLP standards. The study authors calculated corresponding doses of 0, 2.1, 7.1, 22.1, 71.4, and 156 mg/kg-day for males and 0, 2.1, 7.3, 22.4, 79.1, and 151 mg/kg-day for females. Ten rats per sex per dose group were designated for urinalysis, serum chemistry, hematologic, and thyroid function analysis. The remaining 10 animals per sex per dose group were examined for histopathology, organ-weight determinations, and sperm morphology or vaginal cytology studies. Rats were observed twice daily, and body weights were recorded once weekly. Serial blood samples were collected on Days 3 or 4, 15, or 16, and 43 or 44 and analyzed for SDH, alanine aminotransferase (ALT), creatinine, creatine phosphokinase (CPK), γ-glutamyl transpeptidase (GGT), and albumin. Platelet counts were also determined. Sixteen-hour urine samples were collected on Days 17 or 18, 45, or 46, and 88 or 89. Appearance, 16-hour volume, specific gravity, glucose and protein concentrations, ALP and AST activity, and porphyrin concentrations were determined. Animals were necropsied at study termination. The liver, right kidney, right testis, brain, heart, thymus, lungs, and seminal vesicles were weighed.

Hematologic analyses included RBC, total and differential WBC counts, Hgb, Hct MCV, MCH, MCHC, and blood morphology. NTP (1991c) used Jonckheere's test to evaluate the statistical significance of dose-response trends for organ-weight, hematologic, serum chemical, and male reproductive system data. If Jonckheere's test showed significance, Shirley's nonparametric multiple comparison procedure was used to assess the significance of pairwise comparisons between dosed and control groups. Otherwise, Dunn's test was used for pairwise comparisons.

All animals survived to the end of the study. The mean body weights of rats of both sexes in the two highest dose groups were lower than those of controls throughout most of the study; final mean body weights of male rats dosed with 71.4 and 156 mg/kg-day were 10% and 21% lower, respectively, compared to controls. In females, final mean body weights at the 79.1 and 151 mg/kg-day doses were 8% and 16% lower, respectively, compared to controls (see Table B.6). Food consumption in all treated groups was similar to controls. NTP (1991c) reported that compound-related clinical signs included hypoactivity and lethargy. Absolute kidney weights were increased in both sexes at 300 ppm (22.1 mg/kg-day for males or 22.4 mg/kg-day for females), and absolute liver weights were increased at 300 ppm (22.1 mg/kg-day) for males and 100 ppm (7.3 mg/kg-day) for females (see Table B.7). Hct, Hgb, and RBC counts were significantly lower than those of controls for males receiving ≥71.4 mg/kg-day, while platelet counts and serum albumin concentration were significantly increased. Female rats exposed to the two highest doses had significantly lower MCV and significantly increased serum albumin concentrations. Statistically significantly decreased free and total thyroxin concentrations were observed at exposures of 300 ppm (22.1 mg/kg-day) in

males and at 30 ppm (2.1 mg/kg-day) in females (see Table B.8). The study authors reported that triiodothyronine (T3) concentrations were not affected, and thyrotropin concentrations were not recorded for each exposed animals. The study authors reported significant changes in urine parameters for both male and female rats. Statistically significant decreases in right whole and cauda epididymal weights and sperm motility were reported in males in the 22.1 and 156 mg/kg-day dose groups (the 71.4 mg/kg-day dose group was not examined). Estrous cycle length was unaffected by treatment. Compound-related lesions were observed in the kidneys of male and female rats but were more prominent in males. Cortical renal tubular cytoplasmic alterations occurred in male rats in all dose groups and were characterized histologically as intracytoplasmic aggregates of large, eosinophilic, angular inclusions that were increased in number and size compared to controls. Based on the reported histologic findings, the study authors assigned a NOEL of 30 ppm (2.1 mg/kg-day) for males and females. However, based on statistically significantly decreased free serum thyroxin in females, this dose (2.1 mg/kg-day) is considered a 13-week LOAEL.

NTP (1991d)

In addition to the studies in rats, NTP (1991d) also conducted a 13-week, peer-reviewed study in mice. B6C3F_1 mice (10/sex/dose) were provided diets containing 0; 30; 100; 300; 1,000; or 2,000 ppm of 1,2,4,5-TCB (98% pure). The control and treatment diets each contained 1% corn oil. This study was performed in compliance with GLP standards. Based on food consumption and body-weight data, the study authors calculated corresponding average daily doses of 0, 4.5, 14.6, 45.2, 150, and 278 mg/kg-day for males and 0, 6.0, 19.7, 56.6, 143, and 302 mg/kg-day for females. Ten mice per sex per dose were designated for serum chemistry, hematologic, and thyroid function analysis and urinalysis. The remaining 10 animals per sex per dose group were evaluated for histopathological changes, organ-weight determinations, and sperm morphology or vaginal cytology studies. Mice were observed twice daily, and body weights were recorded once weekly. Serial blood samples were collected on Days 3 or 4, 17, or 18, and 45 or 46 and analyzed for SDH, ALT, creatinine, CPK, GGT, and albumin. Sixteen-hour urine samples were collected on Days 17 or 18, 45 or 46, and 88 or 89. Mouse appearance, 16-hour urine volume, specific gravity, glucose and protein concentrations, ALP, and AST activity, and porphyrin concentrations were determined. Animals were necropsied at study termination. The liver, right kidney, right testis, brain, heart, thymus, lungs, and seminal vesicles were weighed.

Hematologic analyses included RBC, total and differential WBC counts, Hgb, Hct, MCV, MCH, MCHC, and blood morphology. NTP (1991d) used Jonckheere's test to evaluate the significance of dose-response trends for organ weight, hematologic, serum chemical, and male reproductive system data. If this test showed significance, then Shirley's test (nonparametric multiple comparison procedure) was used to assess the significance of pairwise comparisons between dosed and control groups. Otherwise, Dunn's test was used for pairwise comparisons.

Two of the 10 female mice in the highest dose group were sacrificed in a moribund condition before the end of the study. Mice of both sexes in the 1,000 and 2,000 ppm dose groups lost weight during Week 1 of the study. Mean body weights of males from all dose groups and females in the highest dose group were notably lower than those of the controls; however, data tables in the NTP (1991d) report did not list which dose groups were statistically significantly different from the control group (see Table B.9).

Mice of both sexes in the two highest dose groups had statistically significantly increased platelet counts. NTP (1991d) also noted statistically significantly lower values for Hgb, MCH, Hct, and MCV at the high-dose groups. Male and female mice in the high-dose group also had significantly higher values for serum ALT activity and serum albumin concentration, with serum SDH activity for males and females increased at the two highest doses. The length of the estrous cycle was significantly increased in females from the highest dose group compared to controls; however, estrous cyclicity was not investigated at other doses. The study authors reported that no treatment-related effects were seen on male reproductive organ weights or on sperm evaluations.

Males exposed to 14.6 mg/kg-day exhibited biologically (>10%) and statistically significant increases in absolute and relative liver weights compared to controls (see Table B.10). While females had a statistically significant increase in absolute liver weight at the lowest dose tested (4.5 mg/kg-day), this increase did not exceed 10% until the 45.2 mg/kg-day dose and there was no dose-response. Compound-related lesions were present in the liver of exposed animals of each sex (see Table B.11). NTP (1991d) reported compound-related clinical signs that included tremors in females and prostration, lethargy, hunched position, and rough hair coats in males and females at the two highest dose. Based on increased relative liver weight in males (>10% compared to controls), a NOAEL of 4.5 mg/kg-day and a LOAEL of 14.6 mg/kg-day are identified.

Dow (1982a)

In a subchronic oral toxicity study, Dow (1982a) administered 1,2,4,5-TCB (>99% pure) to beagle dogs (2/sex/group) in diet at concentrations of 0, 0.0001, 0.001, or 0.01% for 92 days. The study authors calculated the following equivalent average daily doses based on body weight and food consumption: 0.028, 0.29, and 2.63 mg/kg-day for male dogs and 0.027, 0.27, and 2.86 mg/kg-day for females. The available copy of this document is of poor quality and largely illegible. The study authors examined the general appearance and the behavior of the animals at unspecified intervals. Animals were weighed weekly, and food consumption was measured throughout treatment. Hematological parameters (PCV, Hgb, RBC, total and differential WBC) and clinical chemistry parameters (blood urea nitrogen [BUN], ALP, AST, ALT, and bromosulfophthalein retention) were measured before study initiation (baseline) and at Days 8, 15, 23, 57, and 77. At necropsy, the following organs were excised, weighed, and processed for histopathological evaluation: lungs, heart, liver, kidneys, spleen, testes, and brain. In addition, the following organs were examined histopathologically: thyroid, adrenals, lymph node, pancreas, aorta, skeletal muscle, prostate, spinal cord, peripheral nerve, pituitary, thymus, gall bladder, trachea, esophagus, stomach, colon, cecum, small intestine, urinary bladder, uterus, and ovary.

Dow (1982a) observed no dose-related changes in food consumption, clinical signs, body weight, hematologic or clinical chemistry parameters, organ weights, or pathological lesions in either sex. A NOAEL of 2.63 mg/kg-day is identified based on the lack of observed effects in male dogs, with no corresponding LOAEL.

Dow (1982b)

Dow (1982b) also conducted a 144-day dietary feeding study in beagle dogs. The available copy of this report is largely illegible, and any illegible information is noted as such. The study authors administered 1,2,4,5-TCB (99.2% pure) in diet at doses of 0, 5, 10, and

20 mg/kg-day to male and female beagle dogs (2/sex/dose) for 144 days. The control and treatment diets each contained 1.0% peanut oil. The study authors stated that the target concentrations were verified using an unspecified analysis. Male and female dogs were housed two per cage with animals of the same sex and dose group housed together. Food and water was provided ad libitum. Dogs were sacrificed at the conclusion of the study.

Dow (1982b) recorded body weight and food consumption weekly. Hematological parameters (PCV, Hgb, RBC, total and differential WBC) were measured before study initiation (baseline) and on Days 33, 81, and 137. The total concentration of 1,2,4,5-TCB in the blood was measured in the high-dose group only. Urinalysis parameters (specific gravity, pH, sugar, albumin, WBC, RBC, epithelial cells, casts, crystals, bacteria, mucus) were recorded before study initiation and following the 144-day exposure period. Clinical chemistry parameters were measured, including BUN, SGOT, SGPT, AP, and BSP retention. Urinary concentrations of coproporphyrin, uroporphyrin, and creatinine were measured after 116 days of treatment.

Dow (1982b) reported no statistically significant effects on body-weight gain, food consumption, hematology, BUN, AST, and bromsulphalein retention. The study authors reported ALP was elevated (statistical significance not reported) at the high dose and for one dog of each sex at the mid dose. The study authors also reported elevated ALT in one dog in the high-dose group. Liver weight was statistically increased in both male and female dogs at the high-dose group and female dogs at the mid-dose group. An increased number of inflammatory cells in the hepatic sinusoids and slight necrosis of hepatocytes were observed in females at the high-dose group. One female in each of the high- and mid-dose group experienced minimal cytoplasmic swelling of hepatocytes. Data tables of elevated parameters are illegible and are not reported. Although the small number of animals per group makes identification of a NOAEL or LOAEL somewhat uncertain, a 144-day NOAEL of 5 mg/kg-day and a LOAEL of 10 mg/kg-day based on liver effects in female dogs are considered.

Dow (1984b)

In addition to the studies summarized previously in the Subchronic Studies section, Dow (1984b) also performed another subchronic feeding study in rats. Male and female rats (5/sex/dose; strain not specified) were administered diets containing 0, 0.1, 0.3, 1, 3, or 10 ppm of 1,2,4,5-TCB (>99.5% pure) in ground Purina Laboratory Chow for 101 days. The adjusted daily doses are calculated from study data as 0, 0.01, 0.03, 0.08, 0.25, or 0.82 mg/kg-day for males and 0, 0.01, 0.03, 0.10, 0.29, or 0.97 mg/kg-day for females. Animals were weighed twice weekly for the first month and once weekly thereafter. Gross clinical observations for appearance and behavior were made at unspecified intervals. The lungs, heart, liver, spleen, and testes were removed and weighed after necropsy, and portions were removed and prepared for histological examination.

Male rats showed no evidence of treatment-related effects in gross appearance, behavior, growth, food consumption, mortality, final average organ and body weights, or gross examination of the tissues at any dose tested. Upon microscopic examination, male rats exhibited liver central lobular necrosis and renal tubular epithelial degeneration and necrosis at 0.25 mg/kg-day. Beginning at 0.10 mg/kg-day, Dow (1984b) reported centrilobular necrosis and slight generalized swelling of the hepatocytes and kidneys with degeneration and necrosis of the renal tubular epithelium among female rats. The study authors did not report severity and incidence of the liver and kidney lesions, and no further results were reported. Based on the

observed liver central lobular necrosis and renal tubular epithelial degeneration and necrosis in female rats, a LOAEL of 0.10 mg/kg-day is identified, with a corresponding NOAEL of 0.03 mg/kg-day.

Chronic Studies

Fomenko (1965)

In an article published in Russian, the study authors administered 1,2,4,5-TCB by gavage to albino rats at doses of 0, 0.001, 0.005, or 0.05 mg/kg-day for 8 months. Data from the publication were obtained from a secondary source (IPCS, 1991), and the reported treatment-related effects included increased content of sulfhydryl groups in serum, increased hemoglobin and reticulocytes (≥0.005 mg/kg-day), and disorders of the glycogen-forming function of the liver. The study report was also reviewed by IRIS (U.S. EPA, 1991) and judged unsuitable due to insufficient reporting of data. Thus, identification of a NOAEL or LOAEL is precluded.

