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TOXICOLOGICAL REVIEW

OF

1,2,3-TRICHLOROPROPANE

(CAS No. 96-18-4)

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

September 2009

U.S. Environmental Protection Agency
Washington DC

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LIST OF ABBREVIATIONS AND ACRONYMS

ACPC	N-acetyl-S-(3-chloro-2-hydroxypropyl)-L-cysteine
ADAF	age-dependent adjustment factor
AIC	Akaike Information Criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMC	benchmark concentration
BMD	benchmark dose
BMDL	Benchmark dose, 95% lower bound
BMDS	Benchmark Dose Software
BMR	benchmark response
BSO	1-buthionine-(R,S)-sulfoximine
CASRN	Chemical Abstracts Service Registry Number
CHO	Chinese hamster ovary
CPC	S-(3-chloro-2-hydroxypropyl)-L-cysteine
CYP450	cytochrome P-450
DAF	dosimetric adjustment factor
DCA	1,3-dichloroacetone
GMA	(S-glutathionyl)malonic acid
GSH	reduced glutathione
HEC	human equivalent concentration
IRIS	Integrated Risk Information System
i.p.	intraperitoneal
i.v.	intravenous
K_{ow}	octanol/water partition coefficient
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect-level
MLE	maximum likelihood estimate
MTD	maximum tolerated dose
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect-level
NRC	National Research Council
NTP	National Toxicology Program
PBPK	physiologically based pharmacokinetic
PBTK	physiologically-based toxicokinetic
RACB	Reproductive Assessment by Continuous Breeding
RfC	reference concentration
RfD	reference dose
RfV	reference value
SD	standard deviation
SDH	sorbitol dehydrogenase
SMART	somatic mutation and recombination test
TSCA	Toxic Substances Control Act
UCL	upper confidence limit
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to 1,2,3-trichloropropane. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 1,2,3-trichloropropane.

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

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

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 1,2,3-trichloropropane. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

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

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

Development of these hazard identification and dose-response assessments for 1,2,3-trichloropropane has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size*

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

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through June 2009.

2. CHEMICAL AND PHYSICAL INFORMATION

1,2,3-Trichloropropane (CASRN 96-18-4) is a three-carbon alkane with a single chlorine atom attached to each carbon atom in the chain (Figure 2-1). Synonyms for the compound include glyceryl trichlorohydrin, glycerol trichlorohydrin, and allyl trichloride. Some physical and chemical properties are shown in Table 2-1 (HSDB, 2005).

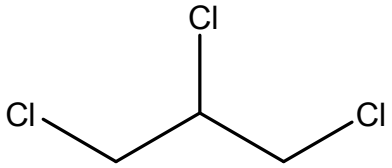


Figure 2-1. 1,2,3-Trichloropropane.

Table 2-1. Physical properties and chemical identity of 1,2,3-trichloro-propane

CASRN 96-18-4	
Chemical formula	$C_3H_5Cl_3$
Molecular weight	147.43
Melting point	14.7°C
Boiling point	156.85°C
Density at 20°C	1.3889 g/mL
Water solubility at 25°C	1,750 mg/L
Log K_{ow}	1.98/2.27
Vapor pressure at 25°C	3.1/3.69 mm Hg
Henry's law constant	3.43×10^{-4} atm-m^3/mol
Conversion factors	1 ppm = 6.13 mg/m^3; 1 mg/m^3 = 0.16 ppm

Sources: ATSDR (1992); HSDB (2005).

1,2,3-Trichloropropane is used in the chemical industry as a solvent for oils and fats, waxes, and resins (HSDB, 2005; ATSDR, 1992). The compound has also been used in paint thinner and varnish remover, and as a degreasing agent. 1,2,3-trichloropropane is generated as a byproduct of the production of other chlorinated compounds such as epichlorohydrin (WHO, 2003). The compound is also used as an intermediate in the production of some pesticides and polymers, such as polysulfide rubbers. The commercially available product is >98–99.9% pure.

3. TOXICOKINETICS

No reports are available that address the toxicokinetics of 1,2,3-trichloropropane in humans by any route of exposure. Experimental studies in rats and mice have demonstrated that absorption of the compound via the oral route results in rapid distribution, extensive metabolism, and clearance within 60 hours (Mahmood et al., 1991), with a half-life in male rats of 23 hours (Gingell et al., 1987). The toxicokinetic data also demonstrate the ability of 1,2,3-trichloropropane or metabolites to bind to intracellular macromolecules such as proteins and nucleic acids (Mahmood et al., 1991; Weber and Sipes, 1990).

3.1. ABSORPTION

Data on the quantitative absorption of 1,2,3-trichloropropane from exposure via the inhalation or dermal routes have not been reported. Quantitative data on the absorption, distribution, and excretion following oral exposure to 1,2,3-trichloropropane were obtained from a study in which rats and mice were treated with ^{14}C-labeled compound by corn oil gavage (Mahmood et al., 1991). Doses of 30 mg/kg (8–10 μCi) [^{14}C]-1,2,3-trichloropropane were administered to 9 male and 12 female Fischer rats, and either 30 or 60 mg/kg to B6C3F1 male mice (three/group). By sacrificing the animals at intervals up to 60 hours, the researchers collected information on the time-dependent distribution of radiolabel in urine, feces, breath, the principal organs and tissues, and bile.

Estimates for the percent absorption of the oral dose can be made by summing the mean values for the radiolabel recovered in the urine and exhaled as CO_2, as well as the radiolabel distributed in the blood, liver, kidney, skin, adipose tissue, and muscle (Table 3-1). By this approach, estimates of the absorbed oral load are 75% in male rats, 68% in female rats, and 84% in male mice. The percent recovered from feces was not used in this calculation because it is likely to contain both an absorbed and non-absorbed fraction. However, the true extent of intestinal absorption is likely to have been greater than the presented 75-84%, because a portion of the radiolabel that appeared in feces, which was not included in the above absorption estimates, would also have been absorbed.

Table 3-1. Distribution and excretion of radiolabeled 1,2,3-trichloropropane (30 mg/kg) 60 hours after oral (gavage) administration

Tissue	Male rats	Female rats	Male mice
Urine	57.1 ± 6.2[a]	49.8 ± 4.3[a]	64.0 ± 5.5[a]
Feces	21.1 ± 4.9	19.4 ± 2.2	16.0 ± 6.0
CO_2	17.7 ± 0.4	18.5 ± 0.6	20.2 ± 1.8
Volatiles	1.5 ± 0.5	1.4 ± 0.8	0.6 ± 0.4
Blood	0.6 ± 0.1	0.9 ± 0.2	0.1 ± 0.04
Liver	1.4 ± 0.2	1.2 ± 0.3	0.6 ± 0.03
Kidney	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.01
Skin	1.1 ± 0.1	1.0 ± 0.1	0.5 ± 0.1
Adipose tissue	0.4 ± 0.1	0.6 ± 0.3	0.2 ± 0.1
Muscle	1.1 ± 0.3	1.0 ± 0.4	1.0 ± 0.2

[a]Percent of total dose (data are mean ± standard deviation [SD] from three rats or mice).

Source: Mahmood et al. (1991).

3.2. DISTRIBUTION

Mahmood et al. (1991) examined the deposition of 30 mg/kg [2-^{14}C]-1,2,3-trichloropropane in rats and mice at three time points: 6, 24, and 60 hours post-administration in corn oil gavage. After 6 hours, most of the radiolabel was found in the forestomach and glandular stomach with smaller quantities in the intestines, adipose tissue, liver, and kidney. At 24 hours, the concentrations of radiolabel in the forestomach, intestines, liver, and kidney were similar. By hour 60, the majority of the radiolabel had been excreted in the urine or feces with some residual radioactivity sequestered predominantly in the liver, kidney, skin, muscle, and adipose tissue (see Table 3-1). The radiolabel detected in tissues after 60 hours was generally not extractable, suggesting that it was bound to macromolecules (Mahmood et al., 1991).

Volp et al. (1984) examined the time-dependent distribution of [1,3-^{14}C]-1,2,3-trichloropropane (2.1 mCi/mmol) in male Fischer rats (three rats per time point) following intravenous (i.v.) injection of 3.6 mg/kg. Animals were maintained in metabolic cages and sacrificed at the following time points: 15 and 30 minutes; 1, 2, 4, and 8 hours; and 1, 2, 4, and 6 days post-administration. Rapid distribution of the radiolabel was observed and 37% of the dose was detected in adipose tissue 15 minutes after administration. After 4 hours, the largest portion (maximum of 7.3% at 1 hour) of the radiolabel was sequestered in the liver, primarily as metabolites, and the kidney contained a maximum of 2.8% of the dose at 2 hours post-administration. The half-lives of phase 1 and phase 2 metabolism of 1,2,3-trichloropropane were 0.57 and 40 hours in the liver, 0.31 and 30 hours in the kidney, 1.8 and 44 hours in the adipose tissue, and 0.29 and 23 hours in the blood, respectively (Volp et al., 1984).

Weber and Sipes (1990) administered intraperitoneal (i.p.) injections of 30 mg/kg (100 μCi/kg) [2-^{14}C]-1,2,3-trichloropropane in vegetable oil to male Fischer rats. Groups of four rats were sacrificed after 1, 4, 24, 48, and 72 hours. Maximal covalent binding of radiolabel to hepatic protein, approximately 600 pmol/mg, was observed at 4 hours post-administration. Maximal covalent binding to hepatic DNA, approximately 250 pmol/mg, occurred at 24 hours. After 72 hours, the amount of radiolabel covalently bound to both hepatic protein and DNA was at or below the levels found 1 hour after administration.

3.3. METABOLISM

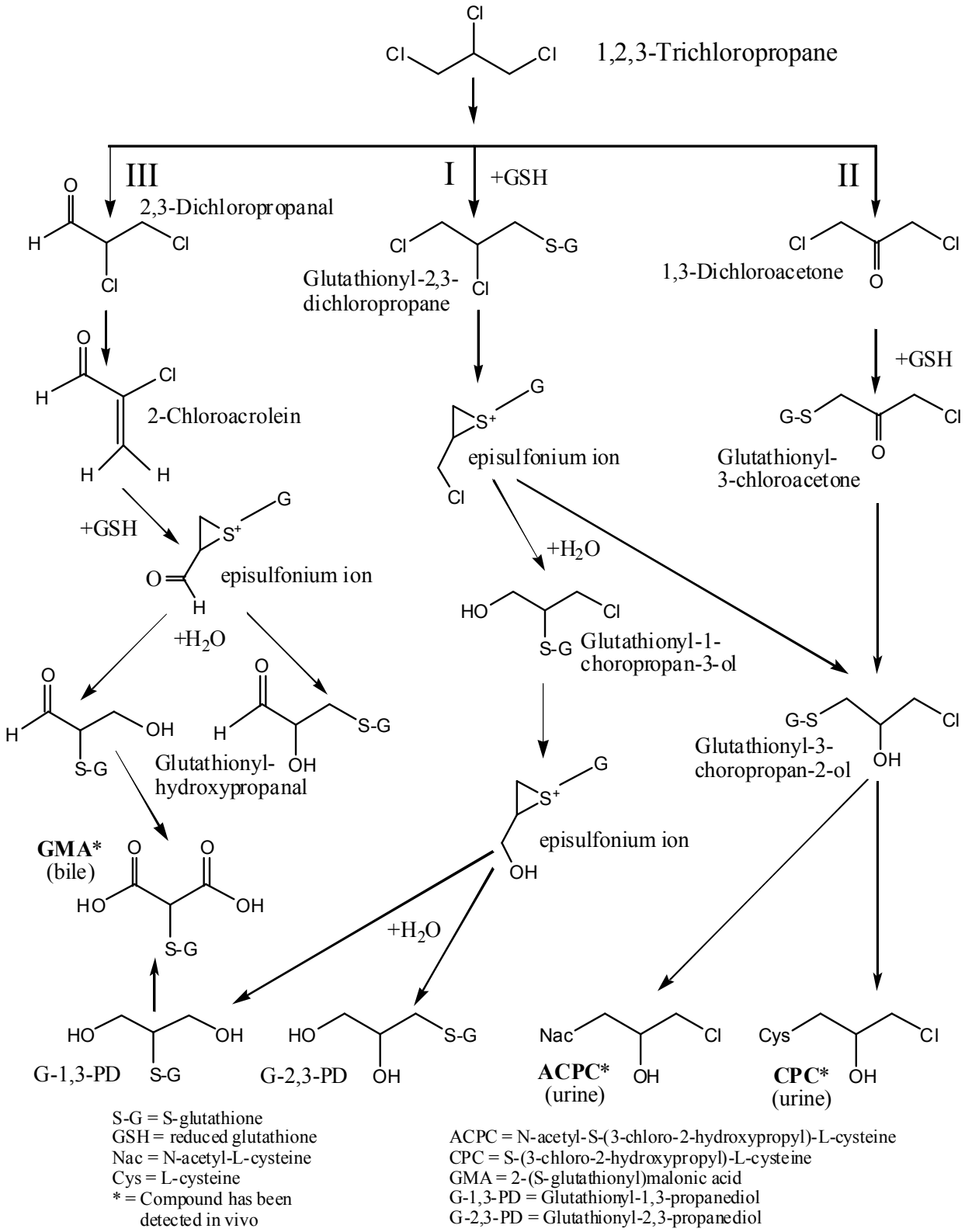
No studies of 1,2,3-trichloropropane metabolism in humans have been reported. In vitro data indicate that human microsomes, in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH), are capable of forming the DNA- reactive chemical, 1,3-dichloroacetone (DCA), from 1,2,3-trichloropropane (Weber and Sipes, 1992).

In rodents, 1,2,3-trichloropropane metabolism appears to involve oxidation catalyzed by cytochrome P-450 (CYP450) or glutathione (GSH) conjugation, but specific details about the metabolic process are unknown. Three potential routes for 1,2,3-trichloropropane metabolism (Figure 3-1) have been proposed by Mahmood et al. (1991):

I) Nucleophilic displacement of a chlorine atom by GSH creates a β-chlorothio ether, and internal displacement of another chlorine creates an episulfonium ion. This reactive ion could hydrolyze to a GSH conjugate that can be cleaved to form N-acetyl-S-(3-chloro-2-hydroxypropyl)-L-cysteine (ACPC) or S-(3-chloro-2-hydroxypropyl)-L-cysteine (CPC). The reactive episulfonium ion could also react with water to form β-chlorothio ether that could form a second episulfonium ion. This second episulfonium ion could form 2-(S-glutathionyl)malonic acid (GMA) through hydrolysis to form a 1,3-dihydroxypropyl GSH conjugate and subsequent oxidation.

II) Oxidation of 1,2,3-trichloropropane at the C2 position, possibly by CYP450 enzymes, could lead to the formation of 1,3-dichloroacetone. Displacement of chlorine from 1,3-dichloroacetone by GSH and reduction of the keto group can result in the formation of ACPC and CPC.

III) Oxidation, possibly by CYP450 enzymes, of 1,2,3-trichloropropane at the C1 position to form 2,3-dichloropropanal. This chlorohydrin could undergo loss of HCl to form chloroacrolein, and then rearrange with GSH to form an episulfonium ion. This ion could then form GMA after the oxidation of the C2 and C3 carbon atoms to form carboxylic acids.



Sources: WHO, 2003; Mahmood et al., 1991.

Figure 3-1. Possible metabolic pathways for 1,2,3-trichloropropane in rats

Evidence for the involvement of CYP450 in 1,2,3-trichloropropane metabolism is provided by the in vitro formation of 1,3-dichloroacetone when isolated rat or human hepatic microsomes were incubated with 1,2,3-trichloropropane (Weber and Sipes, 1992). The formation of 1,3-dichloroacetone, an intermediate in the formation of ACPC and CPC, occurred only in the presence of NADPH and was enhanced by the addition of such CYP450 inducers as phenobarbital and dexamethasone. Conversely, formation of 1,3-dichloroacetone was blocked by the CYP450 inhibitors SKF-525A and 1-aminobenzotriazol. This investigation also demonstrated that rat hepatic microsomes formed 1,3-dichloroacetone from 1,2,3-trichloropropane at a rate of 0.27 nmol/minute/mg protein, which was 10 times faster than the rate of formation of 1,3-dichloroacetone by human hepatic microsomes (0.026 nmol/minute/mg protein) (Weber and Sipes, 1992).

3.4. ELIMINATION

Mahmood et al. (1991) and Volp et al. (1984) demonstrated that urine is the primary route of the excretion of 1,2,3-trichloropropane metabolites in rats and mice. Mahmood et al. (1991) analyzed the urine of F344/N rats and male B6C3F1 mice treated with [2-^{14}C]-1,2,3-trichloropropane by corn oil gavage and found that the parent compound was extensively metabolized to either ACPC or CPC. These investigators also documented that the principal biliary metabolite was GMA. In rats, ACPC was the major urinary metabolite found 6 hours after exposure, accounting for approximately 40% of the radiolabel recovered in males and 10% in females. The urinary metabolite associated with the largest fraction of radiolabel in both males and females 24 hours post-administration could not be identified. However, substantial amounts of radiolabeled ACPC and CPC were detected in urine at 24 hours. In male mice, ACPC accounted for only 3% of the radiolabel at 6 hours (females were not tested). The major metabolites in male mice at both 6 and 24 hours were not identified.

Volp et al. (1984) examined the time-dependent distribution of [1,3-^{14}C]-1,2,3-trichloropropane in male Fischer rats (three rats per time point) following i.v. injection of 3.6 mg/kg. The data from this study demonstrated rapid excretion of the radiolabeled metabolite; after 24 hours, 30% of the initial radiolabel had been exhaled, 40% had been released in the urine, and 18% was excreted in feces. Unchanged 1,2,3-trichloropropane was not detected in the urine.

Weber (1991) conducted a detailed analysis of urinary metabolites by employing proton decoupled and two-dimensional homonuclear correlated nuclear magnetic resonance spectroscopy following the i.p. coadministration of [1,2,3-^{13}C]-trichloropropane and [2-^{14}C]-trichloropropane in soybean oil to male F344/N rats. This investigator identified N-acetyl-S-(2-hydroxy-3-chloropropyl)cysteine, 1,3-(2-propanol)-bis-S-(N-acetylcysteine), N-acetyl-S-(2-hydroxy-2-carboxyethyl)cysteine, 2,3-dichloropropionic acid, 2-chloroethanol, ethylene glycol,

and oxalic acid as potential urinary metabolites of 1,2,3-trichloropropane. It is unknown where in the metabolic pathway these additional urinary metabolites may form.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELING

Volp et al. (1984) developed a physiologically based toxicokinetic (PBTK) model to describe the time-dependent appearance of 1,2,3-trichloropropane and its metabolites in rat tissues. The model consists of compartment-specific mass balance equations for tissues that have physiological significance in storage, transport, and clearance. The model, which accurately predicted the concentration versus time curves for the selected tissues, contains seven compartments: blood, liver, kidney, adipose tissue, muscle, skin, and remaining distribution volume. The model describes the rapid disappearance of 1,2,3-trichloropropane from the blood with biotransformation products concurrently appearing in the urine, bile, and expired air. High concentrations of metabolites were also found in the liver and kidney, and the half-lives for trichloropropane clearance from blood and liver were 23 and 40 hours, respectively (Volp et al., 1984). The tissue distribution of 1,2,3-trichloropropane and metabolites is presented in Section 3.2.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS

Limited information from an acute inhalation study in humans (n = 12) demonstrated that 15-minute exposures to 100 ppm trichloropropane (purity unknown) resulted in irritation of the nose, eyes, and throat of all subjects tested (Silverman et al., 1946). No occupational, epidemiology, or case study data were identified that were applicable to 1,2,3-trichloropropane exposure in humans.

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

4.2.1. Oral Exposure

4.2.1.1. Subchronic Studies

Hazelton Laboratories (1983a, b) conducted a series of subchronic toxicity studies of 1,2,3-trichloropropane in F344/N rats and B6C3F1 mice. The findings of these subchronic studies were included in the National Toxicology Program (NTP) technical report on the toxicology and carcinogenesis of the compound and published in the peer-reviewed literature (NTP, 1993).

The same protocol was used for both the rat and the mouse studies. 1,2,3-Trichloropropane was administered by corn oil gavage 5 days/week for 120 days at doses of 0 (vehicle control), 8, 16, 32, 63, 125, or 250 mg/kg-day. Treatment groups contained 20 animals/sex and the vehicle control group contained 30 animals/ sex, and the animals were approximately 6 weeks old when the studies began. Half of the animals in each group were sacrificed after 8 weeks, and the rest were maintained until week 17. Animals were examined twice daily for clinical signs of toxic stress. Animals were weighed at the start of the study and at weekly intervals during the course of the study. Blood and urine samples were obtained from animals during weeks 8 and 17. Blood samples were analyzed for hematocrit, hemoglobin, and blood cell counts. A limited suite of clinical chemistry parameters was also evaluated. Specific gravity of the urine specimens was determined. Necropsies were performed on all animals with complete histopathologic examinations performed on all animals that had died during the study, moribund animals that were sacrificed during the study, all rats receiving a dose of 125 mg/kg-day, and all controls. A number of organs and tissues were excised and collected from all animals. Tissue weights were reported for the 17-week study only.

In the rat study, 12 males that received 250 mg/kg-day died, or were sacrificed moribund, during the first week of treatment. Six males died during the second week and the remaining two animals were terminated in weeks 3 and 5. Sixteen females in the 250 mg/kg-day dose

group died, or were sacrificed moribund, during the first week. The remaining four animals in this treatment group died during the second week. One male and four female rats that received 125 mg/kg-day 1,2,3-trichloropropane died, or were sacrificed moribund, during the study.

During their brief survival period, rats in the 250 mg/kg-day treatment group were noted to have been emaciated, lethargic, and debilitated. No clinical signs of toxicosis were observed in any of the other treatment groups. Dose-dependent reductions in body weight gain were observed in both males and females. Mean final body weights were significantly reduced for male rats that received 63 and 125 mg/kg-day and females treated with 125 mg/kg-day 1,2,3-trichloropropane. Whole-body and tissue weights were not reported for the 250 mg/kg-day treatment groups. At the 17-week sacrifice, mean weight gain in the 125 mg/kg-day treatment group was reduced by 43 and 60% for males and females, respectively, compared with controls.

A dose-dependent increase in relative liver and kidney weights (organ weight/body weight) was observed in male and female rats, but increases in absolute liver weights and kidney weights were not dose-dependent, other than absolute liver weight in female rats. Mean relative liver weights were statistically ($p \leq 0.01$) significantly increased in males that received 32, 63, or 125 mg/kg-day by 24, 47, and 78%, respectively, compared with controls (Table 4-1), while absolute liver weights statistically significantly ($p \leq 0.01$) increased 10–26% in males receiving 8–125 mg/kg-day (Table 4-2). Mean relative liver weights, when compared with controls, were statistically ($p \leq 0.01$) significantly increased by 12, 18, 37, and 105% in females receiving 16, 32, 63, or 125 mg/kg-day, respectively (Table 4-1), while absolute liver weights statistically significantly ($p \leq 0.05$) increased 17–61% in females receiving 16–125 mg/kg-day (Table 4-2). Mean relative right kidney weights were statistically significantly increased in males that received 32, 63, or 125 mg/kg-day by 12% ($p \leq 0.05$), 26% ($p \leq 0.01$), and 54% ($p \leq 0.01$), respectively, compared with controls (Table 4-3), while absolute right kidney weights were statistically significantly ($p \leq 0.01$) increased 5–19% in males receiving 32, 63, or 125 mg/kg-day (Table 4-4). In females that received 63 and 125 mg/kg-day 1,2,3-trichloropropane, mean relative right kidney weights were statistically ($p \leq 0.01$) significantly increased 32 and 43%, respectively (Table 4-3), while absolute right kidney weights statistically significantly ($p \leq 0.01$) increased 11–25% in females receiving 63–125 mg/kg-day (Table 4-4). Absolute heart weight was statistically significantly ($p \leq 0.01$) decreased 14 and 21% in male rats at 63 and 125 mg/kg-day, respectively. NTP (1993) considered the changes in relative brain and heart weights to be associated with the change in body weight, and not with organ toxicity.

Table 4-1. Relative liver weights (mg organ weight/g body weight) and percent change in F344/N rats exposed to 1,2,3-trichloropropane by gavage for 17 weeks

		Males			Females	
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	24.6 ± 0.5	–	10	25.7 ± 0.4	–
8	10	26.8 ± 0.4	9%	10	27.5 ± 0.6	7%
16	10	27.6 ± 0.5	12%	10	28.9 ± 0.4[c]	12%
32	10	30.5 ± 0.7[c]	24%	10	30.2 ± 0.6[c]	18%
63	10	36.2 ± 1.9[c]	47%	10	35.2 ± 0.8[c]	37%
125	9	43.7 ± 1.6[c]	78%	6	52.6 ± 2.3[c]	105%
250	–	NR[b]		–	NR	

[a]Calculated as the percent change from the control mean.
[b]NR = Due to the rapid onset of mortality, organ weights were not recorded for the high-dose group.
[c]Showing statistically significant differences ($p \leq 0.01$) from the control group by Williams' or Dunnett's test.

Source: NTP (1993).

Table 4-2. Absolute liver weights (g) and percent change in F344/N rats exposed to 1,2,3-trichloropropane by gavage for 17 weeks

		Males			Females	
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	8.87 ± 0.14	–	10	5.14 ± 0.1	–
8	10	9.82 ± 0.21	11%[b]	10	5.49 ± 0.09	7%
16	10	9.72 ± 0.38	10%[b]	10	6.07 ± 0.16	18%[b]
32	10	11.20 ± 0.20	26%[b]	10	6.00 ± 0.09	17%[c]
63	10	10.93 ± 0.23	23%[b]	10	6.79 ± 0.17	32%[b]
125	9	12.07 ± 0.13	19%[b]	6	8.25 ± 0.2	61%[b]
250	NR[d]			NR		

[a]Calculated as the percent change from the control mean.
[b]Showing statistically significant differences ($p \leq 0.01$) from the control group by Williams' or Dunnett's test.
[c]Showing statistically significant differences ($p \leq 0.05$) from the control group by Williams' or Dunnett's test.
[d]NR = Due to the rapid onset of mortality, organ weights were not recorded for the high-dose group.

Source: NTP (1993).

Table 4-3. Relative kidney weights (mg organ weight/g body weight) and percent change in F344/N rats exposed to 1,2,3-trichloropropane by gavage for 17 weeks

	Males			Females		
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	3.00 ± 0.03	–	10	3.16 ± 0.07	–
8	10	2.97 ± 0.04	-1%	10	3.37 ± 0.19	7%
16	10	3.14 ± 0.04	5%	10	3.38 ± 0.06	7%
32	10	3.37 ± 0.03[b]	12%	10	3.49 ± 0.05	10%
63	10	3.77 ± 0.24[c]	26%	10	4.16 ± 0.17[c]	32%
125	9	4.63 ± 0.16[c]	54%	6	4.52 ± 0.19[c]	43%
250	–	NR[d]		–	NR	

[a]Calculated as the percent change from the control mean.
[b]Showing statistically significant differences ($p \leq 0.05$) from the control group by Williams' or Dunnett's test.
[c]Showing statistically significant differences ($p \leq 0.01$) from the control group by Williams' or Dunnett's test.
[d]NR = Due to the rapid onset of mortality, organ weights were not recorded for the high dose group.

Source: NTP (1993).

Table 4-4. Absolute kidney weights (g) and percent change in F344/N rats exposed to 1,2,3-trichloropropane by gavage for 17 weeks

	Males			Females		
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	1.08 ± 0.03	–	10	0.64 ± 0.01	–
8	10	1.09 ± 0.02	1%[a]	10	0.67 ± 0.03	5%[a]
16	10	1.10 ± 0.03	2%	10	0.71 ± 0.02	11%
32	10	1.24 ± 0.02[b]	15%	10	0.70 ± 0.02	11%
63	10	1.13 ± 0.02[b]	5%	10	0.80 ± 0.03[c]	25%
125	9	1.28 ± 0.02[b]	19%	6	0.71 ± 0.02[c]	11%
250	–	NR[d]		–	NR	

[a]Calculated as the percent change from the control mean.
[b]showing statistically significant differences ($p \leq 0.01$) from the control group by Williams' or Dunnett's test.
[c]showing statistically significant differences ($p \leq 0.05$) from the control group by Williams' or Dunnett's test.
[d]NR = Due to the rapid onset of mortality, organ weights were not recorded for the high dose group.

Source: NTP (1993).

An increased incidence of lesions, as described below, was observed in the liver, kidney, and nasal turbinates of rats receiving 125 mg/kg-day 1,2,3-trichloropropane for 120 days (Table 4-5). A time-dependent increase in the number of lesions was noted between the 8- and 17-week

evaluations in the 125 mg/kg-day treatment group. This same pattern was not observed in the 250 mg/kg-day treatment group since the majority of animals did not survive more than 1 week.

Table 4-5. Incidence of liver, kidney, and nasal turbinate lesions in male and female F344/N rats in a 17-week study

Endpoint	Dose (mg/kg-d)					
	0	8	16	32	63	125
Males						
Liver necrosis[a]	0/20	0/10	0/10	1/10	1/10	1/10
Kidney necrosis[a]	0/20	0/10	0/10	0/10	0/10	1/10
Epithelial necrosis of nasal turbinates[a]	0/20	0/10	0/10	0/10	0/10	3/9[b]
Females						
Liver necrosis[a]	0/20	0/10	0/10	0/10	0/10	11/11[c]
Kidney necrosis[a]	0/20	0/10	0/10	0/10	0/10	0/11
Epithelial necrosis of nasal turbinates[a]	0/10	0/10	0/10	0/10	0/10	2/11

[a]Incidence is the number of animals in which lesion was found/number of animals in which tissue was examined.
[b]Showing statistically significant differences ($p \leq 0.05$) from the control group by Fisher exact test.
[c]Showing statistically significant differences ($p \leq 0.01$) from the control group by Fisher exact test.

Source: NTP (1993).

The liver lesions in rats were characterized by multifocal, centrilobular hepatocellular necrosis, with karyomegaly, hemorrhage, and bile duct hyperplasia. Hepatic necrosis was observed in female rats (7/9) receiving 125 mg/kg-day and in all of the rats receiving 250 mg/kg-day 1,2,3-trichloropropane (20/20 males and 20/20 females) at the time of their death. In the 17-week evaluation, hepatic necrosis was observed at terminal sacrifice in 1/10 males and 11/11 females treated with a dose of 125 mg/kg-day, with liver necrosis also evident in 1/10 male rats at 32 and 63 mg/kg-day.

The kidney lesions in the rats were characterized by early diffuse acute tubule necrosis or regenerative hyperplasia, karyomegaly of epithelial cells, and multifocal necrosis. Renal tubular necrosis was observed during the 8-week interim evaluation in 14/20 males and 20/20 females treated with 250 mg/kg-day that died at or before the interim sacrifice. At the 17-week evaluation, renal necrosis was observed in 1/10 males and 0/11 females treated with a dose of 125 mg/kg-day.

Lesions of the nasal turbinates included multifocal necrosis and epithelial attenuation, subepithelial fibrosis, and inflammation. Epithelial necrosis of the nasal turbinates was observed during the 8-week interim evaluation in 14/20 males and 19/20 females treated with 250 mg/kg-day that died at or before the interim sacrifice. At the time of death or at the 17-week evaluation,

epithelial necrosis of the nasal turbinates was observed in 3/9 males and 2/11 females treated with 125 mg/kg-day.

A number of clinical chemistry parameters in rats were statistically significantly affected upon exposure to 1,2,3-trichloropropane. Blood samples were not obtained from animals in the 250 mg/kg-day treatment group. Effects observed were predominantly biomarkers for liver damage. At the 8-week interim evaluation, the activities of alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), and aspartate aminotransferase (AST), were all statistically ($p \leq 0.01$) significantly elevated, 1,200, 433, and 1,000%, respectively, over controls in females that received 125 mg/kg-day. Total bilirubin levels in female rats at the 8-week evaluation increased 50 and 150% at doses of 63 and 125 mg/kg-day, respectively. At the 17-week evaluation, ALT and SDH activities were statistically [($p \leq 0.05$) and ($p \leq 0.01$), respectively] significantly elevated, 248 and 317%, respectively, over controls in females treated with 125 mg/kg-day.

The activity of ALT was statistically ($p \leq 0.05$) significantly elevated in males treated with 125 mg/kg-day at week 8 but not at week 17, while the activity of SDH in males at 17 weeks was statistically significantly ($p \leq 0.05$) increased 25 and 12.5% at 63 and 125 mg/kg-day, respectively. NTP (1993) stated that the increase in ALT and SDH was indicative of hepatocellular damage with subsequent enzyme leakage. The only clinical chemistry parameter that was consistently impacted in both males and females at both time points was pseudocholinesterase (serum carboxylesterase). Activity of this hepatic enzyme decreased in both species with increasing dose and NTP (1993) suggested that the depressed synthesis of pseudocholinesterase was due to hepatocellular damage. A statistically significant decrease was observed at both time points (8 and 17 weeks) evaluated in females at the lowest dose tested, 21 and 14% at 8 mg/kg-day ($p \leq 0.01$), and 9 and 8% in males that received 32 mg/kg-day ($p \leq 0.05$). The authors observed a dose-dependent decrease in pseudocholinesterase of 14–77% at 8 through 125 mg/kg-day in female rats and 8–21% at 32–125 mg/kg-day in male rats at 17 weeks.

In rats, hematocrit, hemoglobin, and erythrocyte counts were statistically significantly decreased by 1,2,3-trichloropropane treatment. At the 8-week sacrifice, hematocrit and red blood cell counts were significantly depressed by 13–23 and 10–18%, respectively, in males that received doses of ≥16 mg/kg-day and in females that received doses of ≥8 mg/kg-day. Hemoglobin was statistically significantly decreased 5-9% in male rats that received doses of ≥16 mg/kg-day and female rats that received ≥63 mg/kg-day.

The 17-week, less-than-lifetime rat study was conducted to determine appropriate doses for the 2-year, 1,2,3-trichloropropane study in rats (NTP, 1993), described later in this document. NTP considered the dose-response of the increased liver and kidney weights to be consistent with the clinical pathological and histopathological findings in the liver and kidney. The NOAEL and LOAEL for hepatocellular necrosis in male rats at 17-weeks were 16 and 32 mg/kg-day, and in females rats at 17-weeks were 63 and 125 mg/kg-day. The NOAEL and LOAEL for

increased SDH in male rats were 32 and 63 mg/kg-day, respectively. A decrease in pseudocholinesterase (serum carboxylesterase) activity in males presented a NOAEL of 16 mg/kg-day and LOAEL of 32 mg/kg-day, whereas females had a LOAEL of 8 mg/kg-day. The NOAEL and LOAEL for renal tubular necrosis in male rats at 17 weeks were 63 and 125 mg/kg-day, respectively, while the NOAEL for renal tubular necrosis in females was 125 mg/kg-day. For epithelial necrosis of the nasal turbinates, the NOAEL and LOAEL in male and female rats at 17 weeks were 63 and 125 mg/kg-day, respectively.

In the NTP (1993) subchronic, B6C3F1 mouse study, which used the same protocol as the rat study above, 16 males that received 250 mg/kg-day 1,2,3- trichloropropane died, or were sacrificed moribund, by week 4. Among the females that received a dose of 250 mg/kg-day, seven died by week 2, and there was an additional death in week 17 (prior to the terminal sacrifice). One male mouse and six female mice were sacrificed at the 8-week interim evaluation. At the end of the 17-week evaluation, 2 out of 10 males at the highest dose were still alive, whereas 7 out of 10 females, tallied before the death of single female during week 17, survived the full evaluation period.

Mean body weight gain in male mice at 250 mg/kg-day was significantly reduced, although the overall mean weight gains among male and female mice at the various doses were similar. At week 17, a statistically significant ($p \leq 0.01$) increase in absolute and relative liver weights was observed in males and females that received a dose of ≥125 mg/kg-day. Mean relative liver weights were increased by 10 and 30% in males receiving 125 and 250 mg/kg-day, respectively, compared to controls (Table 4-6), while absolute liver weights were statistically significantly ($p \leq 0.05$) increased 14, 4, 22, and 25% at 32, 63, 125, and 250 mg/kg-day (Table 4-7). Mean relative liver weights were increased by 12 and 22% in females receiving 125 and 250 mg/kg-day, respectively, compared to controls (Table 4-6), while absolute liver weights were statistically significantly ($p \leq 0.05$) increased at 125 and 250 mg/kg-day by 24% at both doses (Table 4-7). Mean relative right kidney weights in female mice were statistically significantly ($p \leq 0.01$) decreased 17, 13, 11, 17, and 14% at 16, 32, 63, 125, and 250 mg/kg-day, respectively, after 120 days (Table 4-8), while absolute right kidney weights were statistically significantly ($p \leq 0.05$) decreased 13% at 250 mg/kg-day (Table 4-9). The changes in relative and absolute right kidney weights in male mice did not follow a clear dose-response pattern.

Table 4-6. Relative liver weights (mg organ weight/g body weight) and percent change in B6C3F$_1$ mice exposed to 1,2,3-trichloropropane by gavage for 17 weeks

Dose (mg/kg-day)	Males			Females		
	n	Mean ± SE	% Percentage change[a]	n	Mean ± SE	% Percentage change[a]
0	10	39.9 ± 1.0	-–	10	43.3 ± 1.1	-–
8	10	40.3 ± 0.8	1%	10	43.7 ± 0.8	1%
16	10	38.8 ± 0.6	-3%	7	39.9 ± 1.8	-8%
32	10	42.0 ± 0.5	5%	10	44.5 ± 0.5	3%
63	10	39.9 ± 0.8	0%	9	45.2 ± 0.7	4%
125	8	44.0 ± 1.3[c]	10%	9	48.7 ± 1.0[b]	12%
250	2	51.8 ± 1.0[c]	30%	6	52.7 ± 2.2[b]	22%

[a]Calculated as the percent change from the control mean.
[b]Showing statistically significant differences ($p \leq 0.01$) from the control group by Williams' or Dunnett's test.

Source: NTP (1993).

Table 4-7. Absolute liver weights (g) and percent change in B6C3F$_1$ mice exposed to 1,2,3-trichloropropane by gavage for 17 weeks

Dose (mg/kg-day)	Males			Females		
	n	Mean ± SE	Percentage% change[a]	n	Mean ± SE	Percentage% change[a]
0	10	1.06 ± 0.03	-–	10	0.898 ± 0.037	-–
8	10	1.14 ± 0.04	8%	10	0.899 ± 0.035	0%
16	10	1.09 ± 0.02	3%	7	0.938 ± 0.037	4%
32	10	1.21 ± 0.03 [b]	14%	10	0.947 ± 0.016	5%
63	10	1.10 ± 0.03 [b]	4%	9	0.994 ± 0.048	11%
125	8	1.29 ± 0.04 [c]	22%	9	1.118 ± 0.029[c]	24%
250	2	1.32 ± 0.00 [c]	25%	6	1.112 ± 0.053[c]	24%

[a]Calculated as the percent change from the control mean.
[b]Showing statistically significant differences ($p \leq 0.05$) from the control group by Williams' or Dunnett's test.
[c]Showing statistically significant differences ($p \leq 0.01$) from the control group by Williams' or Dunnett's test.

Source: NTP (1993).

Table 4-8. Relative kidney weights (mg organ weight/g body weight) and percent change in B6C3F$_1$ mice exposed to 1,2,3-trichloropropane by gavage for 17 weeks

	Males			Females		
Dose (mg/kg-day)	n	Mean ± SE	% Percentage change[a]	n	Mean ± SE	Percentage% change[a]
0	10	8.75 ± 0.25	–-	10	8.23 ± 0.18	–-
8	10	8.97 ± 0.15	3%	10	8.07 ± 0.17	-2%
16	10	8.85 ± 0.22	1%	7	6.86 ± 0.54[c]	-17%
32	10	9.19 ± 0.14	5%	10	7.18 ± 0.13[c]	-13%
63	10	7.86 ± 0.31	-10%	9	7.31 ± 0.23[c]	-11%
125	7	8.44 ± 0.58	-3%	9	6.87 ± 0.17[c]	-17%
250	2	8.83 ± 0.37	1%	6	7.04 ± 0.30[c]	-14%

[a]Calculated as the percent change from the control mean.
[b]Showing statistically significant differences ($p \leq 0.05$) from the control group by Williams' or Dunnett's test.
[c]Showing statistically significant differences ($p \leq 0.01$) from the control group by Williams' or Dunnett's test.

Source: NTP (1993).

Table 4-9. Absolute kidney weights (g) and percent change in B6C3F$_1$ mice exposed to 1,2,3-trichloropropane by gavage for 17 weeks

	Males			Females		
Dose (mg/kg-day)	n	Mean ± SE	% Percentage change[a]	n	Mean ± SE	% Percentage change[a]
0	10	0.232 ± 0.006	–-	10	0.170 ± 0.005	–-
8	10	0.253 ± 0.007	9%	10	0.166 ± 0.004	-2%
16	10	0.248 ± 0.008	7%	7	0.164 ± 0.010	-2%
32	10	0.265 ± 0.008	14%	10	0.153 ± 0.005	-10%
63	10	0.215 ± 0.008	-7%	9	0.160 ± 0.007	-6%
125	7	0.247 ± 0.011	6%	9	0.158 ± 0.005	-7%
250	2	0.225 ± 0.005	-3%	6	0.148 ± 0.006[b]	-13%

[a]Calculated as the percent change from the control mean.
[b]Showing statistically significant differences ($p \leq 0.05$) from the control group by Williams' or Dunnett's test.

Source: NTP (1993).

Mean relative heart weights in males were statistically significantly ($p \leq 0.05$) decreased 14, 14, 11, 19, 22 and 22% at 8, 16, 32, 63, 125, and 250 mg/kg-day, respectively, compared to controls. Absolute heart weights in males were statistically significantly ($p \leq 0.01$) reduced by 14–25% at ≥63 mg/kg-day and higher. Relative brain weights in male mice were statistically significantly ($p \leq 0.05$) decreased at 16–125 mg/kg-day, with the decrease ranging from 6–11%. Mean relative heart weights in females were statistically significantly ($p \leq 0.05$) reduced 19, 17,

11, 19, and 27% at 16, 32, 63, 125, and 250 mg/kg-day. Absolute heart weights in females were statistically significantly ($p \leq 0.01$) decreased 25% at 250 mg/kg-day. Absolute and relative brain weights were statistically significantly ($p \leq 0.01$) decreased 6–15% in females receiving 16 mg/kg-day or more.

Complete histopathological examinations were conducted on all control animals and mice receiving 125 or 250 mg/kg-day, and mice designated for the interim evaluation that died during the study were included in the group of animals examined at the end of the 17-week study. Forestomach and lung lesions in mice were observed at both the 8-week interim evaluation and the 17-week terminal evaluation (Table 4-10). At the 8-week evaluation, male mice displayed lung and forestomach lesions at 125 mg/kg-day in 1/8 and 6/8 mice, respectively, whereas female mice displayed lung and forestomach lesions at 250 mg/kg-day in 5/6 and 6/6 mice, respectively. Lung and forestomach lesions were found in the one mouse from the 250 mg/kg-day dose group that was examined at the 8-week interim sacrifice.

Table 4-10. Incidence of liver, lung, and forestomach lesions in male and female B6C3F$_1$ mice in a 17-week study

Endpoint	Dose (mg/kg-d)						
	0	8	16	32	63	125	250
Males							
Liver necrosis[a]	1/10	0/10	0/10	0/10	0/10	1/12	14/19[b]
Liver karyomegaly[a]	0/10	0/10	0/10	0/10	0/10	1/12	11/19[b]
Lung lesions-regenerative[a]	0/10	0/10	0/10	0/10	0/10	9/12[b]	14/19[b]
Hyperkeratosis of the forestomach[a]	0/10	0/10	0/10	0/10	0/10	7/12[b]	4/19
Females							
Liver necrosis[a]	0/10	0/10	0/10	0/10	0/9	1/12	5/14[c]
Liver karyomegaly[a]	0/10	0/10	0/10	0/10	0/9	0/12	1/14
Lung lesions-regenerative[a]	0/10	0/10	0/10	0/10	7/9[b]	10/12[b]	7/14[b]
Hyperkeratosis of the forestomach[a]	0/10	0/10	0/10	0/10	7/9[b]	9/12[b]	8/14[b]

[a]Incidence is the number of animals in which lesion was found/number of animals in which tissue was examined.
[b]Showing statistically significant differences ($p \leq 0.01$) from the control group by Fisher exact test.
[c]Showing statistically significant differences ($p \leq 0.05$) from the control group by Fisher exact test.

Source: NTP (1993).

Regenerative lung lesions were observed in 9/12 male mice and 10/12 female mice, and hyperkeratosis of the forestomach in 7/12 male mice and 9/12 female mice receiving 125 mg/kg-day 1,2,3-trichloropropane at the 17-week evaluation. Lung lesions in male and female mice at

250 mg/kg-day 1,2,3-trichloropropane were observed in 14/19 males and 7/14 females, while forestomach lesions in the same dose group were observed in 4/19 males and 8/14 females. At 63 mg/kg-day, female mice displayed lung lesions (7/9) and forestomach lesions (7/9). Hyperkeratosis of the forestomach was attributed to continued irritation resulting from the gavage treatments and was not considered to be life-threatening (Hazelton Laboratories, 1983b). Focal or multifocal desquamation of necrotic cells in the airways, flattened epithelium with loss of differentiated cells, and thickened epithelium with an increase in goblet cells (hyperplasia) were characteristic of the regenerative lung lesions (NTP, 1993).

Liver lesions were observed at both the 8-week interim and 17-week terminal sacrifice. At the 8-week evaluation, liver lesions were not observed in the only examined male mouse that received 250 mg/kg-day 1,2,3-trichloropropane, but hepatic necrosis was observed in 4/6 females that received this dose. Hepatic necrosis at the 8-week evaluation was observed in 6/8 males and 0/8 females that received 125 mg/kg-day. No liver lesions were observed in the 8-week controls.

At the 17-week evaluation, liver necrosis was observed in 14/19 males, most of which died prior to 8-week evaluation, and 5/14 females that received 250 mg/kg-day, and 1/10 male and 0/10 female controls (Table 4-10). Hepatocelluar degeneration associated with fatty change and karyomegaly was also observed in 11/19 males and 1/14 females of the high-dose group. Also at the 17-week evaluation, liver lesions in mice at the 125 mg/kg-day dose occurred in 1/12 males and 1/12 females.

Differences in clinical chemistry parameters in mice administered 1,2,3-trichloropropane for 17 weeks were not considered by the NTP investigators to be treatment related. Several statistically significant changes were observed among hematological parameters; however, these changes were not considered to be biologically significant and failed to follow a consistent dose-response pattern. Hematocrit values were statistically significantly decreased at week 8 in female mice that received 8 and 250 mg/kg-day. At week 17, hematocrit values were statistically significantly decreased in female mice that received 16, 32, 125, or 250 mg/kg-day 1,2,3-trichloropropane. In male mice, a statistically significant decrease in hematocrit values was observed only at week 8 in the 63 and 125 mg/kg-day treatment groups.

The 17-week, less-than-lifetime mouse study was conducted to determine appropriate doses for the 2-year study in mice (NTP, 1993), described later in this document. The dose-related increased liver weights were consistent with the histopathological results, while the hematological data were not associated with 1,2,3-trichloropropane administration (NTP, 1993). The NOAEL and LOAEL for regenerative lung lesions at the 17-week evaluation were 63 and 125 mg/kg-day for male mice and 32 and 63 mg/kg-day for female mice. The NOAEL and LOAEL for liver lesions at the 17-week evaluation were 63 and 125 mg/kg-day for both male

and female mice. A NOAEL of 63 mg/kg-day and LOAEL of 125 mg/kg-day for liver necrosis in male and female mice were identified.

Merrick et al. (1991) administered 1,2,3-trichloropropane in corn oil to Sprague-Dawley rats by gavage for 90 days. Groups of 10 males and 10 females received 0, 1.5, 7.4, 15, or 60 mg/kg-day. Animals that received 60 mg/kg-day exhibited a 14–19% reduction in mean body weight gain when compared to controls. Relative liver weights were statistically ($p \leq 0.05$) significantly increased after 90 days in animals that received 15 or 60 mg/kg-day, and relative kidney weights were statistically ($p \leq 0.05$) significantly increased after 90 days in males that received 60 mg/kg-day and females that received 15 or 60 mg/kg-day. Relative brain and testes weights were statistically ($p \leq 0.05$) significantly increased in males from the high dose group. Organ/body weight ratios were reported graphically.

Female rats that received 60 mg/kg-day 1,2,3-trichloropropane exhibited elevated ALT and AST levels. Mean serum concentrations for these two enzymes appeared to be approximately doubled in the high-dose females, but the actual magnitude of this effect could not reliably be estimated from the graphical presentation of the data. Hematological parameters, which included hemoglobin, hematocrit, and erythrocyte counts, were stated to be unremarkable (data not provided).

An increased incidence of inflammation-associated myocardial necrosis was observed in 6/10 males and 7/10 females that received 60 mg/kg-day 1,2,3-trichloropropane (Table 4-11). These lesions were marked by intense eosinophilic staining with necrotic cells containing granulated or vacuolated cytoplasm and associated macrophages or polynuclear leukocytes. Myocardial necrosis was also observed in a smaller number of animals from all other treatment groups; no myocardial lesions were observed in the control group.

Table 4-11. Incidence of myocardial necrosis in male and female Sprague-Dawley rats following 90-day 1,2,3-trichloropropane exposure

Endpoint	Sex	Dose				
		0	1.5	7.4	15	60
Myocardial necrosis	Male	0/10[a]	2/10	1/10	2/10	6/10
	Female	0/10	0/10	1/10	0/10	7/10

[a]Incidence is the number of animals in which lesion was found/number of animals in which tissue was examined.

Source: Merrick et al. (1991).

Bile duct hyperplasia was observed in the livers of one control male and 4/10 males and 8/10 females in the high-dose group. Other proliferative and neoplastic lesions observed in high-dose animals included a forestomach squamous cell papilloma, forestomach squamous cell hyperplasia, a hepatocellular adenoma, and plasma cell hyperplasia in the mandibular lymph

node, with the latter lacking a dose-response relationship in male rats (2/10, 0/10, 1/10, 0/10, 9/10) and displaying an increased dose-response relationship in female rats (1/10, 1/10, 2/10, 3/10, 5/10).

Inflammation-associated myocardial necrosis was seen in all male dose groups, and the LOAEL selected for this effect in male rats is 1.5 mg/kg-day. The NOAEL and LOAEL for bile duct hyperplasia were 15 and 60 mg/kg-day. The NOAEL and LOAEL for plasma cell hyperplasia in the mandibular lymph node was 1.5 and 7.4 mg/kg-day for male rats, while the LOAEL was 1.5 mg/kg-day for female rats.