Braun et al. (1978a,b)

Braun and colleagues (1978a,b) published the same information on the pharmacokinetic evaluation of 1,2,4,5,-TCB in dogs in two different journal articles in 1978. The study authors administered 1,2,4,5-TCB in diet to beagle dogs (2/sex) at a dose of 5 mg/kg-day for 2 years followed by a 20-month observation period. No controls were reported in the study. The study authors reported slightly increased serum ALP and bilirubin concentrations (compared to historical controls) at 24 months. No other clinical data were reported. Due to the lack of concurrent controls, identification of the single 5 mg/kg-day dose as a NOAEL or LOAEL is precluded.

Developmental Studies

Kacew et al. (1984)

Kacew et al. (1984) conducted a peer-reviewed gavage study to examine the developmental effects of 1,2,4,5-TCB. Pregnant female Sprague-Dawley rats (10/dose) were administered 0, 50, 100, or 200 mg/kg-day 1,2,4,5-TCB (99.5% pure, recrystallized from 95% ethanol) in corn oil vehicle via gavage on Gestational Days (GDs) 6−15. Food and water were provided ad libitum. Animals were weighed and sacrificed on GD 21.

Kacew et al. (1984) removed and weighed all fetuses, then reweighed the whole body, liver, brain, kidney, perirenal fat, spleen, and heart of the dams. The study authors measured the following hematological endpoints in dams: Hgb, Hct, RBC, total and differential WBC, MCV, MCHC, and MCH. The study authors also measured the following clinical chemistry endpoints: sodium, potassium, inorganic phosphorus, total bilirubin, ALP, AST, total protein, calcium, cholesterol, glucose, uric acid, and LDH. Aniline hydroxylase (AH) activity was also measured. Residue analysis for 1,2,4,5-TCB was conducted on the maternal kidney, brain, spleen, heart, liver, and perirenal fat as well as one fetus from each treatment group and the brain and liver from another fetus within each treatment group. All fetuses were observed for gross birth defects, but only live fetuses were counted and examined for skeletal and visceral examination. The study authors completed histopathology on the heart, brain, pituitary, eye, thyroid, parathyroid, trachea, bronchi, lung, thymus, stomach, small and large intestine, pancreas, liver, kidney, spleen, adrenal, skeletal muscle, peripheral nerve, skin, bone marrow, ovary, uterus, and

bladder in the dams. The study authors determined statistical significance by one-way ANOVA. Duncan's Multiple Range Test was conducted on all endpoints that showed significant results from the ANOVA test.

Kacew et al. (1984) reported that 9/10 dams in the high-dose (200 mg/kg) group only survived for an average of 6.5 days after the start of exposure. The study authors reported the cause of death to be circulatory collapse, and these animals showed signs of severe alimentary toxemia with uterine vascular hemorrhages. The study authors reported no statistically significant effects on the absolute or relative weight of the brain, heart, kidney, liver, or spleen of the dams from the other dose groups. The hematological parameters tested were deemed to be in the normal range for pregnant rats. No statistically significant changes in leukocyte differential counts were observed, but the study authors noted an increase in leukocyte counts with increasing dose. Serum cholesterol and hepatic supernate were statistically significantly increased in dams of the mid- and high-dose groups. Hepatic alkaline phosphatase (AP) activity was statistically significantly increased in the low-dose group but not in the mid- or high-dose group. The total number of fetuses per dam was significantly decreased in the low-dose group but not in the mid- or high-dose group (see Table B.12). The study authors could not provide an explanation for this effect because tissue residue analysis indicated higher levels of test material in the mid- and high-dose groups compared to the low-dose group. Based on mortality in dams, a maternal frank effect level (FEL) of 200 mg/kg-day is identified. A maternal NOAEL of 50 mg/kg-day and a LOAEL of 100 mg/kg-day are identified based on increased serum cholesterol and hepatic AH activity in rats.

Kitchin and Ebron (1983)

Kitchin and Ebron conducted a peer-reviewed gavage study to examine the maternal reproductive and developmental effects of 1,2,4,5-TCB. 1,2,4,5-TCB (>98% pure) was ground to a fine power via mortar and pestle, suspended in 1.5% gum tragacanth, and administered via gavage in a volume of 2 mL/kg. Timed-pregnant female Sprague-Dawley rats were administered 0; 30; 100; 300; or 1,000 mg/kg-day 1,2,4,5-TCB on GDs 9−13, where GD 1 is established by the day that sperm were detected in the vaginal smear. The number of animals per dose group was not specifically reported but ranged between 6−8 rats per group. Food and water were provided ad libitum. All animals were sacrificed on GD 14.

Kitchin and Ebron (1983) weighed the livers of all dams immediately after sacrifice, and sections were processed for histopathology. In an unreported number of animals, the study authors removed the uterus and examined the fetuses for "growth and differentiation parameters"; however, the study authors did not delineate the exact parameters examined. Fetal abnormalities included differences in the presence of a beating heart, somite number, and fetal size compared to controls. Tabulated data indicate that fetal death, abnormalities, head length, crown–rump length, somites, and protein content were examined. The study authors assayed maternal rat liver for cytochrome P450, NADPH-cytochrome c-reductase, aminopyrine *N*-demethylase, and ethoxyresorufin *O*-deethylase. The study authors used an ANOVA test to determine the statistical significance of treatment compared to control. Additional tests conducted by the study authors included the William's test for dose-related effects, and the Fisher's Exact test for enumerative data.

Kitchin and Ebron (1983) reported no statistically significant treatment-related effects on maternal mortality, absolute or relative liver weight, or hepatic microsomal protein content. Maternal body-weight gain, reported as the body weight on Day 14 minus the body weight at Day 8, was statistically significantly decreased in the high-dose group only. The study authors reported no significant liver histology effects except the incidence of slight centrilobular hypertrophy in the high-dose group. The study authors reported significantly increased cytochrome P450 in the high-dose group. The study authors did not observe any differences in NADPH-cytochrome c-reductase between control and treatment animals but did find significantly increased *O*-deethylation of ethoxyresorufin compared to control at all doses and increased aminopyrine in the two highest dose groups (see Table B.13). The study authors reported that 1,2,4,5-TCB exposure did not significantly alter resorptions, fetal deaths, abnormalities, protein, somite number, crown-to-rump length, or head length compared to control. A statistically significant decrease in implantations in the high-dose group was observed (data for all other dose groups were not reported). Based on significantly decreased body-weight gain, a maternal LOAEL of 1,000 mg/kg-day and a corresponding NOAEL of 300 mg/kg-day are identified. Based on the lack of any observed developmental effects, a developmental NOAEL of 1,000 mg/kg-day (the highest dose tested) is identified.

Fisher et al. (1990a)

In a report available only as an abstract, Fisher et al. (1990) investigated the maternal and developmental effects of 1,2,4,5-TCB administered to rats and rabbits. The results in rats are denoted as Fisher et al. (1990a), while the results in rabbits are denoted as Fisher et al. (1990b). Pregnant F344 rats (25/dose) were administered 1,2,4,5-TCB (purity not reported) by gavage at doses of 0, 25, 75, and 125 mg/kg-day on GDs 6−15, and dams were sacrificed on GD 21. The study authors reported maternal toxicity at the high dose (125 mg/kg-day) including reduced body-weight gain, reduced food consumption, and clinical signs of toxicity (urine stains, audible respiration, and ocular and nasal discharge). In the fetuses, reduced ossification was observed in all treatment groups, but the study authors noted that ossification was minimal at 25 mg/kg-day. A maternal LOAEL of 125 mg/kg-day based on reduced body-weight gain, reduced food consumption, and clinical signs of toxicity and a corresponding NOAEL of 75 mg/kg-day are identified. A developmental LOAEL of 25 mg/kg-day is identified based on slightly reduced ossification with no developmental NOAEL.

Fisher et al. (1990b)

Pregnant New Zealand White rabbits (15/dose) were administered 1,2,4,5-TCB (purity not reported) by gavage at doses of 0, 5, 15, and 25 mg/kg-day on GDs 6−18. Dams were sacrificed on GD 29. The study authors reported maternal toxicity at all doses including mortality at 5 and 25 mg/kg-day, fetal loss at 5 mg/kg-day, and transient body-weight gain reductions at 5 and 15 mg/kg-day. The study authors reported fetotoxicity (increased incidence of one visceral and one skeletal variation in the cranial region) at the two lowest doses (5 and 15 mg/kg-day) but not at the high dose. Based on the limited reported data, 5 mg/kg-day is identified as a FEL for maternal mortality and fetal loss.

Reproductive Studies

Tyl and Neeper-Bradley (1989)

In an unpublished, non-peer-reviewed, two-generation reproductive toxicity study, Tyl and Neeper-Bradley (1989) administered 1,2,4,5-TCB (~97.6% pure) to male and female F0 Sprague-Dawley rats (28/sex/dose) in diet at concentrations of 0; 30; 300; or 1,000 ppm for a

10-week period prior to mating. The control and treatment diets each contained 1% corn oil. Based on food consumption and body-weight data, the study authors calculated corresponding average daily doses of 2.2, 21.1, and 70.3 mg/kg-day for F0 males and 2.6, 25.5, and 82.5 for F0 females. Males and females within each dose group were randomly paired 1:1 for 21 days for mating. A satellite group of 10 females per concentration were also administered control and treatment diet for 10 weeks. Males that did not successfully mate within the first week of pairing were mated with satellite females for 1 week. Parental F0 males were necropsied following the satellite mating period. F0 females were administered the control or 1,2,4,5-TCB diet prior to mating and throughout mating, gestation, parturition, and lactation. F0 mated females were weighed on GDs 0, 7, 13, and 20, and females with litters were weighed on PND 0, 4, 7, 14, and 21. F1 weanlings (28/sex/group) were randomly selected to produce the F2 generation, with intra-litter matings avoided whenever possible. Remaining F1 weanlings were necropsied and examined grossly. The F1 parental animals (28/sex/dose) were fed the 1,2,4,5-TCB diet (0, 30, or 300 ppm) for 11 weeks prior to mating. All F1 weanlings from the 1,000-ppm group died; therefore, no F2 generation was bred for this treatment group. Mating and fertility indices were calculated for F0 and F1 males and females. For F1 and F2 litters, the following indices were calculated: gestational index, live birth index, 4-day survival index, 7-day survival index, 14-day survival index, 21-day survival index, and lactation index.

Tyl and Neeper-Bradley (1989) conducted histopathological analysis of the following tissues from parental F0 and F1 control and high-dose animals: pituitary, liver, kidneys, vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, and other tissues with gross lesions. Kidneys from all F0 and F1 parental males were examined for the presence of hyaline droplets by staining with Mallory-Heidenhain stain. Immunostaining to verify the presence of alpha-2u protein was not done. Satellite females were examined for number of uterine implantation sites but did not undergo further histopathological examination. Statistical analyses were performed with the litter as the unit of comparison. Continuous variables were compared among control and treatment groups using Levene's test for equal variance, ANOVA, and *t*-tests. If variance was homogenous and ANOVA was significant, a pooled *t*-test was used for pairwise comparison. If variance was heterogeneous by Levene's test, all groups were compared by ANOVA for unequal variance followed by a separate variance *t*-test for pairwise comparison when necessary. Bonferroni's correction was used to correct for multiple comparisons. Nonparametric data were evaluated by the Kruskal-Wallis test, followed by the Mann-Whitney U test. Incidence data were compared with Fisher's exact test.

In the F0 generation, body weights in males and females in the high-dose group were significantly reduced compared to controls during most of the 10-week period prior to mating. Food consumption in F0 males and females in the high-dose group was also significantly reduced for the entire period prior to mating. Tyl and Neeper-Bradley (1989) also observed transient reductions in body-weight gain and food consumption at various intervals. No clinical signs of toxicity were observed in F0 males; however, F0 females exhibited hypoactivity, ataxia, emaciation, dehydration, unkempt appearance, urine stains, and labored breathing during the period prior to mating.

During the F0 generation mating period, no treatment-related effects on reproductive parameters (gestational length, percentage of pregnancies, percentage of males-siring litters, mating index, fertility index) were observed. In the low- and high-dose groups, the total number of pups born per litter and number of live pups born per litter were statistically significantly

reduced (see Table B.14). All pups in the high-dose group died by Postnatal Day (PND) 14. Litter sizes in the low-dose group were reduced through PND 4, and litter size in the mid-dose group was reduced on PND 14 and 21. Pup body weights were significantly reduced in the mid-dose group on PNDs 7, 14, and 21 and in the high-dose group on PND 0 and 4. Tyl and Neeper-Bradley (1989) observed no treatment-related findings during gross necropsy of F1 pups not selected as parental or satellite breeding animals for the F2 generation.

Necropsy of the F0 male parental animals revealed increased liver size, increased kidney size, and color change in the pancreatic lymph nodes at the high dose, and diffuse color change in kidneys at the mid and high doses. In F0 parental females in the high-dose group, color change of the jejunum was observed. Treatment-related findings in the liver included eosinophilic cytoplasmic inclusions (males) and vacuolation (females) in the high-dose groups; hepatocellular hypertrophy was also observed in the mid- and high-dose groups of both sexes. In kidneys of F0 males, the study authors observed increased incidences of hyaline droplet nephrosis (severity scored using the Mallory-Heidenhain stain) in all dose groups, and tubular proteinosis, granular cast formation in tubules, interstitial nephritis, and interstitial fibrosis at the mid and high doses. In the kidneys of F0 females, only proteinosis was observed in the high-dose group. Final body weight of F0 males at the high dose was significantly decreased (~15% compared to control) while body weights of females remained unaffected by treatment. Increased absolute organ weights (>10% compared to control values) were observed in male livers and kidneys at the mid and high dose and in female livers at the high dose (see Table B.14). Tyl and Neeper-Bradley (1989) reported that females in the high-dose group experienced increased absolute brain weight; however, values reported in the study data tables indicated a decrease at the high-dose. Increased organ weights relative to body weight were also observed in kidneys of all treated male animals; livers and testes in males at the mid and high dose; brains in males at the high dose; livers in females at the mid and high dose; and kidneys in females at the high dose.

Because all pups in the F1 high-dose group died by PND 14, only two groups were bred to produce the F2 generation. The parents for the F2 generation were treated for up to 11 weeks during a prebreed period followed by the same treatment protocol for the F1 generation. Based on body weight and food consumption data, Tyl and Neeper-Bradley (1989) calculated average daily doses for 0, 30, and 300 ppm as 0, 2.0, and 21.3 for F1 males and 0, 2.5, and 25.4 for F1 females. No treatment-related clinical signs or effects on reproductive parameters were observed for the F1 parental generation. Gestational body weights in F1 parental females were comparable to control values. No reduction in litter size was observed in any F2 litters; however, F2 pup body weights were reduced on PND 7, 14, and 21 in the 300-ppm (25.5-mg/kg-day) group. Perinatal deaths (PND 0−4) and deaths at PND 14 were also increased in this dose group. No treatment-related findings were observed during gross necropsy of F2 pups.

Necropsy of the F1 parental generation demonstrated gross and microscopic changes in the kidney and liver similar to the F0 parental generation. Increased absolute liver weight and liver weight relative to body weight was observed in males at the high dose (21.3 mg/kg-day). Positive staining for alpha-2u protein in kidneys was also observed in males at the high dose (21.3 mg/kg-day). In females, Tyl and Neeper-Bradley (1989) reported reduced brain weights and increased relative liver weight at the high dose (25.4 mg/kg-day).

Based on kidney effects in F1 males, a parental LOAEL of 2.0 mg/kg-day is identified, with no corresponding NOAEL. A reproductive LOAEL of 25.5 mg/kg-day and NOAEL of 2.6 mg/kg-day are identified based on reduced survival of F1 offspring.

NTP (1991e)

NTP (1991e) published a GLP-compliant reproductive toxicity study that exposed male and female CD-1 mice to 1,2,4,5-TCB (≥99% pure) in feed (suspended in corn oil). This study was completed using the tasks described in the Reproductive Assessment by Continuous Breeding Protocol (RACB) as follows: Task 1: a 14-day dose-setting study with 5 doses and a control group (8 animals per sex per group); Task 2: a continuous breeding phase with a control group (40 breeding pairs) and 3 dose groups (20 breeding pairs per dose group); Task 3: a 1-week crossover mating trial using 3 groups of 20 pairs conducted if Task 2 is positive for reproductive effects and after the Task 2 litter was weaned; and Task 4: an offspring assessment (conducted if Task 2 is negative for reproductive effects). Under Task 2, the F0 generation of mice (20 per sex per group) was administered 280; 720; and 1,800 ppm 1,2,4,5-TCB in the diet (mixed in corn oil). A control group consisting of 40 males and 40 females received feed containing 1% corn oil. Adjusted daily doses under Task 2, as reported by the study authors, were 42, 109, and 246 mg/kg-day for the F0 males and 43, 108, and 253 mg/kg-day for the F0 females. The adjusted daily doses under Task 4, as reported by NTP (1991e), were 0 and 108 mg/kg-day for F1 males and 0 and 127 mg/kg-day for F1 females. Food and water were provided ad libitum. F0 animals were exposed to 1,2,4,5-TCB for 1 week prior to mating, 14 weeks of cohabitation, and 3 weeks after cohabitation (18 weeks total). The study authors measured body weight and food consumption weekly. Fertility, litters per breeding pair, number and proportion of live pups per litter, sex ratio of live pups, and mean male and female pup body weights per litter were measured throughout the study. The study authors did not report details on the sacrifice or necropsy of the F0 generation after the delivery of the pups. The study authors reported that 19 of the 20 high-dose F0 females died or were humanely sacrificed (18 animals died during parturition). The study authors feared that the high-dose males would not survive until the scheduled necropsy and sacrificed the males sooner than planned. At Week 11 of the study, an additional 33 male CD-1 mice (140−150 days old) were purchased from Charles River Laboratories (Portage, MI) and served as a substitute necropsy group to match the unscheduled dosing group sacrifice. The original control animals were maintained through the end of the study.

The last litters born from Task 2 were used for Task 4. The animals in Task 4 were dosed at the same doses as the F0 generation after weaning. At approximately 74 days (±10 days), animals were housed 2 per cage. Once the mice reached sexual maturity, 20 animals per sex per group were cohabitated for 7 days. NTP (1991e) checked for a vaginal copulatory plug and all other endpoints listed in Task 2. At necropsy, the following endpoints were measured: organ weights, body weights, epididymal sperm motility, sperm count and morphology, and estrual cyclicity (measured by vaginal lavage for 12 days before sacrifice).

NTP (1991e) used the Cochran-Armitage test on all data presented as a proportion. Under Task 2, Shirley's Test was used to compare the mean values of dose groups and the control. In cases where a trend was detected, Jonckheere's test was used. All other data were analyzed with Dunn's Test. Under Task 4, Wilcoxon's test was used to evaluate each dose group compared to control.

NTP (1991e) reported that 19/20 F0 females in the high-dose group died before Week 12 of the study period. Under Task 2, the study authors reported a significantly decreased number of live pups per litter in the 720-ppm group (see Table B.15). Also in the 720-ppm group, the number of total live pups was significantly decreased in Litters 1, 2, and 4, the number of live male pups and proportion of males was significantly decreased in Litters 1 and 2; and the adjusted weight of total and live and female pups was significantly decreased in Litter 5. In the 280-ppm group, the male–to-female sex ratio was significantly decreased in Litter 1. No other adverse reproductive endpoints were reported. The body weights of F0 females were within 10% of control values. Food consumption was significantly decreased in Weeks 1 and 2 for both males and females in the high-dose group. The study authors reported significantly increased body weight, liver, right cauda, and seminal vesicle weight in the 1,800 ppm group in F0 males (see Table B.16). Relative liver weight was two times the control value in 1,800-ppm males. Abnormal sperm was also significantly higher in males of this high-dose group. During the holding period between Task 2 and Task 4, live pup weight was significantly decreased in males and females at PND 21 in the 720-ppm group.

Historically, the Task 4 protocol includes control and high-dose group animals only, yet due to the high mortality in high-dose females, results considered under Task 4 in NTP (1991e) were modified to include the control and mid-dose group. Individual male and female food consumption and body weight were not significantly decreased. However, combined male and female food consumption was significantly decreased in the mid-dose group compared to control. NTP (1991e) reported no treatment-related effects on reproductive parameters. However, body weight and relative liver and kidney/adrenal weight were significantly increased in males and females in the mid-dose group (see Tables B.17 and B.18). Additionally, relative right testis weight was significantly increased in males in the mid-dose group. The study authors reported no significant clinical signs of toxicity. The study authors reported cystic ovaries in five females, dilation of the uterus in two females, and nodules on the liver in one female in the mid-dose group. The study authors reported the presence of cytomegaly and karyomegaly of hepatocytes in males and females in the mid-dose group, with severity ranging from mild to moderate and slightly more severe effects in females compared to males. The study authors reported no significant lesions in the testis or epididymis of male mice (see Tables B.19 and B.20).

NTP (1991e) concluded that the unexpected mortality in the high dose females under Task 2 of the study may be due to effects of 1,2,4,5-TCB and the stress of parturition. The study authors noted the discrepancy between the decreased live pups under Task 2 and the absence of the same effect under Task 4. Overall, the study authors concluded that 1,2,4,5-TCB caused mild reproductive toxicity along with parental toxicity evidenced by significantly increased relative liver and kidney/adrenal weights in both male and female mice at the mid-dose group. Based on increased organ weights and decreased number of live pups, a LOAEL for parental and reproductive toxicity, respectively, is identified as 108 mg/kg-day in females with a corresponding NOAEL of 43 mg/kg-day.

Carcinogenicity Studies

No oral carcinogenicity studies were identified and none of the available short-term-, subchronic-, or chronic-duration studies reported tumors in the animals tested (see Table 3).

Other Studies
No studies were identified.

Inhalation Exposures
No studies were identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Table 4A and Table 4B summarize genotoxicity studies and other studies conducted with 1,2,4,5-TCB.

Table 4A. Summary of 1,2,4,5-TCB Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with or without S9 activation	0; 10; 33; 100; 333; 1,000; or 1,333 µg/plate	−	−	NA	Haworth et al. (1983)
SOS repair induction	ND					
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	ND					
Recombination induction	ND					
Chromosomal aberration	ND					
Chromosomal malsegregation	ND					
Mitotic arrest	ND					
Genotoxicity studies in mammalian cells—in vitro						
Mutation	ND					
Chromosomal aberrations	Chinese Hamster Ovary cells with or without S9 metabolic activation	Highest dose tested: 150 µg/mL	−	−	Precipitate observed at 150 µg/mL	Loveday et al. (1990)
Sister chromatid exchange (SCE)	Chinese Hamster Ovary cells with or without S9 metabolic activation	Highest dose tested: 76.5 µg/mL	−	−	Precipitate observed at 76 µg/mL	Loveday et al. (1990)
DNA damage	ND					

Table 4A. Summary of 1,2,4,5-TCB Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
DNA adducts	ND					
Genotoxicity studies in mammals—in vivo						
Chromosomal aberrations and DNA damage	5/5, SPF Han (Bor:NMRI) mouse, single i.p. injection, micronuclei of femoral marrow examined 16, 24, or 48 hr following administration	5,000 mg/kg	−	NA	No indications of a clastogenic effect were observed	Bayer (1993)
	8/0, B6C3F$_1$ mouse, single i.p. injection, bone marrow cells harvested at 17 or 36 hr following administration and examined for chromosomal aberrations	NV	−	NA	NA	Shelby and Witt (1995)
Sister chromatid exchange (SCE)	ND					
DNA damage	ND					
DNA adducts	ND					
Mouse biochemical or visible specific locus test	ND					
Dominant lethal	ND					
Genotoxicity studies in subcellular systems						
DNA binding	ND					

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = positive, ± = equivocal or weakly positive, − = negative, NA = not applicable, NV = not available, ND = no data.

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	S-D rat (5 males); single gavage; [^{14}C]-labeled 1,2,4,5-TCB (0.4 µCi/mg)	Radioactivity detected in urine and feces with most detected in urine; 8% of dose excreted in 48 hr and continued at steady rate until 7 d when 21% of dose excreted; identified 2,3,5,6-tetrachlorophenol as main metabolite.	1,2,4,5-TCB is minimally metabolized (21% detected in excreta after 7 d), with most excretion occurring in urine.	Chu et al. (1984b)
Metabolism/ toxicokinetic	Beagle dog (2/sex); diet; 5 mg/kg-d for 2 yr followed by 20-mo recovery phase	Increased serum ALP and bilirubin (compared to historical control data) at end of 2-yr treatment; test material had more affinity to fat than plasma.	Study authors concluded that changes in clinical chemistry were reversible, and compound administration resulted in slight impairment of liver function. The fat–to-plasma ratio of test material increased rapidly after cessation of treatment.	Braun et al. (1978a,b)
Metabolism/ toxicokinetic	Squirrel monkey (4 males); single gavage twice/wk for 3 wk; [^{14}C]-labeled 1,2,4,5-TCB, 50 mg/kg (0.027 µCi/mg)	No metabolites identified and >99% radioactivity detected was unchanged parent compound; main route of excretion through feces where 18% of administered dose excreted at 48 hr postdose; <0.1% excreted in urine at 48 hr.	Study authors concluded that the major route of excretion in monkeys is through feces, and only 18% of the administered dose was detected at 48 hr postdosing.	Schwartz et al. (1987)
Mode of action/ mechanistic	F344 rat (18 males/treated group, 12 males in control group); medium-term liver focus bioassay initiated with single i.p. injection of 200 mg/kg diethylnitrosamine (DEN) followed by daily gavage of 0.1 or 0.4 mmol/kg-d for 6 wk; partial hepatectomy 3 wk after initiation with DEN; measured induction of glutathione-S-transferase	Significant increase in number and area of GST-P positive foci in liver following DEN initiation plus administration of test material by gavage.	1,2,4,5-TCB promoted preneoplastic foci in liver of rats initiated with DEN.	Gustafson et al. (1998)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
	placental form (GST-P) foci			
Mode of action/ mechanistic	F344 rat (18 males/treated group, 12 males in control group); medium-term liver focus bioassay initiated with single i.p. injection of 200 mg/kg diethylnitrosamine (DEN) followed by daily gavage of 0.1 or 0.4 mmol/kg-d for 6 wk; partial hepatectomy 3 wk after initiation with DEN; evaluated liver weights, histopathology, reduced and oxidized glutathione levels (GSH, GSSG), CYP450 induction, and nonfocal GSTP1-1 and *c-fos* induction	Increased final liver weights, hepatocellular centrilobular hypertrophy, karyomegaly, anisocytosis; 50% decrease in GSSG, no change in GSH compared to control; induced CYP1A2, CYP2B1/2, and CYP2E1; no induction of nonfocal GSTP1-1; induction of *c-fos*.	The study provides data on the molecular and cellular changes that inform mechanisms of hepatocarcinogenic potential of 1,2,4,5-TCB following an initiating dose of DEN and partial hepatectomy.	Gustafson et al. (2000)
Mode of action/ mechanistic	Male F344 rats; initiation with DEN (200 mg/kg i.p.); daily gavage beginning 2 wk following initiation of 0.1 mol/kg 1,2,4,5-TCB for 6 wk; partial hepatectomy; evaluated liver weight, normal hepatocyte division rates, and the number and volume of GST-P positive foci at 23, 26, 28, 47, and 56 d after initiation with DEN (200 mg/kg i.p.)	Induction of GST-P foci; no increase in hepatocyte division rates.	The study provides data on the molecular and cellular changes that inform mechanisms of hepatocarcinogenic potential of 1,2,4,5-TCB following an initiating dose of DEN and partial hepatectomy.	Ou et al. (2003)

Metabolism/Toxicokinetic Studies

Metabolism studies in Sprague-Dawley rats, beagle dogs, and squirrel monkeys indicate that 1,2,4,5-TCB is not extensively metabolized (Chu et al., 1984b, Braun et al., 1978a,b; Schwartz et al., 1987), with no more than 21% of the administered dose was subsequently recovered in excreta up to 7 days postdosing in rats. In squirrel monkeys, >99% of the parent compound was excreted, and no metabolites were detected (Schwartz et al., 1987). Chu et al. (1984b) identified 2,3,5,6-tetrachlorophenol as a main metabolite excreted in urine of rats. Further study details are presented in Table 4B.