Villeneuve et al. (1985) administered 1,2,3-trichloropropane in drinking water to Sprague-Dawley rats. Ten rats/sex/group were exposed 7 days/week for 90 days to 0, 1, 10, 100, or 1,000 mg/L. Drinking water contained 0.5% Emulphor to assure adequate solubility of the test chemical. Two groups of control animals were employed; one received tap water and the other received a 0.5% Emulphor solution. Body weight and water intake values were used for females in the 100 and 1,000 mg/L exposure groups to calculate delivered doses of 18 and 149 mg/kg-day, respectively. The delivered dose for males in the 1,000 mg/L exposure group was calculated to be 113 mg/kg-day. Clinical signs were monitored daily, and body weights were recorded weekly. At termination, the brain, liver, kidney, heart, and spleen were excised and weighed. A number of hematological and clinical chemistry parameters were evaluated in blood samples obtained at sacrifice. Each animal was subjected to a full necropsy, and tissues and organs were obtained for histopathologic examination. In addition, the specific activities of some mixed-function oxidases, including aniline hydroxylase and aminopyrine demethylase, were measured in liver homogenates.

Three animals died during the course of the study, but their deaths were not considered to be treatment-related. Mean body weight gain was reduced by approximately 30% in male and female rats that were exposed to 1,000 mg/L 1,2,3-trichloropropane, when compared with both controls ($p \leq 0.05$) and vehicle controls ($p \leq 0.05$). No difference in absolute organ weights was observed. Relative liver and kidney weights were reportedly increased in males that were exposed to 1,000 mg/L by 22 and 27%, respectively, when compared to vehicle controls. Mean relative liver weights were apparently increased 6 and 17% in females that were exposed to 100 and 1,000 mg/L, respectively. Mean relative kidney weights in females were reportedly increased 14 and 34% in the 100 and 1,000 mg/L treatment groups, respectively. Mean relative brain weights for the 1,000 mg/L exposure groups were reportedly increased by 21 and 23% in males and females, respectively. Mean serum cholesterol levels were apparently increased 55% in female rats exposed to 1,000 mg/L and no effect on cholesterol was observed in males. Hepatic aminopyrine demethylase activity was reportedly significantly increased in males and females that were exposed to 1,000 mg/L. Aniline hydroxylase activity was apparently significantly increased in males that received 1,000 mg/L.

Mild, but significant, histomorphological changes were reported in the liver, including anisokaryosis, accentuated zonation, and fatty vacuolation; kidney, including eosinophilic inclusions, pyknosis, nuclear displacement, fine glomerular adhesions and interstitial reactions and histologic proteinuria; and thyroid, including angular collapse of follicles, reduction in colloid density, and increased epithelial height, of both sexes of rats in the highest exposure group, although the number of affected animals was not reported. Biliary hyperplasia was also noted in females at 1,000 mg/L. Treatment with 1,2,3-trichloropropane also caused liver and kidney enlargement, as well as increased serum cholesterol levels and hepatic mixed-function oxidase activity. Mean lymphocyte and neutrophil counts were depressed by approximately 40% in male rats exposed to 1,000 mg/L, but were still within the historical reference range for Sprague-Dawley rats from the laboratory. A NOAEL of approximately 18 mg/kg-day and a LOAEL of 113-149 mg/kg-day was identified for both increased relative liver weight in males and females and serum cholesterol levels in females.

4.2.1.2. Chronic Studies

NTP (1993) conducted a 2-year study of the toxicity and carcinogenicity of 1,2,3-trichloropropane in F344/N rats, the data of which were also published in Irwin et al. (1995). The chemical was administered by corn oil gavage to 60 rats/sex/group. The rats were approximately 6 weeks old when the study was initiated. Rats received doses of 0 (vehicle control), 3, 10, or 30 mg/kg-day, and after 15 months (65–67 weeks), 8–10 rats per group were sacrificed to allow an interim evaluation of all toxicological parameters and histopathology. Due to high mortality in rats receiving 30 mg/kg at the interim evaluation, the remaining survivors in that group were sacrificed at week 67 (females) and week 77 (males). Due to the early termination of this treatment group, organ weights and hematology data were only obtained at the 15-month interim sacrifices.

Clinical observations were made twice daily, while body weights were recorded weekly for 13 weeks and then monthly (NTP, 1993). As mentioned above, up to 10 rats/group were sacrificed at month 15. From this interim sacrifice blood samples were obtained for hematology and clinical chemistry analyses. Hematological parameters included hematocrit, hemoglobin, and counts of erythrocytes, leukocytes, and differential leukocytes. Clinical chemistry parameters included the serum levels of ALT, AST, creatine kinase, lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), and 5'-nucleotidase. Whether at the planned sacrifice or as each rat died or became moribund, all rats were subjected to a gross necropsy, and a full range of organs and tissues was processed for histopathologic examination. Hematology, clinical chemistry, and tissue weight data were obtained only from rats that were sacrificed at the 15-month interim because the majority of treated animals died prior to the end of the study.

Survival rates were statistically significantly reduced ($p < 0.001$) in rats that received 10 or 30 mg/kg-day 1,2,3-trichloropropane (Table 4-12). An effect on survival was apparent, as the 10 and 30 mg/kg-day groups of rats died or were sacrificed moribund prior to or soon following the 15-month interim evaluation. The mortality in rats was attributed to cancer associated with chemical exposure (NTP, 1993).

Table 4-12. Survival rates and percent probability of survival for F344/N rats exposed to 1,2,3-trichloropropane by gavage for 2 years

F344/N rats				
Dose (mg/kg-d)	**Males**		**Females**	
0	34/49[a]	70[b]	31/50[b]	62[a]
3	32/50	64	30/49	62
10	14/48	30[c]	8/52	16[c]
30	0/52	0[c]	0/52	0[c]

[a]Animals surviving to study termination and number of animals in the treatment group. Accidental deaths were excluded and censored from survival analysis.
[b]Kaplan-Meier determinations of percent probability of survival at end of study.
[c]$p < 0.001$.

Source: NTP (1993).

In rats, the mean body weights of males and females receiving doses of 3 or 10 mg/kg-day, observed throughout the study, appeared similar to the mean body weights of corresponding control rats; the mean body weights of the high-dose males and females, however, appeared lower than the control rat body weights (NTP, 1993). Statistically significant increases ($p \leq 0.05$) in absolute liver weights were observed in male and female rats exposed for 15 months to doses of ≥3 mg/kg-day 1,2,3-trichloropropane. Mean relative liver weights were significantly increased by 15 and 28% in male rats that received doses of 10 or 30 mg/kg-day, respectively, when compared with controls (Table 4-13a). Mean relative liver weights in female rats that received doses of 10 or 30 mg/kg-day were increased 12 and 40%, respectively (Table 4-13a). Absolute liver weights were significantly increased by 10, 18, and 28% in male rats and 14, 16, and 34% in female rats that received doses of 3, 10, and 30 mg/kg-day, respectively (Table 4-13b).

Table 4-13a. Relative liver weights (mg organ weight/g body weight) and percent change in F344/N rats chronically exposed to 1,2,3-trichloropropane by gavage at the 15-month interim evaluation

F344/N						
		Males			Females	
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	31.2 ± 0.6	–	10	30.8 ± 0.8	–
3	10	33.1 ± 0.7	6%	10	30.9 ± 0.6	0%
10	10	36.0 ± 0.7[b]	15%	8	34.6 ± 1.0[b]	12%
30	8	39.8 ± 0.9[b]	28%	8	43.2 ± 0.7[b]	40%

[a]Percent change relative to control.
[b]$p \leq 0.01$.

Source: NTP (1993).

Table 4-13b. Absolute liver weights (g) and percent change in F344/N rats chronically exposed to 1,2,3-trichloropropane by gavage at the 15-month interim evaluation

F344/N						
		Males			Females	
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	14.27 ± 0.37	–	10	7.79 ± 0.13	–
3	10	15.63 ± 0.37[b]	10%	10	8.87 ± 0.31[c]	14%
10	10	16.8 ± 0.48[c]	18%	8	9.00 ± 0.28[c]	16%
30	8	18.23 ± 0.52[c]	28%	8	10.40 ± 0.37[c]	34%

[a]Percent change relative to control.
[b]$p \leq 0.01$.
[c]$p \leq 0.05$ by Williams' or Dunnett's test.

Source: NTP (1993).

Statistically significant increases ($p \leq 0.05$) in absolute right kidney weights were observed in male rats exposed for 15 months to doses of ≥3 mg/kg-day 1,2,3-trichloropropane and female rats exposed to doses of ≥10 mg/kg-day. Mean relative kidney weights in males from these treatment groups were increased by 4, 10 and 29%, respectively (Table 4-14a). Mean relative kidney weights of females in the 10 and 30 mg/kg-day treatment groups were increased by 8 and 31% (Table 4-14a). Absolute kidney weights were significantly increased by 8, 12, and 30% in male rats that received doses of 3, 10, and 30 mg/kg-day, and significantly increased by 11 and 24% in female rats that received doses of 10 and 30 mg/kg-day (Table 4-14b).

Table 4-14a. Relative right kidney weights (mg organ weight/g body weight) and percent change in F344/N Rats chronically exposed to 1,2,3-trichloro-propane by gavage at the 15-month interim evaluation

F344/N						
		Males			Females	
Dose (mg/kg-day)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	2.96 ± 0.04	–	10	3.08 ± 0.07	–
3	10	3.09 ± 0.09	4%	10	2.93 ± 0.07	-5%
10	10	3.25 ± 0.05[b]	10%	8	3.34 ± 0.06[c]	8%
30	8	3.82 ± 0.05[b]	29%	8	4.04 ± 0.12[b]	31%

[a]Percent change relative to control.
[b]$p \leq 0.01$.
[c]$p \leq 0.05$ by Williams' or Dunnett's test.

Source: NTP (1993).

Table 4-14b. Absolute right kidney weights (grams) and percent change in F344/N Rats chronically exposed to 1,2,3-trichloropropane by gavage at the 15-month interim evaluation

F344/N						
		Males			Females	
Dose (mg/kg-day)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	1.35 ± 0.03	–	10	0.786 ± 0.015	–
3	10	1.46 ± 0.04[b]	8%	10	0.839 ± 0.023	7%
10	10	1.51 ± 0.03[c]	12%	8	0.869 ± 0.019[b]	11%
30	8	1.75 ± 0.05[c]	30%	8	0.971 ± 0.034[c]	24%

[a]Percent change relative to control.
[b]$p \leq 0.05$ by Williams' or Dunnett's test.
[c]$p \leq 0.01$.

Source: NTP (1993).

The data for clinical chemistry parameters was sporadic, with ALT and 5'-nucleotidase levels statistically ($p \leq 0.05$) significantly decreased 31 and 13%, respectively, in males that received 30 mg/kg-day.

Treatment-related effects were detected among the hematological parameters in rats; however, the effects were not considered to be biologically relevant. Rats that received 30 mg/kg-day displayed mean hematocrit values that were statistically ($p \leq 0.05$) significantly decreased by 5 and 7% for males and females, respectively, when compared with controls. The

mean hemoglobin concentration was decreased by 4% in male rats that received either 3 ($p \leq 0.01$) or 30 ($p \leq 0.05$) mg/kg-day. Both males and female rats in the high dose group had statistically ($p \leq 0.01$) significantly elevated counts of leukocytes and segmented neutrophils, but not in the 10 mg/kg-day group. NTP (1993) stated that the decreased hematocrit may have been associated with depressed erythropoeisis or with blood loss from neoplasms in the forestomach or oral mucosa, and that the increase in leukocytes was likely due to inflammation associated with the chemical-induced neoplasms (NTP, 1993).

At the 2-year evaluation, hepatocellular necrosis was observed in 1/49 and 4/52 female rats at 3 and 30 mg/kg-day, respectively. Hepatocellular necrosis was not observed in male rats. In addition, a dose-dependent increase in bile duct hyperplasia was observed in male rats at 30 mg/kg-day. Nonneoplastic effects were also observed in the forestomach, kidney, and pancreas of male and female rats at the 2-year evaluation. The incidence of basal cell and squamous hyperplasia of the forestomach was increased at 3, 10, and 30 mg/kg-day in both males (28/50, 13/49, and 6/52) and females (5/50, 8/49, and 7/52) (NTP, 1993). A dose-dependent increase in the incidence of renal tubule hyperplasia was observed in males (1/50, 21/49, and 29/52) exposed to 3, 10, or 30 mg/kg-day and in females (2/47, 3/52, and 10/51) exposed to 3, 10, or 30 mg/kg-day (NTP, 1993). Increased severity of nephropathy was also observed in males at 10 and 30 mg/kg-day, with mean severity scores of 2.6 and 2.4, respectively, compared to the control and low dose, with mean severity scores of 2.0. In addition, a dose-dependent increase in acinar hyperplasia of the pancreas was observed at 3, 10, and 30 mg/kg-day in male (44/50, 46/49, and 48/52) and in female rats (14/49, 24/52, and 9/52) (NTP, 1993).

An increase in the incidence of forestomach tumors was observed in all rat treatment groups (Table 4-15), regardless of sex. However, the incidences of forestomach neoplasms were generally higher in males than in females at the same dose levels. All male treatment groups also had increased incidence of pancreatic tumors (Table 4-15). Male and female rats that received doses of ≥10 mg/kg-day 1,2,3-trichloropropane had an increase in the incidence of oral cavity tumors (Table 4-15). In each male group that received doses of ≥10 mg/kg-day, an increased incidence of renal tumors was observed. An increase was observed in females at both 10 and 30 mg/kg-day for the clitoral gland tumors and at the 10 and 30 mg/kg-day for mammary gland tumors (Table 4-15). In the 30 mg/kg-day treatment group, an increased incidence of Zymbal's gland tumors was observed in females and an increased incidence of preputial gland tumors was observed in males at 30 mg/kg-day (Table 4-15). Forestomach tumors were described in the NTP (1993) report as follows:

> The masses were squamous cell papillomas or squamous cell carcinomas arising from the stratified squamous cell epithelium of the forestomach. Multiple squamous cell papillomas or carcinomas often occurred in the same rat, and in

some rats, the neoplasms were so extensive that it was difficult to discern if they represented a single neoplasm or the confluent growth of multiple neoplasms.

Table 4-15. Incidence of neoplasms in F344/N rats chronically exposed to 1,2,3-trichloropropane by gavage

	Tumor incidence[a]							
	Males (mg/kg-d)				Females (mg/kg-d)			
Tissue site/tumor type	0	3	10	30	0	3	10	30
Oral cavity								
Papillomas or carcinomas	1/60	4/60	19/59[b]	43/60[b]	1/60	6/59	28/60[b]	37/60[b]
Forestomach								
Papillomas or carcinomas	0/60	35/60[b]	46/59[b]	51/60[b]	0/60	17/59[b]	42/59[b]	27/60[b]
Pancreas (acinar)								
Adenomas or adenocarcinomas	5/60	21/60[b]	37/59[b]	31/60[b]	0/60	0/59	2/60	0/60
Kidney (renal tubules)								
Adenomas or adenocarcinomas	0/60	2/60	20/59[b]	26/60[b]	0/60	0/57	0/60	1/59
Preputial gland								
Adenomas or carcinomas	5/59	6/57	9/59	17/58[c]	–	–	–	–
Clitoral gland								
Adenomas or carcinomas	–	–	–	–	5/56	11/56	18/58[b]	17/59[c]
Mammary gland								
Adenocarcinomas	–	–	–	–	1/60	6/59	12/60[b]	22/60[b]
Zymbal's gland								
Carcinomas	0/60	0/60	0/59	3/60	0/60	1/59	0/60	4/60[c]

[a]Values are pooled results from the outcome of histopathologic examinations of animals at the interim and terminal sacrifices.
[b]$p < 0.001$ by life table or logistic regression test.
[c]$p < 0.05$ by life table or logistic regression test.

Source: NTP (1993).

Forestomach tumors were accompanied by an increased incidence of focal hyperplasia of the stratified squamous cell epithelium. The hyperplasia, squamous cell papilloma, and squamous cell carcinoma of the forestomach were said to constitute a morphological continuum and the squamous cell papillomas and carcinomas were noted to be similar to those of the oral mucosa (NTP, 1993).

The NOAEL and LOAEL for relative liver weight change in male and female rats is 3 and 10 mg/kg-day, respectively, while the LOAEL for absolute liver weight change in male and female rats was 3 mg/kg-day. The NOAEL and LOAEL for relative right kidney weight in male and female rats were 3 and 10 mg/kg-day, respectively, while the LOAEL for absolute right kidney weight in male rats was 3 mg/kg-day and the NOAEL and LOAEL in female rats were 3

and 10 mg/kg-day, respectively. Tumors were evident in the oral cavity, forestomach, pancreas, kidney, and Zymbal's gland of male and female rats, along with preputial gland tumors in males and clitoral gland and mammary gland tumors in females.

NTP (1993) conducted a 2-year study of the toxicity and carcinogenicity of 1,2,3-trichloropropane in B6C3F1 mice. The chemical was administered by corn oil gavage to 60 mice/sex/group, and the mice were approximately 6 weeks old when the study began. Mice were treated with 0 (vehicle control), 6, 20, or 60 mg/kg-day, and after 15 months (65–67 weeks), 8–10 mice per group were sacrificed to allow an interim evaluation of all toxicological parameters and histopathology. Due to high mortality in the mice receiving 60 mg/kg-day, surviving mice were evaluated at week 73 (females) and week 79 (males). Due to the early termination of this treatment group, organ weights and hematology data were only obtained at the 15-month interim sacrifices.

Clinical observations were made twice daily, while body weights were recorded weekly for 13 weeks and then monthly (NTP, 1993). As mentioned above, up to 10 mice/group were sacrificed at month 15. From this interim sacrifice, blood samples were obtained for hematology and clinical chemistry analyses. Hematological parameters included hematocrit, hemoglobin, and counts of erythrocytes, leukocytes, and differential leukocytes. Clinical chemistry parameters included the serum levels of ALT, AST, creatine kinase, LDH, SDH, and 5'-nucleotidase. Whether at the planned sacrifice or as each mouse died or became moribund, all mice were subjected to a gross necropsy, and a full range of organs and tissues was processed for histopathologic examination. Hematology, clinical chemistry, and organ weight data were obtained only from mice that were sacrificed at the 15-month interim because the majority of treated mice died prior to the end of the study.

Survival rates were statistically significantly reduced ($p < 0.001$) in mice that received doses of ≥6 mg/kg-day (Table 4-16). An effect on survival was apparent in all dose groups at the 15-month interim evaluation. The mortality in mice was attributed to cancer associated with chemical exposure (NTP, 1993).

Table 4-16. Survival rates and percent probability of survival for B6C3F$_1$ mice exposed to 1,2,3-trichloropropane by gavage for 2 years

Dose (mg/kg-d)	Males		Females	
0	42/52[a]	81[b]	41/50[a]	82[b]
6	18/51	36[c]	13/50	26[c]
20	0/54	0[c]	0/50	0[c]
60	0/56	0[c]	0/55	0[c]

[a]Animals surviving to study termination and number of animals in the treatment group. Accidental deaths were excluded and censored from survival analysis.
[b]Kaplan-Meier determinations of percent probability of survival at end of study.
[c]$p < 0.001$.

Source: NTP (1993).

In mice, final mean body weights were significantly decreased by 17 and 18% in males and females, respectively, after a dose of 60 mg/kg-day, when compared to controls. Mean relative liver weights were increased by 32% in males and 40% in females that received 60 mg/kg-day (Table 4-17a). Other significant changes in organ weights among mice that received this dose included increased relative kidney weights in females (21%) (Table 4-18a), and increased relative brain weights in males (20%) and females (25%). Absolute liver and right kidney weight changes were sporadic, and no consistent pattern of treatment-related effects was apparent (Tables 4-17b and 4-18b).

Table 4-17a. Relative liver weights (mg organ weight/g body weight) and percent change in B6C3F$_1$ mice chronically exposed to 1,2,3-trichloropropane by gavage

Dose (mg/kg-d)	Males			Females		
	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	38.9 ± 1.9	–	10	34.4 ± 0.8	–
6	9	36.2 ± 1.5	-7%	10	34.7 ± 1.1	1%
20	8	44.6 ± 6.2	15%	9	35.7 ± 0.6	4%
60	5	51.2 ± 4.8[b]	32%	5	48.3 ± 2.8[c]	40%

[a]Percent change relative to control.
[b]$p \leq 0.05$ by Williams' or Dunnett's test.
[c]$p \leq 0.01$.

Source: NTP (1993).

Table 4-17b. Absolute liver weights (g) and percent change in B6C3F$_1$ mice chronically exposed to 1,2,3-trichloropropane by gavage

		Males			Females	
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	1.72 ± 0.09	–	10	1.49 ± 0.03	–
6	9	1.63 ± 0.08	-5%	10	1.33 ± 0.03[b]	-11%
20	8	1.76 ± 0.19	2%	9	1.50 ± 0.04	1%
60	5	1.92 ± 0.14	12%	5	1.69 ± 0.18	13%

[a]Percent change relative to control.
[b]$p \leq 0.05$ by Williams' or Dunnett's test.

Source: NTP (1993).

Table 4-18a. Relative right kidney weights (mg organ weight/g body weight) and percent change in B6C3F$_1$ mice chronically exposed to 1,2,3-trichloropropane by gavage

		Males			Females	
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	8.0 ± 0.25	–	10	4.99 ± 0.09	–
6	9	7.67 ± 0.41	-4%	10	5.27 ± 0.14	6%
20	8	7.81 ± 0.18	-2%	9	5.19 ± 0.14	4%
60	5	8.4 ± 0.59	5%	5	6.02 ± 0.11[b]	21%

[a]Percent change relative to control.
[b]$p \leq 0.01$.

Source: NTP (1993).

Table 4-18b. Absolute right kidney weights (g) and percent change in B6C3F$_1$ mice chronically exposed to 1,2,3-trichloropropane by gavage

		Males			Females	
Dose (mg/kg-d)	n	Mean ± SE	Percentage change[a]	n	Mean ± SE	Percentage change[a]
0	10	0.353 ± 0.011	–	10	0.217 ± 0.006	–
6	9	0.344 ± 0.019	-3%	10	0.203 ± 0.006	-6%
20	8	0.314 ± 0.013	-11%	9	0.217 ± 0.006	0
60	5	0.317 ± 0.022	-10%	5	0.210 ± 0.015	-3%

[a]Percent change relative to control.

Source: NTP (1993).

In mice, creatine kinase was statistically ($p \leq 0.05$) significantly elevated 235% in males that received 60 mg/kg-day, and SDH was statistically ($p \leq 0.05$) significantly elevated 72% in females that received the same dose. However, clinical chemistry differences between dose groups and control animals were not considered to be directly related to 1,2,3-trichloropropane administration (NTP, 1993).

Treatment-related effects were detected among the hematological parameters, but the effects were indirectly related 1,2,3-trichloropropane toxicity. Mean hematocrit values were decreased by 5 and 4% in male and female mice, respectively, that received 20 mg/kg-day. Mean hematocrit values were statistically ($p \leq 0.01$) decreased by 10 and 11% in males and females, respectively, that received 60 mg/kg-day. Similar statistically ($p \leq 0.01$) significant dose dependent changes in hemoglobin concentration and the number of erythrocytes were observed in female mice that received doses of 20 or 60 mg/kg-day. Female mice in the high-dose group also had statistically ($p \leq 0.01$) significantly elevated numbers of leukocytes, segmented neutrophils, and lymphocytes. NTP stated that the decreased hematocrit may be associated with depressed hematopoeisis or to blood loss from neoplasms in the forestomach, and the increased number of leukocytes was likely due to inflammation associated with the chemically-induced neoplasms (NTP, 1993).

At the 2-year evaluation, an increase in hepatocellular necrosis was observed in male mice (1/52, 2/51, 11/54, and 8/56) and female mice (1/50, 6/50, 5/51, and 10/55) in the vehicle control, 6, 20, and 60 mg/kg-day, respectively (NTP, 1993). In addition, a dose-dependent increase in eosinophilic foci was observed in the livers of both males and females. Nonneoplastic effects were also observed in the forestomach of male and female mice at the 15-month and 2-year evaluation. The incidence of squamous hyperplasia in the forestomach increased at 3, 10, and 30 mg/kg-day in male (29/51, 27/54, and 34/56) and female mice (15/49, 14/51, and 31/55), respectively (NTP, 1993).

In mice, the sites of statistically ($p \leq 0.001$) significant neoplasm formation for both sexes were the forestomach and liver (Table 4-19). Incidences of Harderian gland tumors were increased in males at 20 and 60 mg/kg-day, and the increase in incidence of oral cavity tumors was statistically significant in females at the highest dose. The incidence of uterine/cervical tumors in female mice was increased at 6, 20, and 60 mg/kg-day. The highest incidence of neoplasms and most marked dose-response effect for both species was in the forestomach. A 97% incidence of tumors of the forestomach was evident in male mice at the lowest dose tested (90% in females). These data suggest that an elevated incidence of tumors in the forestomach might occur at doses lower than those employed in this study. NTP (1993) noted that:

> In contrast to dosed rats, there were few neoplasms of the oral mucosa in dosed mice. Nevertheless, squamous cell carcinomas arising from the pharyngeal or lingual mucosa

were observed in one 20 mg/kg and five 60 mg/kg females, and none were seen in controls.

Table 4-19. Incidence of neoplasms in B6C3F$_1$ mice chronically exposed to 1,2,3-trichloropropane by gavage

Tissue site/tumor type	Tumor incidence[a]							
	Males (mg/kg-d)				Females (mg/kg-d)			
	0	6	20	60	0	6	20	60
Oral cavity								
Papillomas or carcinomas	0/60	0/59	0/60	2/60	1/60	0/60	2/60	5/60[b]
Forestomach								
Papillomas or carcinomas	3/60	57/59[c]	57/60[c]	59/60[c]	0/60	54/60[c]	59/60[c]	59/60[c]
Liver								
Adenomas or carcinomas	14/60	24/59[b]	25/60[b]	33/60[c]	8/60	11/60	9/60	36/60[c]
Harderian gland								
Adenomas	1/60	2/59	10/60[b]	11/60[b]	3/60	6/60	7/60	10/60
Uterine/cervical								
Adenomas or adenocarcinomas	–	–	–	–	0/50	5/50[b]	3/51[b]	9/54[b]

[a]Values are pooled results from the outcome of histopathologic examinations of animals at the interim and terminal sacrifices.
[b]$p < 0.05$ by life table or logistic regression test.
[c]$p < 0.001$ by life table or logistic regression test.

Source: NTP (1993).

The exophytic, or outward growing, papillary or nodular masses in the forestomach of mice were similar to those observed in rats. Moreover, the extensive neoplastic growth observed in rats was also noted in mice. The study authors suggested that the hyperplasia, squamous cell papilloma, and squamous cell carcinoma of the forestomach observed in the B6C3F1 mice were on a morphological continuum as in the F344/N rats (NTP, 1993).

The NOAEL and LOAEL for relative liver weight change in male and female mice were 20 and 60 mg/kg-day, respectively; however, the NOAEL for absolute liver weight change was 60 mg/kg-day in male and female mice. The NOAEL and LOAEL for relative right kidney weight change in female mice were 20 and 60 mg/kg-day, respectively; while the NOAEL in male mice for relative right kidney weight change was 60 mg/kg-day. The NOAEL for absolute right kidney weight was 60 mg/kg-day for both sexes. It should be noted that the high mortality associated with chemical exposure led to the early termination of the 20 and 60 mg/kg-day dose groups. Tumors were evident in the oral cavity, forestomach, liver, and Harderian gland of both male and female mice, and in the uterine/cervical tissue in females. The critical effect for noncancer data was weight change in the liver and right kidney, while the critical effect for the cancer data was tumor development in the aforementioned organs of mice.

4.2.2. Inhalation Exposure

4.2.2.1. Subchronic Studies

Johannsen et al. (1988) conducted a series of prechronic and subchronic inhalation studies in 7-week-old CD rats. The initial subchronic study was followed by a second subchronic study that used lower doses because lesions were observed in all three exposure groups in the initial bioassay.

In a range-finding study, five CD rats/sex/group were exposed in 1 m^3 stainless steel and glass chambers to nominal concentrations of 0, 100, 300, 600, or 900 ppm 1,2,3-trichloropropane vapor (0, 600, 1,800, 3,600, and 5,400 mg/m^3) 6 hours/day, 5 days/week, for up to 4 weeks. At the highest concentration, all but one of the rats died after a single exposure. Three animals exposed to 600 ppm and one exposed to 300 ppm died prior to study termination. Surviving rats exposed to 600 ppm trichloropropane became prostrated during exposure periods. Males that were exposed to 600 ppm initially lost weight, but returned to their pre-exposure weights by the end of the experiment. Females exposed to this concentration showed a similar pattern but did not regain the initial weights. Weight gain was statistically significantly reduced ($p \leq 0.05$) in rats exposed to 300 ppm and appeared depressed, but was not significantly different from controls for animals exposed to 100 ppm. Relative and absolute liver weights were statistically significantly elevated ($p \leq 0.05$) in males for all treatment groups and for females in the 300 and 600 ppm groups ($p \leq 0.05$) for relative liver weight and in the 300 ppm group for absolute liver weight. Absolute brain and kidney weights and organ/body ratios were increased in the 300 and 600 ppm treatment groups. Absolute ovary weights and organ/weight ratios were decreased in the 300 and 600 ppm groups, and absolute spleen weights and organ/weight ratios and absolute testis weights were decreased in the 600 ppm treatment group. The magnitude of change in body and tissue weights was not reported.

The results of the 4-week range finding study were used to establish target concentrations 0, 5, 15, or 50 ppm (0, 30, 90, or 300 mg/m^3) as the exposure concentrations for a 13-week study, with analytical concentrations of 4.5 ± 0.2, 15 ± 0.3, and 49 ± 1.0 ppm. Each exposure group contained 15 CD rats/sex. Blood samples were taken for clinical chemistry and hematological parameters at week 7 from controls and the animals that were exposed to 50 ppm, and at termination from all surviving animals. A gross pathological examination was conducted on all animals and the weights of all major organs were recorded. Portions of the major organs and tissues were processed for histopathologic examination. The results of these examinations are described in the following paragraphs.

There were no treatment-related deaths in the 13-week study. Daily observation of treated animals revealed a general, dose-dependent pattern of respiratory tract and conjunctival irritation, including red nasal discharge and excessive lacrimation. An increased incidence of yellow staining of the anogenital fur was also observed.

A number of statistically significant changes were reported for whole body and organ weights; however, the magnitudes of change in the body and tissue weights was not reported by Johannsen et al. (1988) but were provided by the initial investigating group, Biodynamics, Inc. (1979). Statistically significant reductions in terminal body weight were observed in females exposed to 15 (7%) and 50 (9%) ppm. No effect on body weight was observed in males. Mean absolute and relative liver weights (Table 4-20) were statistically significantly elevated 13-21% in the male rat exposure groups. Mean absolute liver weights were statistically significantly elevated 10% in females exposed to 50 ppm ($p \leq 0.01$), and relative liver weights were statistically significantly ($p \leq 0.01$) increased 8 and 20% in females at 15 and 50 ppm, respectively. Relative lung weights (Table 4-21) were also statistically significantly ($p \leq 0.01$) increased 14 and 13%, respectively, in female rats at doses of 15 and 50 ppm, although no effect was evident in male rats. The mean relative kidney weight of males exposed to 50 ppm was significantly increased approximately 10%.

Table 4-20. Absolute and relative liver weights and percent change in CD rats exposed to 1,2,3-trichloropropane by inhalation, 6 hours/day, 5 days/week, for 13 weeks

Dose (ppm)	n	Absolute[a]		Relative[b]	
Male					
0	15	13.8 ± 1.06	-–	3.14 ± 0.128	-–
5	15	16.7 ± 1.58 [c]	21%	3.56 ± 0.258 [c]	13%
15	15	16.3 ± 1.48 [c]	18%	3.57 ± 0.207 [c]	14%
50	14	16.4 ± 1.51 [c]	19%	3.79 ± 0.260 [c]	21%
Female					
0	15	10.6 ± 0.81	-–	3.4 ± 0.126	-–
5	15	10.9 ± 0.76	3%	3.6 ± 0.213	6%
15	15	10.7 ± 1.05	1%	3.7 ± 0.216 [c]	8%
50	15	11.7 ± 1.06 [c]	10%	4.1 ± 0.266 [c]	20%

[a] Mean ± SD.
[b] Percent increase relative to control.
[c] $p \leq 0.01$.

Source: Biodynamics, Inc. (1979).

Table 4-21. Absolute and relative lung weights and percent change in CD rats exposed to 1,2,3-trichloropropane by inhalation, 6 hours/day, 5 days/week, for 13 weeks

Dose (ppm)	n	Absolute		Relative	
Male					
0	14	1.49 ± 0.162[a]	-–	0.340 ± 0.029[a]	-–
5	15	1.62 ± 0.192	9%[b]	0.345 ± 0.036	1%[b]
15	15	1.58 ± 0.100	6%	0.347 ± 0.030	2%
50	14	1.51 ± 0.102	1%	0.351 ± 0.028	3%
Female					
0	15	1.27 ± 0.126[a]	-–	0.406 ± 0.031[a]	-–
5	15	1.31 ± 0.124	4%[b]	0.430 ± 0.040	6%[b]
15	15	1.34 ± 0.107	6%	0.461 ± 0.033[c]	14%
50	15	1.31 ± 0.129	3%	0.460 ± 0.051[c]	13%

[a]Mean ± SD.
[b]Percent increase relative to control.
[c]$p \leq 0.01$.

Source: Biodynamics, Inc. (1979).

A number of histopathologic lesions were observed (Table 4-22), including an increased incidence of mild to marked peribronchial lymphoid hyperplasia at 5, 15, and 50 ppm. The peribronchial lymphoid hyperplasia in the 15-ppm male rats was of equal severity to the 50-ppm group, but the hyperplasia in the 15-ppm female rats and that evident in the 5-ppm males and females were less severe. Hepatocellular hypertrophy in males at 5, 15, and 50 ppm appeared to be at mild centrilobular to midzonal levels, but was not evident in the highest dose group females. Treated females appeared to show a dose-dependent increase in extramedullary hematopoiesis of the spleen. Statistical analysis was not conducted on these results.

Table 4-22. Incidence of histopathologic lesions in CD rats exposed via inhalation to 1,2,3-trichloropropane, 6 hours/day, 5 days/week for 13 weeks

Response	0	0.5	1.5	5	15	50
Male rats (ppm via inhalation)						
Peribronchial lymphoid hyperplasia	0/15	0/15	0/15	6/15[a]	11/15[a]	10/15[a]
Hepatocellular hypertrophy	0/15	0/15	0/15	13/15	15/15	15/15
Hematopoiesis of the spleen	0/15	0/15	0/15	No data	No data	5/15
Female rats (ppm via inhalation)						
Peribronchial lymphoid hyperplasia	1/15	0/15	0/15	5/15	4/15	6/15[b]
Hepatocellular hypertrophy	0/15	0/15	0/15	No data	No data	0/15
Hematopoiesis of the spleen	5/15	0/15	0/15	7/15	9/15	13/15

[a] $p \leq 0.0001$, trend test conducted by EPA.
[b] $p \leq 0.001$, trend test comducted by EPA.

Sources: Biodynamics, Inc. (1979); Johannsen et al. (1988).

There were no significant dose-related changes in any of the hematological or clinical chemistry parameters evaluated (Johannsen et al., 1988).

The NOAEL and LOAEL for decreased terminal body weight in female rats were 5 and 15 ppm, respectively, while the NOAEL for decreased terminal body weight in male rats was 50 ppm. The LOAEL for increased absolute and relative liver weight in male rats was 5 ppm, while the NOAEL and LOAEL for increased absolute liver weight in female rats were 15 and 50 ppm, respectively, and the NOAEL and LOAEL for increased relative liver weight in females were 5 and 15 ppm, respectively. The NOAEL and LOAEL for increased relative lung weights in female rats is 5 and 15 ppm, respectively, and the NOAEL and LOAEL for increased relative kidney weights in males is 15 and 50 ppm, respectively. A LOAEL of 5 ppm was designated for peribronchial lymphoid hyperplasia in male CD rats, as well as for hepatocellular hypertrophy in male rats and hematopoiesis of the spleen in female rats.

The presence of lesions in animals from all exposure groups of the 13-week study prompted the initiation of a follow-up study using lower exposure concentrations (Johannsen et al., 1988). In the second 13-week study, the investigators employed a very similar experimental protocol with exposure concentrations of 0, 0.5, or 1.5 ppm (0, 3, or 9 mg/m^3). The protocol for the second study did not include urinalysis and the histopathological evaluation was limited to bone, brain, gonads, kidneys, liver, lungs, lymph nodes, nasal turbinates, and spleen in control and high-dose (1.5 ppm) rats. It also included two additional hematological and a few clinical chemistry parameters.

Small increases in mean absolute and relative ovarian weights were observed in females in the 1.5 ppm dose group, but microscopic results to support this as a treatment-related effect were not found and this effect was not observed in the previous 13-week study with doses up to

50 ppm. Treatment-related histopathological findings at 0.5 or 1.5 ppm were not observed in any tissue examined (Table 4-22).

In the follow-up study sporadic changes were observed in some hematological and clinical chemistry parameters, including apparently increased platelets in females exposed to 1.5 ppm for 7 weeks and increased fasting glucose levels in females exposed to 1.5 ppm for 13 weeks. In the absence of an apparent dose-response pattern these changes were considered by the investigators to be unrelated to the 1,2,3-trichloropropane exposures. All other hematology and clinical chemistry parameters measured were unremarkable and displayed no apparent effect from 1,2,3-trichloropropane exposure.

This investigation by Johannsen et al. (1988) identified a NOAEL of 1.5 ppm, with regards to body or organ weight changes and histopathological effects, such as those evident in the first study by Johannsen et al.

4.2.2.2. Chronic Studies

No studies were identified that examined the chronic toxicity of 1,2,3-trichloropropane via inhalation.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Studies

NTP (1990) conducted a reproduction and fertility assessment of 1,2,3-trichloropropane in CD-1 mice using the Reproductive Assessment by Continuous Breeding (RACB) protocol. This assessment consisted of four tasks/studies: (1) a range-finding study, (2) a continuous breeding study, (3) a determination of the affected sex, and (4) an offspring assessment. All treatments were administered by corn oil gavage.

In Task 1, mice (eight/sex/group) received 0, 12.5, 25, 50, 100, and 200 mg/kg-day for 14 days. No effect on weight gain or clinical signs of toxicity was observed. One male in the high dose group died. The results of this study were used to select the doses for Task 2.

Task 2 was a continuous breeding study in which 20 breeding pairs received 0, 30, 60, or 120 mg/kg-day for 126 days. Endpoints monitored for this task included clinical signs of toxicity, parental body weight, water consumption, fertility, litters/pair, live pups/litter, proportion of pups born alive, sex of live pups, and pup weights at birth. Pups were not monitored for physical abnormalities. The last litter (F_1) born during the holding period following the continuous breeding phase was reared by each dam until weaning, and was then used in the assessment of second generation fertility in Task 4.

The parental body weights, both male and female, were within 10% of the corresponding control values, except for the 120 mg/kg-day females, which exhibited an increase in body weight greater than 10%. Water consumption was significantly increased in weeks 6, 10, and

14; however, consumption was calculated per cage, and, up until week 14, male and female mice were housed in the same cage. At terminal necropsy, absolute and relative liver weights were statistically significantly increased in the 120 mg/kg-day male and female mice, but data analysis for the intermediate dose groups is unavailable.

Statistically significant reduction in fertility was evident at the 4th and 5th breedings (Table 4-23). A statistically significant ($p \leq 0.05$) reduction in fertility was evident from the decrease in the number of pregnancies per fertile mouse pair at the fourth breeding (89%), but not the fifth breeding (68%), at 60 mg/kg-day group, and the third, fourth, and fifth breedings (89, 68, and 42%, respectively) at 120 mg/kg-day. A dose-related decrease in fertility from the fourth to fifth breeding at 60 mg/kg-day was observed, but this decrease did not reach statistical significance. A statistically significant ($p \leq 0.05$) reduction in the number of live mouse pups/litter was observed, when compared with controls, in the second through the fifth breedings at the highest dose (120 mg/kg-day) and at the fifth breeding at 60 mg/kg-day (Table 4-23). The 120 mg/kg-day group displays a dose- and time-related decrease in the number of live pups/litter. However, the decrease in the number of pups/litter in the fifth breeding at 60 mg/kg-day is statistically significant due to an increase in the number of pups/litter in the controls during the fifth breeding. The number of live pups/litter increases from the fourth to the fifth breeding at 60 mg/kg-day and does not follow a dose- or time-related response.

Table 4-23. Fertility indices and number of live pups/litter in breeding pairs of CD-1 mice exposed to 1,2,3-trichloropropane by gavage

	Dose group (mg/kg-d)							
	0		30		60		120	
Litter	Fertility[a]	Live pups/litter	Fertility[a]	Live pups/litter	Fertility[a]	Live pups/litter	Fertility[a]	Live pups/litter
1st	38/38 (100)	11.1 ± 0.6	18/18 (100)	10.3 ± 1.0	19/19 (100)	10.5 ± 0.9	18/19 (95)	11.5 ± 0.8
2nd	38/38 (100)	12.6 ± 0.4	18/18 (100)	10.8 ± 1.2	19/19 (100)	10.7 ± 1.1	18/19 (95)	5.2 ± 0.6[b]
3rd	38/38 (100)	12.4 ± 0.5	18/18 (100)	11.3 ± 1.0	19/19 (100)	11.0 ± 1.2	17/19[b] (89)	6.7 ± 1.0[b]
4th	38/38 (100)	11.8 ± 0.6	17/18 (94)	11.2 ± 0.7	17/19[b] (89)	9.9 ± 1.0	13/19[b] (68)	2.9 ± 0.6[b]
5th	33/38 (87)	12.8 ± 0.4	14/18 (78)	12.1 ± 0.7	13/19 (68)	11.3 ± 0.8[b]	8/19[b] (42)	2.5 ± 0.6[b]

[a]Fertility data are the number of fertile pairs/number of cohabiting pairs (percent fertile).
[b]Significantly different ($p < 0.05$) from the control group by Cochran-Armitage trend test.

Source: NTP (1990).

The cumulative days to litter were statistically significantly longer than control values for the third breeding (12%) at 60 mg/kg-day and the fourth (6.5%) and fifth (3.3%) breedings at 120 mg/kg-day. The proportion of male pups born alive in the fifth breedings appeared to decrease in a dose-dependent manner. The proportion of males in the fifth breeding of the 120

mg/kg-day treatment group was 0.27 versus 0.53 for the controls, with proportions in the fifth breeding at 30 and 60 mg/kg of 0.43 and 0.42, respectively. Live pup weights were slightly, statistically significantly increased at the highest dose, 120 mg/kg-day. However, when adjusted for average litter size ± standard error, the increase in live pup weights was eliminated in male pups at 120 mg/kg-day, and a decrease, although not statistically significantly, in female pups and combined pups was visible.

Task 3, a 1-week crossover mating trial, was conducted with the same adult mice from the control and 120 mg/kg-day treatment groups from Task 2. Three groups of 20 breeding pairs (control males × control females, control males × high-dose females, and control females × high-dose males) were evaluated for fertility and the presence of morphological and histopathologic changes to the reproductive organs. At termination, F_0 mice were necropsied and major organs were excised and weighed. Treated F_0 mice of both sexes displayed statistically significantly ($p \leq 0.05$) increased absolute and relative liver weights, 19 and 20% in males and 25 and 22% in females, respectively, compared with controls. The weights of the right epididymis and cauda epididymis in F_0 males were statistically significantly ($p \leq 0.05$) lower, 5 and 8%, respectively, than those of controls. The absolute kidney weights of treated F_0 females were statistically significantly ($p \leq 0.05$) reduced (5%) compared with controls. All F_0 males were evaluated for epididymal sperm parameters, and no differences in motility, count, or abnormal sperm numbers were detected. The 120 mg/kg-day treated females delivered fewer live pups (~50%) than untreated females, with decreased body weight (9%) in male offspring and fewer live male pups per litter than controls.

In Task 4, members of the last set of litters (F_1) to be born in Task 2 were reared, weaned, and allowed to reach sexual maturity before being paired individually with a member of the opposite sex from a separate litter but within the same treatment group. Breeding pairs were assessed for the same mating endpoints as in Task 2 and the same terminal endpoints as in Task 3. There were statistically significant ($p \leq 0.05$) decreases, 78 and 43% of controls, in the indices for mating (number of females with plug/number of cohabiting pairs) and fertility (number of fertile pairs/number of females with plug), respectively, for the 120 mg/kg-day group. The estrous cycles for F_1 females of all treatment groups were statistically significantly longer than in controls ($p \leq 0.05$), and may be associated with an increase in the infertile period of metestrus.

At necropsy, F_1 male and female terminal body weights were statistically significantly ($p \leq 0.05$) increased, 5–11%, in the 60 and 120 mg/kg-day groups. There was a statistically significant ($p \leq 0.05$) increase, 17–50%, in absolute and relative liver weights in males and females at 60 and 120 mg/kg-day, and a statistically significant ($p \leq 0.05$) increase, 6–27%, in absolute kidney weights in male and female mice at 60 and 120 mg/kg-day. A statistically significant ($p \leq 0.05$) 34% decrease in absolute right ovary weight was evident at the highest dose level, with a statistically significant ($p \leq 0.05$) decrease in relative ovary weight at 60 and

120 mg/kg-day of 15 and 39%, respectively. Histopathological examination of tissues from 10 females from each group revealed no difference between the groups in the incidence and severity of lesions.

Based on the decreased number of fertile pairs and live pups/litter among the cohabiting pairs in the 120 mg/kg-day treatment group, the investigators concluded that 1,2,3-trichloropropane treatment could impair fertility and reproduction (NTP, 1990). In Task 2, a NOAEL and LOAEL of 30 and 60 mg/kg-day, respectively, were identified for a decrease in the number of pregnancies per fertile mouse pair at the fourth and fifth breeding. A reduction in the number of live mouse pups/litter was observed across doses in the second through the fifth breedings from breeding pairs at the highest dose (120 mg/kg-day) and at the fifth breeding at 60 mg/kg-day; which provided a NOAEL of 30 mg/kg-day and a LOAEL of 60 mg/kg-day at the fifth breeding and a NOAEL of 60 mg/kg-day and LOAEL of 120 mg/kg-day for the first through the fourth breedings. The LOAEL for the decreased proportion of males in the fifth breeding was 30 mg/kg-day. Task 3, a cross-over mating trial, identified a LOAEL of 120 mg/kg-day in treated females for decreased pups/litter, decreased male pup weight, and decreased proportion of males/litter. A NOAEL and LOAEL of 60 and 120 mg/kg-day, respectively, for decreased fertility and mating indices were identified from Task 4. A LOAEL of 30 mg/kg-day for lengthened estrous cycle was also apparent.

4.3.2. Inhalation Studies

Johannsen et al. (1988) reported the results of a single-generation reproductive study using 10 male and 20 female CD rats/group conducted in two dosing studies. In the first study, animals in 1 m^3 stainless steel and glass chambers were exposed to target vapor concentrations of 0, 5, or 15 ppm (0, 30, or 90 mg/m^3), with measured concentrations of 4.6 ± 0.2 and 15 ± 0.2, 1,2,3-trichloropropane 6 hours/day, 5 days/week, for a 10-week pre-mating period, a mating period (not to exceed 40 days), and for gestation days 0-14 for females. Male and female rats were housed in a ratio of 1:2, respectively, nightly during the mating period. Females that were not impregnated after the 10 days were paired with a different male for 10 days until pregnant. In the second study, the same numbers of rats were exposed to target concentrations of 0, 0.5, or 1.5 ppm (0, 3, or 9 mg/m^3) using a similar protocol (mating period not to exceed 30 days). Females delivered and all litters were weaned on postnatal day 21. Animals were examined daily for clinical signs and received a weekly physical exam when body weights were recorded, with mated females weighed through gestation and lactation. Pups were weighed at birth, on postnatal days 4 and 14, and when they were sacrificed on postnatal day 21. At termination, all F_0 parents were necropsied, and sections of their reproductive organs were processed for histopathologic examination.

In the first study, females exposed to 15 ppm had lower body weights during gestation and lactation, although weight gains were consistent with the controls. Both sexes exposed to 15 ppm exhibited decreases in weight and weight gain during the premating period of exposure. All groups of female rats exhibited low mating performance, 16 females out of 20 mated at 5 ppm and 10 females out of 20 mated at 15 ppm, compared with 37 females out of 40 mated in the control group (Table 4-24); although fewer females in the high concentration group mated, statistical significance was not demonstrated by Johannsen et al. (1988). The decrease in the number of females that mated was statistically significant ($p < 0.02$) at 15 ppm compared to the control group (Fisher Exact test conducted by EPA). The decrease in mating performance was also statistically significant ($p = 0.01$) for linear trend (χ^2 test conducted by EPA). Male rats in both treated and control groups displayed apparently lower mating performance, 4/10, 6/10 and 3/10 for control, 5 ppm and 15 ppm groups, respectively, but not statistically significant mating indices. Fertility indices were unaffected by trichloropropane exposure. There was no treatment-related effect on litter or pup data. Histopathological evaluation of the testes, epididymis, and ovaries did not identify any treatment-related changes.

Table 4-24. Decreased mating performance in female CD rats following inhalation of 1,2,3-trichloropropane for 6 hours/day, 5 days/week, for a 10-week pre-mating period, a mating period (not to exceed 40 days), and gestation days 0–14

	Dose (ppm)				
	0	**0.5**	**1.5**	**5**	**15**
Mated	37	19	20	16	10[a]
Failed to mate	3	1	0	4	10[a]
n	40	20	20	20	20

[a]Statistically significant ($p < 0.02$) compared to control group in the Fisher Exact test conducted by EPA.

In the second study, adverse effects on mating performance and fertility indices due to 1,2,3-trichloropropane were not observed (Table 4-24). Lesions of the testes, epididymides, and ovaries were not evident. Consistent or obviously treatment-related reproductive effects were not observed in any of the experimental groups in either generation.

This study identified a NOAEL of 15 ppm for low mating performance and fertility indices. For decreased body weight in females during gestation and lactation and for decreased body weights and weight gain in both sexes during the premating period, a NOAEL of 5 and LOAEL of 15 were identified.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Data

In the rat, oral LD_{50} values ranging from 150 to 500 mg/kg 1,2,3-trichloropropane have been reported (Greim, 1993). A 4-hour LC_{50} of approximately 500 ppm (3,000 mg/m^3) has been determined for rats and mice (Greim, 1993). McOmie and Barnes (1949) identified an LC_{50} of approximately 30 ppm in mice exposed to vapor for 20 minutes, while Reyna (1987) could not determine an LC_{50} in Sprague-Dawley rats, but suggested that the LC_{50} was greater than 4.8 mg/L air.