Mode-of-Action/Mechanistic Studies

Several medium-term bioassays focused on the liver have been conducted with 1,2,4,5-TCB in rats administered an initiating dose of diethylnitrosamine (200 mg/kg i.p.) and evaluated for the presence of GST-P foci in liver after partial hepatectomy. Results indicate that 1,2,4,5-TCB induced GST-P positive foci in rat liver. However, the available data do not provide a definitive conclusion on a mode-of-action for 1,2,4,5-TCB. Study-specific data are provided in Table 4B.

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 present a summary of noncancer and cancer reference values, respectively. IRIS data also are indicated in the table.

Table 5. Summary of Noncancer Reference Values for 1,2,4,5-TCB (CASRN 95-94-3)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M and F	Thyroid histopathology	3×10^{-5}	$LOAEL_{HED}$	0.0098	300	Chu et al. (1983)
Chronic RfD (mg/kg-d) IRIS (U.S. EPA, 1991)	Rat/M	Kidney lesions	3×10^{-4}	NOAEL	0.34 (Note: IRIS did not apply DAF methodology)	1,000	Chu et al. (1984a)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

NDr = not determined.

Table 6. Summary of Cancer Values for 1,2,4,5-TCB (CASRN 95-94-3)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	NDr			
p-IUR	NDr			

NDr = not determined.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

The toxicity database for effects induced by 1,2,4,5-TCB following oral exposure is robust and includes effects observed in rats, mice, dogs, and rabbits (tabulated in Table 7 and provided graphically in Figure 2). Potential targets of 1,2,4,5-TCB oral toxicity include the thyroid, liver, and kidney. Body weight depression, mortality, and developmental and reproductive effects were also observed.

Table 7. Response Array Information

Citation	Species	Study Type	Sex	Critical Effect
Dow (1982a)	Dog	Subchronic	Male and Female	No observed effects
Fisher et al. (1990a)	Rat	Developmental	Male and Female	Delayed ossification
Chu et al. (1984a) (IRIS principal study)	Rat	Subchronic	Male	Kidney pathology
Dow (1984a)	Rat	Subchronic (30 d)	Male	Kidney pathology
Dow (1984b)	Rat	Subchronic	Female	Kidney pathology
Tyl and Neeper-Bradley (1989)	Rat	Reproductive	Male	Kidney pathology
Dow (1982b)	Dog	Subchronic	Female	Liver pathology
NTP (1991d)	Mouse	Subchronic	Male	Increased liver weight
Kacew et al. (1984)	Rat	Developmental	Female (dams)	Increased serum cholesterol, hepatic aniline hydroxylase (AH)
Chu et al. (1983)	Rat	Subchronic	Male	Liver pathology
Kitchin and Ebron (1983)	Rat	Reproductive	Female (dams)	Decreased body weight
NTP (1991e)	Mouse	Reproductive	Female	Reduced live pups
Chu et al. (1983) (principal study for subchronic p-RfD)	**Rat**	**Subchronic**	**Male**	**Thyroid pathology**
NTP (1991c)	Rat	Subchronic	Female	Decreased free serum thyroxin

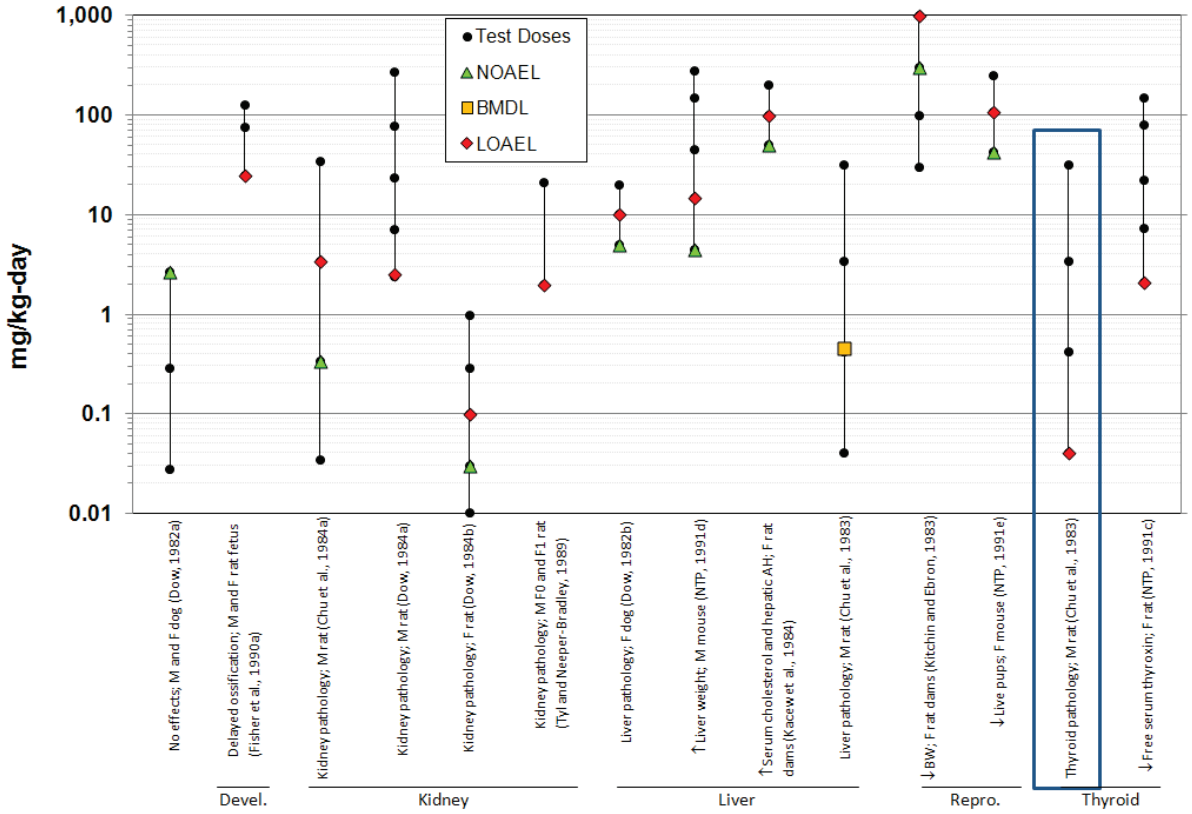

Figure 2. 1,2,4,5-TCB Exposure-Response Array

Several oral studies are candidates for derivation of the subchronic p-RfD. **The Chu et al. (1983) study is selected as the principal study for the derivation of the subchronic p-RfD**. The most sensitive effect following 1,2,4,5-TCB exposure evident in the database was thyroid toxicity in male rats (Chu et al. 1983), with a LOAEL at the lowest tested dose of 0.041 mg/kg-day, and it is selected as the critical effect. This observed thyroid toxicity consisted of histopathological changes noted as moderate-to-severe at the high dose, and increased epithelial height, angular collapse of thyroid follicles, and reduction in colloid density at all doses. Female rats also exhibited thyroid histopathology at 0.59 mg/kg-day. The NTP (1991c) study observed a statistically significant decrease in free serum thyroxin in female rats at the lowest dose tested (2.1 mg/kg-day) supporting that thyroid toxicity could be occurring at lower doses in that study (see Table B.8). Although other studies at higher doses and longer durations (chronic) did not report information on thyroid histopathology or biochemistry, there was no information to the contrary.

Kidney toxicity was also observed in several studies at doses as low as 0.1 mg/kg-day (DOW, 1984a). Kidney histopathology was also observed in male rats in the principal study (Chu et al., 1983) at doses ≥3.4 mg/kg-day. The study authors suggested that the kidney histopathology could be related to alpha-2u nephropathy, but immunostaining to verify the presence of alpha-2u protein was not done. A very thorough reproductive study (Tyl and Neeper-Bradley, 1989) identified kidney effects in rats but at a dose (2 mg/kg-day) considerably higher than the observed thyroid effects. The IRIS chronic RfD was based on kidney lesions at

0.34 mg/kg-day in the male rat (Chu et al., 1984a). However, all the kidney effects were observed at doses greater than the selected critical effect (thyroid histopathology). Additionally, Chu et al. (1983) qualitatively observed lung lesions occurring at 0.041 mg/kg-day in male rats but did not elaborate on this finding. No other studies in the database reported lung lesions, so this effect was not considered significant in context.

The dose-response data on thyroid histopathology for male rats in the Chu et al. (1983) study could not be successfully modeled by BMDS, thus, a NOAEL/LOAEL approach was employed to identify a potential point of departure (POD). Histopathological changes in the liver (males) observed in the Chu et al. (1983) study were also modeled with BMDS (Appendix C) but provided a BMDL (0.456 mg/kg-day) over 10-fold higher than the LOAEL for thyroid effects. Therefore, the LOAEL of 0.041 mg/kg-day based on thyroid histopathology in male rats (Chu et al., 1983) is chosen as the POD to derive a subchronic p-RfD for 1,2,4,5-TCB.

EPA endorses body-weight scaling to the ¾ power ($BW^{3/4}$) to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD under certain exposure conditions. The use of $BW^{3/4}$ scaling for deriving an RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite but not for portal-of-entry or developmental endpoints.

Following U.S. EPA (2011b) guidance, the POD for thyroid histopathology in adult rats is converted to an HED through application of a DAF as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

Where:

DAF = dosimetric adjustment factor
BW_a = animals body weight
BW_h = human body weight

Using a BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans (U.S. EPA, 1988) identified for the critical effect yields a POD_{HED} as follows:

$$POD_{HED} = \text{POD (mg/kg-day)} \times DAF$$
$$= 0.041 \text{ mg/kg-day} \times 0.24$$
$$= 0.0098 \text{ mg/kg-day}$$

$$\textbf{Subchronic p-RfD} = LOAEL_{HED} \div UF_C$$
$$= 0.0098 \text{ mg/kg-day} \div 300$$
$$= \mathbf{3 \times 10^{-5} \text{ mg/kg-day}}$$

The composite uncertainty factor (UF_C) of 300 is described in Table 8.

Table 8. Uncertainty Factors for Subchronic p-RfD of 1,2,4,5-TCB

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) has been applied to account for uncertainty in characterizing the toxicodynamic differences between rats and humans following oral 1,2,4,5-TCB exposure. The toxicokinetic uncertainty has been accounted for by calculation of a human equivalent dose (HED) through application of a dosimetric adjustment factor (DAF) as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	1	A UF_D of 1 has been applied because the database includes one acceptable two-generation reproductive toxicity study rats (Tyl and Neeper-Bradley, 1989) and two acceptable developmental toxicity studies in rats (Kitchin and Ebron, 1983; Kacew et al., 1984) via the oral route.
UF_H	10	A UF_H of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 1,2,4,5-TCB in humans.
UF_L	10	A UF_L of 10 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL.
UF_S	1	A UF_S of 1 has been applied because a subchronic-duration study was selected as the principal study.
UF_C	300	Composite uncertainty factor.

NA = not applicable.

The confidence of the subchronic p-RfD for 1,2,4,5-TCB is low as explained in Table 9 below.

Table 9. Confidence Descriptors for Subchronic p-RfD for 1,2,4,5-TCB

Confidence Categories	Designation[a]	Discussion
Confidence in principal study	M	Although compliance with GLP standards was not stated by study authors, the study is presented in a peer-reviewed journal and otherwise meets the standards of study design and performance, with numbers of animals, examination of potential toxicity endpoints and presentation of information.
Confidence in database	M	The database includes acceptable reproductive and developmental toxicity studies and chronic-duration studies. However, the chronic-duration studies in rodents and dogs are not comprehensive and of high quality. The database also encompasses other studies which did not report thyroid effects, indicating some inconsistency in the overall toxicity profile.
Confidence in subchronic p-RfD[b]	M	The overall confidence in the subchronic p-RfD is medium

[a]L = low, M = medium, H = high.
[b]The overall confidence cannot be greater than lowest entry in table.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

A chronic RfD of 3×10^{-4} mg/kg-day is available on IRIS based on the subchronic, 90-day feeding study by Chu et al. (1984a) that identified an increased incidence of kidney lesions in male rats. At the time of the IRIS assessment, a DAF was not applied to the POD used for derivation of the RfD. Additionally, subsequent to the IRIS file, additional studies have become available, and these studies have been summarized herein. The IRIS database should be checked to determine when changes are made. No chronic p-RfD is developed in this document.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

No adequate inhalation studies in humans or animals are identified for the derivation of a provisional reference concentration. Therefore, derivation of a subchronic p-RfC is precluded.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

No adequate inhalation studies in humans or animals are identified for the derivation of a provisional reference concentration. Therefore, derivation of a chronic p-RfC is precluded.

CANCER WEIGHT-OF-EVIDENCE (WOE) DESCRIPTOR

Table 10 identifies the cancer WOE descriptor for 1,2,4,5-TCB.

Table 10. Cancer WOE Descriptor for 1,2,4,5-TCB

Possible WOE Descriptor	Designation	Route of entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	NA
"Likely to Be Carcinogenic to Humans"	NS	NA	NA
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	NA
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**No studies on the carcinogenic potential of 1,2,4,5-TCB in animals or humans via the oral or inhalation route are available in the literature.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	NA

NS = not selected, NA = not applicable.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

There are insufficient data to assess the carcinogenic potential of 1,2,4,5-TCB via the oral route; therefore, derivation of a p-OSF is precluded.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

There are insufficient data to assess the carcinogenic potential of 1,2,4,5-TCB via the inhalation route; therefore, derivation of a p-IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

No provisional screening values are provided for 1,2,4,5-TCB.

APPENDIX B. DATA TABLES

Table B.1. Chromosome Aberrations in Peripheral Blood Lymphocytes of Workers Exposed to 1,2,4,5-TCB[a]

Groups	Normal Control		Factory Employees Control		1,2,4,5-TCB	
	No.	%	No.	%	No.	%
Frequency of Chromatid-Type Chromosome Aberrations						
Mitoses examined	2,523		838		1,360	
Gap	73	2.89	46	5.48	81	5.95
Isogap	19	0.75	2	0.23	30	2.20
Break	40	1.59	26	3.10	55	4.04
Isobreak	17	0.67	18	2.14	32	2.35
Exchange	-	-	-	-	2	0.15
Total	149	5.90	92	10.97	198	14.70
Frequency of Labile Chromosome-Type Aberrations						
Mitoses examined	2,523		838		1,360	
Acentric fragment	9	0.35	8	0.95	19	1.40
Ring chromosome	-	-	-	-	2	0.15
Dicentric chromosome	-	-	-	-	2	0.15
Total	9	0.35	8	0.95	23	1.69
Frequency of Stable Chromosome-Type Aberrations						
Karyotypes examined	460		144		237	
Deletion	19	4.13	10	6.94	27	11.39
Inversion	4	0.87	1	0.69	4	1.68
Translocation	3	0.65	2	1.38	5	2.10
Total	26	5.65	13	9.02	36	15.18

[a]Kiraly et al. (1979).

Table B.2. Relative Liver Weight and Serum Cholesterol of Sprague-Dawley Rats Administered 1,2,4,5-TCB in Diet for 28 Days[a,b]

Male	**Control**	**500 ppm (32 mg/kg-d)**
Relative liver weight (% of body weight)	4.5 ± 0.54	5.9 ± 0.42* (131)
Serum cholesterol (mg/100 mL)	86 ± 9.5	112 ± 9.8* (130)
Female	**Control**	**500 ppm (56 mg/kg-d)**
Relative liver weight (% of body weight)	4.4 ± 0.30	5.3 ± 0.51* (120)
Serum cholesterol (mg/100 mL)	86 ± 13	110 ± 7* (128)

[a]Chu et al. (1983).
[b]Values represent mean ± SD (% of control); % of control is calculated; $n = 9-10$ per group.
*Significantly different from control, $p < 0.05$. Study authors reported liver weights and serum cholesterol only for the control and highest dose group (500 ppm) and no intermediate dose groups.

Table B.3. Hepatic Mixed-Function Oxidase Activities of Rats Administered 1,2,4,5-TCB in Diet for 28 Days[a,b]

Parameter[c]	**Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)**				
Male	**0.0 (Control)**	**0.5 (0.041)**	**5.0 (0.42)**	**50 (3.4)**	**500 (32)**
AH (nmol PAP/h × mg protein)	12 ± 5.1	15 ± 1.9 (125)	14 ± 3.6 (117)	19 ± 4.0 (158)	33 ± 6.5* (275)
ER (nmol/min × mg protein)	0.02 ± 0.02	0.10 ± 0.07 (500)	0.14 ± 0.16 (700)	0.22 ± 0.27 (1,100)	0.23 ± 0.25* (1,150)
APDM (nmol HCHO/h × mg protein)	20 ± 7.1	26 ± 6.8 (130)	26 ± 6.9 (130)	35 ± 7.6* (175)	64 ± 18* (320)
Females	**0.0 (Control)**	**0.5 (0.059)**	**5.0 (0.61)**	**50 (6.2)**	**500 (56)**
AH (nmol PAP/h × mg protein)	20 ± 6.9	22 ± 7.9 (110)	22 ± 7.6 (110)	23 ± 7.2 (115)	34 ± 1.5 (170)
EROD (nmol/min × mg protein)	0.09 ± 0.08	0.12 ± 0.06 (133)	0.13 ± 0.08 (144)	0.14 ± 0.06 (156)	0.18 ± 0.04 (200)
APDM (nmol HCHO/h × mg protein)	16 ± 3.0	16 ± 4.2 (100)	16 ± 2.0 (100)	17 ± 2.7 (106)	36 ± 8.8* (225)

[a]Chu et al. (1983).
[b]Values represent mean ± SD (% of control); % of control is calculated; $n = 8-10$ per group.
[c]AH = aniline hydroxylase; APDM = aminopyrine demethylase; ERD = ethoxyresorufin *O*-deethylase
*Significantly different from control ($p < 0.05$).

Table B.4. Incidence of Selected Histopathological Lesions in Sprague-Dawley Rats Administered 1,2,4,5-TCB in Diet for 28 Days[a,b]

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)				
Male	**0.0 (Control)**	**0.5 (0.041)**	**5.0 (0.42)**	**50 (3.4)**	**500 (32)**
Liver	0/10	2/10	0/10	4/10	10/10
Thyroid	0/10	5/10	3/10	4/10	9/10
Kidney	1/10	0/10	0/10	5/10	8/10
Lung	0/10	4/10	5/10	2/10	6/10
Female	**0.0 (Control)**	**0.5 (0.059)**	**5.0 (0.61)**	**50 (6.2)**	**500 (56)**
Liver	0/10	1/10	1/10	3/10	10/10
Thyroid	0/10	2/10	3/10	4/10	6/10
Kidney	4/10	1/10	2/10	3/10	3/10
Lung	3/10	NE	NE	NE	2/10

[a]Chu et al. (1983).
[b]Values expressed as animals showing lesions/animals examined.
NE = not examined.

Table B.5. Average Body Weights and Relative Organ Weights of Male and Female Rats Administered 1,2,4,5-TCB (Recrystallized) in Diet for 90 Days[a]

	Exposure Group, % in Diet (Average Daily Dose, mg/kg-d)		
Males	**0 (0)**	**30 (2.7)**	**100 (9.0)**
Number of animals	10	9	9
Average body weight (g)	325	325 (100)	329 (101)
Average relative organ weights (g/100 g body weight)			
Lung	0.63	0.61 (97)	0.55 (87)
Heart	0.33	0.32 (97)	0.32 (97)
Liver	2.76	2.70 (98)	2.84 (103)
Kidney	0.75	0.76 (101)	0.80 (107)
Spleen	0.30	0.29 (97)	0.26 (87)
Testes	0.91	0.91 (100)	0.93 (102)
Females	**0 (0)**	**30 (3.0)**	**100 (10.1)**
Number of animals	10	9	10
Average body weight (g)	193	201 (104)	199 (103)
Average relative organ weights (g/100 g body weight)			
Lung	0.71	0.68 (96)	0.69 (97)
Heart	0.38	0.37 (97)	0.36 (95)
Liver	2.88	2.93 (102)	3.11* (108)
Kidney	0.78	0.80 (103)	0.80 (103)
Spleen	0.35	0.33 (94)	0.34 (97)

[a]Dow Chemical Co. (1984b).
[b]Study authors provided only mean values and no variance data; (% of control in parentheses is calculated).
*Significantly different from control ($p < 0.01$).

Table B.6. Survival, Mean Body Weights, and Food Consumption of Rats Administered 1,2,4,5-TCB in Diet for 13 Weeks[a]

	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)					
Male	**0 (0.0)**	**30 (2.1)**	**100 (7.1)**	**300 (22.1)**	**1000 (71.4)**	**2000 (156)**
Survival[b]	10/10	10/10	10/10	10/10	10/10	10/10
Mean body weight (g)[c]						
Initial	116 ± 5	122 ± 5	117± 5	119 ± 4	121 ± 4	119 ± 4
Final	334 ± 10	331 ± 4	336 ± 4	316 ± 6	299 ± 6	265 ± 4
Change	218 ± 7	210 ± 7	219 ± 4	197 ± 7	178 ± 3	146 ± 3
Final weight relative to controls (%)	-	99	101	95	90	79
Food consumption (g/animal/d)	16	16	16	16	15	15
Female	**0 (0.0)**	**30 (2.1)**	**100 (7.3)**	**300 (22.4)**	**1000 (79.1)**	**2000 (151)**
Survival[b]	10/10	10/10	10/10	10/10	10/10	10/10
Mean body weight (g)						
Initial	101 ± 3	99 ± 3	100 ± 3	98 ± 3	95 ± 3	97 ± 3
Final	200 ± 3	193 ± 4	203 ± 3	197 ± 3	183 ± 3	168 ± 3
Change	100 ± 4	93 ± 2	103 ± 2	99 ± 2	88 ± 3	71 ± 2
Final weight relative to controls (%)	-	97	102	99	92	84
Food consumption (g/animal/d)	22	20	11	11	11	10

[a]NTP (1991c).
[b]Expressed as number of animals surviving/number of animals in group.
[c]Values represent mean ± SE.

Table B.7. Liver and Kidney Weights of F344 Rats Administered 1,2,4,5-TCB in Diet for 13 Weeks[a,b]

	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)					
Male	**0 (0.0)**	**30 (2.1)**	**100 (7.1)**	**300 (22.1)**	**1,000 (71.4)**	**2,000 (156)**
Body weight (g)	347 ± 8.3	340 ± 4.2 (98)	296 ± 16.2* (85)	333 ± 5.8 (96)	299 ± 6.5** (86)	283 ± 4.9** (82)
Right kidney						
Absolute	1,312 ± 39	1,268 ± 18 (97)	1,263 ± 27 (96)	1,608 ± 39** (123)	1,970± 68** (150)	1,849 ± 74** (141)
Relative	3.8 ± 0.07	3.7 ± 0.04 (97)	4.3 ± 0.15** (113)	4.8 ± 0.07** (126)	6.6 ± 0.15** (174)	6.5 ± 0.16** (171)
Liver						
Absolute	12,660 ± 380	12,670 ± 180 (100)	10,590 ± 1,060 (84)	14,270 ± 370* (113)	17,230 ± 530** (136)	19,170 ± 520** (151)
Relative	36.4 ± 0.52	37.2 ± 0.27 (102)	35.0 ± 1.75 (96)	42.9 ± 0.68** (118)	57.6 ± 1.20** (158)	67.6 ± 1.17** (186)
Female	**0 (0.0)**	**30 (2.1)**	**100 (7.3)**	**300 (22.4)**	**1,000 (79.1)**	**2,000 (151)**
Body weight (g)	201 ± 2.9	191 ± 4.4 (95)	207 ± 2.9 (103)	199 ± 3.3 (99)	188 ± 3.0* (94)	173 ± 2.7** (86)
Right kidney						
Absolute	776 ± 18	734 ± 20 (95)	821 ± 16 (106)	844 ± 15* (109)	838 ± 24* (108)	871 ± 25* (112)
Relative	3.9 ± 0.07	3.8 ± 0.06 (97)	4.0 ± 0.08 (103)	4.2 ± 0.06** (108)	4.5 ± 0.08 ** (115)	5.0 ± 0.12** (128)
Liver						
Absolute	6,445 ± 137	6,610 ± 239 (103)	7,304 ± 151** (113)	7,515 ± 164** (117)	9,512 ± 215** (148)	11,908 ± 312 (185)
Relative	32.1 ± 0.43	34.5 ± 0.84* (107)	35.3 ± 0.63** (110)	37.7 ± 0.50** (117)	50.6 ± 0.83** (158)	68.9 ± 1.55** (215)

[a]NTP (1991c).
[b]Values represent mean ± SE (absolute organ weight in mg, relative in mg/g) for groups of 10 animals (% of control); % of control is calculated.
*$p < 0.05$, Dunn's test or Shirley's test.
**$p < 0.01$, Dunn's test or Shirley's test.