Lag et al. (1991) conducted an acute study in rats that investigated the nephrotoxicity of short-chain halogenated alkanes. 1,2,3-Trichloropropane was administered via a single, i.p. injection to five male MOL:WIST rats per dose group at doses of 147, 294, and 441 mg/kg. After 48 hours, the rats were weighed and euthanized, and their kidneys were removed, weighed, and preserved. Dose-dependent increases in mortality, kidney/body weight ratio, and urea excretion were evident. Histopathological examination detected moderate kidney necrosis in one of the two surviving rats at the highest dose level tested.

4.4.2. Short-term Toxicity Data

Miller et al. (1987a, b) conducted two inhalation studies of male and female F344/N rats and B6C3F1 mice. Following the results of the first investigation, the exposures were decreased in the second bioassay. These unpublished studies were submitted to the EPA under the Toxic Substances Control Act (TSCA). In the first rat and mouse study (Miller et al., 1987a), 5/sex/group were exposed to target concentrations of 0, 10, 30, and 100 ppm 6 hours/day, 5 days/week, for 9 days, with measured concentration of 0, 13 ± 0.5, 40 ± 0.4, or 132 ± 0.6 ppm (0, 78, 241, and 796 mg/m^3). Endpoints evaluated included body weight, urinalysis, clinical chemistry, hematology, and gross pathology and histopathology.

Rats in the high-exposure group were less active than controls and did not eat or drink normally after treatment. An exposure and time-dependent reduction in weight gain was observed in treated rats. Terminal body weights in rats were statistically significantly ($p \leq 0.05$) decreased 14 and 10% in males and females, respectively, in the high-exposure group when compared with controls. In male and female rats exposed to 40 ppm, relative liver weights were statistically significantly ($p \leq 0.05$) increased 7 and 9%, respectively. At 132 ppm, absolute and relative liver weights were statistically significantly ($p \leq 0.05$) increased 10 and 21%, respectively, in males and 27 and 42%, respectively, in females.

The concentrations of serum albumin and total protein in male rats and serum albumin in female rats were statistically significantly ($p \leq 0.05$) increased in the high-exposure group, but were not considered by the investigators to be toxicologically significant. No exposure-related changes were observed among any of the hematology parameters, although a statistically

significant ($p \leq 0.05$) increase (6%) in packed cell volume (hematocrit) and hemoglobin (5%), were noted in female rats that were exposed to 40 ppm.

Several pathological changes in rats were associated with 1,2,3-trichloropropane exposure. Gross observation suggested a decrease in thymus size among rats, but the study authors considered this observation to be "secondary to stress." Very slight hepatocellular necrosis and very slight depletion of lymphoid elements in the spleen were observed in all male rats exposed to 132 ppm.

Miller et al. (1987a) also noted a dose-dependent increase in incidence and severity of degeneration and decreased thickness of the olfactory epithelium in the nasal turbinates of rats exposed to 13, 40, or 132 ppm 1,2,3-trichloropropane (Table 4-25). Inflammation in the olfactory epithelium was also evident in rats exposed to 13, 40, or 132 ppm 1,2,3-trichloropropane, and was accompanied by the exudation of inflammatory cells into the nasal cavity lumen (Table 4-26).

Table 4-25. Incidence and severity of decreased thickness and degeneration of the olfactory epithelium in the nasal turbinates of F344/N rats exposed via inhalation to 1,2,3-trichloropropane

	Males (ppm)							Females (ppm)						
Severity[a]	**0**	**1**	**3**	**10**	**13**	**40**	**132**	**0**	**1**	**3**	**10**	**13**	**40**	**132**
Very slight	0	0	5	5	0	0	0	0	0	5	5	1	1	0
Slight	0	0	0	0	5	0	0	0	0	0	0	4	1	0
Moderate	0	0	0	0	0	5	0	0	0	0	0	0	2	2
Severe	0	0	0	0	0	0	5	0	0	0	0	0	1	3
Combined incidence	0	0	5	5	5	5	5	0	0	5	5	5	5	5
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5

[a]Decreased thickness, bilateral, and multifocal, or degeneration, bilateral, and multifocal.

Sources: Miller et al. (1987a, b).

Table 4-26. Incidence and severity of inflammation of the olfactory epithelium in the nasal turbinates of F344/N rats exposed via inhalation to 1,2,3-trichloropropane

	Males (ppm)							Females (ppm)						
Severity[a]	0	1	3	10	13	40	132	0	1	3	10	13	40	132
Very slight	3	1	0	5	2	0	0	3	3	2	5	4	1	0
Slight	0	0	0	0	3	4	1	0	0	0	0	1	4	1
Moderate	0	0	0	0	0	1	4	0	0	0	0	0	0	4
Combined incidence	3	1	0	5	5	5	5	3	3	2	5	5	5	5
Exudate into lumen	0	0	0	2	2	3	1	0	0	0	2	4	4	5
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5

[a]Inflammation, bilateral, and multifocal.

Sources: Miller et al. (1987a, b).

Mice in the high-exposure group were less active than controls and did not eat or drink normally after treatment; however, no effect on weight gain was observed in mice. Absolute liver weights were statistically significantly ($p \leq 0.05$) increased 67 and 73% in male and female mice, respectively, exposed to 132 ppm. Relative liver weights in the high-exposure group were statistically significantly ($p \leq 0.05$) increased by 55 and 60% in males and females, respectively, compared with controls. Male mice also displayed statistically significantly decreased absolute and relative testes weights, 9 and 16%, respectively, in the highest exposure group, but histopathological changes were not observed.

The concentrations of serum albumin and total protein were statistically significantly ($p \leq 0.05$) increased in both sexes of mice, but were not considered by the investigators to be toxicologically significant. There were no dose-related changes among any of the hematological parameters, but the number of platelets was statistically significantly ($p \leq 0.05$) increased 25 and 42% in male and female mice at 132 ppm.

Several pathological changes in mice were associated with 1,2,3-trichloropropane exposure. A moderate increase in hepatocyte size was noted in all male and female mice exposed to 132 ppm, and a slight or very slight depletion of lymphoid elements in the spleen was also reported in all mice at this dose. A dose-dependent increase in the incidence and severity of decreased thickness and degeneration of the olfactory epithelium in the nasal turbinates of mice was also observed (Table 4-27). There was a dose-related increase in inflammation in the olfactory epithelium of the nasal turbinates (Table 4-28) accompanied by the exudation of inflammatory cells into the nasal cavity lumen.

Table 4-27. Incidence and severity of decreased thickness and degeneration of the olfactory epithelium in the nasal turbinates in B6C3F$_1$ mice exposed via inhalation to 1,2,3-trichloropropane

	Males (ppm)							Females (ppm)						
Severity[a]	0	1	3	10	13	40	132	0	1	3	10	13	40	132
Very slight	0	0	0	5	5	4	0	0	0	0	5	5	2	0
Slight	0	0	0	0	0	1	2	0	0	0	0	0	3	0
Moderate	0	0	0	0	0	0	3	0	0	0	0	0	0	5
Combined incidence	0	0	0	5	5	5	5	0	0	0	5	5	5	5
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5

[a]Decreased thickness, bilateral, and multifocal, or degeneration, bilateral, and multifocal.

Sources: Miller et al. (1987a, b).

Table 4-28. Incidence and severity of inflammation of the olfactory epithelium in the nasal turbinates of B6C3F$_1$ rats exposed via inhalation to 1,2,3-trichloropropane

	Males (ppm)							Females (ppm)						
Severity[a]	0	1	3	10	13	40	132	0	1	3	10	13	40	132
Very slight	0	0	0	2	2	4	0	0	0	0	5	1	3	0
Slight	0	0	0	0	0	1	2	0	0	0	0	0	2	0
Moderate	0	0	0	0	0	0	3	0	0	0	0	0	0	5
Combined incidence	0	0	0	2	2	5	5	0	0	0	5	1	5	5
Exudate into lumen	0	0	0	1	1	1	5	0	0	0	0	0	2	5
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5

[a]Inflammation, bilateral, and multifocal

Sources: Miller et al. (1987a, b).

Since changes to the nasal epithelium were observed in the 13, 40, and 132 ppm exposure groups, a follow-up study (Miller et al., 1987b) was initiated using the same study protocol and target exposure concentrations of 0, 1, 3, and 10 ppm, with measured concentrations of 0, 1.0 ± 0.0, 2.9 ± 0.2, or 9.7 ± 0.3 ppm (0, 6, 18, or 60 mg/m^3). Body weights and organ weights of both sexes of rats and mice were not affected at any concentration level. Very slight decreased thickness and degeneration of the olfactory epithelium in the nasal turbinates was observed in male and female rats that were exposed to 3 and 10 ppm (Table 4-25). Very slight inflammation in the olfactory epithelium was also evident in rats exposed to 0, 1, 3, and 10 ppm (Table 4-26). The exudation of inflammatory cells into the nasal cavity lumen was observed in two male and two female rats at 10 ppm.

A very slight decrease in thickness of the olfactory epithelium in the nasal turbinates was observed in both sexes of mice that were exposed to 10 ppm (Table 4-27). Very slight inflammation in the olfactory epithelium was also observed in mice at 10 ppm (Table 4-28). The exudation of inflammatory cells into the nasal cavity lumen was observed in a single male mouse at 10 ppm. No other exposure-related effects were detected.

4.4.3. Aquatic Species Studies

NTP (2005) conducted a toxicity study in 220 male and female guppies (Poecilia reticulate) and 340 male and female medaka (Oryzias latipes) maintained in aquaria water containing 0, 4.5, 9, or 18 mg/L. The guppies were exposed for 16 months and the medaka for 13 months. Ten of each species at each dose group were sacrificed at 9 months for histopathologic analysis. Approximately one-third of the fish that survived until the 9-month evaluation were transferred to chemical-free water at that time and evaluated at study termination. These fish are described as the stop-exposure group.

In the medaka study, survival at 9 months was decreased in the 9 and 18 mg/L groups (NTP, 2005). At the 9-month evaluation, the incidence of choliangiocarcinomas was significantly increased in 9 and 18 mg/L males. The incidence of choliangiocarcinomas was significantly increased in all male and female 1,2,3-trichloropropane treatment groups after 13 months, while the incidence of hepatocholangiocarcinomas was significantly increased only in the fish exposed to 18 mg/L 1,2,3-trichloropropane. The incidence of papillary adenomas of the gallbladder was significantly increased in the 9 and 18 mg/L males after the 13-month exposure. In the stop exposure component of the study, the incidence of papillary adenomas in males was significantly increased only at the highest exposure concentration.

Reduced survival was evident in the guppies at 6 months at the highest concentration tested (18 mg/L) and at 7 months in the 4.5 and 9 mg/L concentrations as well. Survival was significantly reduced in the 18 mg/L guppies at about 8 months (NTP, 2005). At the 9-month interim evaluation, there was an increased incidence of bile duct and hepatocellular neoplasms in the exposed male and female guppies at all concentrations tested. In the stop-exposure component of the study, hepatocellular neoplasms were evident in 18 mg/L males, and bile duct neoplasms were evident in 18 mg/L females.

1,2,3-Trichloropropane was characterized as carcinogenic at concentrations up to 18 mg/L in both sexes of guppies and medaka based on the increased incidence of liver neoplasms and papillary adenoma of the gallbladder (NTP, 2005). Studies of toxicity in aquatic species such as the medaka and guppy are increasingly being used as screening studies for tumor formation and other endpoints of toxicity.

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

4.5.1. Mode of Action Studies

Weber and Sipes (1990) conducted a series of experiments that examined the covalent binding of 1,2,3-trichloropropane to hepatic macromolecules in male F344/N rats. In a preliminary experiment, binding of [^{14}C]-1,2,3-trichloropropane to hepatic protein, DNA, and RNA was measured 4 hours after i.p. administration of 30 mg/kg (100 μCi/kg). Similar amounts of radioactivity were bound to hepatic protein, 418 ± 19 pmol [^{14}C]-1,2,3-trichloropropane equivalents/mg, and RNA, 432 ± 74 pmol [^{14}C] -1,2,3-trichloropropane equivalents/mg, and approximately half as much was bound to DNA, 244 ± 29 pmol [^{14}C] -1,2,3-trichloropropane equivalents/mg. Because of methodological problems, the binding to RNA was not characterized further in this investigation.

In a subsequent time-course study, male rats (4/group) were sacrificed at 1, 4, 24, 48, and 72 hours post i.p. administration of 30 mg/kg [^{14}C]-1,2,3-trichloropropane (100 μCi/kg). To examine the influence of various metabolic pathways, the investigators also administered 1,2,3-trichloropropane to four additional groups (each containing four rats) that had been pretreated as follows:

- 80 mg/kg-day phenobarbital, a CYP450 (CYP2B, CYP3A) inducer, in 0.9% NaCl (i.p.) for 4 days with 1,2,3-trichloropropane treatment on day 5;
- 40 mg/kg-day β-naphthoflavone, a CYP450 inducer (CYP1A), (i.p.) in vegetable oil for 3 days, followed by treatment with 1,2,3-trichloropropane on day 4;
- 75 mg/kg SKF 525-A, an inhibitor of CYP450, in phosphate-buffered saline (pH 5.0) administered (i.p.) 2 hours prior to treatment with 1,2,3-trichloropropane;
- 2 g/kg 1-buthionine-(R,S)-sulfoximine (BSO), which causes a depletion of hepatic GSH, administered in two doses (i.p.) spaced 1.5 hours apart, followed by 1,2,3-trichloropropane treatment 3 hours later.

All rats in the metabolic study were sacrificed 4 hours after treatment with 1,2,3-trichloropropane.

In the time-course study, maximum trichloropropane-equivalent covalent binding to hepatic proteins (approximately 600 pmol/mg) was observed 4 hours after trichloropropane administration and was approximately 2.5-fold greater than at 1 hour post-administration. Maximal covalent binding to hepatic DNA (approximately 250 pmol/mg) was observed 24 hours after administration. By 72 hours, the amount of radioactivity bound to both protein and DNA had returned to levels below those measured 1 hour post administration. At the point of maximal

binding, the amount of [^{14}C]-1,2,3-trichloropropane-derived radioactivity bound to hepatic proteins was more than double the amount bound to hepatic DNA.

Administration of three consecutive doses each of 30 mg/kg 1,2,3-trichloropropane, separated by 24 hours, produced a linear increase in the amount of [^{14}C]-1,2,3-trichloropropane-derived radioactivity bound to hepatic proteins. Repeated dosing did not affect the amount of the chemical equivalent bound to DNA until the third dose at which point the amount of bound radioactivity doubled.

In the metabolic study, induction of CYP450 (CYP) isozymes with phenobarbital pretreatment significantly reduced chemical binding to hepatic protein and DNA by 70 and 64%, respectively, when compared with controls. However, induction of CYP450 isozymes with β-naphthoflavone pretreatment did not significantly alter binding to either macromolecule. Depletion of GSH by BSO pretreatment increased binding to hepatic proteins by 342% and decreased binding to DNA by 44% when compared with controls, with the increased covalent binding due to decreased GSH conjugation of a 1,2,3-trichloropropane metabolite. Inhibition of CYP450 isozymes with SKF 525-A significantly increased binding to hepatic protein and DNA by 58 and 42%, respectively, compared with controls. The decrease in GSH appears to lead to increased levels of a reactive metabolite that does not require GSH to bind with proteins, as evidenced by the increased binding to hepatic proteins when GSH levels were reduced by BSO pretreatment. 1,2,3-Trichloropropane metabolite(s) appear to conjugate with GSH and produce compounds, such as episulfonium ions, that may covalently interact with hepatic DNA.

To further explore the effect of 1,2,3-trichloropropane on GSH, two additional experiments were conducted by Weber and Sipes (1990): hepatic GSH levels were measured in rats receiving 30, 100, and 300 mg/kg 1,2,3-trichloropropane (four rats per dose); GSH levels of control and treated animals were evaluated with and without phenobarbital pretreatment. 1,2,3-Trichloropropane treatment caused a dose-dependent, statistically significant decrease in GSH levels 2 hours after exposure. Phenobarbital pretreatment, on the other hand, did not increase the trichloropropane-induced reduction in hepatic GSH concentrations.

La et al. (1995) investigated the formation of DNA adducts in animals treated with 1,2,3-trichloropropane by using the same route of administration and some of the doses used in the NTP (1993) chronic bioassay. A single dose of either 3 or 30 mg/kg 1,2,3-trichloropropane containing [^{14}C]-1,2,3-trichloropropane (1 mCi) was administered by gavage to male F344/N rats and 6 or 60 mg/kg [^{14}C]-1,2,3-trichloropropane to male B6C3F1 mice. Animals were sacrificed after 6 hours, and DNA adducts were hydrolyzed by neutral thermal or mild acid treatment and separated by cation exchange high performance liquid chromatography. Peaks were characterized by using electrospray ionization mass spectrometry, and their identity was verified with synthesized standards.

The elution profile of the labeled DNA indicated that a single, major DNA adduct was formed (La et al., 1995). The adduct was determined to be S-[1-(hydroxymethyl)-2-(N^7-guanyl)-ethyl]glutathione and was widely distributed among the organs examined (La et al., 1995). A proposed formation pathway involves the biological activation, possibly by conjugation with GSH, of 1,2,3-trichloropropane and intramolecular rearrangement to form episulfonium ions that covalently bind to DNA. The formation of the S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]-glutathione adduct was detected in the forestomach, glandular stomach, kidney, liver, pancreas, and tongue (oral) of F344/N rats, and in the forestomach, glandular stomach, kidney, and liver of B6C3F1 mice. The concentrations of this adduct formed in the target organs (expressed as μmol/mol guanine) showed some correlation with the tumor incidence from the NTP (1993) study (Table 4-29). For example, dose-dependent adduct formation was demonstrated in the forestomach of F344/N rats and B6C3F1 mice, and the forestomach was a primary site of tumor formation in both animal models in the NTP (1993) study. Conversely, dose-dependent adduct formation was apparent in the liver and glandular stomach in both species, although NTP (1993) detected no tumor formation at this tissue site. Adduct formation in the spleen of rats and mice, when compared to other organs, appeared lower, and NTP (1993) did not detect tumors in the spleens of rats and mice.

Table 4-29. Comparison of tumor incidence and DNA-adduct formation in male F344/N rats and B6C3F$_1$ mice

Organ	Dose	Tumor incidence[a]	Adduct level (μmol/mol guanine)[b]
Male rats			
Forestomach[c]	3	33/50	3.7
	30	43/52	14.6
Kidney[c]	3	2/50	6.6 ± 1.4
	30	21/52	38.9 ± 5.0
Pancreas[c]	3	21/50	5.3 ± 1.0
	30	29/52	37.8 ± 12.8
Preputial gland	3	6/47	Not detected
	30	16/50	Not detected
Oral[c]	3	2/50	4.0
	30	37/52	20.4
Glandular stomach	3	0/50	3.8
	30	0/52	20.4
Liver	3	1/50	5.4 ± 0.7
	30	3/52	47.6 ± 21.0
Male mice			
Forestomach[c]	6	50/51	19.8
	60	55/56	41.0
Liver[c]	6	24/51	12.1 ± 4.6
	60	31/56	59.3 ± 21.7
Lung	6	11/51	0.77 ± 0.16
	60	6/56	5.3 ± 0.2
Glandular stomach	6	0/51	28.1
	60	0/56	208.1
Kidney	6	0/51	4.4 ± 2.9
	60	0/56	32.5 ± 11.3

[a]From NTP (1993) and tallied in La et al. (1995).
[b]From La et al. (1995); expressed as mean ± standard deviation from four animals with statistical significance not analyzed.
[c]Statistically significant increase in tumor formation from NTP (1993).

Source: La et al. (1995).

The S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione adduct indentified by La et al. (1995) is an N^7-guanyl adduct shown in Figure 4-1. The adduct crosslinks a physiological oligopeptide, reduced GSH, to DNA by a chemical carcinogen (Ozawa and Guengerich, 1983). The N^7 position on the guanine is a highly electrophilic nitrogen atom that is located in an accessible position on the DNA polymer (Gasparutto et al., 2005). N^7-guanyl adducts generally have an inhibitory effect on sequence-specific DNA binding by regulatory proteins, due to a destabilization of the guanine nucleobase and spontaneous degradation (Gasparutto et al., 2005;

Ezaz-Nikpay and Verdine, 1994). However, the exact role of the N^7-guanyl adducts is unknown (Gasparutto et al., 2005). This DNA adduct lends evidence of the involvement of the episulfonium ion in DNA binding, as the episulfonium ion interacts with reduced GSH and binds to DNA at the N^7 of guanine. The formation of additional DNA adducts could potentially be through the 1,3-dichloroacetone and 2-chloroacrolein pathways of metabolism.

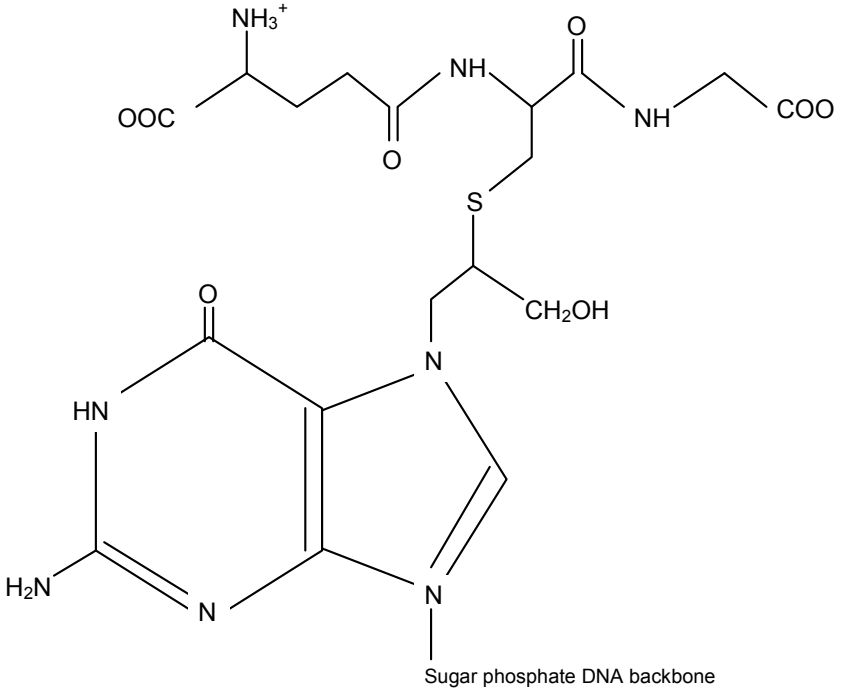


Figure 4-1. Structure of the DNA adduct S-[1-(hydroxymethyl)-2-(N^7-guanyl)-ethyl]glutathione.

In a subsequent publication, the DNA adduct-forming capacity of 1,2,3-trichloropropane in male B6C3F1 mice (n = 15) which received equivalent doses of [^{14}C]-1,2,3-trichloropropane via either corn oil gavage or drinking water was compared (La et al., 1996). The mice were administered 6 mg/kg-day for 5 days via gavage or drinking water. As shown in Table 4-30, a greater amount of DNA adduct was extracted from tissues of those animals receiving 1,2,3-trichloropropane via gavage when compared to those exposed via drinking water, although the only statistically significant differences were in the liver. Similarly, the authors observed little, if any, cellular proliferation in the tissues of animals exposed to 1,2,3-trichloropropane in drinking water. By contrast, cellular proliferation appeared to increase in a dose-dependent manner in tissues of animals exposed to 1,2,3-trichloropropane by gavage.

Table 4-30. Formation of DNA adducts by [^{14}C]-1,2,3- trichloropropane (6 mg/kg-day) administered to B6C3F$_1$ mice by gavage or drinking water

Organ	DNA adduct formation (µmol/mol guanine)	
	Drinking water	Gavage
Target organs for tumor formation		
Forestomach	86.8 ± 73.2	123.1 ± 10.3
Liver	185.5 ± 83.9	374.9 ± 109.2[a]
Nontarget organs for tumor formation		
Glandular stomach	43.2 ± 5.9	42.5 ± 4.6
Kidney	81.9 ± 41.5	193.1 ± 64.4[a]

[a]Indicates a statistically significant difference ($p \leq 0.05$) compared to values obtained in the same tissue of animals receiving 1,2,3-trichloropropane via the alternative route of administration, as calculated by the authors.

Source: La et al. (1996).

4.5.2. Genotoxicity Studies

Bacterial mutagenicity assays

Several in vitro genotoxicity studies have demonstrated 1,2,3-trichloropropane to be mutagenic in various Salmonella strains in the presence of metabolic enzymes, also known as S9 fraction (Table 4-31). In a study of 250 individual chemicals, which included 1,2,3-trichloropropane, Haworth et al. (1983) observed a dose-dependent increase in revertant colonies in *Salmonella typhimurium* strains TA100 and TA1535 that were exposed to 10, 33, 100, and 333 µg 1,2,3-trichloropropane/ plate with activation by both rat and hamster S9 fractions. No increases were observed in strains TA98 and TA1537. Shell Oil Co. (1979) observed dose-dependent increases in revertant colonies in the presence of S9 fraction in tester stains: TA98, at 200 and 2,000 µg/plate; TA100, at 20, 200, and 2,000 µg/plate; and TA1537, at 20 and 200 µg/plate. These investigators also detected revertants, at 200 and 2,000 µg/plate in TA1535 both in the presence and in the absence of an S9 fraction, with a greater number of revertants in the plates with microsomal activation.

Table 4-31. Genotoxicity bioassays of 1,2,3-trichloropropane

Test system	Cells/strain	Positive concentrations	Results		Reference
			-S9	+S9	
In vitro gene mutation assays					
Bacterial assays					
S. typhimurium (Ames test)	TA100, A1535	10, 33, 100, 333 µg/plate	–	+	Haworth et al., 1983
	TA1537, TA98	N/A	–	–	
	TA98	200, 2,000 µg/plate	–	+	Shell Oil Co., 1979
	TA100	20, 200, 2,000 µg/plate	–	+	
	TA1537	20, 200 µg/plate	–	+	
	TA1535	200, 2,000 µg	+	+	
	TA1538	N/A	–	+	
	TA97, TA100, TA1535	10, 33, 100, 333 µg/plate	–	+	NTP, 1993
	TA98	100, 333 µg/plate	–	+	
	TA1537	N/A	–	NP	
	TA100	0. 1, 1 µmol/plate	–	+	Stolzenberg and Hine, 1980
	TA100	0.01, 0.02, 0.04, 0.1 µmol/plate	–	+	Lag et al., 1994
	TA1535, A100	5, 10, 50, 100 µg/plate	–	+	Ratpan and Plaumann, 1988
	TA98, TA1538, TA1537	N/A	–	–	
	TA98, TA100, TA1535	0.02–1.0 mg/plate	–	+	Kier, 1982
	TA1537, TA1538	N/A	–	–	
E. coli (SOS chromotest)	PQ37	N/A	–	–	von der Hude et al., 1988
E. coli (DNA-repair deficient strain)	WP2 uvrA	2,000 µg/plate	–	+	Shell Oil Co., 1979
E. coli (DNA-repair-proficient strain)	WP2	N/A	–	–	
Lower eukaryote					
Saccharomyces cerevisiae (mitotic gene conversion)	JD1	0.1, 0.5, 1.0, 5.0 mg/cm^3	–	+	Shell Oil Co., 1979
Aspergillus nidulans (abberrant mitotic segregation)	P1	N/A	–	NP	Crebelli et al., 1992
Mammalian cell assays					
Mouse Lymphoma	L5178Y	0.01, 0.02, 0.03, 0.04, 0.05, 0.06 µg/mL	–	+	NTP, 1993
	L5178Y	2.4, 3.2, 4.2, 5.6, 7.6, 10, 13, 18 µg/mL	NP	+	Shell Oil Co., 1982

Table 4-31. Genotoxicity bioassays of 1,2,3-trichloropropane

Test system	Cells/strain	Positive concentrations	Results		Reference
			-S9	+S9	
In vitro chromosomal damage assays					
Mammalian cells					
Chromosomal aberrations	Chinese hamster ovary (CHO) cells	59.5, 69.4, 79.2 µg/mL	–	+	NTP, 1993
	Rat liver epithelial	N/A	–	–	Shell Oil Co., 1979
Micronucleus	Human lymphocytes	N/A	–	–	Tafazoli and Kirsch-Volders, 1996
Micronucleus:	AHH-1	0.01, 1, 2, 5 mM	+	NP	Doherty et al., 1996
	MCL-5	1, 2, 5 mM	+	NP	
	H2E1	0.01, 1, 2, 5 mM	+	NP	
Unscheduled DNA synthesis	Male rat hepatocytes (F344/N)	N/A	–	NP	Williams et al., 1989
DNA strand breaks (Comet assay)	Human lymphocytes	2, 4 mM	+	+	Tafazoli and Kirsch-Volders, 1996
	Wistar rat hepatocytes	N/A	–	NP	Holme et al., 1991
DNA Fragmentation	V79	4, 5 mM	+[a]		Eriksson et al., 1991
Sister chromatid exchanges	CHO	14.2, 39.7, 49.6, 59.5 µg/mL	–	+	NTP, 1993
	V79	0.3, 1.0 mM	–	+	von der Hude et al., 1987
In vivo bioassays					
Chromosomal damage: mammalian					
Micronucleus	CD-1 mice, bone marrow erythrocytes	N/A	–		Crebelli et al., 1999
DNA strand breaks (Comet assay)	F344/N male rat hepatocytes	30, 100, 300 mg/kg	+		Weber and Sipes, 1991
	Wistar male rat Kidney	≥375 µmol/kg	+		Lag et al., 1991
DNA adducts	F344/N male rat (multiple organs)	3 or 30 mg/kg	+		La et al., 1995
	$B6C3F_1$ male mice (multiple organs)	6 or 60 mg/kg	+		

Table 4-31. Genotoxicity bioassays of 1,2,3-trichloropropane

Test system	Cells/strain	Positive concentrations	Results		Reference
			-S9	+S9	
Other in vivo assays					
Dominant lethal mutation	Sprague-Dawley male rats, implants and embryos	N/A	–		Saito-Suzuki et al., 1982
Wing spot test	*Drosophila melanogaster*	4.51 µg/L (inhalation)	+		Chroust et al., 2007
Polyploidy	Albino male rat hepatocytes	0.8 mg/L (inhalation)	+		Belyaeva et al., 1974
		0.8, 2.16 mg/L (inhalation)	+		Belyaeva et al., 1977

[a]Metabolic enzyme induction was not specified.

N/A = either chemical had no effect or information is not available (abstracts only); NP = assay was not performed

NTP (1993) tested strains at doses of 3, 10, 33, 100, or 333 µg/plate and 10, 33, 100, 333, 666, 667, or 1,000 µg/plate and observed a dose-dependent increase in the number of revertants in colonies of TA97, TA100, and TA1535 treated with 1,2,3-trichloropropane in the presence of either hamster or rat S9 fraction in repeated experiments. Mutagenic activity was observed in TA98 in the presence of hamster and rat S9 fraction. No mutagenic activity was observed in the TA1537 test strain in the presence of S9. In the absence of an S9 microsomal fraction, no mutagenic activity was detected in any of the Salmonella strains. It should be noted that the NTP (1993) report includes data from the Haworth et al. (1983) article. The descriptions provided here are for trials not included in the earlier report.

Other groups also have demonstrated the mutagenic capability of 1,2,3-trichloropropane by using the Ames test. Stolzenberg and Hine (1980) and Lag et al. (1994) found dose-dependent increases in mutagenic activity in TA100 in the presence of S9 at doses of 14.7 (0.1 µmol/plate) and 147 (1 µmol/plate) µg/plate and ~14.7 (0.1 µmol/plate) µg/plate, respectively. No increases were observed in the nonactivated cultures. A dose dependent, statistically significant increase in mutagenic activity was also demonstrated by Ratpan and Plaumann (1988) in TA1535 and TA100 in the presence of S9 at doses of 5, 10, 50, and 100 µg 1,2,3-trichloropropane/plate, with no mutagenic activity in the same strains in the absence of S9 at the same doses and no mutagenic activity in TA98, TA1537, or TA1538 in the presence and absence of S9 at the same doses. Kier (1982) found mutagenic activity in TA100, TA1535, and TA98 in the presence of S9 fraction at 20–1,000 µg/plate, 20–300 µg/plate, and 100–300 µg/plate, respectively. No mutagenic activity was found in the same strains in the absence of S9 at the same doses nor was mutagenic activity found in TA1537 and TA1538 in the presence and absence of S9 at the same doses.

The mutagenic effects of 1,2,3-trichloropropane have also been examined in other microbial systems with mixed results. von der Hude et al. (1988) showed the compound to be negative for DNA damage in the SOS chromotest using *Escherichia coli* PQ37. 1,2,3-Trichloropropane induced mutations in DNA repair-deficient *E. coli* WP2 uvr A at 2,000 µg/plate, but not in the DNA repair-proficient strain WP2, and induced mitotic gene conversion in *Saccharomyces cerevisiae* after exposure to 0.01, 0.1, 0.5, 1.0, or 5.0 mg/cm^3 1,2,3-trichloropropane in the presence of rat liver S9 (Shell Oil Co., 1979). Increases were not observed in the non-activated cultures. 1,2,3-Trichloropropane tested negative in the *Aspergillus nidulans* diploid strain P1 assay for aberrant mitotic segregation at 0.1 % v:v with 5% survival (Crebelli et al., 1992).

Mammalian cell assays

1,2,3-Trichloropropane has also been shown to induce genotoxic effects in cultured mammalian cells (Table 4-31). NTP (1993) conducted cytogenetic analysis in Chinese hamster ovary (CHO) cells, and the results indicated that 1,2,3-trichloropropane induced both sister chromatid exchanges, at 14.2, 39.7, 49.6, and 59.5 µg/plate and chromosomal aberrations at 59.5, 69.4, and 79.2 µg/plate, in the presence of rat liver S9 fraction. However, 1,2,3-trichloropropane did not induce chromosomal damage in cultured rat liver epithelial cells at doses of 250, 500, or 1,000 µg/mL (Shell Oil Co., 1979), nor did it elicit micronucleus formation in isolated human lymphocytes at doses of 0.1, 2, 4, 6, or 8 mM (0.015, 0.29, 0.59, 0.89, or 1.2 mg/L) (Tafazoli and Kirsch-Volders, 1996). 1,2,3-Trichloropropane induced sister chromatid exchanges in Chinese hamster V79 cells at 0.3 and 1.0 mM with microsomal activation, but did not induce sister chromatic exchanges without microsomal activation (von der Hude et al., 1987). Eriksson et al. (1991) observed DNA fragmentation in Chinese Hamster lung fibroblasts (V79) cells at 4 and 5 mM 1,2,3-trichloropropane, although induction levels were not provided.

1,2,3-Trichloropropane induced micronucleus formation in the mammalian cell lines, AHH-1, MCL-5, and h2E1, in a dose-dependent manner from 0.01 to 5.0 mM for each cell line (Doherty et al., 1996). The human B lymphoblastoid AHH-1 cell line has native cytochrome CYP1A1 activity, the MCL-5 cell line expresses cDNAs encoding human CYP1A2, 2A6, 3A4, and microsomal epoxide hydrolase, and the h2E1 cell line contains CYP1A1 activity and a cDNA for CYP2E1. The increase in micronuclei in AHH-1 and h2E1 was approximately eight-fold, while the increase in MCL-5 was approximately four-fold. The micronuclei of all three cell lines stained both positively and negatively for kinetochore antibody. Although the micronuclei of the MCL-5 cell line stained primarily positive for kinetochore antibody, indicative of aneugenic effects, those induced in the AHH-1 and h2E1 cell lines lacked kinetochore staining, which is indicative of clastogenic effects. The difference in micronucleus formation between AHH-1 and h2E1 and MCL-5 suggests the formation of a less genotoxic or further deactivated metabolite in the MCL-5 line. The MCL-5 cell line endogenously expresses CYP1A1 and contains cDNAs for CYP1A2, 2A6, 3A4, and 2E1, while AHH-1 and h2E1 contain CYP1A1 and

CYP1A1 and 2E1, respectively. The MCL-5 cell line may be capable of metabolizing 1,2,3-trichloropropane to less genotoxic metabolites or less potent inducer of micronuclei.

Use of an alkaline single cell gel electrophoresis test (Comet assay) demonstrated a compound-related increase in the incidence of DNA strand breaks under cytotoxic conditions in isolated human lymphocytes (Tafazoli and Kirsch-Volders, 1996). 1,2,3-Trichloropropane did not induce DNA strand breaks, measured by alkaline elution, in male Wistar rat hepatocytes after a 1-hour exposure to 50 μM (Holme et al., 1991). When tested for genotoxicity in the rat hepatocyte unscheduled DNA synthesis assay, 1,2,3-trichloropropane (10^{-4}% M) was negative for unscheduled DNA synthesis, a general response to DNA damage (Williams et al., 1989).

NTP (1993) found a positive response to 1,2,3-trichloropropane in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells in the presence of rat liver S9 fraction; the lowest effective dose was 0.01 μL. Without S9 activation, no induction of trifluorothymidine resistance was noted at doses below those that produced precipitation of 1,2,3-trichloropropane. Shell Oil Co. (1982) also demonstrated the capacity of the compound to induce forward mutations to confer trifluorothymidine resistance in mouse lymphoma L5178Y cells in the presence of S9 fraction, and an inability to induce forward mutations in the absence of S9 fraction.

In vivo bioassays

In vivo assays provided both positive and negative evidence of genotoxicity (Table 4-31). Chroust et al. (2007) investigated the genotoxic effects of 1,2,3-trichloropropane in the somatic mutation and recombination test (SMART) using *Drosophila melanogaster*. In this bioassay, 72-hour-old larvae were administered 1,2,3-trichloropropane for 48 hours by inhalation, and the wings of the adults were inspected for the presence of wing spots which were characterized as small, large twin, and total spots. The induction of wing spots is caused by genotoxic effects such as somatic mutation, chromosomal rearrangement, or nondisjunction. 1,2,3-Trichloropropane caused a statistically significant (compared to control) increase in the number of total wing spots.

Belyaeva et al. (1974) investigated the effect of 1,2,3-trichloropropane on the ploidy of hepatocytes in rats. Male albino rats inhaled 0.8 mg/L 1,2,3-trichloropropane for 1 week. The percentage of mononuclear tetraploid and octaploid cells was statistically significantly increased, and an increase in ploidy of 16n was also evident. There was also a decrease in the percentage of binuclear cells in concordance with the increase in tetraploid and octaploid. Belyaeve et al., (1977) conducted a similar investigation in order to compare the action of various concentrations of 1,2,3-trichloropropane and 1,2-dichloropropane on the ploidy of hepatocytes. Male albino rats inhaled 1,2,3-trichloropropane at 0.8 or 2.16 mg/L for 1 week, 0.08 mg/L for 2 weeks, and 0.002 mg/L for 3 months. After the 1-week exposure period, the 1,2,3-trichloropropane dosed

animals demonstrated an increase in the number of mononuclear hepatocytes with a nucleus of high ploidy with a decrease in the number of binuclear cells. Following the 2-week exposure, however, the results in the experimental group and control group were indistinguishable. When the exposure time was increased to 3 months and the dose decreased to 0.002 mg/L, a slight increase in nuclei of intermediate ploidy was observed in the 1,2,3-trichloropropane-exposed group. Additional positive evidence of genotoxicity was obtained by Weber and Sipes (1991), who administered single, i.p. injections of 30, 100, or 300 mg/kg 1,2,3-trichloropropane to male F344/N rats, which were then sacrificed 1, 2, 4, 8, 12, 24, and 48 hours post-administration. Using alkaline elution to detect damaged hepatic DNA, they demonstrated that 1,2,3-trichloropropane, or its metabolites, caused the formation of DNA strand breaks. La et al. (1995) characterized the formation of DNA adducts in various organs in both B6C3F1 mice and F344/N rats exposed to 6 or 60 and 3 or 30 mg/kg, respectively. High concentrations of DNA adducts were evident in the tumor-forming organ tissues, including the forestomach, kidney, pancreas, oral cavity, and liver in male rats and the forestomach and liver of male mice, from the NTP (1993) study. DNA adducts were also found in tissues that did not develop tumors, although the increased incidence of tumors and increased mortality in the NTP study may have precluded tumor development in those tissues that formed DNA adducts without tumors. Male MOL:WIST rats were killed 1 hour after receiving 1,2,3-trichloropropane by i.p. administration and the kidney DNA damage was assessed by alkaline elution (Lag et al., 1991). 1,2,3-Trichloropropane was observed to cause DNA breaks in the kidney DNA of rats at doses ≥375 μmol/kg.

Negative results from in vivo assessments were obtained when the compound was included in a survey of 10 aliphatic halogenated hydrocarbons using the CD-1 mouse bone marrow micronucleus test and 1,2,3-trichloropropane doses of 115 and 200 mg/kg (Crebelli et al., 1999). Similarly, 1,2,3-trichloropropane did not induce dominant lethal mutations in male Sprague-Dawley rats when administered by gavage in corn oil at 80 mg/kg-day for 5 days (Saito-Suzuki et al., 1982).

4.5.3. Structural Analog Data—Relationship to 1,2-Dibromo-3-chloropropane and 1,2-Dibromoethane

1,2,3-Trichloropropane is a halogenated propane, with a single chlorine atom attached to each carbon atom in the chain. Halogenated propanes as a class of compounds are generally found to be positive in assays that indicate mutagenicity (Lag et al., 1994; Ratpan and Plaumann, 1988), and there is clear evidence that members of this group, including 1,2-dibromo-3-chloropropane (DBCP) (NTP, 1982a; NCI, 1978) and 1,2-dibromoethane (NTP, 1982b), are carcinogenic in whole animal models. In a study sponsored by the National Cancer Institute (NCI), DBCP was found to be carcinogenic to Osborne-Mendel rats when administered by

gavage in corn oil at 15 or 29 mg/kg-day for up to 78 weeks, and to B6C3F1 mice when administered by gavage in corn oil at 114 and 110 or 219 and 209 mg/kg-day in male and female mice, respectively, for up to 60 weeks (NCI, 1978). A statistically significant increase in the incidence of adenocarcinomas of the mammary gland was observed in female rats. Squamous cell carcinomas in the forestomach resulted in reduced survival in both species. These responses are qualitatively similar to those produced by 1,2,3-trichloropropane.

In addition to the oral bioassay conducted by NCI (1978), an inhalation bioassay of DBCP was conducted by NTP (1982a). NTP administered technical-grade DBCP, which contained trace amounts of epichlorohydrin and 1,2-dibromoethane, to F344/N rats and B6C3F1 mice via inhalation at concentrations of 0.6 or 3.0 ppm for 6 hours/day, 5 days/week for 76–103 weeks. DBCP induced nasal cavity tumors and tumors of the tongue in male and female rats, as well as cortical adenomas in the adrenal glands of female rats (NTP, 1982a). In mice, DBCP induced nasal cavity tumors and lung tumors in both sexes (NTP, 1982a). NTP (1982a) concluded that DBCP was carcinogenic in male and female F344/N rats and B6C3F1 mice. DBCP also forms the same major DNA adduct, S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]-glutathione, as 1,2,3-trichloropropane (Humphreys et al., 1991).

NTP (1982b) also conducted an inhalation cancer bioassay in F344/N rats and B6C3F1 mice, in which test animals inhaled 10 or 40 ppm of 1,2-dibromoethane for 78-103 weeks. In rats, 1,2-dibromoethane inhalation caused an increased incidence of carcinomas, adenocarcinomas, and adenomas and adenomatous polyps of the nasal cavity; hemangiosarcomas of the circulatory system; mesotheliomas of the tunica vaginalis; fibroadenomas of the mammary glands; and alvoelar/bronchiolar adenomas and carcinomas (NTP, 1982b). In mice, 1,2-dibromo-ethane inhalation caused an increased incidence of alveolar/bronchiolar adenomas and carcinomas; hemangiosarcomas of the circulatory system; fibrosarcomas in the subcutaneous tissue; carcinomas of the nasal cavity; and adenocarcinomas of the mammary gland (NTP, 1982b).

In addition to the carcinogenicity data, mode-of-action data for similar compounds support the proposed mode of action for 1,2,3-trichloropropane; specifically, the formation of episulfonium ions and subsequent DNA binding. 1,2-Dibromoethane spontaneously forms the episulfonium ion, thiiranium, following conjugation with GSH, which may then bind to DNA (U.S. EPA, 2004). DNA binding of metabolites of 1,2-dibromoethane and DBCP have been demonstrated in vitro in calf thymus DNA (Inskeep and Guengerich, 1984) and the binding of metabolites of 1,2-dibromopropane to DNA in vivo has been demonstrated in rats following i.p. injection (Kim and Guengerich, 1990).

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

There are no data on the toxicological effects of exposure to 1,2,3-trichloropropane in humans via ingestion. Three subchronic studies in rats and mice (NTP, 1993; Merrick et al., 1991; Villeneuve et al., 1985), a single chronic study in rats and mice (NTP, 1993), and a reproductive study in mice (NTP, 1990) have investigated the effects of oral exposure in animal models.

The NTP (1993) toxicology and carcinogenesis studies conducted in F344/N rats and B6C3F1 mice constitute the database of chronic oral toxicity studies for 1,2,3-trichloropropane. The effects of subchronic oral exposure to 1,2,3-trichloropropane have been investigated by NTP (1993), Merrick et al. (1991), and Villeneuve et al. (1985). A reproductive and fertility assessment investigation of 1,2,3-trichloropropane was conducted with Swiss CD-1 mice (NTP, 1990). 1,2,3-Trichloropropane was administered by corn oil gavage in all of these investigations, except the study by Villeneuve et al. (1985), which provided 1,2,3-trichloropropane to rats via drinking water. Table 4-32 provides the observed effects and corresponding NOAELs and LOAELs for the subchronic, chronic, and reproductive toxicity studies available for 1,2,3-trichloropropane.

Table 4-32. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to 1,2,3-trichloropropane

Effect	Sex	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Subchronic—NTP (1993)			
F344/N rats			
Increased relative liver weight	Female	8	16
Increased absolute liver weight	Male	–	8
Decreased pseudocholinesterase	Female	–	8
Increased SDH	Male	32	63
Increased ALT	Female	63	125
Hepatocellular necrosis	Male	16	32
Increased relative kidney weight	Male	16	32
Increased absolute kidney weight	Male	16	32
Kidney necrosis	Male	63	125
Decreased absolute heart weight	Male	32	63
Nasal turbinate necrosis	Male/female	63	125
B6C3F$_1$ mice			
Increased relative liver weight	Male/female	63	125
Increased absolute liver weight	Male	16	32
Hepatocellular necrosis	Male/female	63	125
Hepatocellular karyomegaly	Male	63	125

Table 4-32. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to 1,2,3-trichloropropane

Effect	Sex	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Increased relative kidney weight	Female	8	16
Increased absolute kidney weight	Female	125	250
Decreased relative heart weight	Male	–	8
Decreased absolute heart weight	Male	32	63
Decreased relative brain weight	Male/female	8	16
Decreased absolute brain weight	Female	8	16
Regenerative lung lesions	Female	32	63
Hyperkeratosis of the forestomach	Female	32	63
Subchronic—Merrick et al. (1991)			
F344/N rats			
Increased ALT	Female	15	60
Increased AST	Female	15	60
Myocardial necrosis	Male	–	1.5
Bile duct hyperplasia	Male/female	15	60
Plasma cell hyperplasia in the mandibular lymph node	Female	–	1.5
Subchronic—Villeneuve et al. (1985)			
F344/N rats			
Increased relative liver weight	Male/female	~18	113–149
Increased serum cholesterol	Female	~18	149
Increased hepatic aminopyrine demethylase	Male/female	~18	149
Aniline hydroxylase	Male	~18	113
Biliary hyperplasia	Female	~18	149
Increased relative kidney weight	Female	~1.8	~18
Chronic—NTP (1993)			
F344/N rats			
Increased relative liver weight	Male/female	3	10
Increased absolute liver weight	Male/female	–	3
Increased 5'-nucleotidase	Male	10	30
Increased relative kidney weight	Male/female	3	10
Increased absolute kidney weight	Male	–	3
Hepatocellular necrosis	Female	3	30
Forestomach hyperplasia	Male/female	–	3
B6C3F$_1$ mice			
Increased relative liver weight	Male/female	20	60
Increased absolute liver weight	Male/female	60	–
Increased relative kidney weight	Female	20	60
Increased absolute kidney weight	Male/female	60	–
Increased creatine kinase	Male	20	60
Hepatocellular necrosis	Female	–	6

Table 4-32. Observed effects and corresponding NOAELs and LOAELs for subchronic, chronic, and reproductive toxicity studies following oral exposure to 1,2,3-trichloropropane

Effect	Sex	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Forestomach hyperplasia	Male/female	–	6
Reproductive—NTP (1990)			
CD-1 mice			
Decrease in number of pregnancies/fertile mouse		30	60
Decrease in number of live pups/litter		30	60
Increased cumulative d to litter		30	60
Decreased proportion of male pups		60	120
Decreased mating indices		60	120
Decreased fertility indices		60	120

The principal finding of the NTP (1993) chronic toxicity studies was a statistically significant elevated incidence of tumors in both rats and mice at multiple sites. The tumorogenic effects of 1,2,3-trichloropropane are discussed in greater detail in Section 4.7. The increased incidence of tumors was accompanied by a significant decrease in survival. The percent probability for survival was significantly decreased in rats receiving a dose of ≥10 mg/kg-day 1,2,3-trichloropropane, and in mice receiving a dose of ≥6 mg/kg-day. Because the decrease in survival was associated with the increased incidence of tumors (NTP, 1993), it was notconsidered a noncancer effect. However, it is important to note that the nonneoplastic changes associated with chronic oral exposure to 1,2,3-trichloropropane occurred at doses that also produced cancer and an associated decrease in the percent chance of survival.

Statistically significant increases in absolute and relative liver and right kidney weights were observed in the subchronic and chronic studies. The increase in liver and kidney weights may be associated with the metabolic role of these organs involving the induction of metabolic enzymes and other proteins in metabolizing 1,2,3-trichloropropane. However, this metabolic role may be combined with the binding of 1,2,3-trichloropropane metabolites to hepatic proteins and DNA in the continuum to liver damage. Corn oil gavage has been shown to increase cell proliferation in hepatocytes (Rusyn, et al., 1999); however, the NTP assay control animals, to which the dose groups were compared, also received corn oil gavage. Organ weight increases were proportionally greater in rats than mice, and increased organ weights were, generally, also more pronounced in females than males. The variation in the effect on organ weights between species and sexes indicates that there may be toxicokinetic and toxicodynamic differences that affect the metabolism of 1,2,3-trichloropropane.

In male rats, a statistically significant decrease in ALT and 5'-nucleotidase was apparent after chronic exposure to 30 mg/kg-day, while in female mice, a statistically significant increase in SDH was evident after chronic exposure to 60 mg/kg-day. Hepatocellular necrosis was

observed in female rats at 3 and 30 mg/kg-day and in male and female mice at all dose groups including controls at the 15-month interim evaluation (not statistically significant).