Table B.8. Serum Thyroid Hormone Concentrations in F344 Rats Administered 1,2,4,5-TCB in Diet for 13 Weeks[a]

Endpoint	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)					
Male	**0 (0.0)**	**30 (2.1)**	**100 (7.1)**	**300 (22.1)**	**1,000 (71.4)**	**2,000 (156)**
Free thyroxin (ng/dL)						
Day 3/4	2.1 ± 0.11	2.0 ± 0.16 (95)	1.9 ± 0.09 (90)	1.3 ± 0.15[c,]** (62)	0.4 ± 0.04** (19)	0.3 ± 0.02** (14)
Day 45/46	2.0 ± 0.19	1.8 ± 0.15 (90)	2.2 ± 0.20 (110)	1.3 ± 0.13** (65)	0.6 ± 0.07** (30)	0.2 ± 0.02** (10)
Day 88/89	1.7 ± 0.13	1.5 ± 0.11 (88)	1.5 ± 0.14 (88)	1.0 ± 0.12** (59)	0.5 ± 0.04** (29)	0.2 ± 0.03** (12)
Total thyroxin (µg/dL)						
Day 17/18	5.1 ± 0.42	5.1 ± 0.49 (100)	4.9 ± 0.38 (96)	3.0 ± 0.16** (59)	1.6 ± 0.13** (31)	1.2 ± 0.11** (24)
Day 45/46	5.1 ± 0.29	4.6 ± 0.33 (90)	5.1 ± 0.25 (100)	3.4 ± 0.19** (67)	2.0 ± 0.15** (39)	1.3 ± 0.08** (25)
Day 88/89	4.2 ± 0.16	4.1 ± 0.39 (98)	4.1 ± 0.37 (98)	3.3 ± 0.32 (79)	1.9 ± 0.14** (45)	1.3 ± 0.05** (31)
Female	**0 (0.0)**	**30 (2.1)**	**100 (7.3)**	**300 (22.4)**	**1,000 (79.1)**	**2,000 (151)**
Free thyroxin (ng/dL)						
Day 3/4	2.0 ± 0.10	1.7 ± 0.09* (85)	1.6 ± 0.09* (80)	1.1 ± 0.08** (55)	0.4 ± 0.03** (20)	0.2 ± 0.02** (10)
Day 45/46	1.6 ± 0.14	0.9 ± 0.10** (56)	1.3 ± 0.18* (81)	1.0 ± 0.07** (63)	0.4 ± 0.04** (25)	0.2 ± 0.03[c,]** (13)
Day 88/89	1.0 ± 0.08	1.0 ± 0.11 (100)	0.8 ± 0.09 (80)	0.6 ± 0.10** (60)	0.3 ± 0.03** (30)	0.2 ± 0.03** (20)
Total thyroxin (µg/dL)						
Day 17/18	4.7 ± 0.32	3.8 ± 0.25 (81)	3.8 ± 0.31 (81)	2.5 ± 0.22** (53)	1.4 ± 0.08** (30)	1.2 ± 0.08** (26)
Day 45/46	4.6 ± 0.24	2.8 ± 0.21** (61)	3.8 ± 0.32** (83)	3.2 ± 0.17** (70)	1.6 ± 0.06** (35)	1.2 ± 0.06** (26)
Day 88/89	2.8 ± 0.21	2.6 ± 0.24 (93)	2.6 ± 0.11 (93)	1.8 ± 0.19** (64)	1.4 ± 0.06** (50)	1.2 ± 0.08** (43)

[a]NTP (1991c).
[b]Data for animals bled sequentially on Days 3 or 4, 17 or 18, 45 or 46, and 88 or 89; values represent mean ± SE for groups of 10 animals unless otherwise specified (% of control); % of control is calculated.
[c]Nine animals were examined.
*$p < 0.05$, Dunn's test or Shirley's test.
**$p < 0.01$, Dunn's test or Shirley's test.

IU = international units.

Table B.9. Survival, Mean Body Weights, and Feed Consumption of B6C3F$_1$ Mice Administered 1,2,4,5-TCB in Diet for 13 Weeks[a]

	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)					
Male	**0 (0.0)**	**30 (4.5)**	**100 (14.6)**	**300 (45.2)**	**1,000 (150)**	**2,000 (278)**
Survival[b]	10/10	10/10	10/10	10/10	10/10	10/10
Mean body weight (g)[c]						
Initial	21.4 ± 0.5	20.8 ± 0.7	21.5 ± 0.4	20.7 ± 0.3	20.5 ± 0.4	20.8 ± 0.6
Final	32.1 ± 0.7	29.7 ± 0.7	31.9 ± 0.8	29.7 ± 0.7	30.0 ± 0.7	29.6 ± 0.8
Change	10.7 ± 0.6	8.8 ± 0.2	10.3 ± 0.6	9.0 ± 0.4	9.5 ± 0.4	8.8 ± 0.6
Final weight relative to controls (%)	-	92.5	99.4	92.5	93.5	92.2
Food consumption (g/animal/d)	3.9	3.8	3.9	3.8	3.8	3.5
Female	**0 (0.0)**	**30 (6.0)**	**100 (19.7)**	**300 (56.6)**	**1,000 (143)**	**2,000 (302)**
Survival[b]	10/10	10/10	10/10	10/10	10/10	9/10[d]
Mean body weight (g)[c]						
Initial	16.8 ± 0.3	16.7 ± 0.4	17.2 ± 0.4	16.9 ± 0.5	16.8 ± 0.3	16.5 ± 0.3
Final	25.4 ± 0.5	25.1 ± 0.5	25.5 ± 0.7	25.5 ± 0.7	25.3 ± 0.6	24.6 ± 0.5
Change	8.6 ± 0.3	8.4 ± 0.3	8.3 ± 0.4	8.6 ± 0.5	8.5 ± 0.4	8.3 ± 0.5
Final weight relative to controls (%)	-	98.8	100.4	100.4	99.6	96.9
Food consumption (g/animal/d)	4.1	4.2	4.2	4.0	3.0	3.1

[a]NTP (1991d).
[b]Expressed as number of animals surviving/number of animals in dose group.
[c]Values represent mean ± SE.
[d]Week of death: 2; an additional animal died during Week 13, after the end of dosing.

Table B.10. Liver and Kidney Weights of B6C3F$_1$ Mice Administered 1,2,4,5-TCB in Diet for 13 Weeks[a]

	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)					
Male	**0 (0.0)**	**30 (4.5)**	**100 (14.6)**	**300 (45.2)**	**1,000 (150)**	**2,000 (278)**
Number of animals	10	10	10	10	10	10
Body weight (g)	32.6 ± 0.96	30.8 ± 0.59 (94)	33.1 ± 0.71 (102)	31.2 ± 0.66 (96)	31.9 ± 0.76 (98)	31.3 ± 0.74 (96)
Liver						
Absolute	1,373 ± 58	1,367 ± 44 (100)	1,546 ± 42* (113)	1,458 ± 60 (106)	2,022 ± 67** (147)	3,700 ± 80** (269)
Relative	42.1 ± 1.31	44.4 ± 1.03 (105)	46.8 ± 1.19* (111)	46.6 ± 1.04* (111)	63.3 ± 1.35** (150)	118.4 ± 2.86** (281)
Female	**0 (0.0)**	**30 (6.0)**	**100 (19.7)**	**300 (56.6)**	**1,000 (143)**	**2,000 (302)**
Number of animals	10	10	10	10	10	10
Body weight (g)	25.6 ± 0.53	26.4 ± 0.55 (103)	26.3 ± 0.78 (103)	26.7 ± 0.67 (104)	27.7 ± 0.69* (108)	26.5 ± 0.29 (104)
Right kidney						
Absolute	190 ± 4	199 ± 3 (105)	206 ± 7* (108)	196 ± 7 (103)	208 ± 5* (109)	214 ± 9* (113)
Relative	7.4 ± 0.16	7.5 ± 0.12 (101)	7.8 ± 0.17 (105)	7.4 ± 0.21 (100)	7.5 ± 0.13 (101)	8.1 ± 0.31 (109)
Liver						
Absolute	1,183 ± 38	1,306 ± 36* (110)	1,273 ± 52 (108)	1,312 ± 63 (111)	2,086 ± 73** (176)	4,171 ± 234** (353)
Relative	46.3 ± 1.41	49.4 ± 0.81 (107)	48.4 ± 1.43 (105)	49.1 ± 1.58 (106)	75.4 ± 1.64** (163)	156.9 ± 7.56** (339)

[a]NTP (1991d).
[b]Values represent mean ± SE (absolute weights in mg, relative weights in mg/g body weight) for groups of 10 animals (% of control); % of control is calculated.
*$p < 0.05$, Dunn's or Shirley's test.
**$p < 0.01$, Dunn's or Shirley's test.

Table B.11. Incidence of Selected Histopathological Lesions in B6C3F$_1$ Mice Administered 1,2,4,5-TCB in Diet for 13 Weeks[a,b]

Male	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)					
	0 (0.0)	**30 (4.5)**	**100 (14.6)**	**300 (45.2)**	**1,000 (150)**	**2,000 (278)**
Liver						
Necrosis	0	0	0	0	0	4 (1.7)
Centrilobular hypertrophy	0	0	0	0	7 (1.0)	10 (1.4)
Degeneration	0	0	0	0	0	9 (1.4)
Heart						
Mineralization	0	0	0	2 (1.5)	0	3 (2.3)
Female	**0 (0.0)**	**30 (6.0)**	**100 (19.7)**	**300 (56.6)**	**1,000 (143)**	**2,000 (302)**
Liver						
Necrosis	0	1 (1.0)	0	0	1 (1.0)	1 (1.0)
Centrilobular hypertrophy	0	0	0	0	7 (1.0)	9 (1.8)
Degeneration	0	0	0	0	0	5 (1.2)

[a]NTP (1991d).
[b]Ten animals were examined in each group. Number in parentheses denotes mean grade of severity: 1 = minimal; 2 = mild; 3 = moderate.

Table B.12. Effects of 1,2,4,5-TCB Exposure to Pregnant Sprague-Dawley Rats from GDs 6-15[a]

Parameters[b]	Exposure Group (mg/kg-d)			
	0	**50**	**100**	**200**
Platelets ($\times 10^6$/mm^3)	13.5 ± 1.6 (*n* = 8)	10.8 ± 1.1 (*n* = 8) (80)	11.7 ± 2.1 (*n* = 7) (87)	10.8 (*n* = 1) (80)
Serum cholesterol (mg/100 mL)	82 ± 9 (*n* = 3)	105 ± 19 (*n* = 7) (128)*	103 ± 20 (*n* = 7) (126)*	ND
Liver AH (µmol/hr/mg protein)	11.9 ± 3.8 (*n* = 3)	19.9 ± 6.4 (*n* = 7) (167)*	19.9 ± 6.0 (*n* = 7) (167)*	32.6 (*n* = 1) (274)
Liver AP (µmol/hr/mg protein)	16.2 ± 2.9 (*n* = 3)	26.5 ± 7.7(164)* (*n* = 7)	24.7 ± 9.3 (*n* = 7) (152)	47.3 (*n* = 1) (292)
Number of fetuses per dam	14.0 ± 4.2 (*n* = 8)	8.3 ± 5.5 (59)* (*n* = 8)	13.5 ± 2.1 (96) (*n* = 7)	12 (*n* = 1)

[a]Kacew et al. (1984).
[b]Values represent mean ± SD (% of control); % of control is calculated.
*Statistically significant at $p \leq 0.05$; Duncan's Multiple Range Test.
ND = not determined. AH = aniline hydroxylase, AP = alkaline phosphatase.

Table B.13. Effects of Exposure to 1,2,4,5-TCB on Female Sprague-Dawley Rats during Gestation Days 9−14[a]

Parameter[b,c]	Exposure Group (mg/kg-d)				
	0	**30**	**100**	**300**	**1,000**
Body-weight gain[d]	34.33 ± 2.50	39.46 ± 4.76 (115)	26.65 ± 4.03 (78)	29.08 ± 5.40 (85)	15.15 ± 4.11 (44)
Liver weight	11.22 ± 0.44	11.77 ± 0.46 (105)	11.57 ± 0.30 (103)	11.68 ± 0.25 (104)	10.39 ± 0.46 (93)
Cytochrome P450	0.52 ± 0.03	0.52 ± 0.05 (100)	0.52 ± 0.02 (100)	0.57 ± 0.05 (110)	0.85 ± 0.19 (163)
NADPH cytochrome c reductase	127.38 ± 9.26	154.14 ± 16.85 (121)	145.11 ± 11.37 (114)	141.97 ± 6.32 (111)	134.21 ± 8.00 (105)
Ethoxyresorufin *O*-deethylase	14.10 ± 1.68	29.95 ± 3.29 (212)	40.03 ± 2.65 (284)	49.71 ± 5.86 (353)	48.96 ± 6.66 (347)
Aminopyrine *N*-demethylase	2.26 ± 0.19	2.47 ± 0.24 (109)	2.51 ± 0.19 (111)	3.41 ± 0.43 (151)	3.47 ± 0.56 (154)

[a]Kitchin and Ebron (1983).
[b]Values represent mean ± SE (% of control); % of control is calculated.
[c]Data are digitized by the GetData Digitizer.
[d]Number of animals ranged from 6−8 (specific numbers per dose group not reported).