In the subchronic studies, there was evidence of hepatocellular damage in both rats and mice. Absolute and relative liver weight increases were observed in male and female rats in several studies (NTP, 1993; Merrick et al., 1991; Villeneuve et al., 1985), and were also evident in male and female mice (NTP, 1993). After subchronic exposure, an increase in the incidence of hepatocellular necrosis was apparent in both rats and mice (NTP, 1993), and the serum concentrations of ALT, AST, and SDH were increased in female rats (NTP, 1993; Merrick et al., 1991). Increased serum cholesterol levels and hepatic aminopyrine demethylase activity were also apparent after subchronic administration (Villeneuve et al., 1985). Serum concentrations of pseudocholinesterase were decreased in male and female rats after subchronic 1,2,3-trichloropropane exposure, and reflected a decrease in pseudocholinesterase synthesis (NTP, 1993). Taken as a whole, the increased incidence of hepatocellular necrosis, the increased ALT, AST, SDH, hepatic aminopyrine demethylase activity, and cholesterol serum concentrations, along with the decreases in pseudocholinesterase synthesis and concentration of 5'-nucleotidase, is indicative of hepatocellular damage due to 1,2,3-trichloropropane exposure. Travlos et al. (1996) reported that treatment-related alteration in clinical chemistry was highly associated with histopathological changes.

Increased absolute and relative kidney weights in male and female rats were apparent in several subchronic studies (NTP, 1993; Merrick et al., 1991; Villeneuve et al., 1985), with an inconsistent dose-response pattern for absolute and relative kidney weight in mice (NTP, 1993). The NTP (1993) chronic study showed an increase in absolute and relative right kidney weights in rats and in relative right kidney weight in female mice, as well as an increased severity of nephropathy and incidence of renal tubule hyperplasia in rats. Overt kidney damage was not evident in these studies.

In addition to the liver and kidney effects, cardiac and respiratory system effects were also observed. After subchronic exposure, a decrease in the absolute heart weight in male rats and in the absolute and relative heart weight in mice was evident (NTP, 1993). Merrick et al. (1991) reported an increased incidence of inflammation-associated myocardial necrosis in rats, and an increase in creatine kinase, an indicator of myocardial damage, was evident in male mice following chronic exposure (NTP, 1993). NTP (1993) also reported epithelial necrosis in the nasal turbinates of rats and regenerative lung lesions in mice following subchronic exposure.

Hyperplasia was also observed in the forestomach (basal cell and squamous), kidney (renal tubule), and pancreas (acinar) of rats and in the forestomach (squamous) of mice following chronic exposure to 1,2,3-trichloropropane (NTP, 1993). However, the necrosis observed in the liver, kidney, nasal turbinates, and heart of rats and liver, forestomach, and lungs of mice following subchronic oral exposure to 1,2,3-trichloropropane was not observed in the chronic

NTP (1993) studies, which employed doses lower than those reported to produce these effects in the subchronic studies. The absence of observable necrosis in the chronic study may have been due to decreased survival attributable to the onset of cancer in the chronic study, the observation time points selected in the chronic NTP study, or the development of a tolerance to 1,2,3-trichloropropane following chronic exposure.

Evidence of hematological effects, including decreased hematocrit values, hemoglobin concentrations, erythrocyte counts, and elevated leukocytes and segmented neutrophils counts were observed in both chronic and subchronic NTP (1993) studies; however, these effects were not considered to be biologically relevant. NTP stated that the decreased hematocrit may be associated with depressed hematopoeisis or to blood loss from neoplasms in the forestomach, and the increased number of leukocytes may likely be due to inflammation associated with the chemically-induced neoplasms (NTP, 1993).

A multigeneration fertility and reproduction assessment (NTP, 1990) found a significant reduction in the number of fertile pairs of cohabiting Swiss CD-1 mice exposed to 60 mg/kg-day 1,2,3-trichloropropane. The reduction in fertility was accompanied by a significant reduction in the number of live pups per litter and in the proportion of male pups born alive in the fifth breedings. The decrease in fertility may be related to the observed increase in metestrus, an infertile period of estrous cycles that was reported during Task 4 of the NTP (1990) study. Male reproductive performance and fertility were not affected.

4.6.2. Inhalation

No inhalation studies of 1,2,3-trichloropropane in humans have been identified. A single study on the acute effects in humans found that all subjects (12/sex) reported irritation (eyes, throat, and odor) following 15-minute exposures to 100 ppm trichloropropane (isomer and purity not reported) (Silverman et al., 1946). The database of inhalation toxicity studies in animals includes two 2-week studies submitted to EPA by Miller et al. (1987a, b), a 4-week range finding study, two 13-week studies, and two single-generation reproductive assessments (Johannsen et al., 1988; Biodynamics, Inc., 1979).

Inhalation exposure to 1,2,3-trichloropropane was associated with the following effects: abnormal physical signs (increased lacrimation, discoloration of the anogenital fur), decreased weight gain, increased organ weights, and increased incidences of nonneoplastic lesions in the nasal epithelium, liver, lungs, and spleen (Johannsen et al., 1988; Miller et al., 1987a, b; Biodynamics, Inc., 1979).

Decreased body weight and weight gain during the pre-mating period was observed in both male and female rats in a single-generation reproductive study (Johannsen et al., 1988). In addition, decreased body weight in female rats was observed during gestation and lactation. All groups of female rats exhibited low mating performance, 16/20 females mated at 5 ppm and

10/20 females mated at 15 ppm, compared with 17/20 females in the control group. Although fewer females in the high-concentration group mated, statistical significance was not demonstrated using the χ^2-square test (Johannsen et al., 1988). The decrease in the number of females that mated was statistically significant ($p < 0.02$) at 15 ppm in the Fisher Exact test conducted by EPA.

Similar to the oral toxicity database, the inhalation studies found statistically significant increases in organ weights. Following the 13-week exposure to 1,2,3-trichloropropane, increased absolute and relative liver weights were observed in male rats exposed to concentrations of 5, 15, or 50 ppm and increased absolute and relative liver weights were observed in female rats exposed to 50 ppm and 15 and 50 ppm, respectively (Johannsen et al., 1988). Increased absolute and relative liver weights were observed following 2-week exposures to concentrations of 40 or 132 ppm in rats, and 132 ppm in mice (Miller et al., 1987a). Increased relative lung weights in female rats exposed to concentrations of 15 or 50 ppm for 13 weeks (Biodynamics, Inc., 1979), and increased relative kidney weights were observed in male rats exposed to concentrations of 50 ppm for 13 weeks (Johannsen et al., 1988).

Increased incidences of nonneoplastic lesions have been observed in the nasal epithelium, liver, lung, and spleen of rats or mice following inhalation exposure to 1,2,3-trichloropropane (Johannsen et al., 1988; Miller et al., 1987a, b; Biodynamics, Inc., 1979). Johanssen et al. (1988) observed peribronchial lymphoid hyperplasia in the three high-dose treatment groups of male and female rats, hepatocellular hypertrophy in the three highest male dose groups, and hematopoiesis of the spleen in the highest dose group of male rats and in the three highest female dose groups in female rats. Miller et al. (1987a, b) reported decreased thickness or degeneration of the olfactory epithelium in rats exposed for 2 weeks to concentrations of 3, 10, 13, 40, or 132 ppm 1,2,3-trichloropropane (Tables 4-25 and 4-26). Similar effects were also observed in mice that were exposed to 10, 13, 40, or 132 ppm concentrations (Tables 4-27 and 4-28).

Johannsen et al. (1988) (Biodynamics, Inc., 1979) found an increased incidence of peribronchial lymphoid hyperplasia in male and female rats that were exposed to 5, 15, or 50 ppm 1,2,3-trichloropropane, but they did not examine epithelial tissue in their investigation. Lesions remote from the respiratory tract were also observed (Table 4-22). Centrilobular to midzonal hepatocellular hypertrophy was seen in nearly all male rats that were exposed for 13 weeks to concentrations of 5, 15, or 50 ppm 1,2,3-trichloropropane. However, no evidence of hepatic effects was found in female rats that were exposed to 50 ppm 1,2,3-trichloropropane. Conversely, a dose-dependent increase in the incidence and severity of extramedullary hematopoiesis of the spleen was observed in female, but not male, rats, although this effect is not biologically relevant. This differential expression of histopathic lesions suggests that for 1,2,3-tri-chloropropane, there may be toxicokinetic or toxicodynamic differences between male and female rats.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,2,3-trichloropropane is "likely to be carcinogenic to humans," based on a statistically significant and dose-related increase in the formation of multiple tumors in both sexes of two species from an NTP (1993) chronic oral bioassay. Statistically significant increases in incidences of tumors of the oral cavity, forestomach, pancreas, kidney, preputial gland, clitoral gland, mammary gland, and Zymbal's gland in rats, and the oral cavity, forestomach, liver, Harderian gland, and uterus in mice were reported.

No human oral exposure studies are available. No information is available on the carcinogenic effects of 1,2,3-trichloropropane via the inhalation route in humans or animals. U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. In addition, the data from the chronic oral study demonstrate that tumors occur in tissues remote from the site of absorption, such as in the pancreas, kidney, preputial gland, clitoral gland, and mammary gland. The presence of nonneoplastic lesions in the liver and spleen of rats and mice following subchronic and shorter inhalation exposure to 1,2,3-trichloropropane (Johannsen et al., 1988; Miller et al., 1987a, b) indicates that the chemical can enter the blood stream from the respiratory tract, but the duration of the inhalation studies was too short to show tumor development. Therefore, in the absence of information to indicate otherwise, 1,2,3-trichloropropane is "likely to be carcinogenic to humans" by the inhalation route of exposure. In addition, DBCP induced nasal cavity tumors and tumors of the tongue in male and female F344/N rats, as well as cortical adenomas in the adrenal glands of female rats, and nasal cavity tumors and lung tumors in both sexes of mice following inhalation exposure (NTP, 1982a). 1,2-Dibromoethane caused an increased incidence of carcinomas, adenocarcinomas, and adenomas and adenomatous polyps of the nasal cavity; hemangiosarcomas of the circulatory system; mesotheliomas of the tunica vaginalis; fibroadenomas of the mammary glands; and alvoelar/bronchiolar adenomas and carcinomas in F344/N rats, as well as alveolar/bronchiolar adenomas and carcinomas; hemangiosarcomas of the circulatory system; fibrosarcomas in the subcutaneous tissue; carcinomas of the nasal cavity; and adenocarcinomas of the mammary gland in male and female B6C3F1 mice (NTP, 1982b).

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

NTP (1993) conducted a 2-year study of the toxicity and carcinogenicity of 1,2,3-trichloropropane in F344/N rats. The chemical was administered by corn oil gavage to 60 rats/sex/group. Rats received doses of 0, 3, 10, or 30 mg/kg-day, and after 15 months (65–67 weeks), 8–10 rats per group were sacrificed to allow an interim evaluation of all toxicological parameters and histopathology. Due to high mortality in rats receiving 30 mg/kg at the interim evaluation, the remaining survivors in that group were sacrificed at week 67 (females) and week 77 (males). In the rats, tumors were evident in the oral cavity, forestomach, pancreas, kidney, Zymbal's gland of males and females, along with preputial gland tumors in males and clitoral gland and mammary gland tumors in females. Tumors in the mice were evident in the oral cavity, forestomach, liver, and Harderian gland of both males and females, and in the uterine/cervical tissue in females.

In addition, 1,2,3-trichloropropane was characterized as carcinogenic at concentrations up to 18 mg/L in both sexes of guppies and medaka based on the increased incidence of liver neoplasms and papillary adenoma of the gallbladder (NTP, 2005). Other evidence that supports the carcinogenic potential of 1,2,3-trichloropropane includes: (1) the demonstration that the metabolically activated compound tested positive in a number of in vitro genotoxicity assays, (2) the demonstrated ability of 1,2,3-trichloropropane metabolites to bind to intracellular protein and DNA and form DNA adducts, (3) and the similar site-specific, multispecies carcinogenicity of a structural analog of 1,2,3-trichloropropane, 1,2-dibromo-3-chloropropane (NTP, 1982a), which produces the same DNA adducts as 1,2,3-trichloropropane (Humphreys et al., 1991).

4.7.3. Mode of Action Analysis

4.7.3.1. Hypothesized Mode of Action

It is hypothesized that 1,2,3-trichloropropane-induced carcinogenicity is through a mutagenic mode of action. Specifically, the data suggest that bioactivated 1,2,3-trichloropropane may bind directly to DNA resulting in a mutagenic event that may lead to tumorigenicity in animals.

In vitro bacterial mutation assays have consistently demonstrated a mutagenic potential, dependent on S9 activation, for 1,2,3-trichloropropane. Mammalian cell in vitro studies have shown chromosomal damage, gene mutation, DNA breakage, and micronucleus formation after 1,2,3-trichloropropane exposure. In addition, in vivo assays have demonstrated the ability of 1,2,3-trichloropropane metabolites to bind to hepatic proteins, DNA, and RNA; form DNA adducts in rats and mice; induce DNA strand breaks in the hepatocytes of rats; and induce wing spots (caused by genotoxic alterations such as somatic mutation, chromosomal rearrangement, or nondisjunction) in *D. melanogaster*. In vivo studies measuring dominant lethal induction or micronucleus formation were nonpositive and limit the confidence in the hypothesized mode of

action. Additional in vivo assays that would provide evidence of mutagenicity, such as mutations in tumor suppressor genes or other mutagenic markers, are unavailable.

4.7.3.2. Experimental Support for the Hypothesized Mode of Action

Strength, consistency, specificity of association

The experimental support for mutagenicity of 1,2,3-trichloropropane is presented in sequence, with the formation of DNA adducts first, followed by the in vitro and in vivo evidence.

Evidence for the direct interaction of 1,2,3-trichloropropane metabolites with DNA was presented in vivo (Weber and Sipes, 1990), in which the ability of 1,2,3-trichloropropane metabolites to form covalent bonds with hepatic DNA, RNA, and proteins in rats following i.p. administration was apparent. However, the levels of radioactivity bound to DNA at 72 hours post administration, were below the level measured for 1 hour post administration, and may reflect cytotoxicity and resultant DNA repair or DNA degradation. The administration of three consecutive i.p. doses, 24 hours apart, of 30 mg/kg 1,2,3-trichloropropane resulted in a doubling of the amount of radioactivity bound to DNA after the third dose. Weber and Sipes (1990) conclude that this investigation demonstrates the ability of 1,2,3-trichloropropane or a reactive metabolite to covalently bind to hepatic DNA, RNA, and protein, and that the covalent binding increases with multiple doses. Weber and Sipes (1991) administered i.p. injections of 1,2,3-trichloropropane to male F344/N rats. Following the extraction of hepatic DNA, they demonstrated that 1,2,3-trichloropropane caused the formation of DNA strand breaks.

The involvement of GSH in the activation and binding of a metabolite of 1,2,3-trichloropropane is supported by the pretreatment of Sprague-Dawley rats with BSO (Weber and Sipes, 1990). BSO pretreatment causes a decrease in hepatic GSH in rats and a subsequent decrease in binding of 1,2,3-trichloropropane or reactive metabolite to DNA. The study authors suggested that an intermediate of 1,2,3-trichloropropane metabolism may rearrange to form an episulfonium ion that may bind covalently to DNA.

In a subsequent study, La et al. (1995) characterized the formation of DNA adducts in various organs in both B6C3F1 mice and F344/N rats, and found high concentrations of DNA adducts in the tumor-forming organ tissues, including the forestomach, kidney, pancreas, and liver in male rats and the forestomach, liver, lung and kidney of male mice, from the NTP (1993) study (Table 4-29). The target organs of 1,2,3-trichloropropane toxicity (liver, kidney, forestomach, and intestine) also contained the highest concentration of covalently bound 1,2,3-trichloropropane and related metabolites (Mahmood et al., 1991). A dose-dependent formation of DNA adducts was also evident in organs in which tumor formation was not observed. However, the interpretation of the target organ specificity is complicated due to the high mortality that was observed in the chronic bioassays. Early mortality may not have allowed

tumors in some tissues to fully develop. The relationship between the adduct-forming tissues of La et al. (1995) and the tumor-forming tissues of NTP (1993) support a mode of action involving DNA adduct formation. However, the biological relevance of the major DNA adducts is not known (La et al., 1995).

The S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione adduct indentified by La et al. (1995) is unusual in that it crosslinks a physiological oligopeptide, reduced GSH, to DNA by a chemical carcinogen, in this case 1,2,3-trichloropropane (Ozawa and Guengerich, 1983). The N^7-guanyl adducts have an inhibitory effect on sequence-specific DNA binding by regulatory proteins, due to a destabilization of the guanine nucleobase and spontaneous degradation (Gasparutto et al., 2005; Ezaz-Nikpay and Verdine, 1994). However, the exact role of the N^7-guanyl adducts is unknown (Gasparutto et al., 2005).

The mutagenic activity of 1,2,3-trichloropropane has been demonstrated in bacterial and mammalian cell systems treated with 1,2,3-trichloropropane and activated with an S9 fraction from chemically-induced rat or hamster livers (Doherty et al., 1996; Tafazoli and Kirsch-Volders, 1996; Lag et al., 1994; NTP, 1993; Ratpan and Plaumann, 1988; von der Hude et al., 1987; Haworth et al., 1983; Kier, 1982; Shell Oil Co., 1982, 1979; Stolzenburg and Hine, 1980). In the absence of the enzyme-rich S9 fraction mutagenic activity is typically not observed. 1,2,3-Trichloropropane was positive in primarily *S. typhimuium* strains that detect base pair mutations (TA1535 and TA100) and frame shift mutations (TA1537 [one assay] and TA98) in the presence of S9 fraction (Lag et al., 1994; NTP, 1993; Ratpan and Plaumann, 1988; Haworth et al., 1983; Kier, 1982; Stolzenburg and Hine, 1980; Shell Oil Co., 1979). Mutagenicity was also evident in *E. coli* WP2 uvr A, in the presence of S9 fraction, after exposure to 1,2,3-trichloropropane (Shell Oil Co., 1979). Chromosomal aberrations and sister chromatid exchanges were evident in CHO cells or V79 assays (NTP, 1993; von der Hude et al., 1987), and trifluorothymidine resistance was induced in mouse lymphoma assays, after 1,2,3-trichloropropane exposure and in the presence of S9 fraction (NTP, 1993; Shell Oil Co., 1982). DNA strand breakage caused by 1,2,3-trichloropropane was measured by the Comet assay (single gel electrophoresis test) in isolated human lymphocytes (Tafazoli and Kirsch-Volders, 1996), and 1,2,3-trichloropropane induced micronucleus formation in the mammalian cell lines, AHH-1, MCL-5, and h2E1 (Doherty et al., 1996). The data also demonstrate that the metabolism of 1,2,3-trichloropropane is necessary to activate the chemical's mutagenic potential.

In an in vivo bioassay in *D. melanogaster*, Chroust et al. (2007) investigated the genotoxic effects of 1,2,3-trichloropropane in the SMART. 1,2,3-Trichloropropane caused a statistically significant (compared to control) increase in the number of total wing spots, which is evidence for genotoxic effects such as somatic mutation, chromosomal rearrangement, or nondisjunction. Belyaeva et al. (1977, 1974) observed an increase in the number of mononuclear hepatocytes with a nucleus of high ploidy and a decrease in the number of binuclear cells

following exposure to 1,2,3-trichloropropane. 1,2,3-Trichloropropane also caused DNA breaks in the DNA from isolated kidney nuclei of rats exposed to 1,2,3-trichloropropane (Lag et al., 1991).

1,2,3-Trichloropropane tested nonpositive in bacterial systems not activated with S9 fraction (NTP, 1993; Ratpan and Plaumann, 1988), in the SOS chromotest in *E. coli* (von der Hude et al., 1988), in the DNA-repair proficient *E. coli* WP2 (Shell Oil Co., 1979), and in the *A. nidulans* diploid strain P1 assay for aberrant mitotic segregation (Crebelli et al., 1992). Mammalian cell in vitro assays in which 1,2,3-trichloropropane tested nonpositive for genotoxicity included: the induction of trifluorothymidine resistance in mouse lymphoma cells not activated with S9 fraction (NTP, 1993; Shell Oil Co., 1982); the induction of chromosomal damage in Carworth Farm E rat liver epithelial cells (Shell Oil Co., 1979); the micronucleus formation assay in human lymphocytes, although numerous chlorinated aliphatics failed to induce a clear dose-dependent increase (Tafazoli and Kirsch-Volders, 1996); the unscheduled DNA synthesis assay in rat hepatocytes (Williams et al., 1989); and the induction of DNA strand breaks in Wistar rat hepatocytes (Holme et al., 1991). The in vivo assays in which 1,2,3-trichloropropane tested nonpositive included the bone marrow micronucleus formation assay in CD-1 mice (Crebelli et al., 1999) and the dominant lethal induction assay in male Sprague-Dawley rats (Saito-Suzuki et al., 1982).

An in vitro assay conducted by Weber and Sipes (1992), utilizing rat and human hepatic cells, demonstrated a dose-dependent increase in the formation of the intermediate metabolite, DCA, which the study authors characterized as a direct-acting mutagen. In this study, the formation of DCA was 10-times faster in in vitro systems with rat hepatic microsomes than in in vitro systems with human hepatic microsomes (Weber and Sipes, 1992). DCA, also referred to as 1,3-dichloropropanone or 1,3-dichloro-2-propanone, has shown mutagenicity in *S. typhimurium* TA100 without microsomal activation (Meier et al., 1985). DCA was also shown to be mutagenic in TA1535 and TA100 with and without metabolic activation, with increased mutagenicity in strains TA1535 and TA100 without microsomal activation compared to the same strains with activation (Merrick et al., 1987).

DCA initiated skin tumors after both single and repeated topical treatment of female SENCAR mice followed by the tumor promoter, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (Robinson et al., 1989). The percentages of tumor-bearing mice after a single initiating dose of 37.5, 75, or 150 mg/kg DCA was 47, 47, and 68%, respectively. The percentages of tumor-bearing mice after repeated doses of 300, 450, or 600 mg/kg DCA was 48, 45, and 32%, respectively. In control mice receiving ethanol, the percentage of tumor-bearing mice observed was 12%. The inverted dose response observed in mice under the repeated dosing regimen may have been the result of localized cellular toxicity, which prevented initiated cells from progressing to papilloma (Robinson et al., 1989). The association between this cellular injury

and the increased incidence of carcinomas in animals receiving repeated doses is uncertain and needs to be investigated (Robinson et al., 1989).

Dose-response concordance

The in vitro studies were positive for genotoxicity or mutagenicity at concentrations ranging from 0.001 to 1,000 μg/plate, and indicate that point mutations are the most consistent type of genetic alteration induced by 1,2,3-trichloropropane and are detectable above background and at lower concentrations than the chromosomal damage.

La et al. (1995) characterized the formation of DNA adducts in various organs in both B6C3F1 mice and F344/N rats, and found high concentrations of DNA adducts at 6 hours postadministration in the tumor-forming organ tissues, including the forestomach, kidney, pancreas, and liver, in male rats at 3 or 30 mg/kg-day and in the forestomach, liver, lung, and kidney of male mice at 6 or 60 mg/kg-day, from the NTP (1993) study. The formation of DNA adducts displayed a dose-dependent increase in the same organs that displayed a similar dose-dependent increase in tumor incidence from the NTP (1993) study.

The binding of 1,2,3-trichloropropane or related metabolites to DNA increased with multiple doses of 30 mg/kg-day administered 24 hours apart (Weber and Sipes, 1990). The binding to DNA did not increase after the second dose, but was approximately doubled after the third dose. Polyploidy was apparent in the hepatocytes of male albino rats dosed with 0.8 and 2.16 mg/L for 2 hours (Belyaeva et al., 1974), and a dose-dependent increase in DNA strand breaks was evident in hepatocytes from F344/N rats at 30-100 mg/kg (Weber and Sipes, 1991) and in kidney cells from male Wistar rats at >375 mmol/kg (Lag et al., 1991). A dose-dependent increase in the incidence of tumors was observed in rats dosed with 3–30 mg/kg-day and in mice dosed with 6–60 mg/kg-day (NTP, 1993). The in vivo data demonstrate an increase in DNA-binding capability, DNA strand breaks, and DNA adducts at doses of 1,2,3-trichloropropane that are similar to the dose levels administered in the NTP (1993) bioassay in which an increased incidence of tumors in multiple organs was observed at all dose levels tested.

Temporal relationship

The temporal relationship for mutagenicity and tumorigenicity has not been adequately studied. However, data indicate that metabolism of 1,2,3-trichloropropane to its metabolites is a necessary event in the mutagenic mode of action. 1,2,3-Trichloropropane metabolism follows three potential routes, each of which involves GSH at different steps in the metabolism process. Two primary routes of metabolism involve the formation of an episulfonium ion, while the third involves the intermediate metabolite, DCA (Mahmood 1991), which is a reported mutagen (Weber and Sipes, 1992).

In addition, there are in vitro and in vivo data that demonstrate metabolism of 1,2,3-trichloropropane, followed by binding of reactive metabolites to DNA, and the ultimate formation of DNA adducts. This sequence of events has been demonstrated in the bacterial and mammalian cell systems assays in which activation with an S9 fraction from chemically-induced rat or hamster livers may be necessary for genotoxicity and potential mutagenicity (Tafazoli and Kirsch-Volders, 1996; Lag et al., 1994; NTP, 1993; Ratpan and Plaumann, 1988; von der Hude et al., 1987; Haworth et al., 1983; Kier, 1982; Shell Oil Co., 1982, 1979; Stolzenburg and Hine, 1980).

Evidence for the direct interaction of 1,2,3-trichloropropane metabolites with DNA, RNA, and hepatic proteins was observed 4 hours following i.p. administration of 1,2,3-trichloropropane (Weber and Sipes, 1990). This investigation demonstrates the ability of 1,2,3-trichloropropane metabolites to bind to hepatic DNA, RNA, and protein, and that the binding increases with multiple doses. DNA strand breaks were evident in the extracted hepatic DNA of male F344/N rats administered 1,2,3-trichloropropane by i.p. injection, thus demonstrating that 1,2,3-trichloropropane metabolites cause the formation of DNA strand breaks (Weber and Sipes, 1991).

La et al. (1995) characterized the formation of DNA adducts in various organs in both B6C3F1 mice and F344/N rats 6 hours following a single dose of 1,2,3-trichloropropane, and found high concentrations of DNA adducts in the tumor-forming organ tissues, including the forestomach, kidney, pancreas, and liver in male rats and the forestomach, liver, lung, and kidney of male mice.

Biological plausibility and coherence

Mutagenicity as a mode of action for carcinogenicity in humans is generally accepted and is a biologically plausible mechanism for tumor induction. The formation of DNA adducts in organs that also displayed an increase in the tumor incidence in rats and mice indicates coherence of the effects and is evidence supporting a mutagenic mode of action (Table 4-29). The proposed mode of action includes bioactivation of 1,2,3-trichloropropane leading to DNA adduct formation, followed by the induction of mutations in cancer-related genes, and eventually resulting in tumor formation. However, the formation of DNA adducts of 1,2,3-trichloropropane in tissues other than those where tumors formed (La et al., 1995) is an area of uncertainty associated with the suggested mutagenic mode of action. DNA adduct formation for some tumor types may be necessary but not sufficient for the induction of tumors and is not an uncommon occurrence as DNA adducts of known direct-acting carcinogens (e.g., benzo[a]pyrene) have been observed in tissues where tumors were not found. The formation of DNA adducts in nontumor forming tissues and organs may signify that DNA adducts by themselves are insufficient to cause

tumors or that the increased mortality in the rats and increased tumor incidence in other organs precluded tumor formation in the nontumor-forming organs.

In addition, the formation of episulfonium ions and subsequent DNA binding by similar compounds supports a mutagenic mode of action of carcinogenesis for 1,2,3-trichloropropane. Specifically, 1,2-dibromoethane spontaneously forms the episulfonium ion, thiiranium, following conjugation with GSH, which may then bind to DNA (U.S. EPA, 2004). DNA binding of metabolites of 1,2-dibromoethane and DBCP has also been demonstrated in vitro in calf thymus DNA (Inskeep and Guengerich, 1984). Binding of metabolites of 1,2-dibromopropane in vivo in rats following i.p. injection (Kim and Guengerich, 1990) also provides support for the proposed mode of action. Holme et al. (1989) found that DBCP induced DNA damage in liver cells at concentrations much lower than concentrations that resulted in cytotoxicity and bacterial (*S. typhimurium*) mutagenicity.

4.7.3.3. Other Possible Modes of Action

Data are not available to make a determination about whether other modes of action, such as cytotoxicity with tissue repair due to DNA degradation or disruption of cell signaling, are associated with the carcinogenic activity of 1,2,3-trichloropropane. However, the mode of action of 1,2,3-trichloropropane-induced forestomach tumors may include promotion. Specifically, the use of corn oil as a vehicle for the administration of carcinogenic chemicals has been shown to increase the incidence and severity of epithelial cell proliferation of the forestomach in rats (Ghanayem et al., 1986). However, no data demonstrating proliferation in the forestomach following corn oil gavage administration of 1,2,3-trichloropropane are available.

4.7.3.4. Conclusions About the Hypothesized Mode of Action

The proposed mode of action for 1,2,3-trichloropropane tumorigenicity involves mutagenicity via reactive metabolites. The data supporting a mutagenic mode of action include:

- Mutagenic response in short-term bacterial assays (with microsomal activation), indicative of base-pair substitutions and frameshift mutations, and induced chromosomal damage, gene mutations, DNA breakage, micronucleus formation, and enhanced DNA viral transformation in mammalian cell assays;

- Covalent binding of 1,2,3-trichloropropane metabolites to hepatic protein, DNA, and RNA and the induction of DNA strand breaks in the hepatocytes of rats following in vivo exposure, and induced wing spot formation in the SMART in *D. melanogaster*;

- Dose-dependent formation of DNA adducts, including the major adduct S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione, in various organs of both

B6C3F1 mice and F344/N/N rats, with DNA adducts present in tumor-forming organs of male rats and mice; and

- Dose-dependent increase in the formation of the intermediate metabolite, and reported mutagen and tumor initiator, DCA, and the formation of reactive episulfonium ion metabolites.

The available in vitro and in vivo data also indicate that metabolites of 1,2,3-trichloropropane have an affinity for certain nucleic acids and a capacity to form DNA adducts, although in vivo assays that directly measure mutagenicity are unavailable. For example, regular test batteries for different genetic endpoints in vitro and, especially, in vivo, such as micronucleus formation, chromosomal aberrations, unscheduled DNA synthesis, sister chromatid exchanges, Comet assay, and DNA adduct analysis, are limited.

A number of assays have tested nonpositive for DNA reactivity and mutagenicity of 1,2,3-trichloropropane. 1,2,3-Trichloropropane tested nonpositive in studies investigating mutagenic potential (without microsomal activation), micronucleus formation, unscheduled DNA synthesis, and chromosomal damage in vitro. Nonpositive in vivo assays included dominant lethal induction and micronucleus formation. Despite these nonpositive results, other chlorinated aliphatics, while showing a weak response, failed to induce a clear dose-dependent increase in miconucleus formation, which suggests that the Comet assay, in which 1,2,3-trichloropropane induced DNA damage, may be a more suitable and sensitive method for this chemical class (Tafazoli and Kirsch-Volders, 1996). In addition, Crebelli et al. (1999) stated that micronucleus formation in mouse bone marrow is weakly sensitive to the genotoxic effects induced by halogenated hydrocarbons in other test systems, and a negative bone marrow micronucleus assay should not offset the consistently positive in vitro results (Dearfield and Moore, 2005).

Is the hypothesized mode of action sufficiently supported in test animals?

The covalent binding of bioactivated 1,2,3-trichloropropane to hepatic DNA, RNA, and protein was evident in male F344/N rats (Weber and Sipes, 1990). A dose-dependent increase in the amount of 1,2,3-trichloropropane equivalents bound to hepatic DNA and protein was demonstrated.

Weber and Sipes (1991) administered i.p. injections to male F344/N rats. Following the extraction of hepatic DNA, they demonstrated that 1,2,3-trichloropropane, or its metabolites, caused the formation of DNA strand breaks.

La et al. (1995) characterized the formation of DNA adducts in various organs both in B6C3F1 mice and F344/N rats, and found high concentrations of DNA adducts in organ tissues in which tumor formation was observed by NTP (1993). The investigators characterized the DNA adduct, indicating that a single, major 1,2,3-trichloropropane-derived DNA adduct was

formed irrespective of the tissue type, and determined the adduct to be S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione. The formation of this adduct was detected in the forestomach, glandular stomach, kidney, liver, pancreas, and tongue of F344/N rats, and in the forestomach, glandular stomach, kidney, and liver of B6C3F1 mice. The concentrations of adduct formed in the target organs showed correlation with the tumor incidence from the NTP (1993) study.

The target organs of 1,2,3-trichloropropane toxicity (liver, kidney, forestomach, and intestine) also contain the highest concentration of covalently-bound 1,2,3-trichloropropane and related metabolites (Mahmood et al., 1991), which supports a role for metabolic activation and binding in the early stages of carcinogenesis.

In addition to the experimental data for 1,2,3-trichloropropane, halogenated propanes, as a class of compounds, are generally considered to be mutagenic (Lag et al., 1994; Ratpan and Plaumann, 1988).

Is the hypothesized mode of action relevant to humans?

The postulated key events, the metabolism of 1,2,3-trichloropropane to a DNA-reactive compound and the alteration of the genetic material leading to tumor-inducing mutations, are both possible in humans. Mutagenicity as a mode of action for carcinogenicity in humans is generally accepted and is a biologically plausible mechanism for tumor induction. The toxicokinetic and toxicodynamic processes that would enable reactive metabolites to produce mutations in animal models are biologically plausible in humans.

Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?

According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), children exposed to carcinogens with a mutagenic mode of action are assumed to have increased early-life susceptibility. The *Supplemental Guidance* (U.S. EPA, 2005b) recommends the application of age-dependent adjustment factors (ADAFs) for carcinogens that act through a mutagenic mode of action. Given the weight of the available evidence, 1,2,3-trichloropropane acts through a mutagenic mode of carcinogenic action and the ADAFs should be applied.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No studies are available that address the possible adverse effects of 1,2,3-trichloropropane in children. However, there is evidence that 1,2,3-trichloropropane is mutagenic and, therefore, may act through a mutagenic mode of action for carcinogenicity. In accordance with the *Supplemental Guidance* (U.S. EPA, 2005b), the mutagenic mode of carcinogenic action for 1,2,3-trichloropropane would indicate an increased carcinogenic

susceptibility for early-life exposures. Although developmental toxicity studies for 1,2,3-trichloropropane are unavailable, developmental toxicity is a concern due to the genotoxicity of 1,2,3-trichloropropane and the possibility for genetic damage to the germ cells of the F1 generation that could be transmitted to the F2 generation. In addition, the two-generation reproductive assessment by gavage indicates that the developing fetus may be a target of toxicity due to an observed reduction in the number of live mouse pups/litter and in the proportion of male pups born alive following oral exposure.

4.8.2. Possible Gender Differences

The extent to which men and women differ in susceptibility to 1,2,3-trichloropropane is unknown. However, some data may exist that imply a difference between male and female rats in their response to inhalation of the compound. For example, 15/15 male CD rats exposed to 1,2,3-trichloropropane via inhalation (6 hours/day, 5 days/week, for 13 weeks) at a concentration of 50 ppm displayed histopathological lesions in the liver, while 0/15 females displayed this effect at the same concentration (Johannsen et al., 1988). A clear-cut dose-dependent response in this effect was seen in the males, but females showed no response. The biological significance of this finding for lower doses and for other species is unclear.

4.8.3. Other

GSH appears to be necessary for the formation of the DNA adduct, S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione. Individuals with a GSH deficiency may be less susceptible to the carcinogenic effects of 1,2,3-trichloropropane. Conversely, individuals with increased expression of GSH may have an increased susceptibility to the genotoxic effects of 1,2,3-trichloropropane.

5. DOSE RESPONSE ASSESSMENT

5.1. CHRONIC ORAL REFERENCE DOSE (RfD)

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

Data on the health effects of oral exposure to 1,2,3-trichloropropane in humans are not available. The database of chronic and subchronic animal studies included a 2-year gavage study in F344/N rats and B6C3F1 mice (Irwin et al., 1995; NTP, 1993), a 90-day gavage study in Sprague-Dawley rats (Merrick et al., 1991), a 90-day drinking water study in Sprague-Dawley rats (Villeneuve et al., 1985), a 17-week gavage study in F344/N rats (NTP, 1993; Hazleton Laboratories, 1983a), a 17-week gavage study in B6C3F1 mice (NTP 1993; Hazleton Laboratories, 1983b), and a two-generation reproductive/fertility assessment in Swiss CD-1 mice (NTP, 1990). The subchronic (i.e., 90-day study or less) study data were not considered in the selection of a principal study for deriving the chronic RfD because the database contains reliable dose-response data from a chronic study of two species and a two-generation reproductive assessment. The data from the subchronic studies were, however, used to corroborate the findings of the chronic studies.

The dose-dependent, noncancer effects associated with oral exposure to 1,2,3-trichloropropane include increased liver weights (subchronic and chronic); increased kidney weights (subchronic and chronic); hepatic, renal, myocardial, lung, and nasal turbinate epithelial necrosis (subchronic); decreased synthesis of pseudocholinesterase (subchronic); decreased ALT and 5'-nucleotidase levels (chronic); increased ALT, AST, and SDH levels (subchronic and chronic); increased hepatic aminopyrine demethylase and aniline hydroxylase activity (subchronic); elevated creatine kinase (chronic); decreased number of pregnancies per fertile pair, reduced number of live pups/litter; and decreased proportion of male pups born alive (NTP, 1993, 1990; Merrick et al., 1991; Villeneuve et al., 1985).

The NTP (1993) study was selected as the principal study because it was a well-designed chronic study, conducted in both sexes of two rodent species with a sufficient number of animals per dose group. The number of test animals allocated among three dose levels and an untreated control group was acceptable, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Increased liver weight was selected as the critical effect because liver toxicity appeared to be the most sensitive effect. There is evidence of hepatocellular damage, including increased incidence of hepatocellular necrosis and decreased synthesis of pseudo-cholinesterase, from the subchronic NTP (1993) studies, and increased serum concentrations of hepatocellular enzymes (ALT and SDH), decreased concentration of 5'-nucleotidase, and increased incidence of histopathologic liver lesions, including hepatocellular necrosis, from the chronic NTP (1993) studies. Increased liver weight was selected as the critical effect because it

represents the most sensitive effect observed in the liver and occurs early in the process of liver toxicity associated with oral exposure to 1,2,3-trichloropropane. The designation of the liver as a target organ for noncancer effects is also supported by the mechanistic data from Weber and Sipes (1990) that demonstrated the binding of 1,2,3-trichloropropane metabolites to hepatic proteins and nucleic acids.

Other possible critical effects include kidney, respiratory, myocardial, or reproductive toxicity. In the kidney, an increase in organ weight after both subchronic and chronic exposure was accompanied by an increased severity of nephropathy and incidence of renal tubule hyperplasia in rats in the chronic NTP (1993) study. The subchronic NTP (1993) study also demonstrated epithelial necrosis in the nasal turbinates of rats and regenerative lung lesions in mice. Hyperplasia was also observed in the forestomach (basal cell and squamous) and pancreas (acinar) of rats and in the forestomach (squamous) of mice following chronic exposure to 1,2,3-trichloropropane (NTP, 1993). Merrick et al. (1991) showed an increased incidence of inflammation-associated myocardial necrosis in rats, and increased levels of creatine kinase were apparent in the chronic NTP study. NTP (1990) demonstrated a decrease in the number of pregnancies per fertile pair, a reduction in the number of live pups/litter, and a decrease in the proportion of male pups born alive. Although the liver appeared to be the most sensitive indicator of 1,2,3-trichloropropane-induced toxicity, RfDs for the changes in kidney weight, fertility, and pups/litter were also derived for comparison purposes.

5.1.2. Methods of Analysis—Including Models

Benchmark dose (BMD) modeling was conducted using the EPA's Benchmark Dose Software (BMDS, version 1.4.1) to analyze the changes in liver and kidney weight, fertility, and number of pups/litter associated with chronic exposure to 1,2,3-trichloropropane (see Appendix B for details). The software was used to calculate potential points of departure (PODs) for deriving the chronic RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$). For continuous endpoints, the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) states that a minimal level of change in an endpoint that is generally considered to be biologically significant may be used to define the benchmark response (BMR). For analysis of absolute and relative liver and kidney weight changes in both rats and mice, a BMR of 10% was selected, as it is analogous to the 10% change in body weight used to identify maximum tolerated doses (MTDs). A BMR of 1 standard deviation (SD) from the control mean was also included for comparisons with other chemicals in the IRIS database that affect absolute and relative liver weights. In the reproductive toxicity study, a 10% change in fertility rate was selected as the BMR, in accordance with the *Benchmark Dose Technical Guidance Document* (US EPA, 2000c) and a 1% change in mean live pups/litter for the 4^{th} and 5^{th} litters was selected as the BMR due to the frank toxicity of this endpoint.

Absolute and relative liver weight changes were also modeled using a BMR of 1 SD, as recommended by the Benchmark Dose Technical Guidance Document (US EPA, 2000) for continuous endpoints for comparison purposes.

Table 5-1 presents BMDs and their corresponding lower 95% confidence limits (BMDLs) for each observed effect that was considered and amenable to modeling. The candidate BMD for each endpoint was identified by comparing the BMDS outputs from the fitted models for each of the four data sets: male rats, female rats, male mice, and female mice. Adequacy of each model fit was determined by assessing the χ^2 goodness-of-fit statistic using a significance level of $\alpha = 0.1$. The best-fitting models were selected from those exhibiting adequate fit by considering the Akaike Information Criterion (AIC) value of each model and how well the model visually fit the data (see Appendix B).

Table 5-1. Candidate BMDs for chronic and reproductive effects associated with oral exposure to 1,2,3-trichloropropane

Endpoint	Species/sex	Model	BMD[a] (mg/kg-d)	BMDL[a] (mg/kg-d)	BMR
Absolute liver weight	Rat/male	Hill	3.8	1.6	10% change in mean organ weight
Absolute liver weight	Rat/male	Hill	3.2	1.4	1 SD
Relative liver weight	Rat/male	Hill	5.5	3.1	10% change in mean organ weight
Relative liver weight	Rat/male	Hill	3.2	1.8	1 SD
Absolute kidney weight	Rat/female	Hill	9.0	3.4	10% change in mean organ weight
Relative kidney weight	Rat/male	Hill	10.5	6.4	10% change in mean organ weight
Fertility generating 4th litter	Mice	Log-probit (slope ≥ 1)	52.6	37.3	10% change in fertility rate
Fertility generating 5th litter	Mice	Probit	31.2	23.3	10% change in fertility rate
Live pups/litter- 4th litter	Mice	Polynomial	13.8	3.2	1% change in mean live pups/litter
Live pups/litter- 5th litter	Mice	Polynomial	13.6	5.6	1% change in mean live pups/litter

[a]BMDs and BMDLs from the best-fitting models for each endpoint (see Appendix B).

The increases in both absolute and relative liver weights in male rats were fit adequately by the Hill model. Increase in liver weight was chosen as the critical effect and, more specifically, absolute liver weight was selected to represent this increase in liver weight because it was the most sensitive endpoint. The selection of the liver as the critical target organ and increased liver weight as the critical effect representative of this hepatotoxicity is supported by increased serum liver enzyme levels, increased incidence of hepatic necrosis, and decreased pseudocholinesterase. Increased liver weight was selected as the critical effect because it represents the most sensitive endpoint in a spectrum of liver effects and occurs early in the process of liver toxicity associated with oral exposure to 1,2,3-trichloropropane.

The 10% increase in absolute liver weight is a more sensitive endpoint than the 1% decrease in the number of live pups/litter in the 4^{th} and 5^{th} litters. Statistically significant reductions, relative to controls, in the number of live pups/litter were observed in mice in the second through the fifth breedings at the highest dose tested (120 mg/kg-day) and at the fifth breeding at a dose of 60 mg/kg-day. When comparing the BMD modeling results, the lower POD identified for the increase in absolute liver weight ($BMDL_{10\%}$ of 1.6 mg/kg-day) is thus thought to represent a more sensitive endpoint than the decrease in the number of live pups/litter ($BMDL_{1\%}$ of 3.2 mg/kg-day), even though the decrease in live pups/litter is considered a frank effect.

Consideration of the available dose-response data to determine an estimate of oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 2-year gavage study in Fischer rats (NTP, 1993) and increased absolute liver weight in males as the principal study and critical effect, respectively, for deriving the chronic RfD for 1,2,3-trichloropropane. The dose-response relationships between oral exposure to 1,2,3-trichloropropane and impaired fertility in CD-1 mice are also suitable for deriving a chronic RfD, but these endpoints yielded higher BMDLs than the selected critical effect (absolute liver weight) and corresponding BMDL.

The BMDL corresponds to the 95% lower bound on dose associated with a 10% increase in mean absolute liver weight. The BMD_{10} estimated from the Hill model using absolute liver weight change in male F344/N rats is 3.8 mg/kg-day and the corresponding $BMDL_{10}$ is 1.6 mg/kg-day. A BMR of a 10% change in the mean was used in the modeling of absolute and relative liver and kidney weight because the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) recommends using the minimal amount of change in the endpoint that is considered to be biologically significant to define the BMR. Duration-adjustment of the PODs was done to approximate continuous daily exposures by multiplying the BMD_{10} and $BMDL_{10}$ by (5 days)/(7 days) = 0.71; resulting in a BMD_{ADJ} of 2.70 mg/kg-day and a $BMDL_{ADJ}$ of 1.1 mg/kg-day.

5.1.3. Chronic RfD Derivation—Including Application of Uncertainty Factors (UFs)

A $BMDL_{ADJ}$ of 1.1 mg/kg-day for increased absolute liver weight in male rats chronically exposed to 1,2,3-trichloropropane by gavage (NTP, 1993) was used as the POD to calculate the chronic RfD. A total UF of 300 was applied to this effect level: 10 for uncertainty associated with interspecies differences (UF_A: animal to human), 10 to account for intraspecies variation (UF_H: human variability), and 3 for database deficiencies (UF_D: database deficiency). The rationale for application of these UFs is described below.

A 10-fold UF_A was used to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability) because information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans.

A 10-fold UF_H was used to account for variation in susceptibility among members of the human population (i.e., interindividual variability) because information is unavailable to predict potential variability in human susceptibility.

An UF_S was not needed to account for extrapolation from subchronic-to-chronic exposure because a chronic study was used to derive the chronic RfD.

An UF_L for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, a BMR of a 10% change in absolute liver weight was selected under an assumption that it represents a minimal biologically significant change.

A 3-fold UF_D was selected to account for database deficiencies. The database of chronic and subchronic animal studies includes a 2-year gavage study in F344/N rats and B6C3F1 mice (Irwin et al., 1995; NTP, 1993), a 90-day gavage study in Sprague-Dawley rats (Merrick et al., 1991), a 90-day drinking water study in Sprague-Dawley rats (Villeneuve et al., 1985), a 17-week gavage study in F344/N rats (NTP, 1993; Hazleton Laboratories, 1983a), a 17-week gavage study in B6C3F1 mice (NTP 1993; Hazleton Laboratories, 1983b), and a two-generation reproductive/fertility assessment in Swiss CD-1 mice (NTP, 1990). A threefold UF_D for database deficiencies was applied because the database lacks information on developmental toxicity associated with 1,2,3-trichloropropane. In addition, the two-generation reproductive toxicity study indicates that the developing fetus may be a target of toxicity. The lack of a reproductive toxicity study that extends beyond two generations and the absence of a developmental toxicity study are of particular concern due to the genotoxicity of 1,2,3-trichloropropane, which may mean that any resulting genetic damage to the germ cells of the F1 generation may not be detected until the F2 generation.

The chronic RfD for 1,2,3-trichloropropane was calculated as follows:

$$RfD = BMDL_{ADJ} \div UF$$
$$= 1.1 \text{ mg/kg-day} \div 300$$
$$= 4 \times 10^{-3} \text{ mg/kg-day (rounded to one significant figure)}$$

5.1.4. Chronic RfD Comparison Information

Figure 5-1 is an exposure-response array that presents NOAELs, LOAELs, and the dose range tested corresponding to selected health effects, some of which were considered candidates for chronic RfD derivation, from subchronic, chronic, and reproductive toxicity studies. The health effects from the subchronic NTP (1993) study included decreased synthesis of pseudocholinesterase and hepatic necrosis. The health effects from the chronic NTP study included increased absolute and relative liver and kidney weights, and the effects from the NTP reproductive toxicity study included a decrease in the number of pregnancies per fertile pair and a decrease in the number of live pups per litter.

Figure 5-2 presents the POD, applied uncertainty factors, and candidate chronic RfDs for additional endpoints that were modeled using the EPA's BMDS (version 1.4.1) and which appear in Table 5-1. This figure is intended to provide information on additional health effects associated with varying levels of 1,2,3-trichloropropane exposure.

PODs and candidate chronic RfDs that could be derived from the additional health effects identified in Table 5-1 are presented in Figure 5-1 to allow a comparison with the critical effect. For increased relative liver weight, increased absolute and relative kidney weights, decreased fertility generating the 4th and 5th litters, and decreased live pups/litter, the uncertainty factors applied were a 10-fold UF to account for uncertainty in extrapolating from laboratory animals to humans, a 10-fold UF to account for variation in susceptibility among members of the human population, and a threefold UF for database deficiencies.

A change in liver weight is the most sensitive endpoint in a spectrum of liver effects following oral exposure to 1,2,3-trichloropropane, and increased serum liver enzymes and an increased incidence of hepatic necrosis, as well as a decrease in pseudocholinesterase, all indicators of liver damage, provide support for the selection of the liver as the critical target organ. The dose-response relationships between oral exposure to 1,2,3-trichloropropane and impaired fertility in CD-1 mice are also suitable for deriving a chronic RfD, but yield higher BMDLs than the selected critical effect. Thus, the RfD based on absolute liver weight is likely to be protective of any impaired fertility effects. Consideration of the available dose-response data to determine an estimate of oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 2-year gavage study in Fischer rats (NTP, 1993) and increased absolute liver weight in males as the principal study and critical effect, respectively, for deriving the chronic RfD for 1,2,3-trichloropropane.

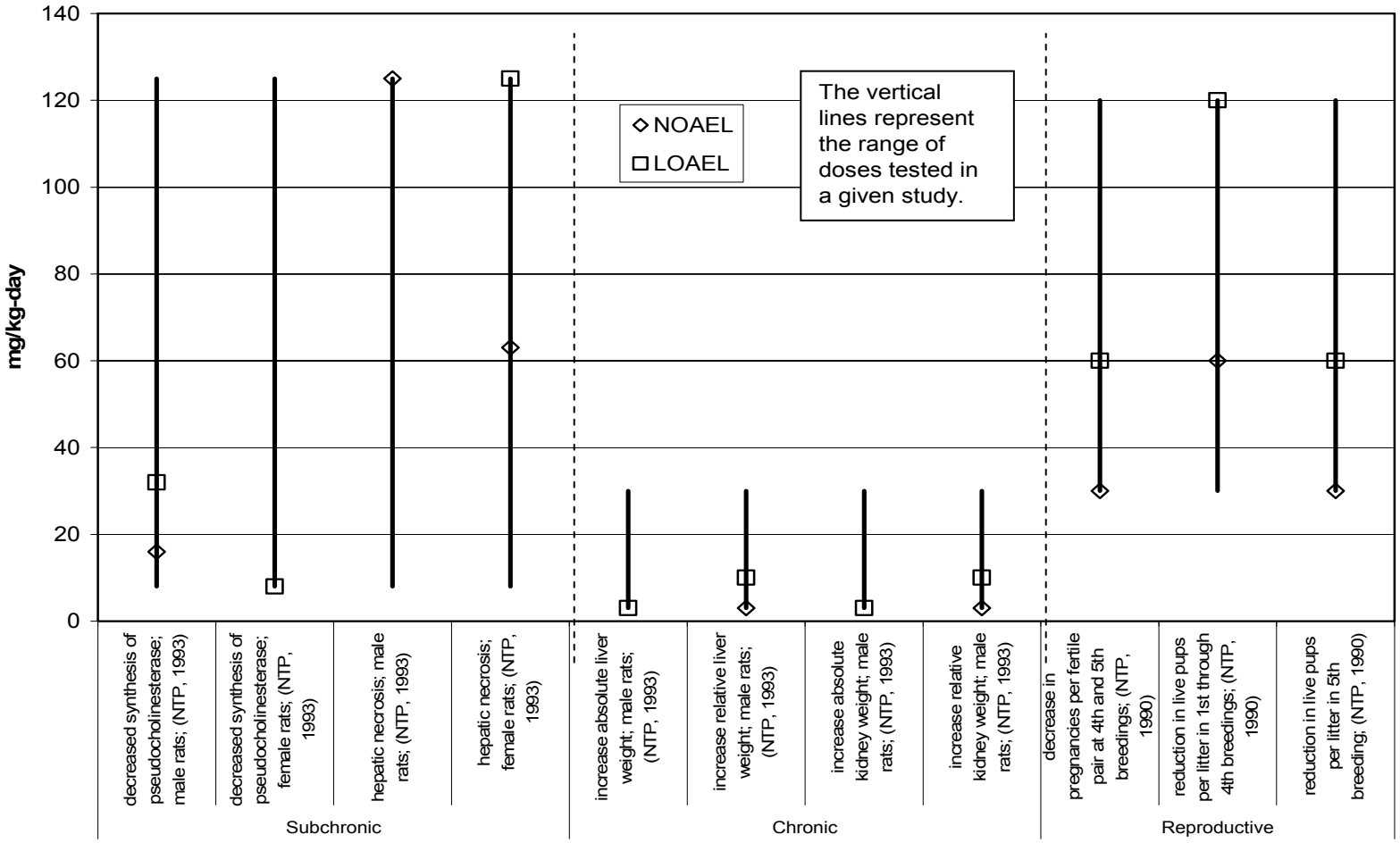


Figure 5-1. Exposure-response array of selected subchronic, chronic, and reproductive toxicity effects.

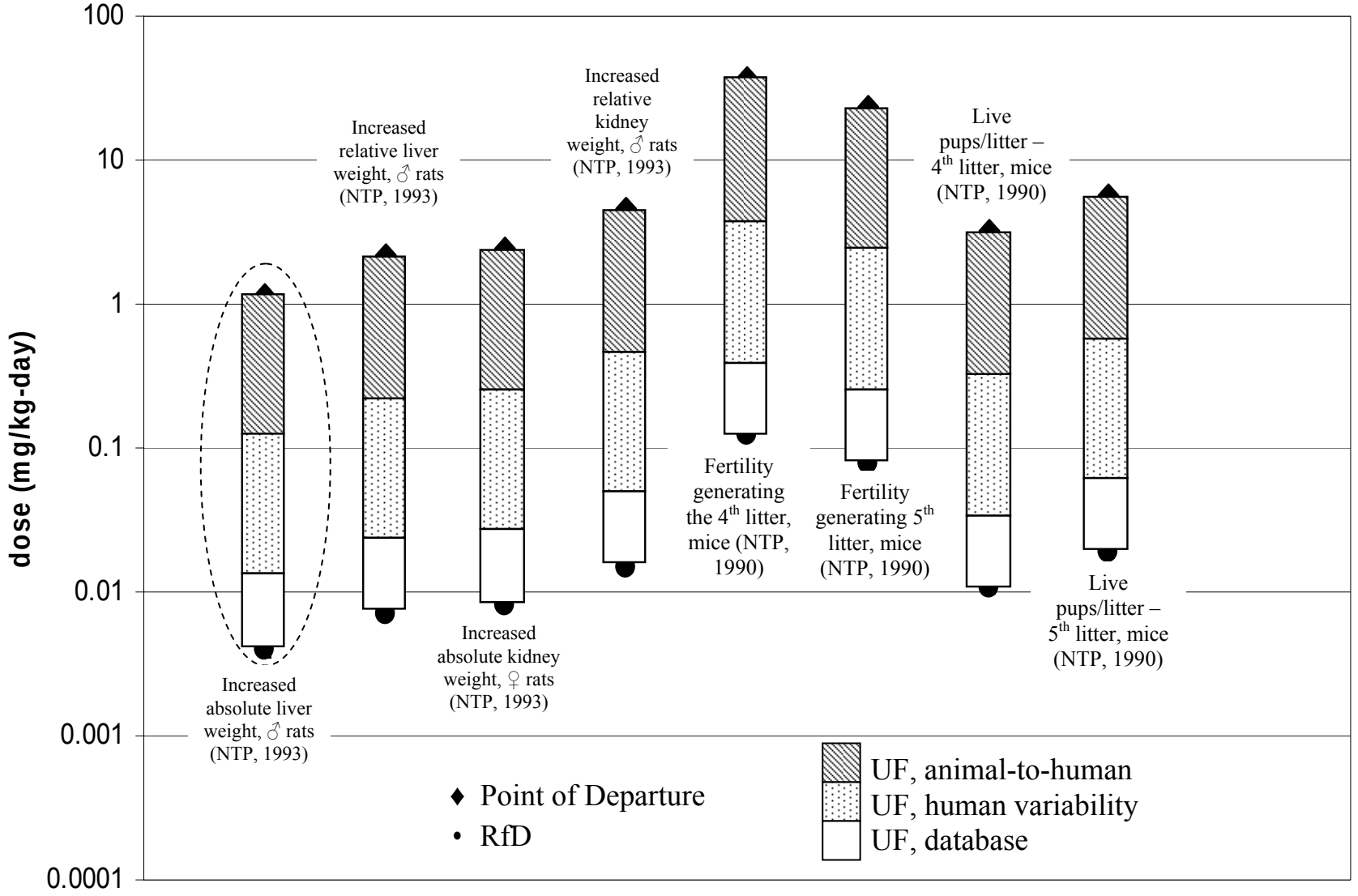


Figure 5-2. PODs for selected endpoints (with critical effect circled) from Table 5-1 with corresponding applied UFs and derived candidate chronic oral RfDs.

5.1.5. Previous Oral Assessment

The previous IRIS assessment for 1,2,3-trichloropropane was entered into the database in 1987 and contains an oral chronic RfD of 6×10^{-3} mg/kg-day. The chronic RfD was based on a duration-adjusted NOAEL of 5.71 mg/kg-day for alterations in clinical chemistry and reduced red blood cell mass in female F344/N rats following a 17-week gavage exposure (NTP, 1983; Hazleton Laboratories, 1983a). A total UF of 1000 was used to account for interspecies extrapolation, human variability, and extrapolation from a subchronic study. This assessment was last updated in 1990 before the publication of the NTP (1993) Technical Report used for this assessment.

5.2. CHRONIC INHALATION REFERENCE CONCENTRATION (RfC)

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

Inhalation studies of 1,2,3-trichloropropane in humans are limited. A single report (Silverman et al., 1946) on the effects in humans found that all subjects (12/sex) experienced irritation (eyes, throat, and odor) following 15-minute exposures to air concentrations of 100 ppm trichloropropane (isomer and purity not reported). The database of inhalation toxicity studies in animals includes two 2-week studies submitted to EPA by Miller et al. (1987a, b), a 4-week range-finding study, two 13-week studies, and two single-generation reproductive assessments (Johannsen et al., 1988; Biodynamics, Inc., 1979).

Increased organ weights and histopathological lesions in rodents have been associated with subchronic inhalation exposure to 1,2,3-trichloropropane. Concentration-dependent increases in absolute and relative liver weight were observed in males and female rats (Johannsen et al., 1988; Miller et al., 1987a; Biodynamics, Inc., 1979). An increase in relative lung weight was also observed in female rats (Biodynamics, Inc., 1979). The histology data demonstrated that 1,2,3-trichloropropane is both a local irritant affecting the nasal epithelium (Miller et al., 1987a, b) and a systemic toxicant producing effects remote from the site of entry, including peribronchial lymphoid hyperplasia, hepatocellular hypertrophy, and extramedullary hematopoiesis (Johannsen et al., 1988; Biodynamics, Inc., 1979).

Johannsen et al. (1988) also conducted two single-generation reproductive toxicity studies using 10 male and 20 female CD rats/group. Female rats exhibited decreased mating performance at 5 ppm, where 16 out of 20 females mated, and at 15 ppm, where 10 out of 20 females mated, compared with 17 out of 20 mated females in the control group. The decrease in the proportion of females that mated was found to be statistically significant ($p < 0.02$) at 15 ppm in the Fisher Exact Test conducted by EPA.

The Johannsen et al. (1988) study was selected as the principal study. The number of test animals allocated among five dose levels and an untreated control group was acceptable, with examination of appropriate toxicological endpoints in both sexes. The critical effect selected for the derivation of the chronic RfC is the development of peribronchial lymphoid hyperplasia in

the lungs of CD rats, with a NOAEL of 1.5 ppm and a LOAEL of 5 ppm, which is supported by the occurrence of this effect in both male and female rats and the possible correlation between the hyperplasia and the observed increased lung weight. Peribronchial lymphoid hyperplasia, also defined as lymphoid hyperplasia of the bronchus-associated lymphoid tissue, is histologically characterized by the presence of hyperplastic lymphoid follicles with reactive germinal centers distributed along the bronchioles and bronchi (Howling et al., 1999; Myers and Kurtin, 1995; Fortoul et al., 1985; Yousem et al., 1985). A NOAEL of 5 ppm and a LOAEL of 15 ppm were identified for the increase in lung weight.. Although an increase in liver and kidney weights was apparent, lesions and serum enzyme levels indicative of liver and kidney damage were not evident. The only pathological endpoint observed in the liver was hepatocellular hypertrophy in male rats at 5, 15, and 50 ppm. The observed liver effects occurred at doses higher than those found for lung effects and , as such, were not considered further for the derivation fo the POD. The hematopoiesis of the spleen in female rats was not considered biologically relevant as there was no change in the clinical chemistry and hematology parameters.

5.2.2. Methods of Analysis—Including Models

Benchmark dose (BMD) modeling was conducted using the EPA's BMDS (version 1.4.1) to analyze the increased incidence of peribronchial lymphoid hyperplasia in CD rats and, for purposes of comparison, the decreased mating performance in female CD rats (see Appendix C for details). The software was used to calculate potential PODs for deriving the chronic RfC by estimating the effective dose at a specified level of response (BMC_x) and its 95% lower bound ($BMCL_x$). For dichotomous endpoints, the *Benchmark Dose Technical Guidance Document* (US EPA, 2000c) states that an excess risk of 10% is the generally recommended BMR in the absence of any specific data on the change in the critical effect that would be considered biologically significant. Therefore, for the analysis of increased incidence of peribronchial lymphoid hyperplasia, a BMR of 10% is selected. In modeling the decreased mating performance, a BMR of 10% was selected, in accordance with the *Benchmark Dose Technical Guidance Document* (US EPA, 2000c).

Table 5-2 presents benchmark concentrations (BMC) and the corresponding lower 95% confidence limits (BMCLs) for each observed effect that was considered and amenable to modeling. The candidate BMCs for each endpoint were identified by examining the BMDS outputs from the fitted models for each of the data sets. Adequacy of model fit was determined by evaluating the χ^2 goodness-of-fit statistic using a significance level of $\alpha = 0.1$. Of the models that exhibited adequate fit, the best-fitting models were selected based on AIC values and how well the model fit the data visually (see Appendix C).

Table 5-2. BMD modeling results used in the derivation of the RfC

Endpoint	Species/sex	Model	BMC (ppm)	BMCL[a] (ppm)	BMR
Peribronchial lymphoid hyperplasia	Rat/male	Log-logistic (slope ≥ 1)	1.6	0.84	10% extra risk
Decreased mating performance	Rat/female	Log-probit (slope ≥ 1)	4.5	3.0	10% extra risk

[a]BMCs and BMCLs from the best-fitting models for each endpoint (see Appendix B).

The 13-week inhalation study in CD rats (Johannsen et al., 1988) was selected as the principal study and increased incidence of peribronchial lymphoid hyperplasia in males was selected as the critical effect for deriving the chronic RfC for 1,2,3-trichloropropane. The dose-response relationship between inhalation exposure to 1,2,3-trichloropropane and decreased mating performance in female CD rats is also suitable for deriving a chronic RfC, but this relationship yields higher BMCLs than the critical effect selected.

The BMCL corresponds to the 95% lower bound on the dose associated with a 10% increase in the incidence of peribronchial lymphoid hyperplasia. The BMD_{10} calculated from the log-logistic model (slope ≥ 1) using the incidence of peribronchial lymphoid hyperplasia in male CD rats is 1.6 mg/m^3 and the corresponding $BMCL_{10}$ is 0.84 mg/ m^3.

Human equivalent concentrations (HECs) were calculated from the candidate PODs. PODs were converted to mg/m^3, adjusted to continuous exposure (7 days/week, 24 hours/day), and multiplied by a dosimetric adjustment factor (DAF) to calculate the HEC. A DAF is a ratio of animal and human physiologic parameters. The specific DAF used depends on the nature of the contaminant (particle or gas) and the target site (e.g., respiratory tract or remote to the portal-of-entry).

The RfC methodology (U.S. EPA, 1994b) classifies gases into three categories based on their water solubility and reactivity with respiratory tract tissue. 1,2,3-Trichloropropane is considered a category 2 gas because it is relatively insoluble in water and demonstrates systemic toxicity. For category 2 gases, HEC values are calculated using methods for both category 1 (portal-of-entry effects) and category 3 (systemic effects) gases (U.S. EPA, 1994b). The DAF for a category 1 gas is based on the animal-to-human ratio of the minute volume (V_e) divided by the surface area (SA) of the region of the respiratory tract where the effect occurs. The DAF for a category 3 gas is based on the ratio of the animal blood:gas partition coefficient ($H_{b/g\text{-}animal}$) and the human blood:gas partition coefficient ($H_{b/g\text{-}human}$).

The critical effect for the chronic RfC is considered a systemic effect because the critical effect is located beyond the lung tissue in the bronchus-associated lymphoid tissue. The HEC for increased incidence of peribronchial lymphoid hyperplasia in rats exposed to 1,2,3-trichloropropane (a category 3 gas) for 6 hours/day, 5 days/week for 13 weeks was calculated

from a $BMDL_{10}$ of 0.84 ppm (0.84 ppm × molecular weight[147.43] / 24.45 = 5.07 mg/m^3). Conversionto a continuous exposure was accomplished as follows:

$$\begin{aligned} BMCL_{ADJ} &= BMCL_{10} \times (6 \text{ hours})/(24 \text{ hours}) \times (5 \text{ days})/(7 \text{ days}) \\ &= 5.07 \text{ mg/m}^3 \times 0.25 \times 0.71 \\ &= 0.90 \text{ mg/m}^3 \end{aligned}$$

The DAF for an extra-respiratory effect of a gas is the ratio of the animal/human blood: air partition coefficients $[(H_{b/g})_A/(H_{b/g})_H]$. However, the human and rat blood partition coefficients for 1,2,3-trichloropropane are not known. In accordance with the RfC methodology (U.S. EPA, 1994b), when these partition coefficients are unknown, a ratio of 1 is used. This allows a $BMDL_{HEC}$ to be derived as follows:

$$\begin{aligned} BMCL_{HEC} &= BMCL_{ADJ}\,(mg/m^3) \times (H_{b/g})_A/(H_{b/g})_H \\ &= BMCL_{ADJ}\,(mg/m^3) \times 1 \\ &= 0.90 \text{ mg/m}^3 \end{aligned}$$

Application of the inhalation dosimetry methods to the incidence of peribronchial lymphoid hyperplasia in the lung resulted in a $BMCL_{HEC}$ of 0.90 mg/m^3.

5.2.3. Chronic RfC Derivation—Including Application of Uncertainty Factors (UFs)

The $BMCL_{HEC}$ value of 0.90 mg/m^3 based on increased incidence of peribronchial lymphoid hyperplasia in the lungs of male CD rats exposed to 1,2,3-trichloropropane via inhalation (Johannsen et al., 1988) was used as the POD to derive the chronic RfC for 1,2,3-trichloropropane. A total UF of 3000 was applied to this POD: 3 for extrapolation from rats to humans (UF_A: animal to human), 10 for consideration of intraspecies variation (UF_H: human variability), 10 for extrapolation from a subchronic to a chronic exposure (UF_S), and 10 for database deficiencies. The rationale for application of these UFs is described below. Figure 5-3 presents the POD, applied UFs, and quantified chronic RfC for the critical effect selected, an increased incidence of peribronchial lymphoid hyperplasia in the lungs of male rats, as well as decreased mating performance in female rats.

An UF_A of 3 was selected to account for uncertainties in extrapolating from rats to humans. This value is adopted by convention where an adjustment from an animal-specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. An UF of 10 is comprised of two components of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed through the application of a human equivalent concentration (HEC) as described in the RfC methodology (U.S. EPA, 1994b). The

toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method. However, a threefold UF is retained to address this component.

A 10-fold UF_H was used to account for variation in susceptibility among members of the human population (i.e., interindividual variability) because insufficient information is available to predict potential variability in susceptibility among the population.

A 10-fold UF_S was used to account for uncertainty in extrapolating from a subchronic to chronic exposure duration. A 10-fold UF_D was used to account for deficiencies in the database. The database of 1,2,3-trichloropropane inhalation studies, which included two 2-week studies submitted to EPA by Miller et al. (1987a, b), a 4-week range finding study, two 13-week studies, and a single-generation reproductive toxicity study (Johannsen et al., 1988; Biodynamics, Inc., 1979), provides reliable dose-response data from subchronic studies in two species and from a single-generation reproductive toxicity study. However, the database is lacking a multigenerational reproductive toxicity study and a developmental toxicity study. The database deficiencies are of particular concern due to the genotoxicity of 1,2,3-trichloropropane, because genetic damage to the germ cells of the F1 generation may not be detected until the F2 generation.

An UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in the incidence of peribronchial lyphoid hyperplasia was selected as the BMR.

The chronic RfC for 1,2,3-trichloropropane was calculated as follows:

$$\begin{aligned} \text{RfC} &= BMCL_{HEC} \div UF \\ &= 0.90 \text{ mg/m}^3 \div 3{,}000 \\ &= 3 \times 10^{-4} \text{ mg/m}^3 \text{ (rounded to one significant figure)} \end{aligned}$$

5.2.4. Chronic RfC Comparison Information

Similar to the oral toxicity studies, the inhalation studies found statistically significant increases in organ weights. An increase in absolute and relative liver weights were observed in male and female rats following subchronic inhalation exposure and in male and female mice following a 2-week exposure to 1,2,3-trichloropropane. Additionally, an increase in relative lung weights was observed in female rats and an increase in relative kidney weights was observed in male rats following subchronic exposure to 1,2,3-trichloropropane. An increased incidence of peribronchial lymphoid hyperplasia was observed in male and female rats exposed to 5, 15, or 50 ppm 1,2,3-trichloropropane, but the investigators did not examine epithelial tissue in this study. Centrilobular to midzonal hepatocellular hypertrophy was seen in nearly all male rats that were exposed for 13 weeks via inhalation to concentrations of 5, 15, or 50 ppm 1,2,3-

trichloropropane. However, no evidence of hepatic effects was found in female rats that were exposed via inhalation to 50 ppm 1,2,3-trichloropropane. Conversely, a dose-dependent increase in the incidence and severity of extramedullary hematopoiesis of the spleen was observed in female, but not male rats, although this effect is not biologically relevant.

The critical effect selected for the derivation of the chronic RfC is the incidence of peribronchial lymphoid hyperplasia in the lungs of male CD rats due to the occurrence of this effect in both male and female rats, and the possible correlation between the hyperplasia and the observed increased lung weight. In addition, the decreased mating performance in female CD rats was considered a potential critical effect and this endpoint was subjected to BMD modeling. PODs and chronic reference concentrations (RfCs) that could be derived from the additional health effects identified in Table 5-2 are presented in Figure 5-3 to allow a comparison with the critical effect. For the increased incidence of peribronchial lymphoid hyperplasia and decreased mating performance, the UFs applied were a 10-fold UF to account for uncertainty in extrapolating from laboratory animals to humans, a 10-fold UF to account for variation in susceptibility among members of the human population, a 10-fold UF to account for subchronic-to-chronic extrapolation, and a 3-fold UF for database deficiencies.

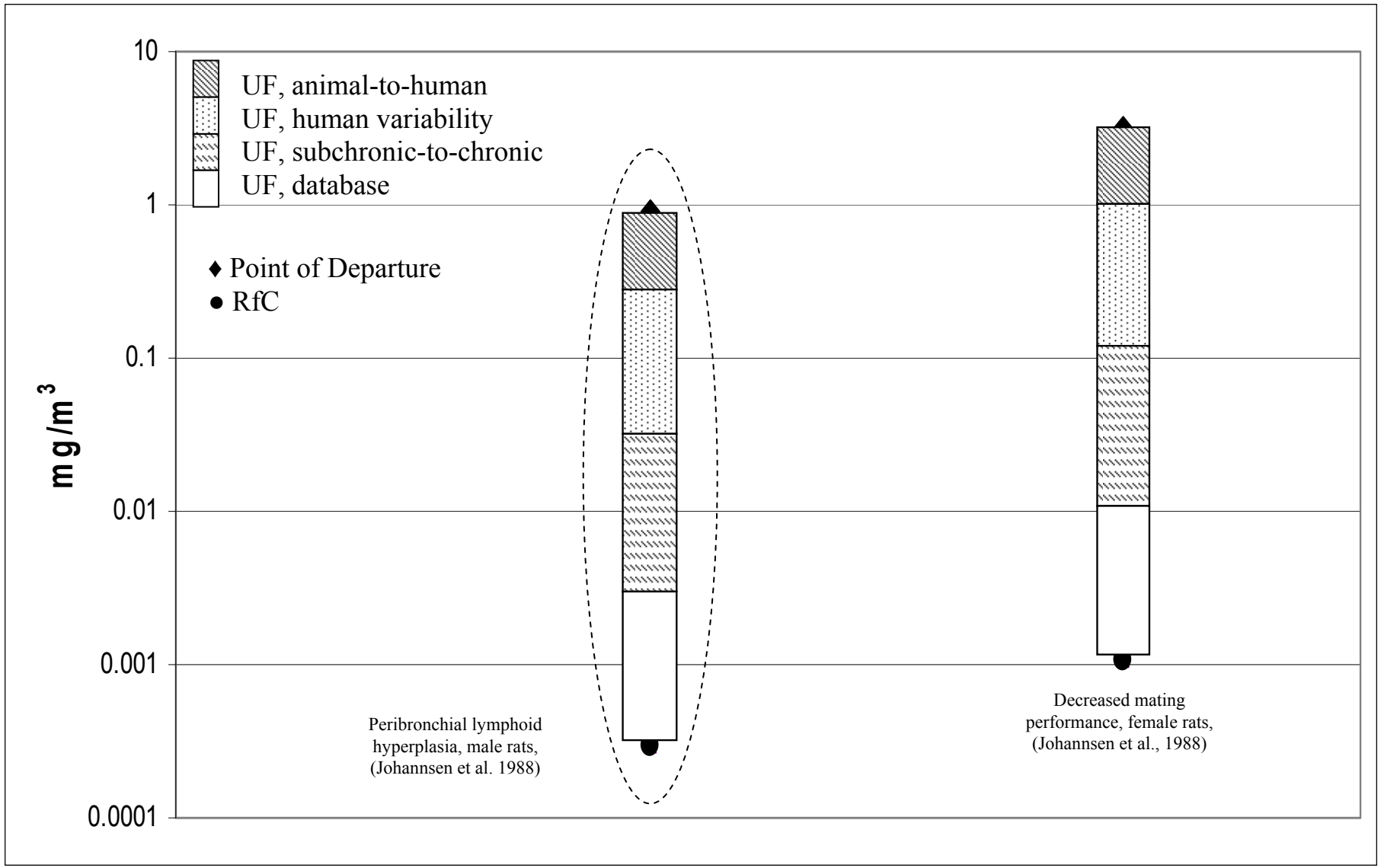


Figure 5-3. PODs for selected endpoints (with critical effect circled) from Table 5-2 with corresponding applied UFs and derived candidate chronic inhalation RfCs.

5.2.5. Previous Inhalation Assessment

A RfC is not available in the current IRIS assessment, which was completed in 1987 (U.S. EPA, 2007).

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

Risk assessments need to portray associated uncertainty. The following discussion identifies uncertainties associated with the chronic RfD and chronic RfC for 1,2,3-trichloropropane. As presented earlier in this chapter (Sections 5.1.2 and 5.1.3; 5.2.2 and 5.2.3), the UF approach, following EPA practices and RfC and RfD guidance (U.S. EPA, 1994b), was applied to a POD, a $BMDL_{HEC}$ for the RfD and a $BMCL_{HEC}$ for the chronic RfC. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal bioassay to human exposure, a diverse population of varying susceptibilities, and database deficiencies. These extrapolations are carried out with default approaches given the paucity of experimental 1,2,3-trichloropropane data to inform individual steps.

An adequate range of animal toxicology data is available for the hazard assessment of 1,2,3-trichloropropane, as described throughout the previous section (Chapter 4). The database of oral toxicity studies includes a chronic gavage study in rats and mice, multiple subchronic gavage and drinking water studies conducted in rats and mice, and a two-generation reproductive/fertility assessment in mice. Toxicity associated with oral exposure to 1,2,3-trichloropropane is observed in the liver, kidney, and reproductive endpoints, including decreased fertility in generating the 4^{th} and 5^{th} litters and decreased number of live pups/litter in the 4^{th} and 5^{th} litters. The database of inhalation toxicity studies in animals includes two 2-week studies submitted to EPA, a 4-week range-finding study, two 13-week studies, and two single-generation reproductive assessments. The inhalation database, however, is lacking a chronic exposure study. Toxicity associated with inhalation exposure to 1,2,3-trichloropropane is observed in the respiratory system as an increased incidence of peribronchial lymphoid hyperplasia. In addition to the oral and inhalation data, there are numerous absorption, distribution, metabolism, and excretion studies, although information on internal or target organ doses of 1,2,3-trichloropropane is not available. Mode of action and genotoxicity studies are also available. Critical data gaps have been identified and uncertainties associated with data deficiencies are more fully discussed below.

Consideration of the available dose-response data to determine an estimate of oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 2-year gavage study in Fischer rats (NTP, 1993) and increased liver weight in males as the principal study and critical effect, respectfully, for deriving the chronic RfD for 1,2,3-trichloropropane. The dose-response relationships between oral exposure to 1,2,3-

trichloropropane and impaired fertility in CD-1 mice are also suitable for use in deriving a chronic RfD, but yields higher BMDLs than the selected critical effect. Thus, the RfD based on an increase in absolute liver weight should be protective of impaired fertility. It should be noted that mice exposed via gavage demonstrated higher DNA adduct formation and cellular proliferation than mice exposed via drinking water (La et al., 1996). Thus, the utilization of the gavage study to derive the RfD, rather than a drinking water or dietary study, may lead to the derivation of a conservative RfD.

In addition, studies have demonstrated that the use of corn oil as a vehicle may increase chemically-induced hepatotoxicity. For example, use of corn oil gavage led to increased hepatotoxicity, measured by altered liver weight, serum chemistry, and histopathological examination, of chloroform when compared to administration via drinking water in F344/N rats (Larson et al., 1995) and B6C3F1 mice (Bull et al., 1986). The role of the corn oil vehicle in the observed hepatotoxicity following gavage exposure to 1,2,3-trichloropropane is unknown. .

The critical effect selected for the derivation of the chronic RfC is based on the increased incidence of peribronchial lymphoid hyperplasia in the lungs of male CD rats. Support for the selection of this critical effect was observed in both male and female rats along with increased lung weight. Although an increase in liver and kidney weights was apparent, serum enzyme level alterations and an increased incidence of histopathological lesions indicative of liver and kidney damage were not observed and the weight changes were observed at doses higher than those observed for the lung effects. The hepatocellular hypertrophy reported in male rats and the hematopoiesis of the spleen in female rats was considered to be of questionable biological significance in the absence of additional overt toxicity in the liver and spleen, as there was no change in the clinical chemistry or hematology parameters. It is important to recognize that the critical effect selected for the derivation of the RfD was increased absolute liver weight, which was supported by additional evidence of hepatotoxicity (i.e., increased serum enzyme levels, cholinesterase levels, and necrosis), and that endpoints indicative of hepatotoxicity were not observed following inhalation of 1,2,3-trichloropropane. In addition, there are toxicokinetic and possible toxicodynamic differences between the two routes of exposure that may account for these differences. A portal-of-entry effect is expected following 1,2,3-trichloropropane exposure via inhalation and a first-pass effect is expected following oral exposure.

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

Similarly, the selection of the BMD model for the quantitation of the chronic RfC does not lead to significant uncertainties in estimating the POD since benchmark effect levels were

within the range of experimental data. However, the selected model, the log-logistic model, does not represent all possible models one might fit, and other models could be selected to yield results that may be either higher or lower than those included in this assessment.

Extrapolating from animals to humans yields further uncertainties. The effect and the magnitude associated with the concentration at the POD in rodents are extrapolated to human response. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not possible for the toxicity observed following oral and inhalation exposure to 1,2,3-trichloropropane. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans, so the 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the chronic RfD. For the chronic RfC, a factor of 3 was adopted by convention where an adjustment from an animal-specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. AnUF of 10 is comprised of two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed through the use of a HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method, but a UF of 3 is retained to account for this component.

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation needs consideration. In the absence of 1,2,3-trichloropropane-specific data on human variation, a factor of 10 was used in the derivation of both the chronic RfD and the chronic RfC. Human variation may be larger or smaller; however, 1,2,3-trichloropropane-specific data to examine the potential magnitude of over- or under-estimation are unavailable.

Data gaps have been identified that yield uncertainties associated with deficiencies regarding the developmental toxicity of 1,2,3-trichloropropane following oral exposure. The two-generation reproductive toxicity study indicated that the developing fetus may be a target of toxicity. In addition, the lack of a multigenerational study, beyond two generations, is of particular concern due to the genotoxicity of 1,2,3-trichloropropane, as genetic damage to the germ cells of the F1 generation may not be detected until the F2 generation. Thus, the absence of a study specifically evaluating developmental toxicity represents an area of uncertainty or gap in the database. Likewise, the database of inhalation studies is lacking a multigenerational reproductive toxicity study and a developmental toxicity study.

5.4. CANCER ASSESSMENT

There are no available studies on cancer in humans associated with exposure to 1,2,3-trichloropropane. NTP (1993) observed 1,2,3-trichloropropane-induced benign and malignant tumors in male and female F344/N rats and male and female $B6C3F_1$ mice in a 2-year gavage cancer bioassay. 1,2,3-Trichloropropane has been reported to be a mutagen in *S. typhimurium* assays (Lag et al., 1994; NTP, 1993; Ratpan and Plaumann, 1988; Haworth et al., 1983; Kier, 1982; Stolzenberg and Hine, 1980; Shell Oil Co., 1979). Studies have also demonstrated the induction of chromosomal aberrations and sister chromatid exchanges in CHO cell assays (NTP, 1993), trifluorothymidine resistance induction in mouse lymphoma assays (NTP, 1993; Shell Oil Co., 1982), DNA strand breakage measured by the Comet assay (single gel electrophoresis test) in isolated human lymphocytes (Tafazoli and Kirsch-Volders, 1996), and the induction of micronucleus formation in the mammalian cell lines, AHH-1, MCL-5, and h2E1 (Doherty et al., 1996).

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,2,3-trichloropropane is "likely to be carcinogenic to humans," based on a statistically significant and dose-related increase in the formation of multiple tumors in both sexes of two species from an NTP (1993) chronic oral bioassay. Statistically significant increases in incidences of tumors of the oral cavity, forestomach, pancreas, kidney, preputial gland, clitoral gland, mammary gland, and Zymbal's gland in rats, and the oral cavity, forestomach, liver, Harderian gland, and uterus in mice, were reported. In the absence of any data on the carcinogenicity of 1,2,3-trichloropropane via the inhalation route, no inhalation unit risk has been derived in this evaluation.

5.4.1. Choice of Study/Data with Rationale and Justification

The study by NTP (1993) was used for development of an oral slope factor. This was a well-designed study, conducted in both sexes in two species, and included examination of appropriate toxicological endpoints in both sexes of rats and mice. Tumor incidences were elevated with increasing exposure level at numerous sites across all sex/species combinations, involving point of contact in the alimentary system and more distant locations. Due to the increased carcinogenic response at all dose levels and the increased mortality in the two high-dose groups in both rats and mice, NTP stated that carcinogenic activity might have been detected at doses lower than those tested in the chronic study (NTP, 1993). The early mortality observed in rats and mice was associated with the development of chemical-related neoplasms, especially in the forestomach (NTP, 1993).

5.4.2. Dose-Response Data

In the NTP (1993) study, groups of 60 male and female F344/N rats and $B6C3F_1$ mice were administered 0, 3, 10, or 30 and 6, 20, or 60 mg/kg-day 1,2,3-trichloropropane, respectively, by gavage, 5 days/week, for 2 years. Ten male and 10 female rats and mice from each dose group were designated for evaluation at 15 months. High mortality in both species in all high-dose groups necessitated early termination of the rat high-dose groups at weeks 77 (males) and 67 (females). All other groups of rats were sacrificed after 2 years (104 weeks). For the mice, mid-dose groups were sacrificed at week 89, and high-dose male and female mice were sacrificed at weeks 79 and 73, respectively. All other groups of mice were sacrificed after week 104.

Dose-related, statistically significant increasing trends in tumors were noted at the following sites:

- Squamous cell carcinomas or papillomas of the alimentary system in male and female rats and mice;
- Zymbal's gland carcinomas in male and female rats;
- Pancreatic acinar cell adenomas or adenocarcinomas, preputial gland adenomas or carcinomas, and kidney tubular cell adenomas in male rats;
- Clitoral gland adenomas or carcinomas, and mammary gland adenocarcinomas in female rats;
- Hepatocellular adenomas or carcinomas, and harderian gland adenomas in male and female mice; and
- Uterine adenomas or adenocarcinomas in female mice.

The tumors generally appeared earlier and showed statistically significantly increasing trends with increasing exposure level (by life table test or logistic regression, $p \leq 0.001$). The data are summarized in Tables 5-3 (male and female rats) and 5-4 (male and female mice). The alimentary system tumors are presented as the combined incidence of squamous papillomas or squamous cell carcinomas of the pharynx/palate, tongue, or forestomach. Additionally, incidences of oral cavity tumors only (squamous papillomas or squamous cell carcinomas of the pharynx/palate or tongue) are presented. Data are not available to indicate whether the malignant tumors developed specifically from progression of the benign tumors. However, etiologically similar tumor types, i.e., benign and malignant tumors of the same cell type, were combined for these tabulations because of the possibility that the benign tumors could progress to the malignant form, as outlined in the 2005 Cancer Guidelines (U.S. EPA, 2005a).

Table 5-3. Tumor incidence and time of first occurrence in male and female F344/N rats following gavage exposure to 1,2,3-trichloropropane

Site		0 mg/kg-d	3 mg/kg-d	10 mg/kg-d	30 mg/kg-d	Trend test *p*-value[a]
Male rats						
Alimentary system, squamous neoplasms	Total[b]	1/59[c] (2%) 104[d]	39/60 (65%) 64	48/57 (84%) 58	58/60 (97%) 47	<0.001
	Oral cavity only	1/59[c] (2%) 104	4/60 (7%) 99	19/57 (33%) 58	43/60 (72%) 47	<0.001
Pancreas: acinar adenoma or adenocarcinoma		5/59 (8%) 104	20/60[e] (33%) 98	36/57 (63%) 67	31/58 (53%) 60	<0.001
Kidney tubular cell: adenoma		0/59 (0%) –	2/60 (3%) 104	18/57[e] (35%) 94	26/58 (45%) 60	<0.001
Preputial gland: adenoma or carcinoma		5/58 (8%) 72	6/57 (11%) 93	9/57 (16%) 58	17/56 (30%) 55	<0.001
Hepatocellular adenoma or carcinoma		1/59 (2%) 105	1/60 2% 105	4/57 7% 96	3/58 5% 65	<0.001 (life) 0.011 (log)
Skin: squamous cell papilloma or carcinoma		0/59 (0%) –	2/60 (3%) 98	1/57 (2%) 86	6/57 (10%) 64	<0.001 (life) 0.014 (log)
Zymbal's gland, carcinoma		0/59 (0%) –	0/60 (0%) –	0/57 (0%) –	3/58 (5%) 56	0.005 (life) 0.058 (log)
Female rats						
Alimentary system, squamous neoplasms	Total[b]	1/60 (2%) 104	22/59 (37%) 73	49/59 (83%) 58	44/58 (76%) 33	<0.001
	Oral cavity only	1/60 (2%) 104	6/59 (10%) 95	28/59 (47%) 58	37/58 (64%) 33	<0.001
Clitoral gland, adenoma or carcinoma		5/56 (9%) 102	11/56 (20%) 66	18/57 (32%) 62	17/51 (33%) 44	<0.001
Mammary gland, adenoma or adenocarcinoma		2/57 (4%) 64	6/56 (10%) 67	14/52 (27%) 61	23/47 (48%) 34	<0.001
Zymbal's gland, carcinoma		0/60 (0%) –	1/59 (2%) 102	0/59 (0%) –	4/45 (9%) 48	<0.001

[a]By both life table test ("live") and logistic regression ("log") unless otherwise noted.
[b]Squamous cell papillomas or carcinomas of the pharynx/palate, tongue, or forestomach.
[c]Numbers of animals at risk (denominators) vary due to missing tissues, or due to deaths either occurring before the first incidence of tumor in that group or before wk 52, whichever was earlier.
[d]Week of first incidence.
[e]NTP (1993) summary tables reported slightly higher incidences—21 low-dose males with pancreatic acinar cell tumors, 20 mid-dose males with kidney tubule adenomas—than noted in individual animal histopathology tables.

Source: NTP (1993).

Table 5-4. Tumor incidence in male and female B6C3F$_1$ mice following gavage exposure to 1,2,3-trichloropropane

Site		0 mg/kg-d	6 mg/kg-d	20 mg/kg-d	60 mg/kg-d	Trend test *p*-value[a]
Male mice						
Alimentary system, squamous neoplasms	Total[b]	3/59[c] (5%) 69[d]	57/59[c] (97%) 61	57/60[c] (95%) 55	59/60[c] (98%) 46	<0.001
	Oral cavity only	0/59 (0%) -	0/59 (0%) -	0/60 (0%) -	2/58 (3%) 68	$P > 0.05$
Liver: adenoma or carcinoma		14/59 (23%) 65	24/59 (41%) 74	25/60 (42%) 59	33/60 (55%) 46	<0.001
Harderian gland adenoma		1/59 (2%) 104	2/59 (3%) 91	10/60 (17%) 72	11/60 (20%) 65	0.001
Female mice						
Alimentary system, squamous neoplasms	Total[b]	0/59[c] (0%) –[d]	54/60[c] (90%) 59	59/60[c] (98%) 45	59/60[c] (98%) 42	<0.001
	Oral cavity only	1/59 (0%) 104	0/59 (0%) -	2/60 (3%) 79	5/60 (8%) 61	<0.001 (life) 0.008 (log)
Liver: adenoma or carcinoma		8/59 (13%) 66	11/60 (18%) 77	9/60 (15%) 65	36/58 (60%) 60	<0.001
Harderian gland adenoma		3/59 (5%) 66	6/59 (10%) 80	7/60 (12%) 78	10/60 (17%) 64	<0.001 (life) 0.004 (log)
Uterus: adenoma or adenocarcinoma		0/59 (0%) –	5/59 (8%) 100	3/59 (5%) 83	11/57 (19%) 66	<0.001

[a]By life table test ("life") and logistic regression ("log") unless otherwise noted.
[b]Squamous papillomas or squamous cell carcinomas of the pharynx/palate, tongue, or forestomach.
[c]Numbers of animals at risk (denominators) vary due to missing tissues, or due to deaths occurring before the first incidence of tumor in that group or before wk 52, whichever was earlier.
[d]Week of first incidence.

Source: NTP (1993).

Risk estimates are generally calculated from the incidence of rodents of the most sensitive species, strain, and sex bearing tumors at any of the sites displaying treatment-attributable increases. For 1,2,3-trichloropropane, mice were the more sensitive species, with 90% of females and 97% of males developing tumors. However, with such a high response, extrapolation to lower, environmentally relevant exposures is more uncertain. Consequently, dose-response modeling was considered for all four species/sex combinations.

NTP noted additional tumor sites with apparent dose-related increases in squamous cell papillomas and carcinomas of the skin and hepatocellular adenomas and carcinomas, both in male rats. NTP concluded that because the incidence in no one group was statistically significantly higher than control, the overall trends were not treatment-related. On the other hand, both endpoints show some consistency with other effects observed in the NTP study. First, the squamous cell papillomas and carcinomas of the skin are the same type as observed in

the alimentary system. While all of these animals also had either squamous cell papillomas or carcinomas of the forestomach, neither site was noted as a metastasis of the other. The skin tumors may reflect a systemic component rather than a strictly site of contact mode of action for this tumor type. Consequently, both of these sites were carried through the dose-response modeling analysis. Statistically significant increases in incidences of tumors of the alimentary system (oral cavity and forestomach), pancreas, kidney, preputial gland, clitoral gland, mammary gland, and Zymbal's gland in F344/N rats and the alimentary system (oral cavity and forestomach), liver, Harderian gland, and uterus in B6C3F1 mice were included in the dose-response analysis to derive the cancer oral slope factor for 1,2,3-trichloropropane.

However, some of the external peer review panel members (see Appendix A: Summary of External Peer Review and Public Comments and Disposition) recommended removing the forestomach tumors observed in rats and mice from the quantitative cancer analysis as humans do not have a forestomach or an organ that is homologous to the rodent forestomach. In addition, several panel members indicated that the bolus dose of the chemical administered by gavage, coupled with the slow emptying of the forestomach lends uncertainty to the actual dose that should be used for quantification.

As noted by the peer reviewers, humans do not have a forestomach; however, squamous epithelial tissues in the oral cavity and the upper two-thirds of the esophagous in humans are comparable to the rodent forestomach (IARC, 2003). In addition, forestomach carcinogens in rodents may affect other tissues in humans. It has been suggested that most genotoxic forestomach carcinogens appear to act through a mutagenic mode of action (IARC, 2003). For multi-site carcinogens that induce forestomach tumors and are genotoxic, these tumors are likely relevant to human carcinogenesis (IARC, 2003; Proctor et al., 2007).

Therefore, in the absence of data to indicate otherwise, EPA considers forestomach tumors to be relevant to humans but recognizes that there is some uncertainty associated with the quantification of the tumors with respect to estimating the dose given the gavage dosing regimen. EPA has included the data for these tumors in the quantitative carcinogenic dose-response analysis for the derivation of the oral slope factor (Section 5.4.4). However, in response to the recommendations of some of the external peer review panel members, Section 5.4.4 also includes the derivation of oral slope factors for rats and mice in which forestomach tumors were excluded from the analysis. In addition, uncertainties associated with the quantification of forestomach tumors as noted by some of the external peer review panelists are discussed in Section 5.4.6 (Uncertainties in Cancer Risk Values).

5.4.3. Dose Adjustments and Extrapolation Methods

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the mode of action of the carcinogen and the shape of the cancer dose-response curve. The dose response is assumed to be linear in the low dose range, when evidence supports a mutagenic mode of action because of DNA reactivity, or if another mode of action that is anticipated to be linear is applicable. A linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,2,3-trichloropropane exposure due to the mutagenic mode of carcinogenic action of 1,2,3-trichloropropane.

Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and early termination of at least one dose group, methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. EPA has generally used a model which incorporates the time at which death-with-tumor occurred as well as the dose; the multistage-Weibull model is multistage in dose and Weibull in time, and has the form:

$$P(d) = 1 - exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t \pm t_0)^z],$$

where $P(d)$ represents the lifetime risk (probability) of cancer at dose d (i.e., human equivalent exposure in this case); parameters $q_i \geq 0$, for i = 0, 1, ..., k; t is the time at which the tumor was observed; and z is a parameter which characterizes the change in response with age. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death, and is generally set to 0 either when all tumors are considered incidental or because of a lack of data to estimate the time reliably. The dose-response analyses were conducted using the computer software program TOX_RISK, version 5.2 (property of ICF, Fairfax, VA), which is based on Weibull models drawn from Krewski et al. (1983). Parameters were estimated using the method of maximum likelihood.

Other characteristics of the observed tumor types were considered prior to modeling, including allowance for different, although possibly unidentified, modes of action, and for relative severity of tumor types. First, etiologically different tumor types were not combined across sites prior to modeling, in order to allow for the possibility that different tumor types can have different dose-response relationships because of varying time courses or other underlying mechanisms or factors. Consequently, all of the tumor types listed separately in Tables 5-3 and 5-4 were modeled separately.

A further consideration allowed by the software program is the distinction between tumor types as being either fatal or incidental, in order to adjust for competing risks. Incidental tumors

are those tumors thought not to have caused the death of an animal, while fatal tumors are thought to have resulted in animal death. NTP (1993) stated that neoplasms of the forestomach and oral mucosa in rats and mammary tumors in female rats were the principal cause of death of most animals dying or killed moribund before the end of the study, but did not report individual causes of death, which would be preferable for time-to-tumor analysis. However, because the likely causes of death were relatively evident in this study, a bounding exercise was carried out for each these two malignant tumor types. For the first analysis, all tumors at these sites were considered incidental (an "incidental" analysis). In the second, the tumors observed at unscheduled deaths (including early group termination because those animals were thought to be in extremis) were considered as fatal and the rest for that site considered incidental (a "fatal" analysis). Analyses treating some tumors as fatal permit estimation of incidental or fatal risk; for the purposes of slope factor estimation, estimating the risk of developing a tumor (incidental risk) is of greater interest than estimating the risk of dying with a tumor (fatal risk). Note that there was a slight overlap of squamous cell carcinomas and mammary adenocarcinomas in female rats, involving one rat in the mid-dose group and six rats in the high-dose group. For all other tumor sites, all tumors were treated as incidental. The data modeled are provided in Tables D-1 through D-4 (Appendix D).

For the fatal analyses, it was feasible to estimate t_0, the time between when a potentially fatal tumor becomes observable and when it causes death, because some early deaths were not accompanied by the malignant form of the tumor being analyzed. In addition, the NTP study had the additional feature of an interim sacrifice of 10 animals per sex and species in each dose group at about week 65, which permitted the observation of tumors at these sites before becoming fatal. For the incidental analyses, t_0 was set to zero.

Specific n-stage Weibull models were selected for the individual tumor types for each sex based on the values of the log-likelihoods according to the strategy used by EPA (U.S. EPA, 2002). If twice the difference in log-likelihoods was less than a χ^2 with degrees of freedom equal to the difference in the number of stages included in the models being compared, the models were considered comparable and the most parsimonious model (i.e., the lowest-stage model) was selected. For tumors treated as incidental, plots of model fits compared with Hoel-Walburg estimates of cumulative incidence were also examined for goodness of fit in the lower exposure region of the observed data (Gart et al., 1986). If a model with one more stage fitted the low-dose data better than the most parsimonious model, then the model with one higher stage was selected.

PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, generally corresponding to 10% extra risk, where extra risk is defined as [P(d) - P(0)]/[1 - P(0)]. Lower risks were used for responses demonstrating less than a 10% response throughout the data range. The lifetime oral cancer slope factor for humans is defined as the

slope of the line from the lower 95% bound on the exposure at the POD. This 95% upper confidence limit (UCL) represents a plausible upper bound on the true risk.

Adjustments for approximating human equivalent slope factors applicable for continuous exposure were also carried out by the dose-response software program. Consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), an adjustment for cross-species scaling was applied by the software program, to address toxicological equivalence across species, after the model-fitting phase. Following EPA's cross-species scaling methodology, the time-weighted daily average doses were converted to human equivalent doses on the basis of $(\text{body weight})^{3/4}$ (U.S. EPA, 1992). It was not necessary to adjust the administered doses for lifetime exposure prior to modeling for the groups terminated early, because the software program used characterizes the tumor incidence as a function of time, from which it provides an extrapolation to lifetime exposure. In addition, TOX_RISK estimated continuous daily exposure by multiplying each slope factor by (5 days)/(7 days) = 0.71.

5.4.4. Oral Slope Factor and Inhalation Unit Risk

The results of applying the multistage-Weibull models to the tumor incidence data for the four sex and species combinations in the NTP study are provided in Table 5-5. An oral slope factor for each of the tumor sites was calculated by dividing the BMR level (usually 10%) by its corresponding BMDL. In the absence of any data on the carcinogenicity of 1,2,3-trichloropropane via the inhalation route, no inhalation unit risk has been derived in this evaluation.

The highest slope factor for each of the four data sets corresponded to squamous cell neoplasms of the alimentary system, whether or not malignant tumors observed at unscheduled deaths were considered incidental or fatal. The incidental analyses led to higher slope factors than did the fatal analysis for female rats and mice, and to generally similar results in the male rats and mice. While the fatal analyses may tend to overestimate the risk estimates, because the number of deaths due to these tumors may have been overestimated, the slope factors in this assessment were derived from the fatal analyses because this tumor context describes these data sets as well as possible given the available information.

The mouse data led to higher risk estimates than did the rat data, with the female mice demonstrating the highest slope factor of all, at 26 per mg/kg-day for the fatal analysis of alimentary system tumors. The highest slope factor based on the male mice data was 5.6 per mg/kg-day for the fatal analysis of alimentary system tumors. For rats, the corresponding estimates were 3.1 and 1.1 per mg/kg-day for males and females, respectively.