Table B.14. Organ Weights and Reproductive Data of Sprague-Dawley (CD) Rats Administered 1,2,4,5-TCB in the Diet during a Two-Generation Reproduction Study[a,b]

	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-d)			
F0 Adult Males[c]	**0 (0.0)**	**30.0 (2.2)**	**300.0 (21.1)**	**1,000.0 (70.3)**
Final body weight (g)	583.6 ± 60.19	561.5 ± 51.33 (96)	562.4 ± 57.59 (96)	495.8 ± 39.64** (85)
Liver (g)	20.846 ± 3.01	20.208 ± 2.77 (97)	23.587 ± 3.21** (113)	30.640 ± 2.73** (147)
Kidney (g)	3.729 ± 0.27	3.852 ± 0.38 (103)	5.288 ± 0.93** (142)	6.101 ± 0.92** (164)
Brain (g)	2.107 ± 0.09	2.108 ± 0.08 (100)	2.118 ± 0.08 (101)	2.091 ± 0.10 (96)
Testes (g)	3.524 ± 0.24	3.491 ± 0.31 (99)	3.687 ± 0.28 (105)	3.751 ± 0.33* (106)
F0 Adult Females[d]	**0 (0.0)**	**30.0 (2.6)**	**300.0 (25.5)**	**1,000.0 (82.5)**
Final body weight (g)	307.9 ± 18.54	308.5 ± 16.84 (100)	306.0 ± 21.47 (99)	294.8 ± 25.79 (96)
Liver (g)	12.570 ± 1.68	13.023 ± 1.36 (104)	13.336 ± 1.13 (106)	15.650 ± 1.04** (125)
Kidney (g)	2.402 ± 0.21	2.380 ± 0.21 (99)	2.467 ± 0.19 (103)	2.458 ± 0.21 (102)
Brain (g)	1.996 ± 0.09	2.003 ± 0.08 (100)	1.947 ± 0.09 (98)	1.933 ± 0.06* (97)
F1 pup parameters	**0**	**30.0**	**300.0**	**1,000.0**
Live birth index	98.8 ± 3.38 (*n* = 24)	98.7 ± 3.83 (*n* = 25)	98.9 ± 3.38 (*n* = 26)	78.9 ± 20.1** (*n* = 24)
7-Day survival index	100 ± 0.0 (*n* = 24)	96 ± 20.0 (*n* = 24)	100 ± 0.0 (*n* = 26)	1.3 ± 4.62** (*n* = 13)
Lactation index	100 ± 0.0 (*n* = 24)	96 ± 10.0 (*n* = 25)	88.5 ± 22.89** (*n* = 26)	0.0 ± 0.0** (*n* = 13)
F2 pup parameters	**0**	**30.0**	**300.0**	**1,000.0**
Live birth index	94.3 ± 21.21 (*n* = 22)	97.0 ± 5.43 (*n* = 19)	93.8 ± 14.55 (*n* = 22)	-
7-Day survival index	100.0 ± 0.0 (*n* = 21)	100.0 ± 0.0 (*n* = 19)	98.2 ± 8.18 (*n* = 21)	-
Lactation index	100.0 ± 0.0 (*n* = 21)	100.0 ± 0.0 (*n* = 19)	84.9 ± 27.37** (*n* = 21)	-

[a]Tyl and Neeper-Bradley (1989).
[b]Values represent mean ± SD for groups of 28 animals (% of control); % of control is calculated.
[c]Sacrificed at Week 15.
[d]Sacrificed at Week 17.
*Significantly different from control, $p < 0.05$.
**Significantly different from control, $p < 0.01$.
"-" indicates that no F2 pups were bred for this dose group due to entire litter loss at the high dose.

Table B.15. Reproductive Parameters of F0 Generation CD-1 Mice Administered 1,2,4,5-TCB in Diet for 18 Weeks[a]

Parameter[b]	Exposure Group, % in Diet[c]		
	0	0.028 (42 or 43 mg/kg-d)	0.072 (109 or 108 mg/kg-d)
Average no. of litters per pair	4.9 ± 0.0	5.0 ± 0.0 (102)	5.0 ± 0.0 (102)
Live pups per litter			
Male	6.0 ± 0.2	5.6 ± 0.3 (93)	5.5 ± 0.2 (92)
Female	6.2 ± 0.3	6.2 ± 0.4 (100)	5.9 ± 0.3 (95)
Combined	12.2 ± 0.4	11.8 ± 0.5 (97)	11.4 ± 0.3* (93)
Proportion of F1 pups born alive	0.96 ± 0.02	0.95 ± 0.03 (99)	1.00 ± 0.00 (104)
Sex of F1 pups born alive (male/total)	0.50 ± 0.01	0.48 ± 0.01* (96)	0.49 ± 0.02 (98)
Live pup weight (g)			
Male	1.55 ± 0.02	1.57 ± 0.02 (101)	1.57 ± 0.02 (101)
Female	1.51 ± 0.02	1.51 ± 0.02 (100)	1.50 ± 0.01 (99)
Combined	1.53 ± 0.02	1.54 ± 0.02 (101)	1.53 ± 0.02 (100)
Adjusted live pup weight (g)			
Male	1.55 ± 0.02	1.57 ± 0.02 (101)	1.57 ± 0.02 (101)
Female	1.52 ± 0.01	1.51 ± 0.02 (99)	1.48 ± 0.02 (97)
Combined	1.53 ± 0.01	1.54 ± 0.02 (101)	1.53 ± 0.02 (100)

[a]NTP (1991e).
[b]Values represent mean ± SE (% of control); % of control is calculated.
[c]Results for the high-dose group (0.18% in diet) are not reported due to high mortality preceding termination of the study.
*Statistically significant at $p \leq 0.05$; Dunnett's test conducted by study authors.

Table B.16. Relative Organ Weights of F0 Generation Following 18 Weeks Exposure to 1,2,4,5-TCB in Male CD-1 Mice[a]

Parameter[b]	Exposure Group, % in Diet	
	0	0.18 (246 or 253 mg/kg-d)
Number of animals	20	20
Liver	51.2 ± 1.1	99.8 ± 2.5 (195)*
Right cauda epididymis	0.43 ± 0.01	0.46 ± 0.01 (107)*
Right epididymis	1.4 ± 0.06	1.3 ± 0.03 (93)*
Kidney/adrenal	20.3 ± 0.60	20.6 ± 0.33 (101)
Prostate	0.61 ±0.03	0.57 ± 0.03 (93)
Seminal vesicles	11.8 ± 0.39	12.3 ± 0.30 (104)
Right testis	3.5 ± 0.08	3.3 ± 0.16 (94)

[a]NTP (1991e).
[b]Values represent mean ± SE (% of control); % of control is calculated.
*Statistically significant at $p \leq 0.05$; Wilcoxon test conducted by study authors.

Table B.17. Relative Organ Weights of F1 Generation Following 18 Weeks Exposure to 1,2,4,5-TCB in Male CD-1 Mice[a]

Parameter[b]	Exposure Group, % in Diet	
	0	0.072 (108 mg/kg-d)
Number of animals	20	20
Liver	50.7 ± 0.85	98.2 ± 1.3 (194)*
Right cauda epididymis	0.46 ± 0.01	0.45 ± 0.81(98)
Right epididymis	1.4 ± 0.03	1.4 ± 0.03 (100)
Kidney/adrenal	20.7 ± 0.86	24.6 ± 0.68 (119)*
Prostate	0.66 ± 0.03	0.61 ± 0.03(92)
Seminal vesicles	11.1 ± 0.25	10.8 ± 0.23(97)
Right testis	3.4 ± 0.11	3.9 ± 0.15(115)*

[a]NTP (1991e).
[b]Data reported as mean (mg/g body weight) ± SE (% of control); % of control is calculated.
*Statistically significant at $p \leq 0.05$; Wilcoxon test conducted by study authors.

Table B.18. Relative Organ Weights of F1 Generation Following 18 Weeks Exposure to 1,2,4,5-TCB in Female CD-1 Mice[a]

Parameter[b]	Exposure Groups, % in Diet	
	0	0.072 (127 mg/kg-d)
Number of animals	20	20
Liver	55.7 ± 1.1	76.9 ± 1.5 (138)*
Kidney/adrenal	15.7 ± 0.32	16.8 ± 0.21 (107)*
Right ovary	0.28 ± 0.02	0.24 ± 0.01 (86)

[a]NTP (1991e).
[b]Data reported as mean (mg/g body weight) ± SE (% of control); % of control is calculated.
*Statistically significant at $p \leq 0.05$; Wilcoxon test conducted by study authors.

Table B.19. Incidence of Histopathologic Lesions of F1 Generation Following 18 Weeks Exposure to 1,2,4,5-TCB in Male CD-1 Mice[a,b]

Organ	Parameter	Exposure group, % in Diet					
		0			0.072 (108 mg/kg-d)		
Number examined		10			10		
Severity		Mild	Mod.	Sev.	Mild	Mod.	Sev.
Liver	Hepatocellular degeneration	1	0	0	0	0	0
	Focal hepatitis	1	0	0	3	0	0
	Cytomegaly and karyomegaly	0	0	0	4	6	0
Testis	Degeneration of seminiferous tubules	2	0	0	0	0	0
Epididymis	Degeneration of epithelial cell	1	0	0	0	0	0
Kidney	Dilation of tubules	0	0	0	4	2	0
	Interstitial nephritis	2	0	0	1	0	0
	Regeneration of tubules	3	0	0	7	0	0

[a]NTP (1991e).
[b]Data reported as number of animals showing effect.

Table B.20. Incidence of Histopathologic Lesions of F1 Generation Following 18 Weeks Exposure to 1,2,4,5-TCB in Female CD-1 Mice[a,b]

Organ	Parameter	Exposure group, % in Diet					
		0			0.072 (127 mg/kg-d)		
Number examined		10			10		
Severity		Mild	Mod.	Sev.	Mild	Mod.	Sev.
Liver	Hepatitis	3	0	0	5	0	0
	Cytomegaly and karyomegaly	0	0	0	0	7	2
Kidney	Tubular dilation	1	0	0	0	0	0
	Tubular degeneration	0	0	0	2	0	0
	Interstitial nephritis	1	0	0	2	0	0
	Tubular regeneration	2	0	0	5	2	0

[a]NTP (1991e).
[b]Data reported as number of animals showing effect.

APPENDIX C. BMD OUTPUTS

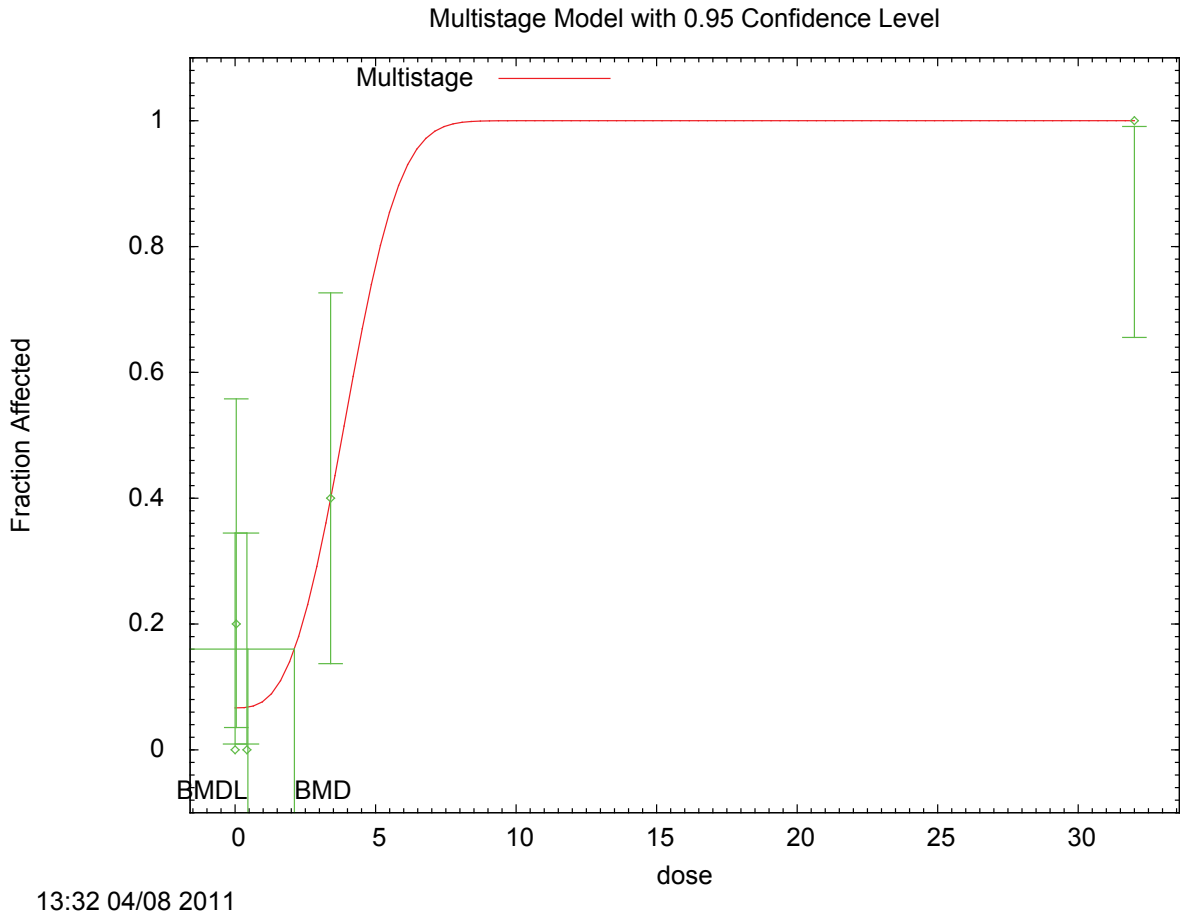

Figure C.1. Multistage (degree = 3) BMD Model for Liver Lesions in Male Rat Data (Chu et al., 1983)