Although the time-to-tumor modeling does help account for competing risks associated with decreased survival times and other tumors, considering the tumor sites individually does not convey the total amount of risk potentially arising from the sensitivity of multiple sites. To get

some indication of the total unit risk from multiple tumor sites, assuming the multiple sites are mechanistically independent, several approaches are available. One approach suggested in the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) would be to estimate cancer risk from tumor-bearing animals. EPA traditionally used this approach until the NRC document *Science and Judgment* (1994) made a case that this approach would tend to underestimate overall risk when tumor types occur in a statistically independent manner. In addition, application of one model to a composite data set does not accommodate biologically relevant information that may vary across sites or may only be available for a subset of sites. For instance, the time courses of the multiple tumor types evaluated varied, as is suggested by the variation in estimates of z (see Table 5-5), from 1.0 (e.g., male rat Zymbal's gland tumors), indicating relatively little effect of age on tumor incidence, to 10 (e.g., female mouse uterine tumors), indicating a much more rapidly increasing response with increasing exposure level. The result of fitting a model with underlying mechanism-related parameters, such as z in the multistage-Weibull model, would be difficult to interpret with composite data. A simpler model could be used for the composite data, such as the multistage model, but available biological information would then be ignored.

Following the recommendations of the NRC (1994) and the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a statistically appropriate upper bound on total risk was estimated in order to gain some understanding of the total risk from multiple tumor sites for each sex/species combination. Note that this upper bound estimate of overall risk describes the risk of developing any combination of the tumor types considered, not just the risk of developing all three simultaneously. Statistical methods which can accommodate the underlying distribution of slope factors are optimal, such as through maximum likelihood estimation or through bootstrapping or Bayesian analysis. However, these methods have not yet been extended to models such as the multistage-Weibull model. Consequently, this analysis used the same method as in the IRIS assessments for 1,3-butadiene (U.S. EPA, 2000d) and 1,2-dibromoethane (U.S. EPA, 2004), which involves assuming that slope factors can be characterized by a normal distribution. Using the results in female mice to illustrate, the overall risk estimate involved the following steps:

1) It was assumed that the tumor types associated with 1,2,3-trichloropropane exposure were statistically independent - that is, that the occurrence of a liver tumor, say, was not dependent upon whether there was a forestomach tumor. This assumption cannot currently be verified, and if not correct could lead to an overestimate of risk from summing across tumor sites. However, NRC (1994) argued that a general assumption of statistical independence of tumor-type occurrences within animals was not likely to introduce substantial error in assessing carcinogenic potency from rodent bioassay data.

2) The models previously fitted to estimate the BMDs and BMDLs were used to extrapolate to a low level of risk (R), in order to reach the region of each estimated dose-response function where the slope was reasonably constant and upper bound estimation was still numerically stable. For these data, a 10^{-3} risk was generally the lowest risk necessary. The oral slope factor for each site was then estimated by $R/BMDL_R$, as for the estimates for each tumor site above.

3) The maximum likelihood estimates (MLE) of unit potency (that is, risk per unit of exposure) estimated by R/BMD_R, were summed across the alimentary system, liver, Harderian gland, and uterus in female mice.

4) An estimate of the 95% upper bound on the summed oral slope factor was calculated by assuming a normal distribution for the individual risk estimates, and deriving the variance of the risk estimate for each tumor site from its 95% upper confidence limit (UCL) according to the formula:

$$95\%\ UCL = MLE + 1.645 \times s.d.,$$

rearranged to:

$$s.d. = (UCL - MLE) / 1.645,$$

where 1.645 is the t-statistic corresponding to a one-sided 95% confidence interval and >120 degrees of freedom, and the standard deviation (s.d.) is the square root of the variance of the MLE. The variances (variance = $s.d.^2$) for each site-specific estimate were summed across tumor sites to obtain the variance of the sum of the MLEs. The 95% UCL on the sum of MLEs was calculated from the expression above for the UCL, using the variance of the sum of the MLE to obtain the relevant s.d (s.d. = $variance^{1/2}$).

Tables 5-5 and D-5 provides a summary of combined risk estimates for all four data sets. The resulting combined upper bound slope factor for female mice was 28 per mg/kg-day, compared with 26 per mg/kg-day for just alimentary system tumors. The difference between these two estimates is insignificant given the approximate nature of low dose extrapolation and because details of the algorithms differ slightly, with the risk values being estimated at a 10% extra risk, and the combined risk estimates being estimated at 10^{-3} extra risk. More importantly, the alimentary system tumors were clearly a much more sensitive response in female mice, and both estimates converge on 30 per mg/kg-day when rounded to one significant digit. Combined risks for the other data sets ranged from 1.6 per mg/kg-day for female rats (an increase of about 50% from 1.1 per mg/kg-day for alimentary system tumors only) to 6.8 per mg/kg-day for male mice (an increase of about 15% from 5.9 per mg/kg-day for alimentary system tumors only). Interestingly, despite the greater number of sites with dose-related increases in male rats, the combined risk of 4.1 per mg/kg-day essentially reflects only the alimentary system and pancreatic tumors (see Tables 5-5 and D-5, proportion of total variance column).

Based on the analyses discussed above, the recommended upper bound estimate on human extra cancer risk from continuous lifetime oral exposure to 1,2,3-trichloropropane is

30 per mg/kg-day. The oral slope factor based on incidences of tumors in the alimentary system (including oral cavity and forestomach tumors), liver, harderian gland and uterus of female mice was recommended because female mice are the most sensitive to tumor induction following exposure to 1,2,3-trichloropropane. The recommended estimate reflects the time-to-tumor dimension of the responses as well as the exposure-response relationships for the multiple tumor sites. This slope factor should not be used with exposures greater than 0.6 mg/kg-day, the human equivalent dose corresponding to the POD for the female mouse alimentary system tumors, because the observed dose-response relationships do not continue linearly above this level and the fitted dose-response models better characterize what is known about the carcinogenicity of 1,2,3-trichloropropane.

Table 5-5. Dose-response modeling summary for tumors associated with oral exposure to 1,2,3-trichloropropane; rat and mouse tumor incidence data

Tumor type and context[a]		Multistage-Weibull model coefficients (MLE)[b]	Human equivalent continuous POD[c], mg/kg-d		Slope factor[d], $(mg/kg\text{-}d)^{-1}$	Overall slope factor, $(mg/kg\text{-}d)^{-1}$
			BMD_{10}	$BMDL_{10}$		
Male rats						
Alimentary system, total squamous neoplasms	Incidental	$q_0 = 1.1 \times 10^{-12}$ $q_1 = 1.9 \times 10^{-11}$ $q_2 = 2.1 \times 10^{-12}$ $z = 5.1$	0.050	0.033	3.0	4.1[e] (3.9)
	Fatal	$q_0 = 2.9 \times 10^{-15}$ $q_1 = 5.9 \times 10^{-14}$ $q_4 = 2.5 \times 10^{-17}$ $z = 6.4$ $t_0 = 29$	0.041	0.032	3.1	
Pancreas: acinar adenoma or adenocarcinoma		$q_0 = 4.5 \times 10^{-19}$ $q_1 = 2.4 \times 10^{-19}$ $q_2 = 1.2 \times 10^{-19}$ $z = 8.7$	0.20	0.10	1.0	
Preputial gland adenoma or carcinoma		$q_0 = 1.1 \times 10^{-4}$ $q_1 = 2.7 \times 10^{-5}$ $z = 1.4$	1.3	0.59	0.17	
Kidney tubular cell adenoma		$q_2 = 2.5 \times 10^{-15}$ $z = 6.2$	0.49	0.32	0.16	
Hepatocellular adenomas or carcinomas		$q_0 = 4.6 \times 10^{-19}$ $q_2 = 3.5 \times 10^{-20}$ $z = 8.2$	0.85[f]	0.53[f]	0.010	
Skin: squamous cell papillomas or carcinomas		$q_1 = 3.6 \times 10^{-6}$ $z = 1.6$	3.4	1.4	0.070	
Zymbal's gland carcinoma		$q_1 = 1.6 \times 10^{-5}$ $z = 1.0$	6.1[f]	2.5[f]	0.021	

Table 5-5. Dose-response modeling summary for tumors associated with oral exposure to 1,2,3-trichloropropane; rat and mouse tumor incidence data

Tumor type and context[a]		Multistage-Weibull model coefficients (MLE)[b]	Human equivalent continuous POD[c], mg/kg-d		Slope factor[d], $(mg/kg\text{-}d)^{-1}$	Overall slope factor, $(mg/kg\text{-}d)^{-1}$
			BMD_{10}	$BMDL_{10}$		
Female rats						
Alimentary system, total squamous neoplasms	Incidental	$q_0 = 2.5 \times 10^{-12}$ $q_1 = 8.1 \times 10^{-12}$ $q_2 = 5.6 \times 10^{-12}$ $z = 4.9$	0.15	0.055	1.8	1.5 (2.4)
	Fatal	$q_0 = 1.7 \times 10^{-11}$ $q_1 = 5.7 \times 10^{-11}$ $q_2 = 2.7 \times 10^{-11}$ $z = 4.5$ $t_0 = 27$	0.17	0.09	1.1	
Clitoral gland adenoma or carcinoma		$q_0 = 3.1 \times 10^{-7}$ $q_1 = 6.5 \times 10^{-7}$ $z = 2.4$	0.31	0.24	0.41	
Mammary gland adeno-carcinoma	Incidental	$q_0 = 3.5 \times 10^{-4}$ $q_1 = 2.9 \times 10^{-4}$ $z = 1$	0.61	0.43	0.24	
	Fatal	$q_0 = 9.8 \times 10^{-13}$ $q_1 = 3.3 \times 10^{-13}$ $q_3 = 7.5 \times 10^{-15}$ $z = 5.3$ $t_0 = 4.7$	0.72	0.34	0.29	
Zymbal's gland carcinoma		$q_1 = 1.4 \times 10^{-5}$ $z = 1.2$	4.9	1.6	0.063	
Male mice						
Alimentary system, total squamous neoplasms	Incidental	$q_0 = 6.5 \times 10^{-9}$ $q_1 = 6.5 \times 10^{-8}$ $z = 3.5$	0.030	0.017	5.9	6.8[e] (6.8)
	Fatal	$q_0 = 2.6 \times 10^{-10}$ $q_1 = 2.8 \times 10^{-9}$ $z = 4.2$ $t_0 = 32$	0.022	0.017	5.9	
Liver: adenoma or carcinoma		$q_0 = 3.4 \times 10^{-10}$ $q_1 = 8.9 \times 10^{-11}$ $z = 4.5$	0.22	0.14	0.73	
Harderian gland adenoma		$q_0 = 2.0 \times 10^{-10}$ $q_2 = 2.2 \times 10^{-10}$ $z = 3.9$	1.1	0.57	0.17	

Table 5-5. Dose-response modeling summary for tumors associated with oral exposure to 1,2,3-trichloropropane; rat and mouse tumor incidence data

Tumor type and context[a]		Multistage-Weibull model coefficients (MLE)[b]	Human equivalent continuous POD[c], mg/kg-d		Slope factor[d], $(mg/kg\text{-}d)^{-1}$	Overall slope factor, $(mg/kg\text{-}d)^{-1}$
			BMD_{10}	$BMDL_{10}$		
Female mice						
Alimentary system, total squamous neoplasms	Incidental	$q_1 = 3.3 \times 10^{-12}$ $z = 6.0$	0.0032	0.00065	150	28 (160)
	Fatal	$q_1 = 7.3 \times 10^{-16}$ $q_2 = 9.6 \times 10^{-17}$ $z = 7.5$ $t_0 = 24$	0.0095	0.0039	26	
Liver: adenoma or carcinoma		$q_0 = 5.6 \times 10^{-18}$ $q_1 = 9.2 \times 10^{-19}$ $q_3 = 5.5 \times 10^{-21}$ $z = 8.2$	0.30	0.14	0.73	
Harderian gland adenoma		$q_0 = 6.9 \times 10^{-12}$ $q_1 = 3.0 \times 10^{-12}$ $z = 4.9$	0.42	0.20	0.50	
Uterus: adenoma or carcinoma		$q_1 = 6.0 \times 10^{-23}$ $q_2 = 2.4 \times 10^{-23}$ $z = 10$	0.42	0.21	0.47	

[a]"Incidental" denotes models treating all tumors of the type listed as incidental to the death of the animal. "Fatal" denotes models treating the tumors (among the type listed) present at unscheduled deaths as causing the death, with the remaining tumors considered incidental. If no context is listed, all tumors were considered incidental.
[b]Multistage-Weibull model: $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t \pm t_0)^z]$, with coefficients estimated in terms of mg/kg-d as administered in bioassay; lower or intermediate stage q_i not listed were estimated to be zero.
[c]POD adjusted to estimate human equivalent continuous exposure, using $BW^{3/4}$ cross-species scaling and by multiplying by (5 d)/(7 d).
[d]Slope factors estimated by dividing the BMR (10% unless specified otherwise) by the BMDL.
[e]Overall slope factor including fatal context for tumors considered under both possibilities. Overall slope factor in parentheses represents incidental context for all tumor types.
[f]BMR = 5%.

BMD_{10} = Concentration at 10% extra risk; $BMDL_{10}$ = 95% lower bound on concentration at 10% extra risk.

Source: NTP (1993).

Oral slope factors derived from tumor incidence data excluding forestomach tumors (see Tables 5-3 and 5-4 for oral cavity tumor incidence data) are presented for purposes of comparision.

Male and female rats and female mice showed statistically significant increasing trends in oral cavity tumor rates.. Male mice did not demonstrate a statistically significant increase in oral cavity tumors, but there was an increasing trend that was modeled for comparison purposes. For time to tumor modeling, the oral cavity tumors were all considered to be incidental to animal

mortality. All of the carcinomas in the oral cavity in the four species/sex combinations occurred concurrently with carcinomas in the forestomach, which the NTP had considered to be largely responsible for the early mortality seen across the study. In all other respects the modeling was performed as described above in this section. Table 5-6 summarizes the results of modeling the oral cavity tumors.

As discussed earlier in this section, considering the tumor sites individually does not convey the total amount of risk potentially arising from the sensitivity of multiple sites. Therefore, the same procedure described previously was used to estimate the total risk for all tumors, excluding those of the forestomach, for each species/sex combination,. The results are shown in the last column of Table 5-6. Higher risk estimates were calculated for the overall slope factors that included analyses of the forestomach tumors in rats and mice as shown in Tables 5-5 and 5-6.

Table 5-6. Dose-response modeling summary for oral cavity squamous cell neoplasia associated with oral exposure to 1,2,3-trichloropropane (NTP, 1993)

Sex, species	Multistage-Weibull model Coefficients (MLE)[a]	Human Equivalent Continuous Point of departure[b], mg/kg-day		Slope factor[c], $(mg/kg\text{-}day)^{-1}$ for oral cavity tumors	Combined slope factor[d], $(mg/kg\text{-}day)^{-1}$
		BMD_{10}	$BMDL_{10}$		
Male rats	$q_0 = 2.2 \times 10^{-5}$ $q_1 = 3.4 \times 10^{-5}$ $q_2 = 2.5 \times 10^{-6}$ $z = 1.4$	0.68	0.41	0.24	1.3
Female rats	$q_0 = 4.3 \times 10^{-9}$ $q_1 = 2.8 \times 10^{-9}$ $q_2 = 2.1 \times 10^{-9}$ $z = 3.3$	0.44	0.24	0.44	0.9
Male mice	$q_3 = 4.6 \times 10^{-19}$ $z = 6.2$	7.7	2.9	0.034	0.9
Female mice	$q_0 = 1.1 \times 10^{-5}$ $q_1 = 2.4 \times 10^{-6}$ $z = 1.5$	4.9	1.1	0.092	1.3

[a] Multistage-Weibull model: $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t \pm t_0)^z]$, with coefficients estimated in terms of mg/kg-day as administered in bioassay; lower or intermediate stage q_i not listed were estimated to be zero.

[b] Point of departure adjusted to estimate human equivalent continuous exposure, using $BW^{3/4}$ cross-species scaling and by multiplying by (5 days)/(7 days).
BMD_{10} = Concentration at 10% extra risk; $BMDL_{10}$ = 95% lower bound on concentration at 10% extra risk.

[c] Slope factors estimated by dividing the BMR (10% unless specified otherwise) by the BMDL.

[d] Slope factor for oral cavity tumors combined with the non-alimentary system tumors listed in Table 5-5, for each species/sex combination.

Therefore, the recommended upper bound estimate on human extra cancer risk from continuous lifetime oral exposure to 1,2,3-trichloropropane is **30 per mg/kg-day**.

5.4.5. Application of Age-Dependent Adjustment Factors

Because a mutagenic mode of action for 1,2,3-trichloropropane carcinogenicity is sufficiently supported in laboratory animals and relevant to humans (Section 4.7.3.4), and in the absence of chemical-specific data to evaluate differences in susceptibility, increased early-life susceptibility is assumed and the age-dependent adjustment factors (ADAFs) should be applied, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The oral slope factor of 30 per mg/kg-day, calculated from data for adult exposures, does not reflect presumed early-life susceptibility for this chemical. Example evaluations of cancer risks based on age at exposure are given in Section 6 of the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

The *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* establishes ADAFs for three specific age groups. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2 to <16 years, and 1 for 16 years and above (U.S. EPA, 2005b). The 10-fold and 3-fold adjustments in slope factor are to be combined with age specific exposure estimates when estimating cancer risks from early life (<16 years age) exposure to 1,2,3-trichloropropane.

To illustrate the use of the ADAFs established in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), sample calculations are presented for three exposure duration scenarios, including full lifetime, assuming a constant 1,2,3-trichloropropane exposure of 0.001 mg/kg-day (Table 5-7).

Table 5-7. Application of ADAFs for a 70-year exposure to 0.001 mg/kg-day 1,2,3-trichloropropane from ages 0 to 70

Age group	ADAF	Unit risk (per mg/kg-d)	Exposure concentration (mg/kg-d)	Duration adjustment	Partial risk
0–<2 yrs	10	30	0.001	2 yrs/ 70 yrs	0.01
2–<16 yrs	3	30	0.001	14 yrs/ 70 yrs	0.02
≥16 yrs	1	30	0.001	54 yrs/ 70 yrs	0.02
				Total risk	0.05

Note that the partial risk for each age group is the product of the values in columns 2–5 [e.g., 10 × 30 × 0.001 × 2/70 = 0.01], and the total risk is the sum of the partial risks. Thus, a 70-year risk estimate for a constant exposure of 0.001 mg /kg-day starting at birth is 0.05, or 5%.

If calculating the cancer risk for a 30-year exposure to a constant 1,2,3-trichloropropane exposure level of 0.001 mg/kg-day from ages 0 to 30, the duration adjustments would be 2/70,

14/70, and 14/70, and the partial risks would be 0.01, 0.02, and 0.01, resulting in a total risk estimate of 0.03, or 3%.

If calculating the cancer risk for a 30-year exposure to a constant 1,2,3-trichloropropane exposure level of 0.001 mg/kg-day from ages 20 to 50, the duration adjustments would be 0/70, 0/70, and 30/70, and the partial risks would be 0, 0, and 0.01, resulting in a total risk estimate of 0.01, or 1%.

5.4.6. Uncertainties in Cancer Risk Values

As in most risk assessments, extrapolation of study data to estimate potential risks to human populations from exposure to 1,2,3-trichloropropane has engendered some uncertainty in the results. Some types of uncertainty, but not all, may be considered quantitatively. Principal uncertainties are summarized below and in Table 5-8.

Table 5-8. Summary of uncertainty in the 1,2,3-trichloropropane cancer risk assessment

Consideration/ approach	Impact on oral slope factor	Decision	Justification
Low-dose extrapolation procedure	Alternatives could ↓ or ↑ slope factor by an unknown extent	Multistage-Weibull model to determine POD, linear low-dose extrapolation from POD (due to mutagenic mode of carcinogenic action)	A linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,2,3-trichloropropane exposure due to the mutagenic mode of carcinogenic action. Linear extrapolation is generally considered to be a health-protective approach (U.S. EPA, 2005a).
Dose metric	Alternatives could ↑ or ↓ slope factor by an unknown extent	Used administered exposure	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not clearly identified. If the responsible metabolites are generated in proportion to administered concentration, the estimated slope factor is an unbiased estimate.
Cross-species scaling	Alternatives could ↓ or ↑ slope factor [e.g., sixfold ↓ (scaling by BW) or ↑ twofold (scaling by $BW^{2/3}$)]	$BW^{3/4}$ (default approach)	There are no data to support alternatives. Because the dose metric was not an area under the curve, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks (U.S. EPA, 1992).
Statistical uncertainty at POD	↓ slope factor 2.5-fold if MLE used rather than lower bound on POD	BMDL (default approach for calculating reasonable upper bound slope factor)	Size of bioassay results in sampling variability; lower bound is 95% confidence interval on administered exposure.

Table 5-8. Summary of uncertainty in the 1,2,3-trichloropropane cancer risk assessment

Consideration/ approach	Impact on oral slope factor	Decision	Justification
Bioassay–exposure issues	Alternative oral exposures better approximating likely human exposure patterns (without corn oil vehicle, regular/ constant exposure rather than daily bolus gavage) could ↓ slope factor, by an unknown extent	NTP study	Alternative bioassays were unavailable.
Species/gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Female mouse tumors (forestomach, pharynx/palate, tongue; liver, Harderian gland, uterus)	It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across species. Generally, direct site concordance is not assumed; consistent with this view, some human tumor types are not found in rodents and rat and mouse tumor types also differ.
Human relevance of rodent tumor data	Lack of human relevance of tumor data would ↓ slope factor	Tumors with significant dose-response considered for estimating potential human cancer response	1,2,3-Trichloropropane is carcinogenic through a mutagenic mode of action and is a multisite carcinogen in rodents; therefore, the carcinogenicity observed in the rodent studies is relevant to human exposure.
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity.

Choice of low-dose extrapolation approach. The mode of action is a key consideration in clarifying how risks should be estimated for low-dose exposure. A linear, low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,2,3-trichloropropane exposure due to the mutagenic mode of carcinogenic action of 1,2,3-trichloropropane. Linear extrapolation is, generally, considered to be a health-protective approach, and, in some cases, may lead to an overestimation of risk, as stated in the 2005 Cancer Guidelines (U.S. EPA, 2005a).

The multistage-Weibull model was used to model the cancer data because it incorporates the time at which death-with-tumor occurred; however, it is unknown how well this model or the linear low-dose extrapolation predicts low-dose risks for 1,2,3-trichloropropane. The selected

model does not represent all possible models one might fit, and other models could conceivably be selected to yield more extreme results consistent with the observed data, both higher and lower than those included in this assessment. Etiologically different tumor types were not combined across sites prior to modeling, in order to allow for the possibility that different tumor types can have different dose-response relationships because of varying time courses or other underlying mechanisms or factors. The human equivalent oral slope factors estimated from the tumor sites with statistically significant increases ranged from 0.010 to 30 per mg/kg-day, a range of about three orders of magnitude.

However, given the multiplicity of tumor sites, basing the oral slope factor on one tumor site may underestimate the carcinogenic potential of 1,2,3-trichloropropane. Following the recommendations of the NRC (1994) and the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a statistically appropriate upper bound on total risk was estimated in order to gain some understanding of the total risk from multiple tumor sites in male and female F344/N rats and B6C3F1 mice (Table 5-5). Note that this estimate of overall risk describes the risk of developing any combination of the tumor types considered, not just the risk of developing all simultaneously. The estimates of the overall oral slope factor ranged from 2 to 30 per mg/kg-day.

Dose metric. 1,2,3-Trichloropropane is known to be metabolized to intermediates with carcinogenic potential. However, it is unknown whether a metabolite or some combination of metabolites is responsible for the observed toxicity. If the actual carcinogenic moiety(ies) is (are) proportional to administered exposure, then use of administered exposure as the dose metric is the least biased choice. On the other hand, if this is not the most relevant dose metric, then the impact on the human equivalent slope factor is unknown; the low-dose cancer risk value may be higher or lower than that estimated, by an unknown amount.

Cross-species scaling. Without data to the contrary, it was assumed that equal risks result from equivalent constant lifetime exposures. An adjustment for cross-species scaling ($BW^{3/4}$) was therefore applied to address toxicological equivalence of internal doses between each rodent species and humans, consistent with the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Because it is unknown whether there are differences in the pharmacokinetic pathways and pharmacodynamic processes in animals and humans following 1,2,3-trichloropropane exposure, it is not possible to estimate the full impact of this uncertainty beyond that associated with other arbitrary choices for default cross-species scaling factors (such as $BW^{2/3}$ or assuming equivalence on a mg/kg-day basis).

Statistical uncertainty at the point of departure. Measures of statistical uncertainty require assuming that the underlying model and associated assumptions are valid for the data under consideration. For the multistage-Weibull model applied to the female mice alimentary tumor data, there is a reasonably typical degree of uncertainty at the 10% extra risk level (the

POD for linear low-dose extrapolation). The $BMDL_{10}$ for female mice is approximately 2.5-fold lower than the BMD_{10}.

Bioassay selection. The study by NTP (1993) was used for development of an oral slope factor. This was a well-designed study, conducted in both sexes in two species. However, the bolus nature of the 1,2,3-trichloropropane gavage exposures in NTP (1993) may lead to more pronounced irritation, inflammation, cell death, and an eventual increase in tumor incidence at portals of entry because of direct contact of the test chemical with the gastroinstestinal tissues. The number of test animals allocated among three dose levels and an untreated control group was greater than the norm at 60 per group, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Alternative bioassays were unavailable. Overall responses across the four species/sex combinations were similarly robust, all involving the alimentary system in particular, and multiple tumor sites generally.

The impact of the corn oil vehicle on the effects observed in the forestomach following 1,2,3-trichloropropane exposures is unknown. The corn oil, combined with the bolus dosing, may enhance cellular proliferation in the forestomach. An increased incidence and severity of epithelial cell proliferation of the forestomach has been demonstrated in rats following the administration of reported forestomach carcinogens in corn oil (Ghanayem et al., 1986). However, forestomach lesions were not observed in vehicle (corn oil) controls for male and female rats and female mice, and were observed only in male mice (3/50). Evidence of irritation, inflammation, or necrosis localized in the forestomach of rats or mice was not observed following the oral administration of 1,2,3-trichloropropane. In addition, while the administration of 1,2,3-trichloropropane in corn oil may increase the residency time in the forestomach, the effect of this increased residency time in the forestomach is unknown. The tumors observed in the forestomach may be the result of direct interaction of the 1,2,3-trichloropropane with the squamous epithelium lining the forestomach, or the result of 1,2,3-trichloropropane absorbed from the intestine back into the forestomach.

For the dose-response analysis, etiologically similar tumor types (i.e., benign and malignant tumors of the same cell type) were combined because of the possibility that the benign tumors could progress to the malignant form as outlined in the 2005 Cancer Guidelines (U.S. EPA, 2005a).

Choice of species/gender. The oral slope factor for 1,2,3-trichloropropane was quantified using the tumor incidence data for female mice, which were thought to be more sensitive than the other experimental rodents to the carcinogenicity of 1,2,3-trichloropropane. The male and female mice tumor incidence data, while clearly demonstrating carcinogenicity, unfortunately missed nearly all of the relevant dose-response range for mice, with both male and female mice having nearly 100% responses at the lowest exposure level. While these responses were higher

than those of the rats at a comparable exposure level, suggesting greater sensitivity of the mice, it is unknown which animal species would be more sensitive at lower exposures.

Relevance to humans. The human relevance of the forestomach tumors has been noted as a concern because humans lack a forestomach, which serves as a food storage organ. However, the *Guidelines for Carcinogen Risk Assessment* (2005) state that site concordance is not a prerequisite for evaluating the implications of animal study results for humans. The oral cavity, pharynx, and glandular stomach are histologically similar to the rat forestomach, but the tissue dose in these human organs is likely different than the tissue dose in the rodent forestomach, due to prolonged exposure from the food storage function of the forestomach in rodents (Proctor et al., 2007).

Chemicals that are genotoxic and cause tumors at multiple sites in the absence of forestomach irritation are likely relevant to human carcinogenesis. Additionally, it has been suggested that most genotoxic forestomach carcinogens appear to act through a mutagenic mode of action (IARC, 2003). 1,2,3-Trichloropropane is carcinogenic through a mutagenic mode of action and is a multisite carcinogen in rodents. Considering all of the available information, the carcinogenicity observed in the rodent studies is considered relevant to human exposure. In addition, the concordance of the alimentary system tumors across rats and mice lends strength to the concern for human carcinogenic potential. The impact of gavage dosing and the potential delayed emptying time in the forestomach lends uncertainty to the derivation of the oral slope factor the extent of which is unknown.

Human population variability. The extent of inter-individual variability in 1,2,3-trichloropropane metabolism has not been characterized. The human variability in response to 1,2,3-trichloropropane is also unknown. Although a mutagenic mode of action would indicate increased early-life susceptibility, the data exploring whether there is differential sensitivity to 1,2,3-trichloropropane carcinogenicity across life stages is unavailable. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty. The uncertainties associated with this lack of data and knowledge about human variability can, at present, only be discussed in qualitative terms; however, EPA has developed ADAFs to quantitatively account for some of the potential differences in age-dependent response to carcinogens with a mutagenic mode of action. ADAFs are to be applied to the slope factors when assessing cancer risks that include childhood exposures (U.S. EPA, 2005b, also see Section 5.4.5).

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

6.1. HUMAN HAZARD POTENTIAL

1,2,3-Trichloropropane (CAS No. 96-18-4) is used in the chemical industry as a solvent for oils and fats, waxes, and resins. The compound also is used industrially in the production of polymers, such as polysulfide rubbers, and of some pesticides. Significant amounts of 1,2,3-trichloropropane are produced as byproducts during the manufacture of other chlorinated compounds, such as epichlorohydrin. The compound is found in consumer products, such as paint thinner and varnish remover.

Toxicokinetic studies in mice and rats have examined the absorption, distribution, metabolism, and elimination of the compound. These studies have documented the rapid metabolism and excretion of the metabolic products in urine or feces, or on the breath (Mahmood et al., 1991; Volp et al., 1984). The absorbed fraction of an administrated dose is almost completely metabolized by a combination of both the phase I and phase II metabolic pathways. Most of the metabolites are rapidly cleared from the body, although a small fraction of the metabolites have been found to bind to intracellular proteins and nucleic acids (Weber, 1991; Weber and Sipes, 1991, 1990).

No epidemiology studies, case reports, or other studies have documented the effects of oral exposure to 1,2,3-trichloropropane in humans. Data from a chronic toxicity test in F344/N rats and B6C3F1 mice (NTP, 1993) and several subchronic studies (NTP, 1993; Merrick et al., 1991; Villeneuve et al., 1985) have identified the liver as a principal target organ for noncancer effects. All non-neoplastic changes reported following chronic oral exposure to 1,2,3-trichloropropane occurred at doses that also produced increased incidences of tumors. A spectrum of hepatic effects has been reported, ranging from cellular necrosis at high doses to significantly increased organ weights at lower doses. An increase in kidney weight was observed following subchronic (NTP, 1993; Merrick et al., 1991; Villeneuve et al. 1985) and chronic exposure (NTP, 1993); however, overt kidney damage was not evident in these studies. Treatment-related effects were detected in rats and mice among the hematological parameters, but the effects were not biologically relevant or related to direct 1,2,3-trichloropropane toxicity (NTP, 1993). Oral exposure has also been shown to reduce fertility in female CD-1 mice (NTP, 1990).

There are very limited data on the effects of 1,2,3-trichloropropane inhalation in humans. An acute inhalation study from the 1940s found that subjects exposed to 5 ppm trichloropropane (isomer and purity not reported) for 15 minutes found the odor objectionable and complained of irritation of the eyes and throat (Silverman et al., 1946). Likewise, there is a limited database of inhalation toxicity studies in animals, which includes two 2-week studies submitted to EPA by

Miller et al. (1987a, b), a 4-week range finding study, two 13-week studies, and two single-generation reproductive assessments (Johannsen et al., 1988; Biodynamics, Inc., 1979).

Increased incidences of nonneoplastic lesions were observed in the nasal epithelium, liver, lungs, and spleen of rats or mice following subchronic inhalation exposure to 1,2,3-trichloropropane (Johannsen et al., 1988; Miller et al., 1987a, b; Biodynamics, Inc., 1979). Miller et al. (1987a, b) reported decreased thickness or degeneration of the olfactory epithelium in rats exposed for 2 weeks to concentrations of ≥3 ppm 1,2,3-trichloropropane (Table 4-25). Similar effects were also observed in mice that were exposed to concentrations of ≥10 ppm 1,2,3-trichloropropane (Table 4-27).

Inhalation exposure to 1,2,3-trichloropropane was also associated with significant increases in organ weights. Increased absolute and relative liver weights were observed in male rats exposed to concentrations of ≥5 ppm 1,2,3-trichloropropane for 13 weeks (Johannsen et al., 1988). Increased liver weights were observed following 2-week exposures to ≥40 ppm in rats and 132 ppm in mice (Miller et al., 1987a). Other organ weight changes included increased relative lung weights in female rats that were exposed to concentrations of ≥15 ppm for 13 weeks (Johannsen et al., 1988), and increased relative kidney and brain weights in male mice exposed to 50 ppm for 13 weeks (Johannsen et al., 1988).

There are no reports of cancer in humans associated with exposure to 1,2,3-trichloropropane. Increased incidence of tumors was observed in rats and mice following oral exposure to 1,2,3-trichloropropane (NTP, 1993). Dose-related increasing trends in tumors were noted at the following sites:

- squamous cell carcinomas or papillomas of the alimentary system in male and female rats and mice;
- Zymbal's gland carcinomas in male and female rats;
- pancreatic acinar cell adenomas or adenocarcinomas, preputial gland adenomas or carcinomas, and kidney tubular cell adenomas in male rats;
- clitoral gland adenomas or carcinomas, and mammary gland adenocarcinomas in female rats;
- hepatocellular adenomas or carcinomas, harderian gland adenomas in male and female mice; and
- uterine/cervical adenomas or adenocarcinomas in female mice.

All of these tumor sites showed statistically significantly positive trends with increasing exposure level (Cochran-Armitage test for trend, $p<0.05$, most with $p\leq.001$) and generally appeared earlier with increasing exposure levels.

The hypothesized mode of action for 1,2,3-trichloropropane induced carcinogenicity is through a mutagenic mode of action. Specifically, the data suggest that bioactivated 1,2,3-

trichloropropane may bind directly to DNA resulting in a mutagenic event that may lead to tumorigenicity in animals.

In vitro bacterial mutation assays have consistently demonstrated a mutagenic potential, dependent on S9 activation, for 1,2,3-trichloropropane. Mammalian cell in vitro studies have shown chromosomal damage, gene mutation, DNA breakage, and micronucleus formation after 1,2,3-trichloropropane exposure. In addition, in vivo assays have demonstrated the ability of 1,2,3-trichloropropane metabolites to bind to hepatic proteins, DNA, and RNA; form DNA adducts in rats and mice; induce DNA strand breaks in the hepatocytes of rats; and to induce wing spots (caused by genotoxic alterations such as somatic mutation, chromosomal rearrangement, or nondisjunction) in *D. melanogaster*. In vivo studies measuring dominant lethal induction or micronucleus formation were non-positive and limit the confidence in the hypothesized mode of action. Additional in vivo assays which would provide evidence of mutagenicity, such as mutations in tumor suppressor genes or other mutagenic markers, are unavailable.

Given the weight of the available evidence, 1,2,3-trichloropropane acts through a mutagenic mode of carcinogenic action andage-dependent adjustment factors (ADAFs) should be applied.

6.2. DOSE RESPONSE

6.2.1. Noncancer—Oral

The NTP (1993) study is selected as the principal study because it was a well-designed chronic study, conducted in both sexes in two species with a sufficient number of animals per dose group. The number of test animals allocated among three dose levels and an untreated control group was acceptable, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Increased liver weight is chosen as the critical effect because liver toxicity appeared to be the most sensitive effect. There is evidence of hepatocellular damage, including increased incidence of hepatic necrosis and decreased synthesis of pseudo-cholinesterase, from the subchronic NTP (1993) study, and increased serum concentrations of hepatocellular enzymes, decreased concentration of 5'-nucleotidase, and increase incidence of histopathologic liver lesions, from the chronic NTP (1993) study. Thus, increased liver weight represents the most sensitive endpoint in a spectrum of liver effects and occurs early in the process of liver toxicity associated with oral exposure to 1,2,3-trichloropropane.

Other effects considered in the selection of the critical effect included kidney, respiratory, myocardial, or reproductive toxicity endpoints. The increase in kidney weights after both subchronic and chronic exposure was accompanied by renal tubular necrosis following subchronic exposure and renal tubule hyperplasia following chronic exposure (NTP, 1993). In addition, the NTP (1993) study demonstrated epithelial necrosis in the nasal turbinates of rats

and regenerative lung lesions in mice following subchronic exposure to 1,2,3-trichloropropane. Pulmonary toxicity including an increased incidence of inflammation-associated myocardial necrosis in rats and increased levels of creatine kinase were also observed (NTP, 1993; Merrick et al., 1991). NTP (1990) demonstrated a decrease in the number of pregnancies per fertile pair, a reduction in the number of live pups/litter, and a decrease in the proportion of male pups born alive. Although the liver appeared to be the most sensitive indicator of 1,2,3-trichloropropane-induced toxicity, RfDs for the changes in kidney weight, fertility, and pups/liter were quantified for comparison purposes.

BMD modeling was conducted to calculate potential PODs for deriving the chronic RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$) for the changes in liver and kidney weight, fertility, and live pups/litter associated with chronic exposure to 1,2,3-trichloropropane. A BMR of 10% was selected for the derivation of the BMDL for liver and kidney weight increases, and the BMR of 1 SD was modeled for comparison purposes. In the developmental study, a 10% decrease in fertility and a 1% change in mean live pups/litter for the 4^{th} and 5^{th} litters were selected as the BMRs due to the frank toxicity of the reproductive toxicity endpoint.

The chronic RfD of 4×10^{-3} mg/kg-day was calculated from a $BMDL_{ADJ}$ of 1.1 mg/kg-day for increased absolute liver weight in male rats chronically exposed to 1,2,3-trichloropropane by gavage (NTP, 1993). A total UF of 300 was used: 10 for interspecies variability, 10 for interindividual variability, and 3 for database uncertainties. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans and the potential variability in human susceptibility; thus, the interspecies and intraspecies UFs of 10 were applied. In addition, a 3-fold database UF was applied due to the lack of information addressing the potential developmental toxicity associated with 1,2,3-trichloropropane. The RfD comparison figure (Figure 6-1) presents the potential PODs, applied UFs, and derived chronic RfD and comparison RfDs for the critical effect and additional endpoints, respectively, from Table 5-1 in Chapter 5.

The overall confidence in the chronic RfD is medium-to-high. Confidence in the principal study (NTP, 1993) is high. Confidence in the database is medium-to-high even though the database lacks a multigenerational developmental toxicity study. The lack of a multigenerational study is of particular concern due to the genotoxicity of 1,2,3-trichloropropane, because genetic damage to the germ cells of the F1 generation may not be detected until the F2 generation. Reflecting high confidence in the principal study and medium-to-high confidence in the database, confidence in the RfD is medium-to-high.

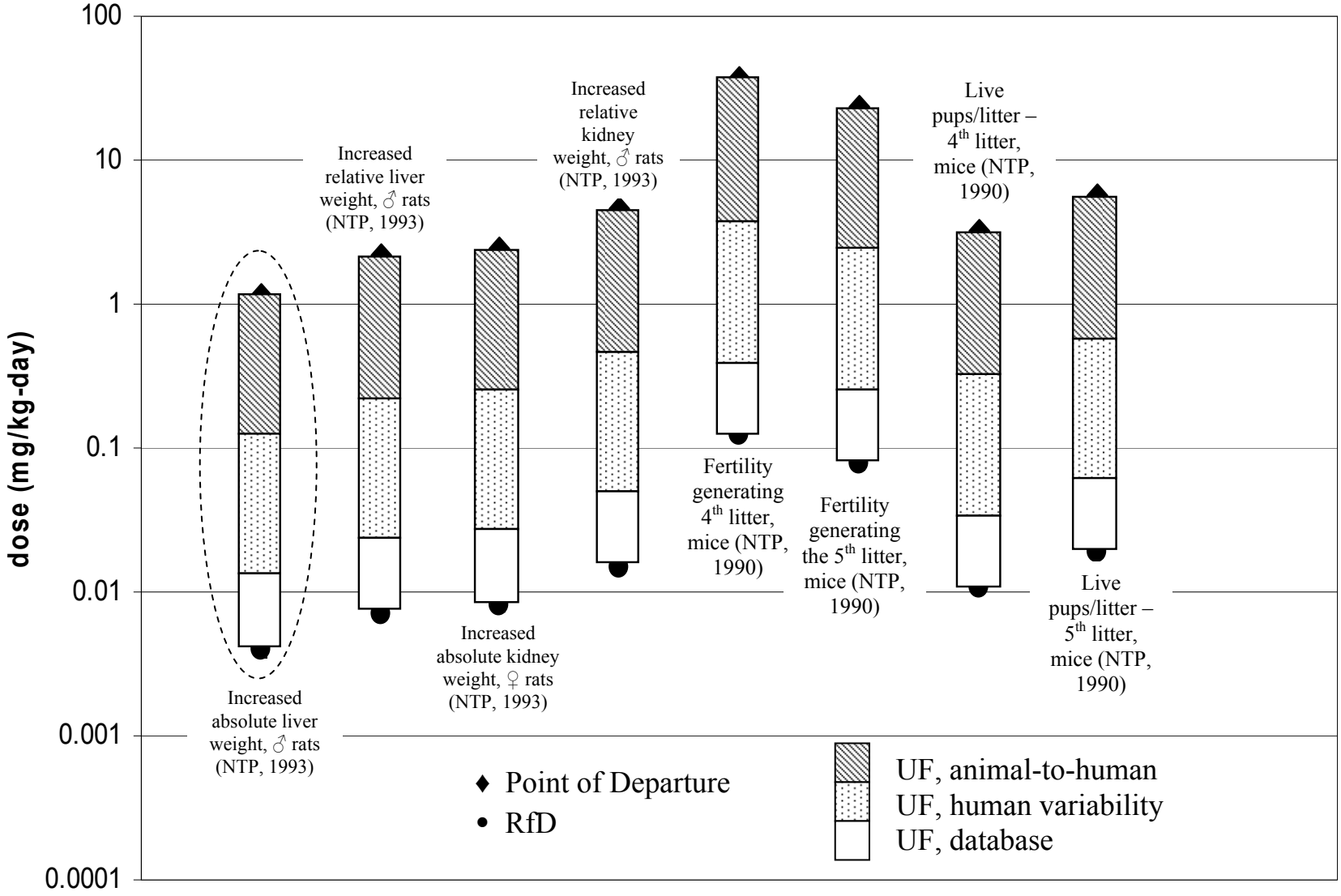


Figure 6-1. PODs for selected endpoints (with critical effect circled) from Table 5-1 with corresponding applied UFs and derived candidate chronic oral RfDs.

6.2.2. Noncancer—Inhalation

The Johannsen et al. (1988) study is selected as the principal study because it was a well-designed subchronic study with a sufficient number of animals per dose group. The number of test animals allocated among five dose levels and an untreated control group was acceptable, with examination of appropriate toxicological endpoints in both sexes of rats and mice. The critical effect selected for the derivation of the chronic RfC is the development of peribronchial lymphoid hyperplasia in the lungs of male CD rats, with a NOAEL of 1.5 ppm and a LOAEL of 5 ppm 1,2,3-trichloropropane, due to the occurrence of this adverse effect in both male and female rats and the possible correlation between the hyperplasia and the observed increased lung weight. Although an increase in liver and kidney weights was apparent, lesions and serum enzyme levels indicative of liver and kidney damage were not evident. The only pathological endpoint observed in the liver was hepatocellular hypertrophy in male rats at 5, 15, and 50 ppm. In the absence of additional toxic effects in the liver (i.e., serum enzyme levels, necrosis), liver weight and hypertrophy observed following inhalation exposure to 1,2,3-trichloropropane were not considered biologically significant.

Johannsen et al. (1988) also conducted two single-generation reproductive toxicity studies using 10 male and 20 female CD rats/group. Female rats exhibited decreased mating performance at 5 ppm, where 16 out of 20 females mated, and at 15 ppm, where 10 out of 20 females mated, compared with 17 out of 20 mated females in the control group. The decrease in the proportion of females that mated was found to be statistically significant ($p<0.02$) at 15 ppm in the Fisher Exact Test conducted by EPA.

BMD modeling was conducted using EPA BMDS version 1.4.1 to analyze the increased incidence of peribronchial lymphoid hyperplasia in CD rats and, for purposes of comparison, the decreased mating performance in female CD rats (see Appendix C for details). The software was used to calculate potential PODs for deriving the chronic RfC by estimating the effective dose at a specified level of response (BMC_x) and its 95% lower bound ($BMCL_x$). For dichotomous endpoints, the *Benchmark Dose Technical Guidance Document* (US EPA, 2000c) states that an excess risk of 10% is generally the default BMR.

HECs were calculated from the candidate POD. HECs were converted to mg/m^3, adjusted to continuous exposure (7 days/week, 24 hours/day), and multiplied by a dosimetric adjustment factor (DAF), a ratio of animal and human physiologic parameters. The specific DAF used depends on the nature of the contaminant (particle or gas) and the target site (e.g., respiratory tract or remote to the portal-of-entry). The DAF for an extra-respiratory effect of a gas is the ratio of the animal/human blood:air partition coefficients $[(H_{b/g})_A/(H_{b/g})_H]$. However, the human and rat blood partition coefficients for 1,2,3-trichloropropane are not known. In accordance with the RfC methodology (U.S. EPA, 1994b) when the partition coefficients are unknown a ratio of 1 is used. The unknown human and rat blood partition coefficients for 1,2,3-

trichloropropane represent a significant data gap, in which the availability of this information would provide for a more accurate HEC calculation.

The chronic RfC of 3×10^{-4} mg/m^3 was calculated from a $BMCL_{HEC}$ of 0.90 mg/m^3 for increased incidence of peribronchial lymphoid hyperplasia in the lungs of male CD rats (Johannsen et al., 1988). A total UF of 3,000 was used: 3 for interspecies variability, 10 for interindividual variability, 10 for extrapolating from a subchronic to chronic exposure duration, and 10 for database deficiencies. A factor of 3 was selected to account for uncertainties in extrapolating from rats to humans, which is adopted by convention where an adjustment from an animal specific $NOAEL_{ADJ}$ to a $NOAEL_{HEC}$ has been incorporated. Insufficient information is available to predict potential variability in susceptibility among the population; thus, the human variability UF of 10 was applied. A 10-fold UF was used to account for uncertainty in extrapolating from a subchronic to chronic exposure duration. A 10-fold UF was used to account for deficiencies in the database. The database of 1,2,3-trichloropropane inhalation studies is lacking a multigenerational reproductive study and a developmental toxicity study. The lack of the multigenerational study is of particular concern due to the genotoxicity of 1,2,3-trichloropropane, because genetic damage to the germ cells of the F1 generation may not be detected until the F2 generation. Figure 6-2 presents the potential PODs, applied UFs, and derived chronic RfC and comparison RfC for the critical effect and additional endpoint, respectively, from Table 5-2 in Chapter 5.

The overall confidence in the chronic RfC is low. Confidence in the principal study (Johannsen et al., 1988) is low-to-medium. Confidence in the database is low as the database lacks a chronic inhalation bioassay and multigenerational reproductive and developmental toxicity studies. The lack of a multigenerational developmental study is of particular concern due to the genotoxicity of 1,2,3-trichloropropane, because genetic damage to the germ cells of the F1 generation may not be detected until the F2 generation. Reflecting low-to-medium confidence in the principal study and low confidence in the database, confidence in the chronic RfC is low.

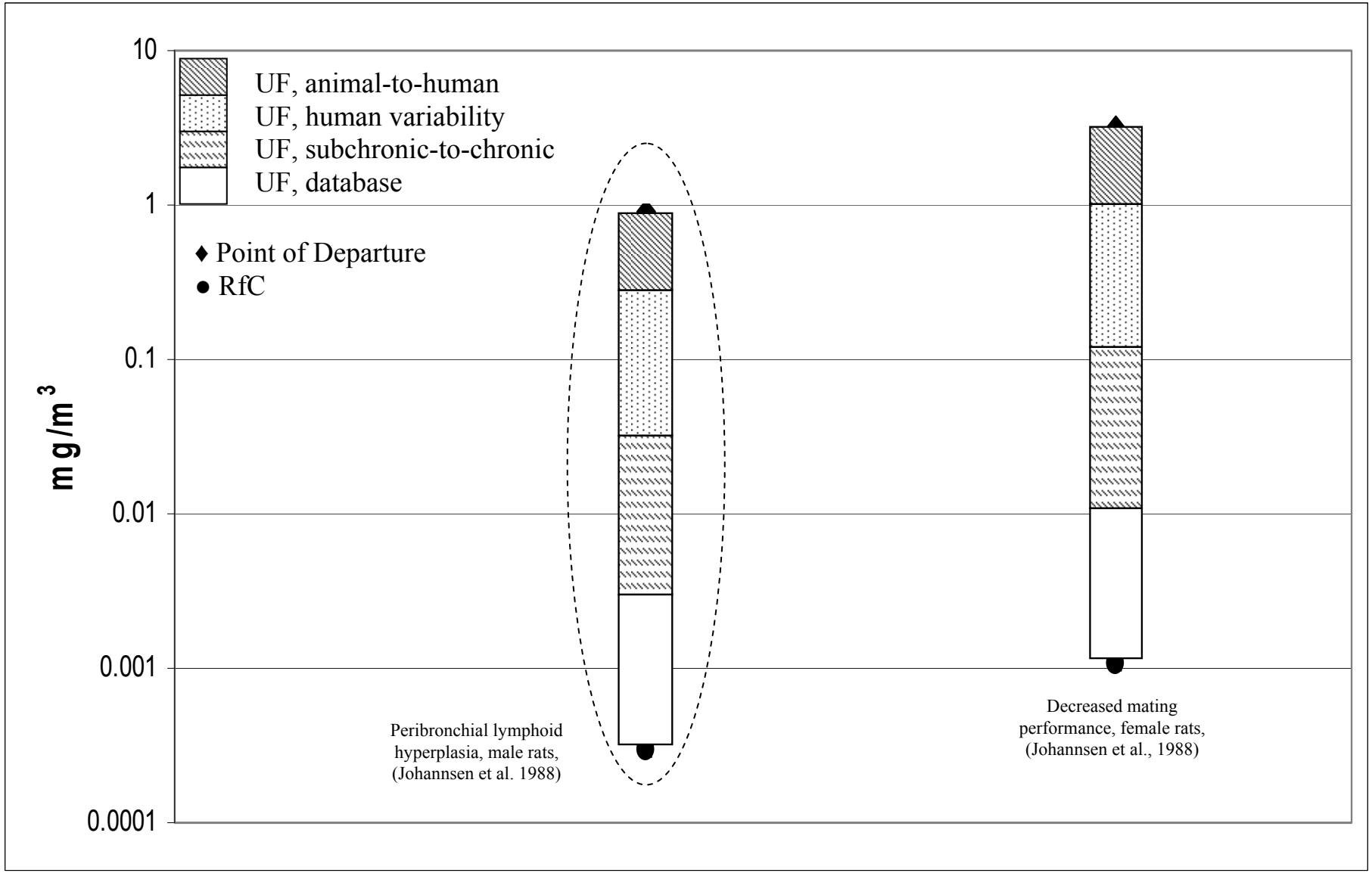


Figure 6-2. PODs for selected endpoints (with critical effect circled) from Table 5-2 with corresponding applied UFs and derived candidatechronic inhalation RfCs.

6.2.3. Cancer—Oral and Inhalation

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,2,3-trichloropropane is *likely to be carcinogenic to humans*, based on the existence of compelling evidence of the compound's tumorigenicity in a single, well-carried-out bioassay in two animal species (Irwin et al., 1995; NTP, 1993). There are no studies that examine the potential carcinogenicity of 1,2,3-trichloropropane in humans. While the use of gavage studies in experimental animals to extrapolate to human exposure to the compound in drinking water may introduce quantitative uncertainty, the consistent dose-dependent formation of tumors, at and remote from the site-of-entry in two animal models, suggests a tumorigenic capacity of 1,2,3-trichloropropane in humans.

A dose-related, statistically significant increasing trend in tumors was observed in the following sites:

- squamous cell carcinomas or papillomas of the alimentary system in male and female rats and mice;
- Zymbal's gland carcinomas in male and female rats;
- pancreatic acinar cell adenomas or adenocarcinomas, preputial gland adenomas or carcinomas, and kidney tubular cell adenomas in male rats;
- clitoral gland adenomas or carcinomas, and mammary gland adenocarcinomas in female rats;
- hepatocellular adenomas or carcinomas, and harderian gland adenomas in male and female mice; and
- uterine/ cervical adenomas or adenocarcinomas in female mice.

These tumors generally appeared earlier with increasing exposure levels, and showed statistically significantly increasing trends with increasing exposure level. Etiologically similar tumor types, benign and malignant tumors of the same cell type, were combined for these tabulations because of the possibility that the benign tumors could progress to the malignant form (U.S. EPA, 2005a). This assumption, if incorrect, has some limited potential to overestimate the carcinogenic potential of 1,2,3-trichloropropane, and is an accepted practice (McConnell et al., 1986).

The mode of action is a key consideration in clarifying how risks should be estimated for low-dose exposure. A linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,2,3-trichloropropane exposures. This approach is supported by the positive evidence of genotoxicity and a mutagenic mode of action.

Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and early termination of at least one dose group, dose-response methods which can reflect the influence of competing risks, and intercurrent mortality on site-specific tumor

incidence rates are preferred. EPA has generally used the multistage-Weibull model in this type of situation, because it incorporates the time at which death-with-tumor occurred and can account for differences in mortality observed between the exposure groups in the rat bioassay. Additionally, etiologically different tumor types were not combined across sites prior to modeling, in order to allow for the possibility that different tumor types can have different dose-response relationships because of varying time courses or other underlying mechanisms or factors.

PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, generally corresponding to 10% extra risk, defined as the extra risk over the background tumor rate. The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD. This 95% UCL represents a plausible upper bound on the true risk.

Adjustments for approximating human equivalent slope factors applicable for continuous exposure were calculated. Following EPA's cross-species scaling methodology, the time-weighted daily average doses were converted to human equivalent doses on the basis of (body weight)$^{3/4}$ (U.S. EPA, 1992) and the estimated continuous daily exposures were calculated by multiplying each slope factor by (5 days)/(7 days) = 0.71. The impact of applying these adjument factors to the slope factor is unknown. The human equivalent oral slope factors estimated from the tumor sites with statistically significant increases ranged from 0.02 to 3.0 per mg/kg-day.

However, given the multiplicity of tumor sites, basing the oral slope factor on one tumor site may underestimate the low-dose carcinogenic potential of 1,2,3-trichloropropane. Following the recommendations of the NRC (1994) and the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a statistically appropriate upper bound on total risk was estimated in order to gain some understanding of the total risk from multiple tumor sites in male F344/N rats (Table 5-7). Note that this estimate of overall risk describes the risk of developing any combination of the tumor types considered, not just the risk of developing all three simultaneously.

The recommended estimate for an upper bound on human extra cancer risk from lifetime oral exposure to 1,2,3-trichloropropane is 30 per mg/kg-day, derived from female mice alimentary system tumors. This estimate reflects the time-to-tumor response as well as the exposure-response relationships for the multiple tumor sites in female rats. The value based on female rats is recommended because female rats are the most sensitive to tumor induction following exposure to 1,2,3-trichloropropane and yield the highest slope factor. Note that this slope factor should not be used with human exposures greater than 0.6 mg/kg-day, since the observed dose-response does not continue linearly above this level. Cancer risk estimates

derived from the other available datasets ranged from 2 (female rats) to 7 per mg/kg-day (male mice).

Because a mutagenic mode of action for 1,2,3-trichloropropane carcinogenicity is sufficiently supported in laboratory animals and relevant to humans (Section 4.7.3.4), and in the absence of chemical-specific data to evaluate differences in susceptibility, increased early-life susceptibility is assumed and the ADAFs should be applied to the slope factor, as appropriate, in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

An inhalation unit risk was not derived in this assessment. Data on the carcinogenicity of the compound via the inhalation route are unavailable, and route-to-route extrapolation was not possible due to the lack of an adequate physiologically based pharmacokinetic model. However, 1,2,3-trichloropropane is likely to be carcinogenic to humans by the inhalation route since the compound is well-absorbed, and induces tumors at sites other than the portal of entry in oral studies.

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

The Toxicological Review of 1,2,3-trichloropropane has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

On April 10, 2008, EPA introduced revisions to the IRIS process for developing chemical assessments. As part of the revised process, the disposition of peer reviewer and public comments, as found in this Appendix, and the revised IRIS Toxicological Review were provided to the external peer review panel members for their comment on April 24, 2009 The external peer reviewers did not provide any additional comments.

EXTERNAL PEER REVIEW PANEL COMMENTS

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

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

A. General Comments

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

> Comments: Several reviewers agreed that the document is logical, clear, and concise. Other reviewers considered the document redundant and confusing due to the repetition of the toxicological data throughout the document instead of presenting a scientific basis for using the described data to make a decision. Additionally, a reviewer questioned whether the evidence has been completely and logically synthesized. Some of the

reviewers recommended improving the presentation of the evidence for hazard by reducing the redundancy of Section 4 and Section 5.

Response: The content of the *Toxicological Review* was consistent with the current outline for IRIS toxicological reviews, although an effort has been made to streamline the document and reduce the redundancy in Chapter 4 and Chapter 5.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of 1,2,3-trichloropropane.

Comments: Several reviewers did not find any additional studies. Studies on the health effects of 1,2,3-trichloropropane that were identified by the reviewers for consideration are presented below. Additionally, a reviewer identified carcinogenesis studies for the structurally related chemicals, ethylene dibromide and dibromochloropropane, that may provide support for the 1,2,3-trichloropropane cancer assessment. One reviewer commented that the IARC (1995) cancer classification for 1,2,3-trichloropropane should be included in the document.

The following studies were identified by the external peer reviewers for consideration:

Chroust, K., M. Pavlova, Z. Prokop, J. Mendel, K. Bozkova, Z. Kubat, V. Zajickova, and J. Damborsky. 2007. Quantitative structure-activity relationships for toxicity and genotoxicity of halogenated aliphatic compounds: wing spot test *of Drosophila melanogastor*. Chemosphere 67(1):152-9

Glutathione-mediated binding of dibromoalkanes to DNA: specificity of rat glutathione-S-transferases and dibromoalkane structure.
Inskeep PB, Guengerich FP. Carcinogenesis. 1984 Jun;5(6):805-8

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Formation of the DNA adduct S-[2-(N7-guanyl)ethyl]glutathione from ethylene dibromide: effects of modulation of glutathione and glutathione S-transferase levels and lack of a role for sulfation. Kim DH, Guengerich FP. Carcinogenesis. 1990 Mar;11(3):419-24.

Direct-acting alkylating and acylating agents. DNA adduct formation, structure-activity, and carcinogenesis. Van Duuren BL. Ann N Y Acad Sci. 1988;534:620-34

Induction of DNA repair in rat spermatocytes and hepatocytes by 1,2-dibromoethane: the role of glutathione conjugation. Working PK, Smith-Oliver T, White RD, Butterworth BE. Carcinogenesis. 1986 Mar;7(3):467-72

Comparative in vivo genotoxicity and acute hepatotoxicity of three 1,2-dihaloethanes. Storer RD, Conolly RB. Carcinogenesis. 1983 Nov;4(11):1491-4

Carcinogenesis Bioassay of 1,2-Dibromoethane (CAS No. 106-93-4) in F344/N Rats and B6C3F1 Mice (Inhalation Study). National Toxicology Program. Natl Toxicol Program Tech Rep Ser. 1982 Mar;210:1-163

Report on carcinogenesis bioassay of 1,2-dibromoethane (EDB).
[No authors listed]
Am Ind Hyg Assoc J. 1979 Feb;40(2):A31-5

Carcinogenesis in rats of combined ethylene dibromide and disulfiram.
Plotnick HB. JAMA. 1978 Apr 21;239(16):1609

Ginsberg, G. L., Pepelko, W. E., Goble, R. L., and Hattis, D. B. "Comparison of Contact Site Cancer Potency Across Dose Routes: Case Study with Epichlorohydrin," Risk Analysis Vol. 16, pp. 667-681, 1996

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International Agency for Research on Cancer. 1995. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 63. Dry cleaning, Some Chlorinated Solvents and Other Industrial Chemicals, World Health Organization, IARC, Lyon

Response: Several of the recommended studies were already included in the Toxicological Review. Chroust et al. (2007) and NTP (2005) were previously included in Sections 4.5.2, *Genotoxicity Studies*, and 4.4.3, *Aquatic Species Studies*, respectively. In addition, the 1982 NTP inhalation study on 1,2-dibromo-3-chloropropane has been expanded in Section 4.5.3, *Structural Analog Data*. The references that have not been added to the Toxicological Review include; Van Duuren, BL (1988), Working et al. (1986), Storer and Conolly (1983), Plotnick, HB (1978), and Ginsberg et al. (1996), as these references do not contribute significant information to the discussion and analysis in the document. With regard to the IARC classification, EPA does not typically include cancer characterizations of other health agencies in IRIS assessments.

Comment: One reviewer stated that the dermal administration of 1,3-dichloroacetone study should be included in the animal cancer section and considered in the weight of evidence evaluation.

Response: The dermal administration study of 1,3-dichloroacetone was included as supporting evidence of the mode of action of carcinogenicity in Section 4.7.3.2, *Experimental Support for the Hypothesized Mode of Action*.

3. Please discuss research that you think would be likely to reduce uncertainty in the toxicity values for future assessments of 1,2,3-trichloropropane.

Comments: Several reviewers suggested additional research to address the data gaps for 1,2,3-trichloropropane. Specifically, several reviewers suggested that studies addressing the identity and role of the metabolic pathways that produce cytotoxic and/or carcinogenic metabolites that could be utilized in the development of a PBPK model. In addition, the reviewers suggested that PBPK modeling studies that investigate the comparative dosimetry in animals and humans would be useful. Mode of action studies, especially gene mutation studies, could also reduce the uncertainty in the cancer assessment. Another reviewer highlighted the need for a mouse study that captures the correct dose range for assessing carcinogenicity. Toxicity studies performed by administering 1,2,3-trichloropropane in drinking water or other vehicle besides corn oil would allow for a more accurate assessment of the toxicological effects resulting from an exposure that resembles the potential human exposure. In addition, an inhalation cancer bioassay and multigenerational reproductive toxicity study, as well as an oral developmental study, were also recommended.

Response: EPA agrees that the above research recommendations would improve future hazard identifications of 1,2,3-trichloropropane.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

The reviewers generally agreed that the uncertainty has been clearly, transparently, and adequately discussed, but several reviewers offered suggestions to more completely

characterize the uncertainty associated with the 1,2,3-trichloropropane database. These comments are presented as follows:

Comment: Two reviewers suggested more complete discussion of the relevance of forestomach tumors in the cancer assessment, especially the potential role of hyperplasia of the forestomach epithelium in the development of cancer.

Response: Additional discussion of the relevance of forestomach tumors in the cancer assessment has been included in Sections 5.4.2, 5.4.4, and 5.4.6. Please also see response to comment under questions D.3 and D.4.

Comment: One reviewer commented that the report underestimates the potential for the corn oil vehicle to influence the carcinogenicity of 1,2,3-trichloropropane.

Response: EPA recognizes that the administration of 1,2,3-trichloropropane in corn oil may enhance the proliferative mechanisms that may follow the genetic changes that were the result of the mutagenic mode of action, and has included text in Section 5.4.6, *Uncertainties in Cancer Risk Values*.

Comment: Several reviewers highlighted the need for consideration and discussion of the mouse tumor data with respect to the most sensitive species being selected for the cancer quantification.

Response: EPA originally excluded the mouse data from the cancer quantitation because the tumor response in the lowest dose was close to a maximum response; however, based on the external peer review comments, EPA has modeled the mouse data using the same methods as were used with the rat data. The analysis of the mouse tumor data (added to Section 5.4) is now used in the derivation of the OSF because the mouse is the most sensitive species.

Comment: One reviewer stated that more discussion of the pharmacokinetic uncertainties would be beneficial.

Response: Text was added to Section 5.4.6, *Uncertainties in Cancer Risk Values*, addressing pharmacokinetic uncertainty.

Comment: One reviewer commented that the uncertainty needs to be based on an improved discussion and utilization of the information that is available. More specifically, data is available that provides estimates of relative rates of formation of a particular adduct with DNA at varying doses that were not fully exploited. This data can be used to develop better insight into the dosimetric aspects of the cancer risk assessment.

Response: Sections 5.3, *Uncertainties in Chronic Oral Reference Dose and Inhalation Reference Concentration*, and 5.4.6, *Uncertainties in Cancer Risk Values*, have been expanded upon and modified. Also, please see response to the third comment under D.2.

B. Oral Reference Dose (RfD) for 1,2,3-Trichloropropane

1. A chronic RfD for 1,2,3-trichloropropane has been derived from a 2-year oral gavage study (NTP, 1993) in rats and mice. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comment: The reviewers generally agreed that the selection of the NTP (1993) report as the principal study was scientifically justified.

Response: No response.

Comment: One reviewer expressed concern that it is problematic to have such a large prediction of cancer risk at the RfD. The reviewer stated that the cancer risk estimated at the RfD of 0.004 mg/kg-day using the proposed EPA potency of 4 per mg/kg-day is 0.016 or 2 in 100. Another reviewer also raised concern over deriving an RfD at doses that are carcinogenic.

Response: Under current Agency practice, an RfD is derived based solely on *noncancer* effects observed in animal or human studies, while an oral cancer slope factor is derived based solely on *cancer* effects seen in animal or human studies. In addition, these two toxicity values are typically derived using different qualitative and quantitative analyses.

2. Increased liver weight was selected as the critical effect. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Is the rationale

for this selection transparently and objectively described in the document? Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect. Please comment on the use of increased absolute liver weight instead of relative liver weight to describe the liver weight change.

Comment: Several reviewers commented that the increase in liver weight as the critical effect has not been scientifically justified and may be considered an adaptive response to 1,2,3-tichloropropane exposures. A reviewer stated that EPA's conclusion that increased liver weight may be part of a continuum of adverse hepatic effects was highly speculative.

Response: EPA considered that, given the available data, increased liver weight represents the most sensitive effect observed in the liver and occurs early in the process of liver toxicity associated with oral exposure to 1,2,3-trichloropropane. In addition to increased liver weight following both subchronic and chronic exposures to 1,2,3-trichloropropane, there is evidence of hepatocellular damage, including increased incidence of hepatocellular necrosis and decreased synthesis of pseudocholinesterase from the subchronic studies at higher doses. Also, increased serum concentrations of hepatocellular enzymes (ALT and SDH), decreased concentration of 5'-nucleotidase, and increased incidence of histopathologic liver lesions, including hepatocellular necrosis, were observed in the chronic study at higher doses. Thus, EPA concluded that increased liver weight may represent the most sensitive effect that occurs early in the process of 1,2,3-trichloropropane-induced hepatoxicity following oral exposure. Additionally, the statement that "increased liver weight may be part of a continuum of adverse hepatic effects*"* has been modified in Section 5.1.1, *Choice of Principal Study and Critical Effect —with Rationale and Justification*, and now reads: "Increased liver weight was selected as the critical effect because it represents the most sensitive effect observed in the liver and occurs early in the process of liver toxicity associated with oral exposure to 1,2,3-trichloropropane."

Comment: A reviewer questioned whether the corn oil gavage may be affecting the pathology observed in the liver of male and female rats and mice following subchronic exposure, and referenced the synergistic affect of corn oil and chloroform on liver pathology.

Response: Text has been added to Section 5.3, *Uncertainties in Chronic Oral Reference Dose and Inhalation Reference Concentration*, of the draft document addressing the effect the corn oil vehicle may have on the derivation of the RfD.

Comment: Reviewers also commented that the data presentation and discussion could be improved by including additional non-neoplastic effects noted by NTP and a side-by-side comparison of the dose response for liver weight increases with the development of pathology or indirect indicators of such pathology.

Response: The sections of the document that present the observed effects of 1,2,3-trichloropropane exposure (Section 4.2, *Subchronic and Chronic Studies and Cancer Bioassays in Animals—Oral and Inhalation*), as well as the analysis of the observed effects (Section 4.6, *Synthesis of Major Noncancer Effects*) and the selection of the critical effect (Section 5.1.1, *Choice of Principal Study and Critical Effect—with Rationale and Justification*) have been modified in an effort to improve the presentation and discussion of the data. Text was added to Section 4.2 and Section 5.1.1 describing additional non-neoplastic effects observed, and a table has been added to Section 4.6.1 that presents the observed effects and corresponding NOAELs and LOAELs for the subchronic, chronic, and reproductive toxicity studies.

Comment: Two reviewers commented that Benchmark Dose modeling should be conducted on additional liver endpoints, such as pseudocholinesterase levels. A reviewer suggested that the modeling of these endpoints would be beneficial to selecting a critical effect by considering all hepatic endpoints together and rounding to derive the point of departure.

Response: EPA concluded that increased liver weight represents the most sensitive effect observed in the liver and occurs early in the process of liver toxicity associated with oral exposure to 1,2,3-trichloropropane. Additional liver endpoints following subchronic exposure in rats were not modeled using Benchmark Dose Software. However, the additional hepatotoxicity endpoints observed were incorporated into Chapter 5 as supporting evidence of the selected critical effect. The supporting evidence of hepatocellular damage includes increased incidence of hepatocellular necrosis and decreased synthesis of pseudocholinesterase, from the subchronic NTP (1993) study, and increased serum concentrations of hepatocellular enzymes (ALT and SDH), decreased concentration of 5'-nucleotidase, and increase incidence of histopathologic liver lesions, including hepatocellular necrosis, from the chronic NTP (1993) study.

Comment: A reviewer recommended that additional consideration be provided for other endpoints that were not modeled, but were considered by NTP to be important. These effects included the increased severity of nephropathy, hyperplasia in the forestomach, pancreas, and kidney.

Response: EPA recognizes that these additional effects were observed in the chronic NTP study, but believes that the modeled endpoints, including increased liver and kidney weight, decreased fertility, and decreased live pups/litter, are representative of the most sensitive toxicological effects observed. Additional effects observed are utilized in the qualitative discussion of the toxicity of 1,2,3-trichloropropane following oral exposure. The incidence of hyperplasia in the forestomach (basal cell and squamous), kidney (renal tubule), and pancreas (acinar) of rats and the incidence of hyperplasia in the forestomach (squamous) of mice following chronic exposure to 1,2,3-trichloropropane (NTP, 1993) were added to Sections 4.2.1.2, *Chronic Studies;* 4.6, *Synthesis of Major Noncancer Effects;* and 5.1.1, *Choice of Principal Study and Critical Effect—with Rationale and Justification*.

3. The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the point of departure (POD). All available models were fit to the data in both rats and mice for increased absolute and relative liver weight, increased absolute and relative kidney weight, fertility generating the 4th and 5th litter, and the number of live pups/litter in the 4th and 5th litters. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and adequately described? Is the benchmark response selected for use in deriving the POD scientifically justified and has it been transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Comment: One reviewer commented that a 10% weight change was too low to use as a benchmark response level in this modeling exercise because BMD modeling is a conservative approach to selecting a point of departure and the critical effect selected has a questionable toxicological significance. Another reviewer stated that the choice of the BMR of 10% was unclear.

Response: The BMR of 10% is analogous to the 10% change in body weight used to identify maximum tolerated doses and is considered to be the minimal level of change

that is biologically significant. The modeling results with a BMR of 1 SD are provided for comparison purposes.

Comment: Two reviewers commented that the benchmark response level of 1% for mean live pups per litter was too low and clearly well below a level of change that could be measured experimentally.

Response: The BMR selected for the mean live pups per litter was selected as the BMR due to the frank toxicity of the reproductive toxicity endpoint, not because the 1% level would be a level of detection for the experiment. The BMR was selected because of the severity of the effect.

Comment: A reviewer commented that comparing the NOAELs and LOAELs for an endpoint of interest to the Benchmark Dose Modeling results would be informative.

Response: Table 4-32 presents the NOAELs and LOAELs identified for the endpoints of interest from the NTP (1993) study and has been added to Section 4.6, *Synthesis of Major Noncancer Effects*. A direct comparison of the NOAELs and LOAELs with the BMDLs was not conducted in Chapter 5 because a comparison of the values from a NOAEL/LOAEL approach and a BMD modeling approach is inappropriate and does not provide confidence in the reference value.

Comment: One reviewer requested that the modeling inputs and results be more comprehensively described. Additionally, a reviewer commented that the modeling outputs for the organ weight changes in the mice should be included in Appendix B.

Response: Appendix B, *Benchmark Dose Modeling Results for the Derivation of the RfD***,** has been expanded to include tables for each endpoint modeled and presents the results for each model output.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfDs. For instance, are they scientifically justified and transparently and objectively described in the document?

Comment: One reviewer commented that the 10-fold interspecies uncertainty factor is not justified (a 3-fold uncertainty factor is justified in the absence of human and animal toxicodynamic data) because the livers of mice and rats have substantially higher

CYP450 and glutathione-S-transferase activities than humans. One reviewer commented that the 10-fold interspecies uncertainty factor was not justified and should be reduced to 3 because the higher DNA adduct formation and cell proliferation observed in mice following oral gavage compared to drinking water.

Response: While the activities of CYP450 and glutathione-S-transferase may be increased in rats compared to humans, we do not know enough about the mode of action or toxicodynamics of 1,2,3-trichloropropane-induced hepatotoxicity to confidently decrease the interspecies uncertainty factor from 10 to 3.

The interspecies uncertainty factor is applied to account for the uncertainty in extrapolating laboratory animal data to average healthy humans. The comparison of DNA adducts formation and cell proliferation in mice following oral gavage and drinking water does not inform the interspecies differences between mice and humans. The DNA adduct data was characterized as an uncertainty in the assessment in Section 5.3, *Uncertainties in Chronic Oral Reference Dose and Inhalation Reference Concentration*.

Comment: One reviewer commented that because the observations in the NTP study were made at 15 months, the point of departure should be adjusted from 15 to 24 months. If an adjustment is not made, a subchronic-to-chronic uncertainty factor should be applied.

Response: EPA considers the 15-month exposure period of the NTP (1993) study to be a chronic exposure.

Comment: A reviewer commented that the BMR of 10% used for the analysis of the liver weight change data should be interpreted as a LOAEL response and that the UF_L should be increased to a value of 3.

Response: The current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, a BMR of a 10% change in absolute liver weight was selected under an assumption that it represents a minimal biologically significant change. When BMD modeling is used to derive the point of departure, a LOAEL-to-NOAEL uncertainty factor is not applied.

5. Please comment on the transparency and scientific rationale and justification for the selection of the database uncertainty factor. Please comment on whether the application of the database

uncertainty factor adequately represents the gap in oral reproductive and developmental toxicity data for 1,2,3-trichloropropane.

Comment: One reviewer commented that the 3-fold database uncertainty factor should be applied to a point of departure from the reproductive/developmental toxicity studies and not applied to a point of departure for liver weight changes. The reviewer commented that application of the 3-fold database uncertainty factor to the POD for reproductive/developmental toxicity is logical because it is a clear effect and could be exacerbated in the next generation. Another reviewer commented that the database UF was not justified because the point of departure for the derived RfD should be protective of the developing fetus (when comparing BMDs and BMDLs for both endpoints). One reviewer suggested that a 10-fold database uncertainty factor be applied because developmental toxicity data is unavailable.

Response: EPA recognizes the adequacy of the available subchronic, chronic, and reproductive toxicity studies following oral exposure to 1,2,3-trichloropropane, and that the critical effect selected may be protective of developmental toxicity. However, the database uncertainty factor is applied to account for the potential for deriving an under-protective reference value as a result of data gaps in the characterization of the chemical's toxicity, and is applied to the entire database of effects unless mode of action information states otherwise. In this instance, the database uncertainty factor of 3 was applied because the available database of subchronic, chronic, and reproductive toxicity studies is lacking a developmental toxicity study and a reproductive toxicity study extended beyond two-generations, due to concern for genetic damage to germ cells. EPA believes that a database uncertainty factor of 3 accounts for the lack of a developmental study and takes into consideration the availability of a two-generation reproductive toxicity study.

C. Inhalation Reference Concentration (RfC) for 1,2,3-Trichloropropane

1. A chronic RfC for 1,2,3-trichloropropane has been derived from the 13 week inhalation study (Johannsen et al., 1988) in rats. Please comment on whether the selection of this study as the principal study is scientifically justified. Is the rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comment: Reviewers commented that the Johannsen et al. (1988) study has not been peer-reviewed.

Response: The Johannsen et al. (1988) study has been peer-reviewed and published in the Journal of Toxicology and Environmental Health, Volume 25, 1988.

Comment: One reviewer commented that the decreased mating performance, which was described by Johannsen et al. (1988) as not statistically significant, was actually statistically significant in a two-group comparison and should be considered further as a potential critical effect. This reviewer also stated that there are other toxicological endpoints from Johannsen et al. (1988) that should be given more consideration as potential critical effects.

Response: Text was added to Section 5.2.1, *Choice of Principal Study and Critical Effect – with Rationale and Justification* describing the decreased mating performance observed in the females exposed to 15 ppm 1,2,3-trichloropropane. EPA conducted a Fisher Exact test and has included the results in Section 5.2.1. With regards to the critical effect selected and additional toxicological effects, please see response to Question 2 below.

2. Peribronchial lymphoid hyperplasia in the lungs of male rats was selected as the critical toxicological effect. Please comment on whether the selection of this critical effect has been scientifically justified. Is the rationale for this selection transparently and objectively described in the document? Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comment: Several reviewers commented that the rationale for selecting peribronchial lymphoid hyperplasia over liver weight change was not well justified, and that it would seem that the liver weight/hepatocellular hypertrophy should be selected as the critical effect. Specifically, when related to achieving consistency between the derivation of the RfD and RfC. A reviewer also questioned the toxicological significance of peribronchial lymphoid hyperplasia. Another reviewer commented that the justification was reasonable, but the argument for its selection is not sufficiently compelling.

Response: In the case of 1,2,3-trichloropropane, the increase in liver weights observed following the inhalation exposure was not dose-related. In the absence of additional

effects in the liver (i.e., serum enzyme levels, necrosis), liver weight and hypertrophy were not considered biologically significant.

In addition, there are toxicokinetic, and as a result possible toxicodynamic, differences between the two routes of exposure. A first-pass effect by the liver is expected for the metabolism of 1,2,3-trichloropropane following oral exposure. The *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b) states that a route-to-route extrapolation, in this case a qualitative extrapolation, should not be conducted when a first-pass effect by the liver is expected.

Peribronchial lymphoid hyperplasia, also defined as lymphoid hyperplasia of the bronchus-associated lymphoid tissue, is histologically characterized by the presence of hyperplastic lymphoid follicles with reactive germinal centers distributed along the bronchioles and bronchi (Howling et al., 1999; Myers and Kurtin, 1995; Fortoul et al., 1985; Yousem et al, 1985). The peribronchial lymphoid hyperplasia is a portal-of-entry effect that is more sensitive than any observed liver effects.

Text was reorganized and added to Section 5.2.1, *Choice of Principal Study and Critical Effect—with Rationale and Justification,* addressing the selection of the critical effect.

Comment: One reviewer commented that the argument that characterizing the liver weight changes observed following inhalation exposure as adaptive is in conflict with selecting increased liver weight as the critical effect following oral exposure.

Response: The increased liver weight following inhalation exposure to 1,2,3-trichloropropane was described as follows: "Although an increase in liver and kidney weights was apparent, lesions and serum enzyme levels indicative of liver and kidney damage were not evident. The only pathological endpoint observed in the liver was hepatocellular hypertrophy in male rats at 5, 15, and 50 ppm. In the absence of a dose-related increase in liver weight and the lack of additional effects in the liver (i.e. serum enzyme levels, necrosis), liver weight and hypertrophy were not considered biologically significant." The liver weight changes following oral exposure to 1,2,3-trichloropropane were considered biologically significant as the change in liver weight was accompanied by increased serum liver enzymes, increased incidence of hepatic necrosis, and a decrease in pseudocholinesterase. All of these effects are indicative of liver damage and provide support for the selection of the liver as the critical target organ. Additionally, a portal-of-entry effect is expected following 1,2,3-trichloropropane exposure via inhalation and a

first-pass effect is expected following oral exposure; indicating that direct comparison between routes is not possible.

3. The chronic RfC has been derived utilizing the NOAEL/LOAEL approach to define the point of departure. Please provide comments with regards to whether this is the best approach for determining the point of departure. Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Comment: Several reviewers commented that benchmark dose modeling should have been attempted, and that, in an effort to establish a good model fit, the highest-dose may be dropped from the modeling.

Response: The increased incidence of peribronchial lymphoid hyperplasia in male and female rats and the decreased mating performance in female rats, following inhalation exposure to 1,2,3-trichloropropane, were modeled using Benchmark Dose Software (1.4.1c). The BMD analysis is now the basis for the POD used in the derivation of the RfC and the model outputs are included in Appendix C.

Comment: One reviewer commented that BMDs and BMDLs or NOAELs and LOAELs should be compared to the NOAEL (or BMDL) for peribronchial lymphoid hyperplasia, in a similar manner as was done for the RfD in Section 5.1.4.

Response: Additional text and Figure 5-3 have been added to Section 5.2.4, *Chronic RfC Comparison Information*, comparing the RfC derived from the BMDL for peribronchial lymphoid hyperplasia in male rats with the RfC derived from the BMDL for decreased mating performance in female rats.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfCs. For instance, are they scientifically justified and transparently and objectively described in the document?

Comment: One reviewer stated that the use of interspecies UF of 3 was not well justified.

Response: In this assessment, the toxicokinetic component of the interspecies uncertainty factor is addressed by the determination of a human equivalent concentration

as described in the RfC methodology (U.S. EPA, 1994b). However, the toxicodynamic component of the interspecies uncertainty factor is only partially accounted for by the use of the applied dosimetry method. The application of this uncertainty factor in the Toxicological Review of 1,2,3-trichloropropane is in line with the guidance outlined in the RfC methodology.

5. EPA concluded that a database uncertainty factor of 10 was appropriate for the derivation of the RfC to account for the lack of a two-generation reproductive toxicity study and a developmental toxicity study. Please comment on whether the selection of the database uncertainty factor for the RfC is scientifically justified and has been transparently and objectively described in the document.

Comment: Several reviewers commented that the 10-fold database uncertainty factor was too excessive and stated that the oral 2-generation reproductive toxicity study should provide adequate information addressing 1,2,3-trichloropropane's ability to alter reproduction. A reviewer also commented that the point of departure selected would probably be protective of reproductive and developmental effects. Conversely, a reviewer questioned whether the database uncertainty was large enough given the lack of a chronic inhalation study of 1,2,3-trichlororpropane.

Response: A qualitative, or quantitative, route-to-route comparison of toxicological effects is not appropriate in this case because a portal-of-entry effect is expected following 1,2,3-trichloropropane exposure via inhalation and a first-pass effect is expected following oral exposure. As such, there are toxicokinetic, and as a result possible toxicodynamic, differences between the two routes of exposure. Thus, the utility of the oral reproductive toxicity study to decrease the database uncertainty factor is limited as there is not enough information regarding the toxicokinetics and toxicodynamics following both inhalation and oral exposure to make this comparison.

The point of departure from the chronic study, increased liver weight, may be protective of reproductive and developmental effects, but the lack of an inhalation two-generation reproductive toxicity study and a developmental toxicity study still represents a major data gap and uncertainty.

The lack of a chronic inhalation study of 1,2,3-trichloropropane is addressed by the application of the subchronic-to-chronic uncertainty factor.

D. Carcinogenicity of 1,2,3-trichloropropane

1. Under the EPA's 2005 Guidelines for carcinogen risk assessment (www.epa.gov/iris/backgr-d.htm), 1,2,3-trichloropropane is likely to be carcinogenic to humans. Please comment on the cancer weight of the evidence characterization. Do the available data support the conclusion that 1,2,3-trichloropropane is a likely human carcinogen? Has the scientific justification for the weight of evidence characterization been sufficiently, transparently, and objectively described? Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described?

> Comment: The reviewers agreed with EPA's conclusion that 1,2,3-trichloropropane is likely to be carcinogenic to humans. The reviewers recommended improving the transparency and objectivity of the cancer assessment by reducing the redundancy in Section 4.7. Reviewers also requested the inclusion of structurally-similar chemicals and identified carcinogenesis studies for ethylene dibromide and dibromochloropropane that may provide an important group of comparisons for the cancer assessment for 1,2,3-trichloropropane.
>
> Response: An effort has been made to streamline and reduce the redundancy in Section 4.7. The carcinogenesis bioassays for 1,2-dibromoethane (ethylene dibromide) and 1,2,-dibromo-3-chloropropane (DBCP) were added to Section 4.5.3, *Structural Analog Data*.

2. Evidence indicating the mode of action of carcinogenicity of 1,2,3-trichloropropane was considered. The proposed mode of action includes bioactivation of 1,2,3-trichloropropane leading to the induction of mutations in cancer-related genes. A conclusion was reached that it is possible that this chemical is operating through a mutagenic mode of action, but the database contains limited evidence of in vivo mutagenic events that could lead to the observed cancer. Please comment on whether the weight of the scientific evidence supports this conclusion. Please comment on whether the rationale for this conclusion has been transparently and objectively described. Please comment on data available for 1,2,3-trichloropropane that may support an alternative mode of action.

> Comment: The reviewers generally indicated that the weight of evidence supports mutagenesis as the primary mode of carcinogenic action. Specifically, the reviewer's comments were:
>
> - A reviewer commented that the weight of evidence supports mutagenesis as the primary mode of action for trichloropropane; mutagenesis and cytotoxicity were

due to reactive metabolites, and chronic irritation and cell death likely play a role in carcinogenesis at the initial portals of entry.

- Another reviewer commented that the basic argument was not adequately justified, although there are sufficient data to indicate that 1,2,3-TCP should be considered a genotoxic carcinogen based upon criteria established under the current risk assessment guidelines.
- Another reviewer commented that the conclusion could be strengthened to "very likely" because of the known mutagenic properties of the episulfonium activated metabolite, the dose response data on the DNA adducts in relation to carcinogenesis, and the analogy with other mutagenic carcinogens [ethylene dibromide (1,2-dibromoethane) and dibromochloropropane] that produce similar or the same type of episulfonium activated intermediates via reactions with glutathione.
- Another reviewer stated that the weight of evidence supports the conclusion that it is possible that 1,2,3-trichloropropane is acting through a mutagenic mode of action, but the database contains limited evidence of in vivo mutagenic events that could lead to the observed cancer. In addition, the reviewer stated that data are unavailable to make a determination that other modes of action, such as cytotoxicity followed by regenerative cell proliferation, are plausible.
- A reviewer commented that there is sufficient converging scientific evidence for a mutagenic mode of action.
- A reviewer commented that the data in support of a genotoxic mechanism of action are limited; however, the lines of evidence presented would seem to make a strong case for genotoxicity.
- A reviewer stated that, although the hypothesis of a likely mutagenic mode of action is strongly supported by the available evidence, the hypothesis had yet to be proven. This reviewer stated that there is much stronger evidence that a mutagenic mode of action is "likely" than evidence that would suggest that 1,2,3-trichloropropane is not acting through a mutagenic mode of action.

Response: Taking into consideration the comments from the external peer reviewers and a reevaluation of the available mode of action data, EPA concluded that 1,2,3-trichloropropane acts through a mutagenic mode of action for carcinogenesis. The evidence supporting a mutagenic mode of carcinogenic action includes: mutagenic response, chromosomal damage, DNA breakage, micronucleus formation, and enhanced DNA viral transformation in in vitro studies, covalent binding of 1,2,3-trichloropropane metabolites to hepatic DNA, RNA, and hepatic proteins, the induction of DNA strand

breaks in hepatocytes, induced wing spot formation, dose-dependent formation of DNA adducts, and the dose-dependent increase in 1,3-dichloroacetone, a reported mutagen and tumor initiator. The text in Section 4.7.3, *Mode of Action Analysis*, was revised.

Comment: A reviewer suggested an improvement in the organization of the mode of action analysis section and that alternative modes of action should be considered, especially with respect to forestomach tumors.

Response: The text in Section 4.7, *Evaluation of Carcinogenicity*, has been reorganized. Alternative modes of action have not been addressed in this document because the available data for 1,2,3-trichloropropane does not support a mode of action other than mutagenicity. Text addressing the potential for enhanced carcinogenic response due to the use of corn oil as the vehicle has been added to Section 4.7.3.3, *Other Possible Modes of Action*.

Comment: One reviewer also proposed modifying the dose response analysis to reflect the likely saturation of the activating metabolism pathway via either depletion of glutathione or the glutathione transferase enzymes. This reviewer stated that the DNA adduct observations from La et al. (1995) indicated some degree of saturation.

Response: Modifying the dose response analysis to reflect the saturation of the activating metabolic pathway was not included in the draft document. Saturation of the metabolic pathway of 1,2,3-trichloropropane may occur, but the evidence for saturation is not sufficient to make this type of dose conversion. The DNA adduct formation data is from an acute gavage dose of 1,2,3-trichloropropane with sacrifice of the exposed rodents 6 hours post-exposure. The comparison of the DNA adduct formation data from the acute study to the incidence of tumors from the chronic NTP study can not be scientifically supported and does not contribute to the development of an oral slope factor with improved confidence. This type of inference is not supported by the available data and would incur additional uncertainty than what is already present.

Comment: One reviewer stated that the decision to not apply the age-dependent adjustment factors (ADAFs), as referenced at the end of the cancer uncertainty section, needed additional explanation. In particular, the data supporting the application of the ADAFs based upon a mutagenic mode of action seems justified based on the Agency's cancer risk assessment guidelines. Another reviewer recommended incorporating the ADAFs because of the very strong likelihood of a mutagenic mode of action. One

reviewer recommended not applying the ADAFs because the data are inconclusive regarding the postulated mode of carcinogenic action for 1,2,3-trichloropropane.

Response: Upon reanalysis of the mode of action data, EPA concluded that 1,2,3-trichloropropane is carcinogenic through a mutagenic mode of action and recommends applying the ADAFs. The recommendation for the application of ADAFs follows the *Supplemental Guidance* (U.S. EPA, 2005b). Section 5.4.5, *Application of Age-Dependent Adjustment Factors,* was added to the document.

Comment: One reviewer commented that the absence of mutations in ras genes that are consistent with the one DNA adduct known to be formed from 1,2,3-trichloropropane provides no substantive insight into the question of whether trichloropropane is carcinogenic via a mutagenic mode of action.

Response: The abstract in which the ras mutations were observed was removed from the document due to a lack of adequate study documentation.

3. A two-year oral gavage cancer bioassay (NTP, 1993) was selected as the principal study for the development of an oral slope factor (OSF). Please comment on the appropriateness of the selection of the principal study. Has the rationale for this choice been transparently and objectively described?

Comment: A reviewer again commented that because of the DNA adduct and cell proliferation results of La et al. (1996), an adjustment could be made to the doses from the NTP (1993) study to better estimate drinking water exposure.

Response: The utilization of DNA adduct formation in tumor-forming organ tissues from an acute exposure to 1,2,3-trichloropropane to transform the administered doses in the NTP (1993) study to "low-dose equivalents" imparts another level of uncertainty in the derivation of the oral slope factor. See response to the third comment under question D.2.

Comment: The reviewers generally agreed with the selection of the NTP (1993) study as the principal study for the development of an oral slope factor, although the reviewers highlighted that this was the only study available for this purpose.

Response: No response.

Comment: One reviewer commented that the NTP (1993) cancer bioassay was limited in characterizing the carcinogenic potency in the test species due to a high incidence of mortality in rats and mice. This reviewer also commented that a major limitation of the NTP (1993) bioassay is that the experimental doses exceed the "maximum tolerated dose".

Response: The mortality in both rats and mice was attributed to cancer associated with 1,2,3-trichloropropane exposures and supports the use of the cancer bioassay for quantitative analysis. Text was added to Section 5.4.1, *Choice of Study/Data with Rationale and Justification*, addressing the increased mortality. The use of time-to-tumor modeling makes greater use of the available data than quantal dose-response models.

Comment: Several reviewers commented that the corn oil vehicle may synergize with carcinogens by acting as a co-carcinogen or tumor promoter, thus leading to an overestimation of the cancer risk. One reviewer commented that the bolus nature of the gavage dose used in the NTP (1993) bioassay should be discussed in the document.

Response: The potential effect of the corn oil vehicle, as well as the bolus nature of the gavage dose, on the effects observed in the forestomach following 1,2,3-trichloropropane exposure has been added to Section 5.4.6, *Uncertainties in Cancer Risk Values*.

Comment: Several reviewers commented that the high frequency of tumors observed in the forestomach, and the questionable significance of these tumors, could lead to an overestimation of the cancer risk.

Response: EPA considers the forestomach tumors observed in rodents to be relevant to humans. Text was added to Sections 5.4.2 and 5.4.4 to further support this conclusion. However, in response to the recommendations of some of the external peer review panel members, Section 5.4.4 also includes the derivation of oral slope factors for rats and mice in which forestomach tumors were excluded from the analysis. Additionally, the uncertainties noted by the reviewers are discussed in Section 5.4.6, *Uncertainties in Cancer Risk Values*.

Comment: Reviewers commented that tumors observed in organs with no human homolog were not relevant to human exposure and could lead to an overestimation of the cancer risk.

Response: EPA considers the tumor incidences for the forestomach, Zymbal's gland, Harderian gland, and preputial gland tumors observed in the NTP (1993) study to be relevant to human exposure. This conclusion is based on a lack of data to indicate otherwiseand the *Guidelines for Carcinogen Risk Assessment* (2005) which state that site concordance is not a prerequisite for evaluating the implications of animal study results for humans. Additional text was added to Section 5.4.6, *Uncertainties in Cancer Risk Values*.

4. Data on tumors in multiple organs in F344/N rats were used to estimate the oral cancer slope factor. Please comment on the scientific justification and transparency of this analysis. Please comment on the combination of etiologically similar tumor types, benign and malignant tumors of the same cell type, for quantitative purposes. Please specifically comment on EPA's inclusion of the data on forestomach tumors for cancer quantitation in rats following the administration of 1,2,3-trichloropropane. Please comment on the estimation of a statistically appropriate upper bound on total risk (combined slope factor), which describes the risk of developing any combination of tumor types considered, and the quantitative process used to calculate the combined slope factor.

Comment: The reviewers generally agreed with the decision to combine benign and malignant tumors of the same cell type of the same organ for quantitative purposes. A reviewer stated that the inclusion of premalignant lesions may lead to an overestimation of risk because the frequency with which premalignant lesions progress to carcinomas can be quite low.

Response: Text was added to the *Bioassay selection* subsection of Section 5.4.6, *Uncertainties in Cancer Risk Values*, that addresses the assumption that benign tumors observed following 1,2,3-trichloropropane exposures progress to malignancy.

Comment: Four reviewers disagreed with the inclusion of forestomach tumors in the cancer quantification, as humans do not have a forestomach or an organ that is homologous to the rodent forestomach and that the quantification should be conducted without forestomach tumors. One reviewer also stated that the absence of any lesions in the forestomach of vehicle controls could alleviate concerns that the use of the corn oil vehicle may have influenced the tumor data.

Two reviewers commented that a different mode of action may be operative for the forestomach because of the absence of DNA adducts in the forestomach and the

mutations found in the forestomach were not consistent with the miscoding properties of the major adduct. Another reviewer stated that in the absence of strong evidence that the effects in the forestomach following gavage exposure overpredicts activities local to the site of compound administration, the forestomach tumors should not be excluded from the assessment.

Response: EPA considers forestomach tumors to be relevant to humans and included the data for these tumors in the quantitative carcinogenic dose-response analysis for the derivation of the oral slope factor. Detailed discussions of human relevance were added to Sections 5.4.2 and 5.4.4 to further support this conclusion. However, as recommended by some of the external peer review panelists, Section 5.4.4 was revised to include the derivation of oral slope factors for rats and mice in which forestomach tumors were excluded from the analyses.

EPA has included text in Section 5.4.6, *Uncertainties in Cancer Risk Values,* that addresses that the bolus administration of 1,2,3-trichloropropane in corn oil. In addition, data supporting an alternative mode of action for the forestomach tumors observed in rodents are lacking, and the effect that the corn oil vehicle and bolus dosing may have on the carcinogenicity of 1,2,3-trichloropropane has been addressed in 5.4.6, *Uncertainties in Cancer Risk Values*. As a reviewer stated, the absence of forestomach lesions in the vehicle controls may alleviate concerns about the use of the corn oil vehicle. EPA agrees and has noted in Section 5.4.6 that forestomach lesions were not observed in vehicle controls for male and female rats and female mice, and were observed only in male mice (3/50), further supporting the inclusion of the forestomach tumors in the cancer assessment.

DNA adducts were identified in the forestomachs of both rats and mice 6 hours following a single oral dose and demonstrated a dose-dependent increase, however, the level of DNA adduct observed was not statistically significantly increased from the low to high dose (La et al., 1995). The study investigating the mutations in the forestomach was removed from the document because only the study abstract was available and critical study information could not be ascertained. In addition, as stated by two reviewers, the absence of mutations in the ras gene that are consistent with an identified DNA adduct does not provide substantive insight into the question of whether or not the compound is a mutagenic carcinogen.

Comment: One reviewer commented that the oral bolus dosing with a corn oil gavage is not relevant to actual human exposures. Another reviewer stated that confounding by

gavage, judging by studies of similar compounds, is unlikely to explain the large cancer effect observed.

Response: Additional text regarding the potential effect of the corn oil vehicle on the forestomach following 1,2,3-trichloropropane exposure has been included in Section 5.4.6, *Uncertainties in Cancer Risk Values*.

Comment: Six reviewers disagreed with the exclusion of the mouse data from the cancer quantification. A reviewer commented that excluding the evidence of carcinogenicity in mice from the oral slope factor quantification was not appropriate, and recommended analysis of the mouse data to provide perspective on the uncertainty regarding the differences in sensitivity of the rats and mice. Additionally, a reviewer commented that a lack of adequate dose-response information was available from the mouse tumor incidence data.

Response: EPA originally excluded the mouse data from the cancer quantitation because the tumor response in the lowest dose was close to a maximum response; however, based on the external peer review comments, EPA has modeled the mouse data using the same methods that were used for the rat data. The analysis of the mouse tumor data (added to Section 5.4) is now used in the derivation of the OSF because the mouse is the most sensitive species.

Text was added to Section 5.4.6, *Uncertainties in Cancer Risk Values*, addressing the near maximal tumor response observed in mice at the lowest dose.

Comment: A reviewer suggested including an analysis using Michaelis-Menten modeling to transform the administered doses to multiples of "low-dose equivalents" when projecting low dose risks. By calculating "low dose equivalents", the effect of high dose metabolic saturation would be removed from the dose response model.

Response: Modifying the dose response analysis to reflect the saturation of the activating metabolism pathway was not included in the draft document. In this case, the DNA adduct formation in tumor-forming organ tissues from an acute exposure to 1,2,3-trichloropropane can not be utilized to transform the administered doses in the NTP (1993) study to "low-dose equivalents." See response to the third comment under question D.2.

Comment: Two reviewers disagreed with the classification of all tumors as 'incidental' and stated that the specific tumor types in the dead and sacrificed moribund animals ought to be modeled as fatal tumors.

Response: EPA has included additional analyses classifying the alimentary system sqamous cell carcinomas (in all four sex-species groups) and mammary adenocarcinomas in female rats as fatal in the unscheduled deaths.

Comment: One reviewer stated that the development of an inhalation unit risk should be considered. One approach would be to consider structurally similar compounds that have similar tumor findings and studies by inhalation routes.

Response: The need for an inhalation unit risk is recognized, but carcinogenicity data following inhalation exposure to 1,2,3-trichloropropane are not available. A route-to route extrapolation of the oral slope factor to an inhalation unit risk would be one method to derive an inhalation unit risk, but this method is not appropriate in this case because a portal-of-entry effect is expected following 1,2,3-trichloropropane exposure via inhalation and a first-pass effect is expected following oral exposure. As such, there are toxicokinetic, and as a result possible toxicodynamic, differences between the two routes of exposure.

In addition, analyses relying on structure-activity relationships with similar chemicals are not typically incorporated in IRIS assessments; thus, this approach was not considered for this assessment.

PUBLIC COMMENTS

A. Oral Reference Dose (RfD) for 1,2,3-Trichloropropane

Comment: One commenter stated that the application of the database uncertainty factor to account for limitations in reproductive and developmental studies did not seem warranted, and recommended a 3-fold lower UF be applied in the derivation of the RfD and RfC.

Response: The application of the database uncertainty factor in the Toxicological Review of 1,2,3-trichloropropane is in concordance with the guidance outlined in the RfC Methodology (US EPA, 1994b) and the Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002). Please see response to comment under Charge question B.5 above.

C. Carcinogenicity of 1,2,3-trichloropropane

Comment: One commenterstated that 1,2,3-trichloropropane is carcinogenic due to the induction of tumors in multiple sites.

Response: Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,2,3-trichloropropane is "likely to be carcinogenic to humans", based on a statistically significant and dose-related increase in the formation of multiple tumors in both sexes of two species from an NTP (1993) chronic oral bioassay.