Text Output for Multistage (degree = 3) BMD Model for Liver Lesions in Male Rat Data (Chu et al., 1983)

```
 ======================================================================
        Multistage Model. (Version: 3.2;  Date: 05/26/2010)
        Input Data File: C:/89/Chu_et_al_1983_liver_lesions_m_Multi3_1.(d)
        Gnuplot Plotting File:  C:/89/Chu_et_al_1983_liver_lesions_m_Multi3_1.plt
                                          Fri Apr 08 13:32:27 2011
 ======================================================================

 Histopath_liver_lesions_in_male_rats
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]
```

```
   The parameter betas are restricted to be positive


   Dependent variable = DiffEff
   Independent variable = Dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


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


                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 3.05267e+015


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

             Background      Beta(3)

Background            1        -0.42

   Beta(3)        -0.42            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0667078           *               *                  *
        Beta(1)                0           *               *                  *
        Beta(2)                0           *               *                  *
        Beta(3)        0.0112085           *               *                  *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -11.7341         5
   Fitted model        -14.0863         2       4.70435      3          0.1948
  Reduced model        -31.3435         1       39.2187      4         <.0001

           AIC:         32.1726
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0667	0.667	0.000	10	-0.845
0.0410	0.0667	0.667	2.000	10	1.689
0.4200	0.0675	0.675	0.000	10	-0.851
3.4000	0.3992	3.992	4.000	10	0.005
32.0000	1.0000	10.000	10.000	10	0.000

Chi^2 = 4.29 d.f. = 3 P-value = 0.2316

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.11046

BMDL = 0.456132

BMDU = 3.3767

Taken together, (0.456132, 3.3767) is a 90 % two-sided confidence interval for the BMD

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2013) 2013 TLVs and BEIs. Based on the documentation of the threshold limit values for chemical substances and physical agents & biological exposure indices. ACGIH, Cincinnati, OH. 1798797

ATSDR (Agency for Toxic Substances and Disease Registry). (2013) Minimal risk levels (MRLs) for hazardous substances. ATSDR, Atlanta, GA. Available online at http://www.atsdr.cdc.gov/mrls/pdfs/atsdr_mrls_july_2013.pdf. 1798743

Bayer, AG. (1993) Micronucleus test on the mouse with 1,2,4,5-tetrachlorobenzene with cover letter dated 070993. Miles Inc., Pittsburgh, PA; 930000327, NTIS No. OTS0537763. 677328.

Braun, WH; Sung, LY; Keyer, DG; Kociba, RJ. (1978a) Pharmacokinetic and toxicological evaluation of dogs fed 1,2,4,5-tetrachlorobenzene in the diet for two years. *J Environ Pathol Toxicol* 2(2):225−234. 677336.

Braun, WH; Sung, LY; Keyes, DG; Kociba, RJ. (1978b) Pharmacokinetic and toxicological evaluation of dogs fed 1,2,4,5-tetrachlorobenzene in the diet for two years. J *Toxicol Environ Health* 4(5−6):727−734. 677337.

Bushy Run Research Center. (1988) Reproduction and fertility study on 1,2,3,4-tetrachlorobenzene with two cover letters dated 012688 and 012088 (draft). Standard Chlorine Chemical Co., Export, PA; NTIS No. OTS0000594. 677329.

Cal/EPA (California Environmental Protection Agency). OEHHA toxicity criteria database. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://www.oehha.ca.gov/risk/ChemicalDB/index.asp. Accessed on 9-26-2013.

Cal/EPA (California Environmental Protection Agency). (2013a) All OEHHA Acute, 8-hour and Chronic Reference Exposure Levels (chRELs) as of August 2013. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://www.oehha.ca.gov/air/allrels.html. 1935906

Cal/EPA (Environmental Protection Agency). (2013b) Current proposition 65 list [09/13/13]. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://oehha.ca.gov/prop65/prop65_list/Newlist.html. 1936438

Cal/EPA (California Environmental Protection Agency). (2009) Hot spots unit risk and cancer potency values. Appendix A. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://www.oehha.ca.gov/air/hot_spots/pdf/CPFs042909.pdf. Accessed on February 14, 2011. 684164.

Chapin, R; Gulati, D; Barnes, L. (1997) 1,2,4,5-Tetrachlorobenzene. *Environ Health Perspect Suppl* 105(1):351−352. Available online at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1470297/pdf/envhper00326-0344.pdf. 710666.

Chu, I; Villeneuve, D; Secours, V; Valli, VE. (1983) Comparative toxicity of 1,2,3,4-, 1,2,4,5-, and 1,2,3,5-tetrachlorobenzene in the rat: results of acute and subacute studies. *J Toxicol Environ Health* 11(4−6):663−677. 677338.

Chu, I; Villeneuve, DC; Valli, VE; Secours, VE. (1984a) Toxicity of 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene in the rat: results of a 90-day feeding study. *Drug Chem Toxicol* 7(2):113−127. Available online at http://dx.doi.org/10.3109/01480548408998410. 677339.

Chu, I; Villeneuve, DC; Viau, A; et al. (1984b) Metabolism of 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene in the rat. *J Toxicol Environ Health* 13(4−6):777−786. 677340.

Dow. (1982a) Results of 92-day dietary feeding studies on 1,2,4,5- tetrachlorobenzene in beagle dogs. Dow Chemical Company, Midland, MI; 878211212, NTIS No. OTS0206149. Submitted under TSCA Section 017945. 677331.

Dow. (1982b) Results of 144 day dietary feeding studies on 1,2,3,4- and 1,2,4,5-tetrachlorobenzene in beagle dogs. Dow Chemical Corp, Midland, MI; 878211086, NTIS No. OTS84003A. 677332.

Dow. (1984a,b) Results of dietary feeding of 1,2,4,5-tetrachlorobenze to rats. Dow Chemical Company, Midland, MI. 677334.

Fisher, LC; Tyl, RW; Butler, BL; et al. (1990a,b) Developmental toxicity evaluation of 1,2,4,5-tetrachlorobenzene (TCB) administered by gavage to Fischer 344 (F-344) rats and New Zealand White (NZW) rabbits [Abstract]. *Teratology* 41(5):556. 659971.

Fomenko, VN. (1965) Determination of the maximum permissible concentration of tetrachlorobenzene in water basins. *Gig Sanit* 30:8−15. 711605.

Gustafson, DL; Coulson, AL; Feng, L; et al. (1998) Use of a medium-term liver focus bioassay to assess the hepatocarcinogenicity of 1,2,4,5-tetrachlorobenzene and 1,4-dichlorobenzene. *Cancer Lett* 129(1):39−44. 677348.

Gustafson, DL; Long, ME; Thomas, RS; et al. (2000) Comparative hepatocarcinogenicity of hexachlorobenzene, pentachlorobenzene, 1,2,4,5-tetrachlorobenzene, and 1,4-dichlorobenzene: application of a medium-term liver focus bioassay and molecular and cellular indices. *Toxicol Sci* 53(2):245−252. 652769.

Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen* 5:3−142. Available online at http://dx.doi.org/10.1002/em.2860050703. 028947.

IARC (International Agency for Research on Cancer). (2013) Monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC. Available online at http://monographs.iarc.fr/ENG/Monographs/vol103/mono103-B02-B03.pdf. 1770074

IPCS (International Programme on Chemical Safety). (1991) Chlorobenzenes other than hexachlorobenzene (Report No. Environmental Health Criteria 128). World Health Organization, Geneva, Switzerland. Available online at http://www.inchem.org/documents/ehc/ehc/ehc128.htm. 081628.

Kacew, S; Ruddick, JA; Parulekar, M; et al. (1984) A teratological evaluation and analysis of fetal tissue levels following administration of tetrachlorobenzene isomers to the rat. *Teratology* 29(1):21−27. Available online at http://dx.doi.org/10.1002/tera.1420290104. 677354.

Kiraly, J; Szentesi, I; Ruzicska, M; Czeize, A. (1979) Chromosome studies in workers producing organophosphate insecticides. *Arch Environ Contam Toxicol* 8(3): 309−319. Available online at http://dx.doi.org/10.1007/BF01056247. 065914.

Kitchin, KT; Ebron, MT. (1983) Maternal hepatic effects of 1,2,4,5-tetrachlorobenzene in the rat. *Environ Res* 32:134−144. Available online at http://dx.doi.org/10.1016/0013-9351(83)90200-1. 677357.

Loveday, KS; Anderson, BE; Resnick, MA; et al. (1990) Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals. *Environ Mol Mutagen* 16(4):272−303. Available online at http://dx.doi.org/10.1002/em.2850160409. 106324.

NIOSH (National Institute for Occupational Safety and Health). (2010) NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare, Atlanta, GA. Available online at http://www.cdc.gov/niosh/npg/npgdcas.html. 1788713

NLM (National Library of Medicine). (2011) Hazardous Substances Data Bank (HSDB). National Institutes of Health, Bethesda, MD. Available online at http://toxnet.nlm.nih.gov. Accessed on February 14, 2011. 783990.

NTP (National Toxicology Program). (1991a,b,c,d) Toxicity studies of 1,2,4,5-tetrachlorobenzene in F344 rats and B6C3F1 mice (feed studies). U.S. Department of Health and Human Services, Publich Health Service, Research Triangle Park, NC; NIH 91-3126. Available online at http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox007.pdf. 677360.

NTP (National Toxicology Program). (1991e) Final report on the reproductive toxicity of 1,2,4,5-tetrachlorobenzene (CAS no. 95-94-3) in CD-1 Swiss mice. Environmental Health Research and Testing, National Toxicology Program, Lexington, KY. Available online at http://www.ntis.gov/search/product.aspx?ABBR=PB92128388. 666710.

NTP (National Toxicology Program). (2011) Report on carcinogens, 12th edition. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available online at http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf. 737606

OSHA (Occupational Safety and Health Administration). (2006) Table Z-1 limits for air contaminants: occupational safety and health standards, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC; OSHA Standard 1910.1000. Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992. 670067

OSHA (Occupational Safety and Health Administration). (2011) Air contaminants: occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC; OSHA Standard 1915.1000. Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10286.

Ou, YC; Conolly, RB; Thomas, RS; et al. (2003) Stochastic simulation of hepatic preneoplastic foci development for four chlorobenzene congeners in a medium-term bioassay. *Toxicol Sci* 73:301−314. Available online at http://dx.doi.org/10.1093/toxsci/kfg078. 660380.

Schwartz, H; Chu, I; Villeneuve, DC; Benoit, FM. (1987) Metabolism of 1, 2, 3, 4-, 1, 2, 3, 5-, and 1, 2, 4, 5-tetrachlorobenzene in the squirrel monkey. *J Toxicol Environ Health* 22(3):341−350. 677364.

Shelby, MD; Witt, KL. (1995) Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ Mol Mutagen* 25(4):302−313. Available online at http://dx.doi.org/10.1002/em.2850250407. 624921.

Tyl, RW; Neeper-Bradley, TL. (1989) Two-generation reproduction study of 1,2,4,5-tetrachlorobenzene administered in the diet to Sprague-Dawley rats with letter dated 4/21/89 from Chemical Manufacturers Association. Bushy Run Research Center, Export, PA; NTIS No. OTS0523029. Submitted under TSCA Section 407029. 677368.

U.S. EPA (Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Cincinnati, OH; EPA/600/6-87/008. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855#Download. 064560

U.S. EPA (Environmental Protection Agency). (1991) Integrated Risk Information System (IRIS) 1,2,4,5-Tetrachlorobenzene. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/iris/subst/0107.htm.

U.S. EPA (Environmental Protection Agency). (1994) Chemical assessments and related activities (CARA). Office of Health and Environmental Assessment, Washington, DC; EPA/600/R-94/904. Available online at http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=60001G8L.txt. 596444.

U.S. EPA (Environmental Protection Agency). (2002) A review of the reference dose and reference concentration processes. Final report. Risk Assessment Forum, Washington, DC; EPA/630/P-02/002F. Available online at http://www.epa.gov/raf/publications/pdfs/rfd-final.pdf. 088824.

U.S. EPA (Environmental Protection Agency). (2011) Health effects assessment summary tables (HEAST). Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response, Washington, DC. Available online at http://epa-heast.ornl.gov/. 1577552

U.S. EPA (Environmental Protection Agency). (2011) Recommended use of body weight$^{3/4}$ as the default method in derivation of the oral reference dose. Office of the Science Advisor, Risk Assessment Forum, Washington, DC; EPA/1000/R-11/0001. Available online at http://www.epa.gov/raf/publications/pdfs/recommended-use-of-bw34.pdf. 752972.

U.S. EPA (Environmental Protection Agency). (2012) 2012 Edition of the drinking water standards and health advisories. Office of Water, Washington, DC; EPA 822-S-12-001. Available online at http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf. 1936016.

Union Carbide Corporation. (1992) Initial submission: 2-generation reproduction study of 1,2,4,5-tetrachlorobenzene administered in the diet to Sprague-Dawley rats with cover letter dated 082792. Bushy Run Research Center, Export, PA; 920009343. Available online at http://www.ntis.gov/search/product.aspx?ABBR=OTS0571095. 677325.

WHO (World Health Organization). (1991) Chlorobenzenes other than hexachlorobenzene. Environmental health criteria 128. Available online at http://www.inchem.org/documents/ehc/ehc/ehc128.htm. Accessed on February 14, 2011. 081628.