Comment: One commenter questioned the applicability of the gavage data in assessing human exposures. The commenter highlighted the data investigating gavage versus drinking water exposures and the possibility for overestimating the cancer risk in humans by using the gavage data.

Response: As discussed above under D.3., the potential affect of the bolus nature of the gavage dose on the tumorigenesis observed following 1,2,3-trichloropropane exposure has been added to the *Bioassay selection* subsection of Section 5.4.6, *Uncertainties in Cancer Risk Values*.

Comment: The commenter also stated that some of the tumors attributed to 1,2,3-trichloropropane exposure are not relevant to humans. The commenter highlighted that the

Zymbal's gland, Harderian gland, preputial gland, and forestomach lack human tissue homologues, and are, therefore, not useful for quantitative dose-response assessment.

Response: As discussed above, EPA considers the tumor incidences for the forestomach, Zymbal's gland, Harderian gland, and preputial gland tumors observed in the NTP (1993) study are relevant to human exposure.. Please see response to comments under question D.3 and D.4.

Comment: The commenter also stated that the corn oil gavage exposures to 1,2,3-trichloropropane utilized in the NTP (1993) bioassay are likely to overstate the cancer potency.

Response: Please see reponses to comments under questions D.3 and D.4.

Comment: The commenter also stated that the consideration of the mode of action of carcinogenesis of 1,2,3-trichloropropane justifies decreases in the cancer potency. The commenter also inferred that if EPA's underlying conclusion that 1,2,3-trichloropropane is operating through a mutagenic mode of carcinogenic action is incorrect, other nonlinear, low-dose models are also plausible and should be acknowledged in the document.

Response: EPA disagrees that adequate data are available suggesting or indicating additional modes of action. The mode of action data available supports a mutagenic mode of action. Thus, the presentation of a nonlinear mode of action is not supported by the available data.

Comment: The commenter recommended the inclusion of 1,2,3-trichloropropane metabolism and kinetics information into the estimate of cancer potency. Specifically, that 1,3-dichloroacetone is generated at a rate ten times faster by rat microsomes than by human microsomes.

Response: The metabolic and kinetic information available for 1,2,3-trichloropropane is suitable for qualitative analysis, but it is not sufficient for use in the quantitative derivation of the oral slope factor for cancer. The in vitro investigation provides useful qualitative information, thus the results of the Weber and Sipes (1992) study that demonstrated that 1,3-dichloroacetone was generated at a rate ten-times faster by rat microsomes than by human microsomes was added to Sections 3.3, *Metabolism*, and 4.7.3.2, *Experimental Support for the Hypothesized Mode of Action*.

Comment: The commenter also stated that the allometric scaling factor used to extrapolate doses from the rats in the NTP bioassay to humans is inconsistent with the presumed mode of action, and recommends applying an allometric scaling factor of one.

Response: The $(\text{body weight})^{3/4}$ adjustment for cross-species scaling (from rodent to human) was applied in concordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), which follows EPA's cross-species scaling methodology which states that the time-weighted daily average doses are converted to human equivalent doses on the basis of $(\text{body weight})^{3/4}$ (U.S. EPA, 1992).

Comment: The commenter also stated that the dose-response model used in the derivation of the oral slope factor is inappropriate for analyzing the 1,2,3-trichloropropane tumor data. Accounting for competing causes of death with the time-to-tumor model is not needed when assessing forestomach tumors because nearly all of the tested animals developed forestomach tumors and these tumors were the primary cause of death.

Response: EPA agrees that competing causes of death are less of an issue at the dose levels tested in the study. However, the analysis was undertaken in order to consider what could be inferred about cancer risks at lower exposures, where forestomach tumors were less often the primary cause of death, especially in rats.

Comment: The commenter also stated that the poor quality of the NTP (1993) bioassay should be articulated. Specifically, the high incidence of early mortality due to point-of-contact tumors in both rats and mice should be noted.

Response: Text was added to Section 5.4.1, *Choice of Study/Data with Rationale and Justification*, highlighting the increased mortality in rats and mice at the intermediate and high doses associated with the development of chemical-related neoplasms in the forestomach.

APPENDIX B: BENCHMARK DOSE MODELING RESULTS FOR THE DERIVATION OF THE RFD

Benchmark dose (BMD) modeling was performed to identify the POD for the derivation of the chronic RfD for 1,2,3-trichloropropane. The modeling was conducted in accordance with the draft EPA guidelines (U.S. EPA, 2000b) using BMDS version 1.4.1. The BMD modeling results for the derivation of the chronic RfD are summarized in Table B-1. In addition, the model output results for all of the models per endpoint are presented in the corresponding tables. The model outputs for the selected models for each endpoint are also presented. A brief discussion of the modeling results is presented below.

The following critical effects were modeled using the BMDS version 1.4.1: increased absolute and relative liver weight, increased absolute and relative kidney weight, decreased fertility in the 4th litter, decreased fertility in the 5th litter, pups/litter in the 4th litter, and pups/litter in the 5th litter. The endpoint being modeled specified which set of models, continuous (liner, polynomial, power, and Hill) or dichotomous (gamma, logistic, multi-stage, probit, and Weibull), would be utilized. Model eligibility was determined by assessing the goodness-of-fit using a value of $\alpha = 0.1$ (when appropriate), visual fit, and ranking by AIC.

For absolute liver weight, the male rat data using the Hill model (BMR of 10% change in mean organ weight) was selected. The male rat data using the Hill model (BMR of 10% change in mean organ weight) was selected as the best fit for the relative liver weight changes. Absolute and relative liver weight changes were also modeled using a BMR of 1 SD, as recommended by the Benchmark Dose Technical Guidance Document (U.S. EPA, 2000b). For absolute kidney weight, the female rat data using the Hill model (BMR of 10% change in mean organ weight) was the best fit. The male rat data using the Hill model (BMR of 10% change in mean organ weight) was selected as the best fit for the change in relative kidney weight. The best model fit for decreased fertility in the 4th litter was the log-Probit model (slope ≥ 1) with a BMF of 10% extra risk. The best model fit for decreased fertility in the 5th litter was the Probit model (BMR of 10% extra risk). The best model fit for the number of pups/litter in the 4th litter, as well as for the number of live pups/litter in the 5th litter, was the polynomial model (BMR of 1% change in mean live pups/litter). The BMD results for the best fit models are summarized in Table B-1.

The critical endpoint selected for the derivation of the chronic RfD was increased liver weight with increased absolute liver weight in male rats as the best representation of this critical effect. The Hill model provided the best fit for this data set. The increase in absolute liver weight was selected as the best representation of the critical effect, as opposed to relative liver weight, which provided a BMDL very similar to the change in absolute liver weight, because it is a more direct measure of liver weight change.

Table B-1. BMD modeling used in the derivation of the RfD; final model selected for each endpoint

Endpoint	Species/sex	Model	Goodness-of-fit *p*-value	BMD	BMDL	BMR
Absolute liver weight	Rat/male	Hill	0.677	3.8	1.6	10% extra risk
				3.2	1.4	1 SD
Relative liver weight	Rat/male	Hill	0.986	5.5	3.1	10% extra risk
				3.2	1.8	1 SD
Absolute kidney weight	Rat/female	Hill	0.359	9.0	3.4	10% extra risk
Relative kidney weight	Rat/male	Hill	0.549	10.5	6.4	10% extra risk
Decreased fertility in the 4th litter	Mice	Log-Probit (slope ≥ 1)	0.9458	52.6	37.3	10% extra risk
Decreased fertility in the 5th litter	Mice	Probit	0.9953	31.2	23.3	10% extra risk
Pups/litter-4th litter	Mice	Polynomial	0.8157	13.8	3.2	1% change in mean live pups/litter
Pups/litter-5th litter	Mice	Polynomial	0.337	13.6	5.6	1% change in mean live pups/litter

Absolute liver weight change

Data set	Model	Goodness-of-fit *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Female rat	Polynomial (linear); power	0.005	31.6	10.6	7.8
	Hill	0.037	27.2	1.7	0.6
Male rat	Polynomial (linear); power	0.046	67.9	12.6	9.6
	Hill	**0.677**	**63.9**	**3.8**	**1.6**
Female mouse	Linear	0.001	-88.6	46.4	22.0
	Polynomial (degrees = 2)	0.004	-91.3	40.7	28.7
	Power	0.001	-89.3	39.9	25.4
Male mouse	Polynomial (linear); power	-0.069	-29.8	42.0	16.1
			-31.2		
			-29.8		
	Hill	0.245	-31.8	Failed	Failed

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                                                Mon Apr 16 12:20:13 2007
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 BMDS MODEL RUN
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   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

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


                  Default Initial Parameter Values
                          alpha =      1.78118
                            rho =            0   Specified
                      intercept =        14.27
                              v =         3.96
                              n =     0.217686
                              k =      13.2906


           Asymptotic Correlation Matrix of Parameter Estimates
```

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

	alpha	intercept	v	k
alpha	1	3.6e-007	9.4e-007	9.9e-007
intercept	3.6e-007	1	-0.0082	0.53
v	9.4e-007	-0.0082	1	0.78
k	9.9e-007	0.53	0.78	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	1.60099	0.367293	0.881113	2.32088
intercept	14.3111	0.393288	13.5403	15.082
v	5.12912	1.17736	2.82153	7.43672
n	1	NA		
k	9.74696	6.65395	-3.29454	22.7885

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	14.3	14.3	1.17	1.27	-0.103
3	10	15.6	15.5	1.17	1.27	0.279
10	10	16.8	16.9	1.52	1.27	-0.271
30	8	18.2	18.2	1.47	1.27	0.106

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-27.854952	5	65.709904
A2	-27.294744	8	70.589488
A3	-27.854952	5	65.709904
fitted	-27.941868	4	63.883737
R	-43.424328	2	90.848657

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)

```
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

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

   Test 1              32.2592          6          <.0001
   Test 2              1.12042          3          0.7721
   Test 3              1.12042          3          0.7721
   Test 4             0.173833          1          0.6767

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

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


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

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


        Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =     Relative risk

Confidence level =           0.95

             BMD =        3.77203

            BMDL =       1.60397
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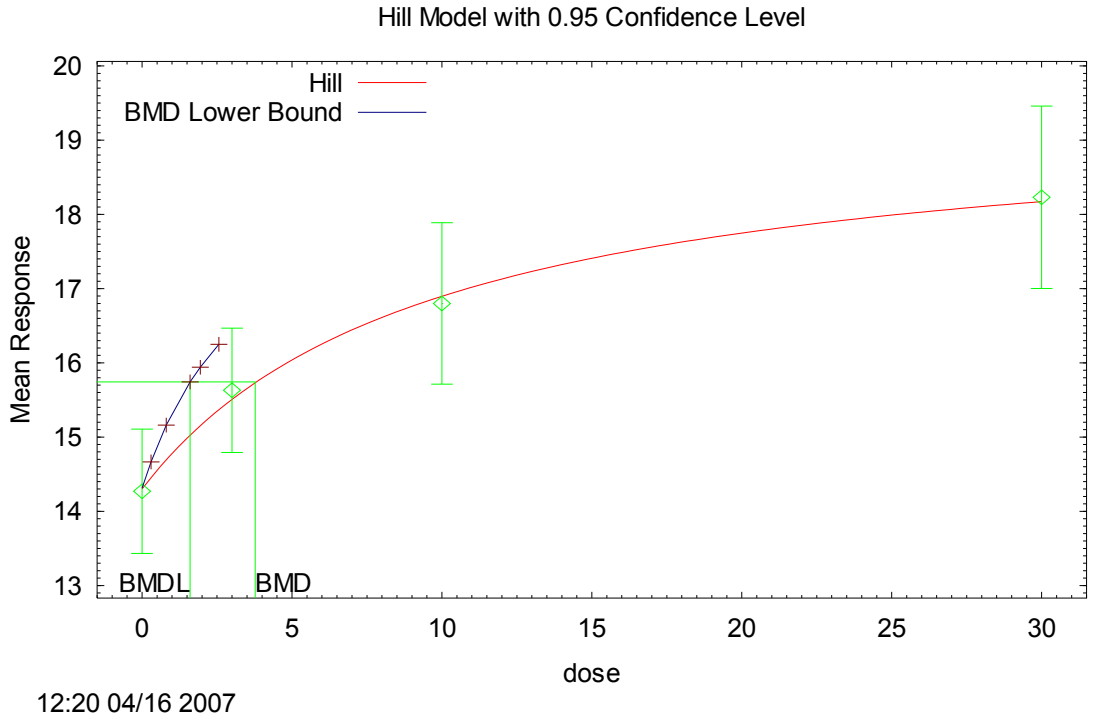


Benchmark Dose Computation

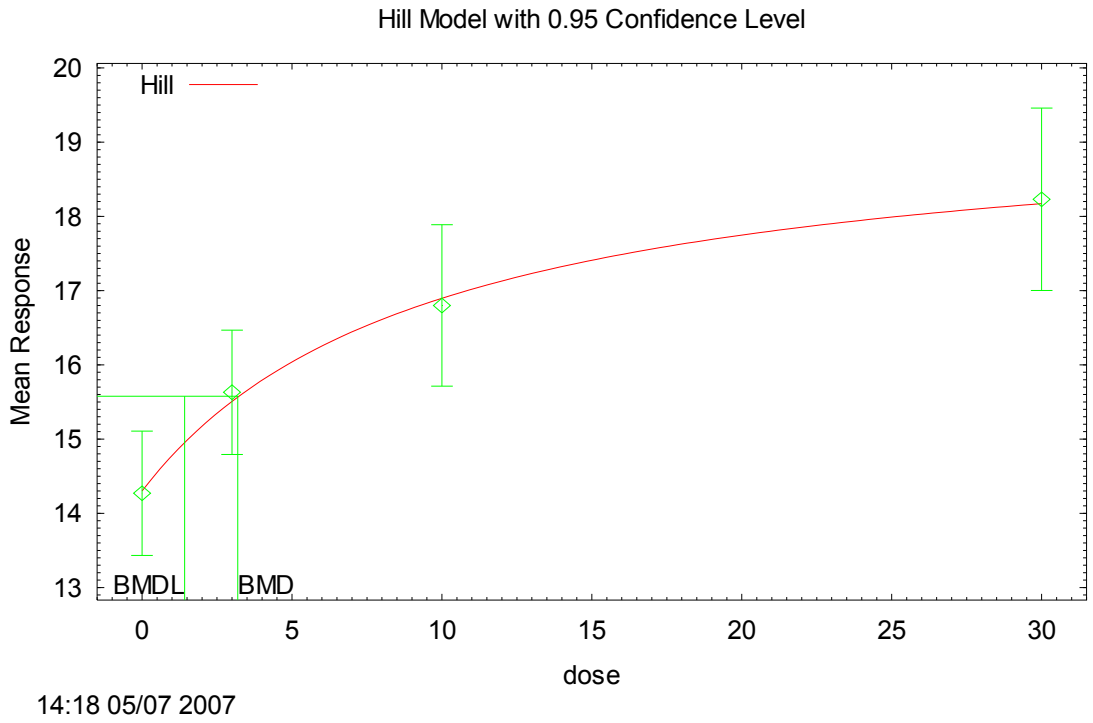
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3.19188

BMDL = 1.42159



Relative liver weight change

Data set	Model	Goodness-of-fit *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Female rat	Linear	0.479	97.7	7.0	6.1
	Polynomial	0.255	99.5	7.9	6.2
	Power	0.297	101.3	8.4	6.2
Male rat	Linear	0.062	100.3	11.8	9.7
	Polynomial	0.018	100.3	11.8	9.7
	Power	0.018	104.3	11.8	9.7
	Hill	**0.986**	**98.8**	**5.5**	**3.1**
Female mouse	Linear	0.033	121.9	15.9	12.4
	Polynomial	0.629	115.3	29.7	20.7
	Power	<0.00001	116.9	34.9	21.9
Male mouse	Linear	0.026	184.0	12.8	7.0
	Polynomial	0.007	184.0	13.4	7.0
	Power	<0.00001	185.7	14.0	7.1

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                                          Mon Apr 16 15:05:35 2007
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 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = intercept + v*doseˆn/(kˆn + doseˆn)


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

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


                  Default Initial Parameter Values
                          alpha =      4.47912
                            rho =            0   Specified
                      intercept =         31.2
                              v =          8.6
                              n =     0.478123
                              k =      11.2069


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	k
alpha	1	-1.8e-008	-3.6e-008	-2.7e-008
intercept	-1.8e-008	1	0.25	0.55
v	-3.6e-008	0.25	1	0.91
k	-2.7e-008	0.55	0.91	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	4.00767	0.919422	2.20563	5.8097
intercept	31.2041	0.591154	30.0455	32.3627
v	14.2018	3.58574	7.17388	21.2297
n	1	NA		
k	19.5753	11.3509	-2.67211	41.8227

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	31.2	31.2	1.9	2	-0.00647
3	10	33.1	33.1	2.2	2	0.0137
10	10	36	36	1.9	2	-0.00949
30	8	39.8	39.8	2.5	2	0.00258

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-45.375808	5	100.751617
A2	-44.937444	8	105.874888
A3	-45.375808	5	100.751617
fitted	-45.375971	4	98.751942
R	-68.896353	2	141.792706

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)

Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	47.9178	6	<.0001
Test 2	0.876729	3	0.831
Test 3	0.876729	3	0.831
Test 4	0.00032515	1	0.9856

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

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

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

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

Benchmark Dose Computation

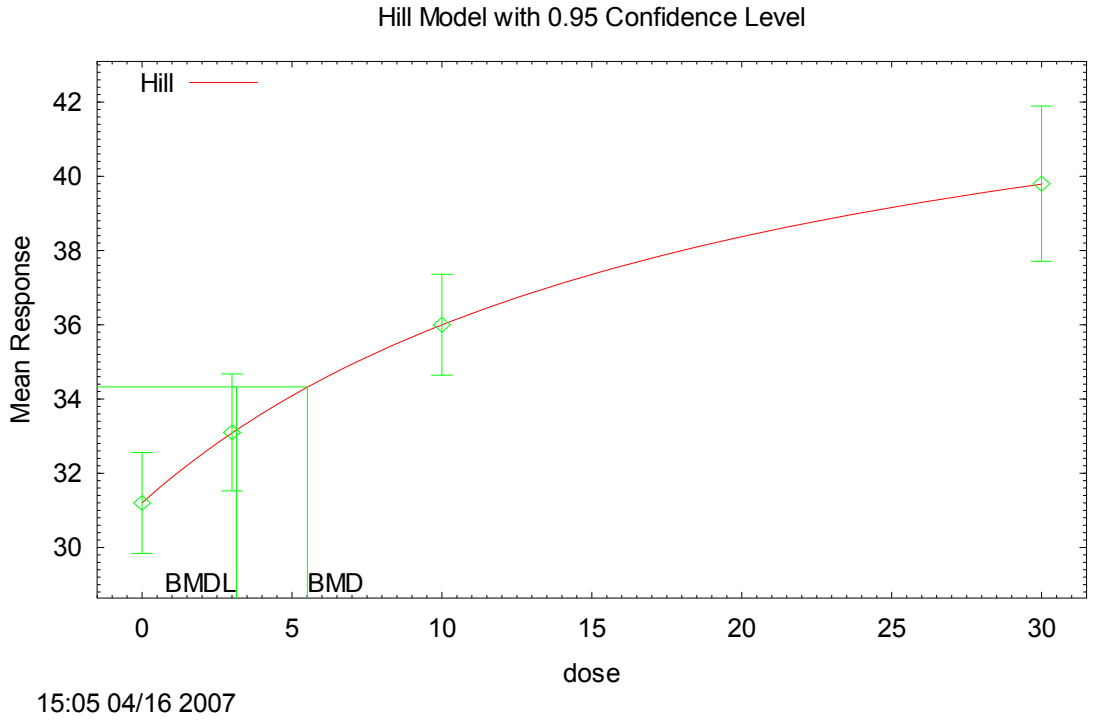
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 5.51221

BMDL = 3.14799



Benchmark Dose Computation

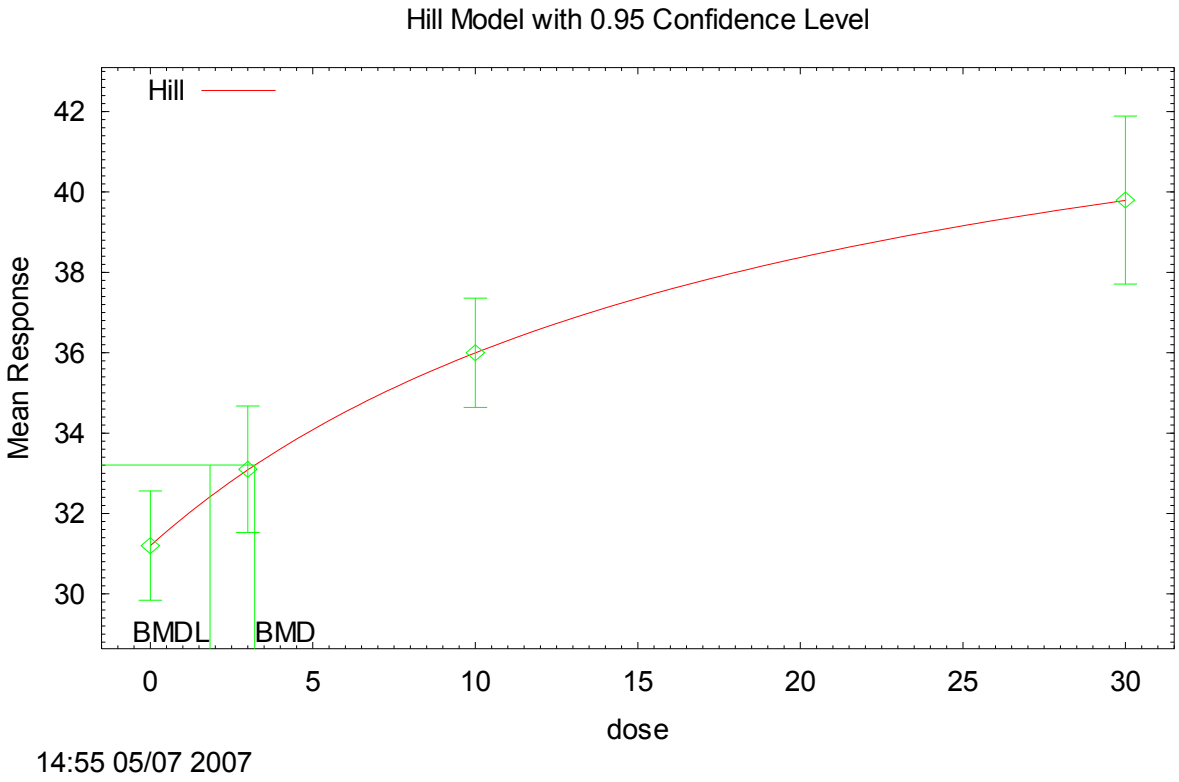
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3.21217

BMDL = 1.83718



Absolute kidney weight

Data set	Model	Goodness-of-fit *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Female rat	Polynomial (linear); power	0.444	-153.0	14.2	10.8
	Hill	**0.359**	**-151.8**	**9.0**	**3.4**
Male rat	Polynomial (linear); power	0.320	-123.6	11.2	9.0
	Hill	0.174	-122.0	8.6	4.1
Female mouse	Data not modeled due to lack of dose-response trend				
Male mouse	Polynomial (linear); power	0.342	-162.9	55.0	28.2

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                                          Thu Apr 19 13:10:47 2007
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 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = intercept + v*doseˆn/(kˆn + doseˆn)


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

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


                  Default Initial Parameter Values
                          alpha =   0.00477394
                            rho =            0   Specified
                      intercept =        0.786
                              v =        0.185
                              n =     0.229976
                              k =      48.1373


           Asymptotic Correlation Matrix of Parameter Estimates

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

                  alpha    intercept            v            k
```

alpha	1	-1e-008	-5.8e-008	-5.5e-008
intercept	-1e-008	1	0.47	0.61
v	-5.8e-008	0.47	1	0.97
k	-5.5e-008	0.61	0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.00434387	0.00102386	0.00233714	0.0063506
intercept	0.793675	0.0198246	0.754819	0.83253
v	0.366433	0.275832	-0.174189	0.907054
n	1	NA		
k	32.6862	46.6525	-58.7509	124.123

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	0.786	0.794	0.047	0.0659	-0.368
3	10	0.839	0.824	0.073	0.0659	0.697
10	8	0.869	0.88	0.054	0.0659	-0.451
30	8	0.971	0.969	0.096	0.0659	0.0841

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	80.322604	5	-150.645208
A2	82.968318	8	-149.936636
A3	80.322604	5	-150.645208
fitted	79.901816	4	-151.803632
R	67.518029	2	-131.036058

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	30.9006	6	<.0001
Test 2	5.29143	3	0.1517
Test 3	5.29143	3	0.1517
Test 4	0.841576	1	0.3589

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

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

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

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

Benchmark Dose Computation

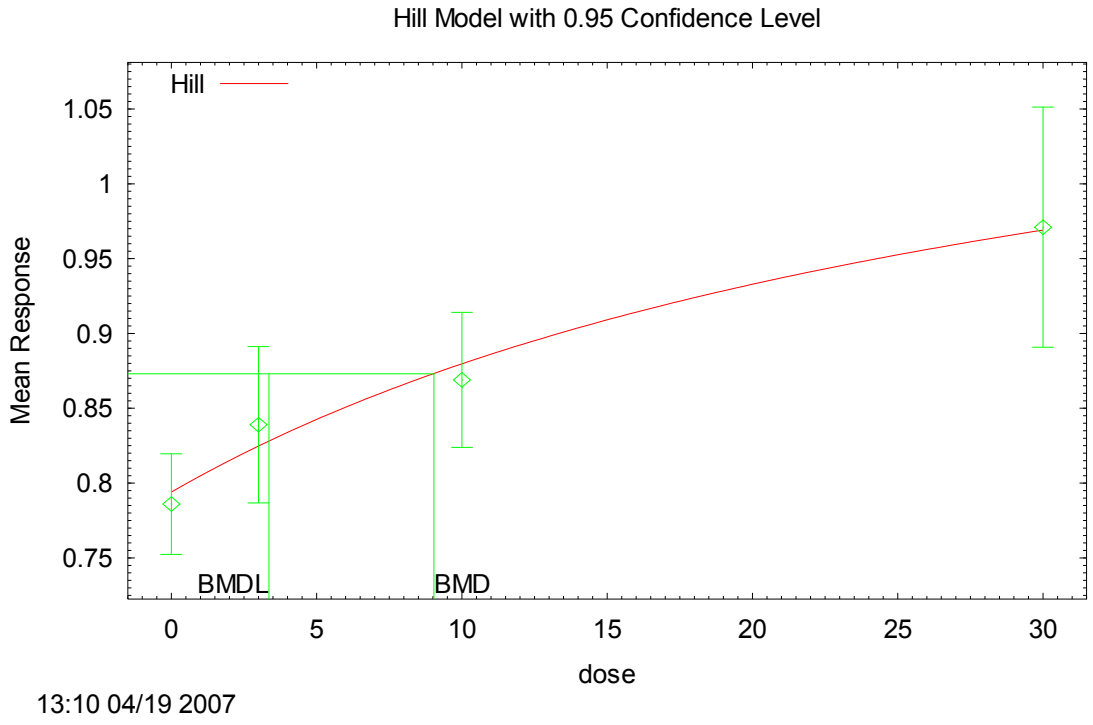
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 9.03706

BMDL = 3.3571



Relative kidney weight

Data set	Model	Goodness-of-fit *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Female rat	Linear	0.052	-60.3	8.4	7.0
	Polynomial	0.020	-58.8	10.7	7.1
	Power	0.027	-57.3	11.1	7.2
Male rat	Linear	0.985	-85.8	10.6	9.2
	Polynomial	0.871	-83.8	10.8	9.2
	Power	0.865	-83.8	10.7	9.2
	Hill	**0.549**	**-84.1**	**10.5**	**6.4**
Female mouse	Linear	0.169	-30.3	32.5	23.8
	Polynomial	0.096	-29.1	41.4	24.9
	Power	0.085	-26.9	43.9	24.6
Male mouse	Linear	0.575	31.1	92.4	34.5
	Polynomial	0.403	30.7	70.3	45.3
	Power	0.430	32.6	61.3	36.8

```
================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\M_R_REL_KIDNEYWT.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\M_R_REL_KIDNEYWT.plt
                                          Thu Apr 19 13:56:54 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

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


                  Default Initial Parameter Values
                          alpha =    0.0360382
                            rho =            0   Specified
                      intercept =         2.96
                              v =         0.86
                              n =     0.542711
                              k =      45.0877


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	intercept	v	k
alpha	1	0.0005	0.00077	0.00077
intercept	0.0005	1	0.65	0.65
v	0.00077	0.65	1	1
k	0.00077	0.65	1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.032551	0.00746772	0.0179146	0.0471875
intercept	2.97723	0.0512988	2.87668	3.07777
v	49.0294	1180.31	-2264.34	2362.4
n	1	NA		
k	1717.72	42099	-80794.8	84230.2

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	2.96	2.98	0.13	0.18	-0.302
3	10	3.09	3.06	0.28	0.18	0.478
10	10	3.25	3.26	0.16	0.18	-0.193
30	8	3.82	3.82	0.14	0.18	0.0184

Model Descriptions for likelihoods calculated

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

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

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

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

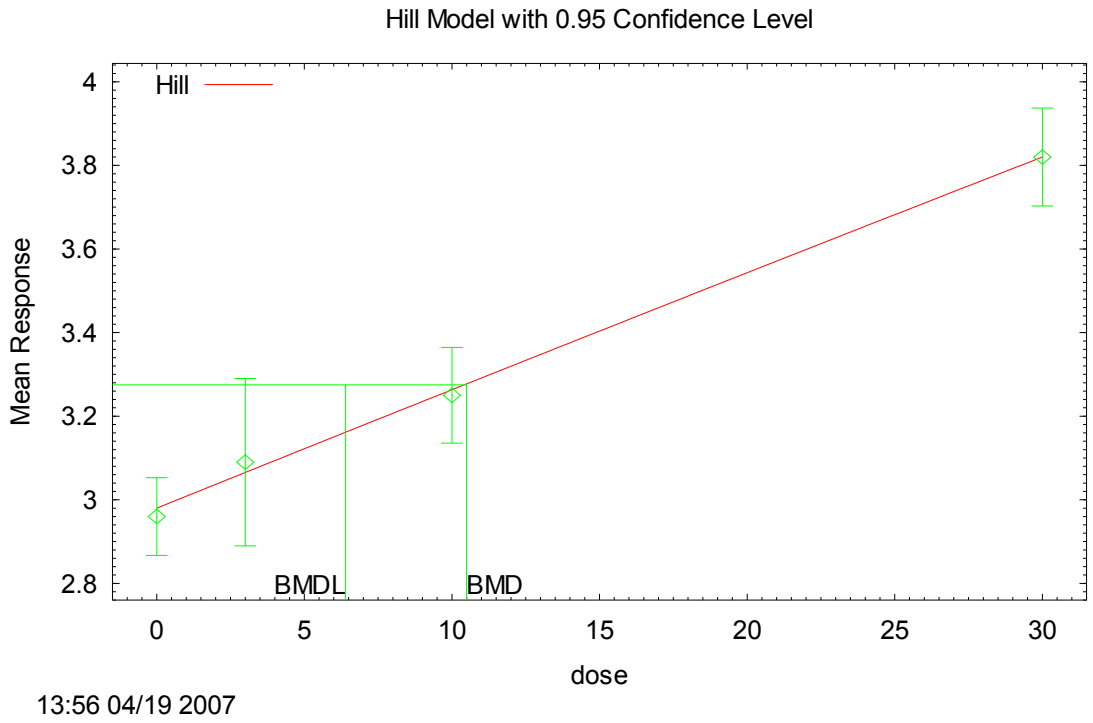
Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	46.253609	5	-82.507217
A2	50.301116	8	-84.602232
A3	46.253609	5	-82.507217
fitted	46.073978	4	-84.147957
R	19.835849	2	-35.671698

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)

```
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

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

   Test 1              60.9305          6          <.0001
   Test 2              8.09501          3          0.04409
   Test 3              8.09501          3          0.04409
   Test 4              0.35926          1           0.5489

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

The p-value for Test 2 is less than .1.  Consider running a
non-homogeneous variance model

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

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


        Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =     Relative risk

Confidence level =           0.95

             BMD =        10.4943

            BMDL =       6.39915
```



Decreased fertility in the 4th litter

Model	Goodness-of-fit *p*-value	AIC	BMD_{10}	$BMDL_{10}$
log-probit (slope > 1)	**0.9458**	**46.54**	**52.6**	**37.3**
Multistage (degree = 1)	0.9058	48.23	54.6	26.1
Weibull (power > 1)	0.9509	48.31	51.7	25.9
Gamma (power > 1)	0.9426	48.33	51.2	25.8
Log-logistic (slope > 1)	0.9372	48.34	51.2	23.6
Probit	0.6667	49.37	68.8	51.7
Logistic	0.6003	49.72	73.5	55.9

```
=============================================================
         Probit Model. (Version: 2.8;  Date: 02/20/2007)
         Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS MOVED\BMD
2\FERTILITY_FOURTH_LITTER.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\BMD 2\FERTILITY_FOURTH_LITTER.plt
                                              Mon Apr 23 10:51:16 2007
 =============================================================

 BMDS MODEL RUN
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   The form of the probability function is:

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

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = Infertile
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

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



   User has chosen the log transformed model


                  Default Initial (and Specified) Parameter Values
                     background =            0
                      intercept =     -5.20395
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept

 intercept            1


                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-5.24473	0.214728	-5.66559	-4.82387
slope	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-22.1049	4			
Fitted model	-22.2676	1	0.325422	3	0.9552
Reduced model	-29.6693	1	15.1288	3	0.00171

AIC: 46.5353

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	38	0.000
30.0000	0.0326	0.587	1	18	0.548
60.0000	0.1250	2.375	2	19	-0.260
120.0000	0.3237	6.151	6	19	-0.074

Chi^2 = 0.37 d.f. = 3 P-value = 0.9458

Benchmark Dose Computation

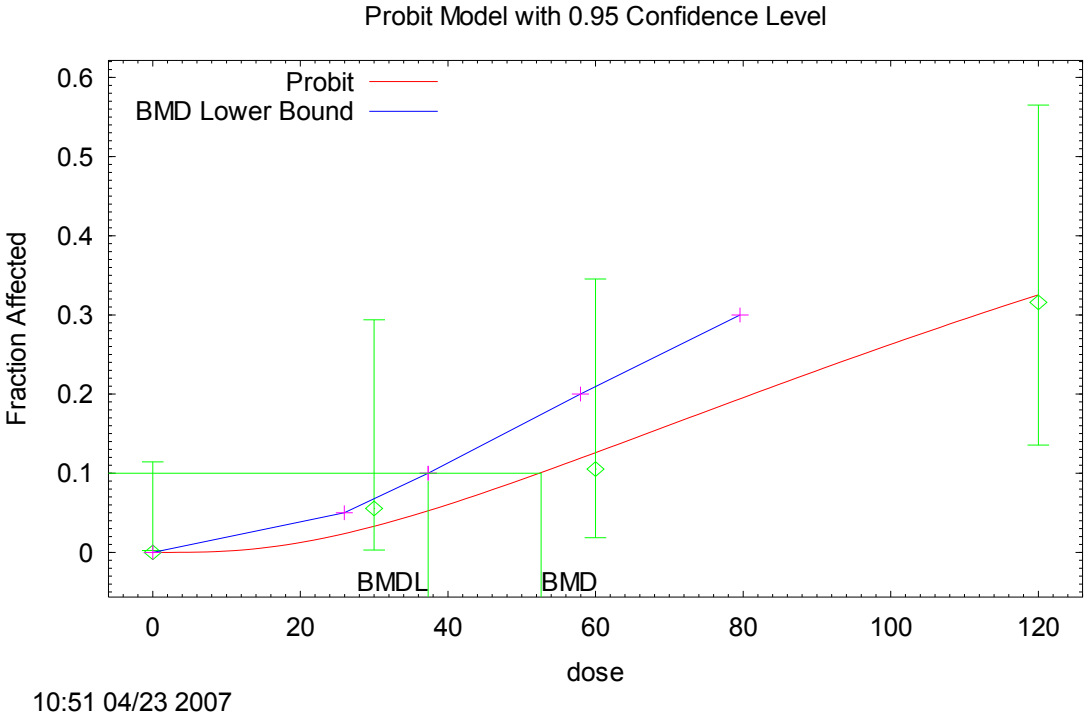
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 52.6244

BMDL = 37.3271


Probit Model with 0.95 Confidence Level
Probit
BMD Lower Bound
Fraction Affected
0.6
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
20
40
60
80
100
120
dose
10:51 04/23 2007

Decreased fertility in the 5th litter

Model	Goodness-of-fit *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Multistage (degree = 1)	0.8078	102.66	20.1	12.6
Weibull (power > 1)	0.8516	104.26	32.8	13
Gamma (power > 1)	0.8244	104.27	33.1	13
Log-logistic (slope > 1)	0.7913	104.30	34	10.3
Log-probit (slope > 1)	0.7351	104.34	34.4	22.3
Probit	**0.9953**	**102.23**	**31.2**	**23.3**
Logistic	0.9925	102.24	33	24.6

```
================================================================
         Probit Model. (Version: 2.8;  Date: 02/20/2007)
         Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\MICE_INFERTILITY.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\MICE_INFERTILITY.plt
                                              Mon Apr 23 10:26:34 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = infertile
   Independent variable = Dose
   Slope parameter is not restricted

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


                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =     -1.10027
                          slope =    0.0107802


           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept        slope

 intercept            1        -0.74

     slope        -0.74            1


                                 Parameter Estimates

                                                95.0% Wald Confidence Interval
```

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
intercept	-1.11544	0.214289	-1.53544	-0.695445
slope	0.0109181	0.00314451	0.00475495	0.0170812

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-49.1124	4			
Fitted model	-49.1172	2	0.00946155	2	0.9953
Reduced model	-55.4327	1	12.6405	3	0.005482

AIC: 102.234

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1323	5.029	5	38	-0.014
30.0000	0.2154	3.877	4	18	0.071
60.0000	0.3226	6.130	6	19	-0.064
120.0000	0.5772	10.967	11	19	0.015

Chi^2 = 0.01 d.f. = 2 P-value = 0.9953

Benchmark Dose Computation

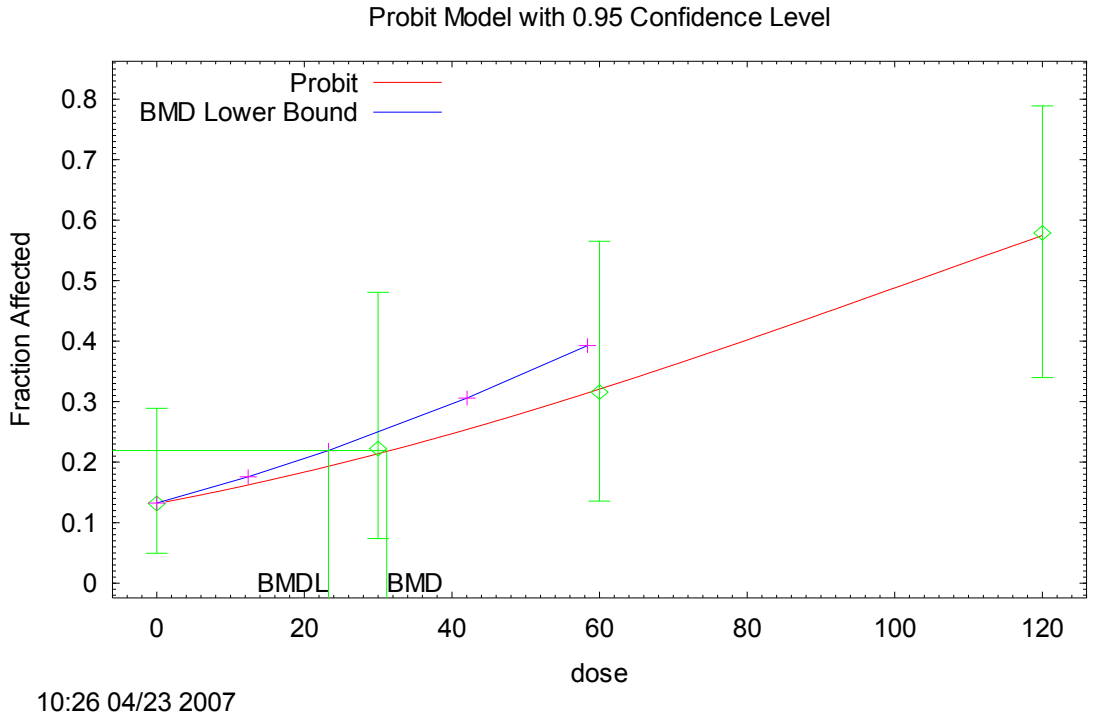
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 31.1591

BMDL = 23.2749



Live pups per litter in the 4th litter

Model	Goodness-of-fit *p*-value	AIC	$BMD_{1\%}$	$BMDL_{1\%}$
Linear	0.006	305.4	1.7	1.5
Polynomial	**0.816**	**295.6**	**13.8**	**3.2**
Power	0.666	297.4	18.7	6.2

```
=================================================================
         Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
         Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS MOVED\BMD
2\LIVE_PUPS_4TH_LITTER.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\BMD 2\LIVE_PUPS_4TH_LITTER.plt
                                              Wed May 02 08:54:44 2007
 =================================================================

 BMDS MODEL RUN
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   The form of the response function is:

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


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

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


                  Default Initial Parameter Values
                         lalpha =       2.4801
                            rho =            0
                         beta_0 =      11.7373
                         beta_1 =            0
                         beta_2 = -0.000679293


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -beta_1
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                 lalpha          rho       beta_0       beta_2

    lalpha            1        -0.98      -0.0063       0.0025

       rho        -0.98            1        0.007      -0.0044

    beta_0      -0.0063        0.007            1        -0.66

    beta_2       0.0025      -0.0044        -0.66            1


                                  Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        lalpha         0.737776         0.731853          -0.696628             2.17218
           rho         0.745657         0.324171           0.110295             1.38102
```

beta_0	11.8652	0.454377	10.9746	12.7557
beta_1	0	NA		
beta_2	-0.00062153	5.41197e-005	-0.000727603	-0.000515458

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	38	11.8	11.9	3.7	3.64	-0.11
30	17	11.2	11.3	2.88	3.57	-0.122
60	17	9.9	9.63	4.12	3.36	0.334
120	13	2.9	2.92	2.17	2.15	-0.0253

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-145.855593	5	301.711185
A2	-142.282446	8	300.564892
A3	-143.607572	6	299.215144
fitted	-143.811261	4	295.622522
R	-171.536421	2	347.072841

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	58.5079	6	<.0001
Test 2	7.14629	3	0.06738
Test 3	2.65025	2	0.2658
Test 4	0.407378	2	0.8157

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

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

The p-value for Test 3 is greater than .1. The modeled variance appears

to be appropriate here

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

Benchmark Dose Computation

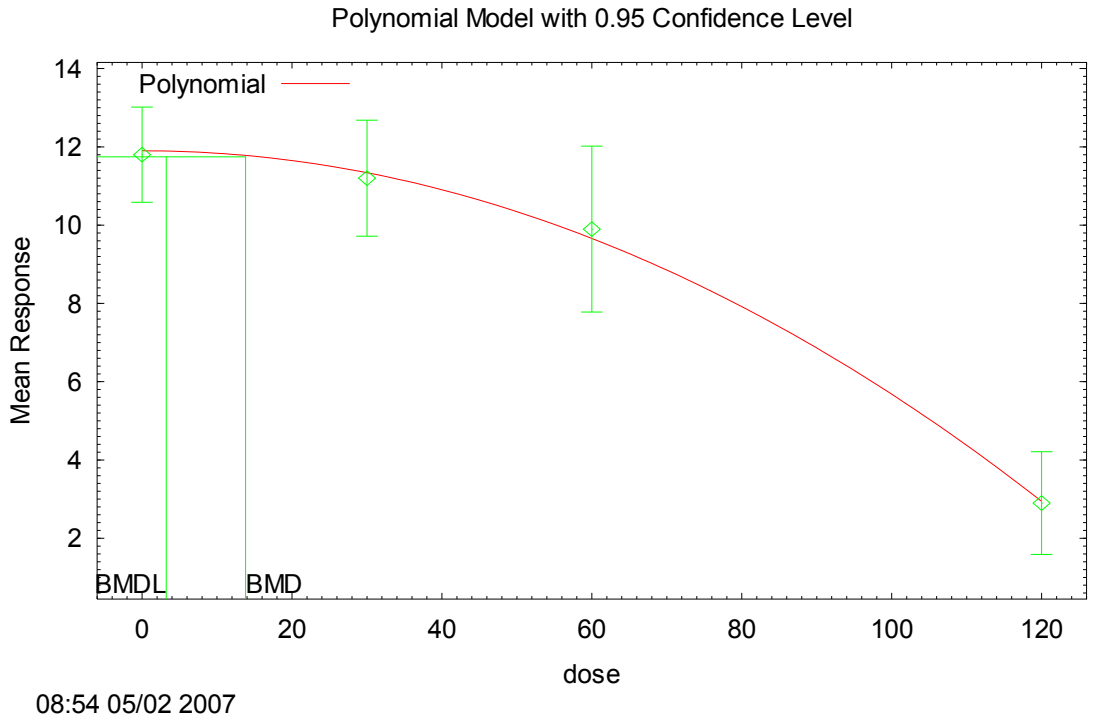
Specified effect = 0.01

Risk Type = Relative risk

Confidence level = 0.95

BMD = 13.8167

BMDL = 3.22598



Live pups per litter in the 5th litter

Model	Goodness-of-fit *p*-value	AIC	$BMD_{1\%}$	$BMDL_{1\%}$
Linear	<0.0001	210.4	1.8	1.6
Polynomial	**0.337**	**193.0**	**13.6**	**5.5**
Power	0.507	193.3	24.5	11.6

```
================================================================
         Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
         Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS MOVED\BMD
2\LIVE_PUPS_5TH_LITTER.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\BMD 2\LIVE_PUPS_5TH_LITTER.plt
                                                Wed May 02 09:00:47 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

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


                  Default Initial Parameter Values
                          alpha =      5.92144
                            rho =            0   Specified
                         beta_0 =      12.6118
                         beta_1 =            0
                         beta_2 = -0.000926768


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -beta_1
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                   alpha       beta_0       beta_2

     alpha             1     1.5e-009     4.5e-009

    beta_0     1.5e-009            1        -0.49

    beta_2     4.5e-009        -0.49            1


                                  Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable        Estimate       Std. Err.     Lower Conf. Limit   Upper Conf. Limit
```

alpha	5.75447	0.986884	3.82022	7.68873
beta_0	12.9649	0.33453	12.3092	13.6205
beta_1	0	NA		
beta_2	-0.000703957	6.43331e-005	-0.000830048	-0.000577866

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	33	12.8	13	2.3	2.4	-0.395
30	14	12.1	12.3	2.62	2.4	-0.361
60	13	11.3	10.4	2.89	2.4	1.31
120	8	2.5	2.83	1.7	2.4	-0.387

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-92.410493	5	194.820986
A2	-90.930911	8	197.861821
A3	-92.410493	5	194.820986
fitted	-93.499230	3	192.998461
R	-128.027125	2	260.054251

Explanation of Tests

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

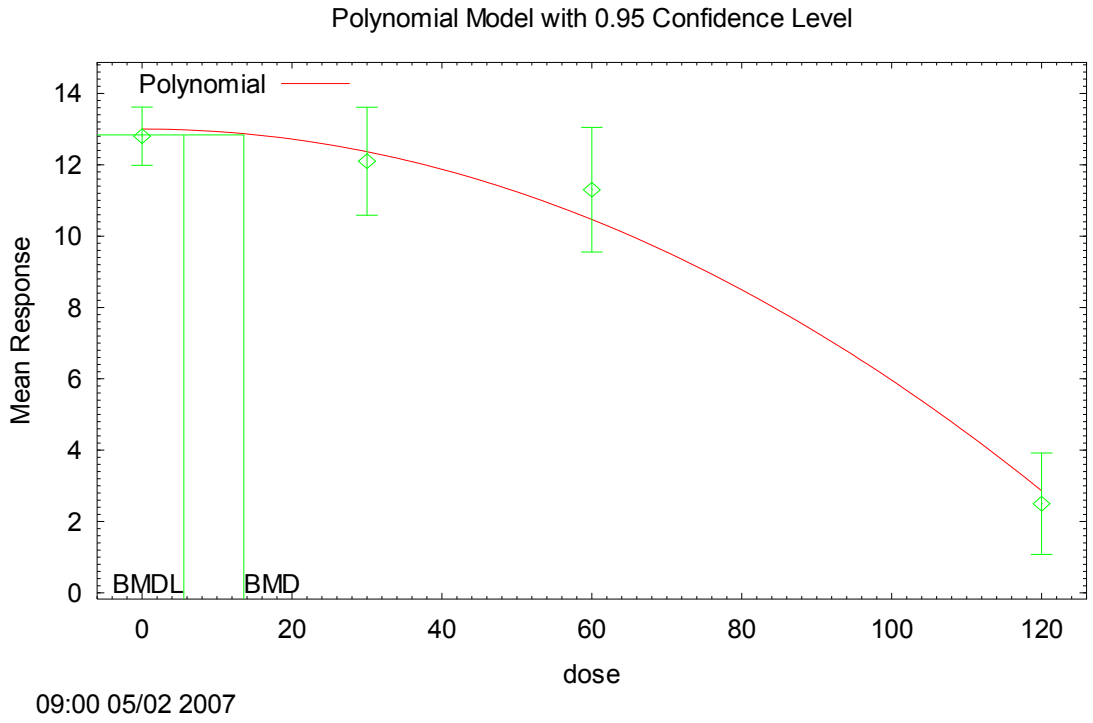
Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	74.1924	6	<.0001
Test 2	2.95916	3	0.398
Test 3	2.95916	3	0.398
Test 4	2.17747	2	0.3366

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

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

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

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


             Benchmark Dose Computation

Specified effect =          0.01

Risk Type        =     Relative risk

Confidence level =          0.95

             BMD =         13.571


            BMDL =         5.5772
```



APPENDIX C: BENCHMARK DOSE MODELING RESULTS FOR THE DERIVATION OF THE RFC

Benchmark dose (BMD) modeling was conducted to identify the POD for the derivation of the chronic RfC for 1,2,3-trichloropropane. The modeling was conducted in accordance with the draft EPA guidelines (U.S. EPA, 2000b) using BMDS version 1.4.1. The BMD modeling results for the derivation of the chronic RfC are summarized in Table C-1. In addition, the model output results for all of the models per endpoint are presented in the corresponding tables, and the model outputs for the selected models for each endpoint are also presented. A brief discussion of the modeling results is presented below.

The following critical effects were modeled using the BMDS version 1.4.1: increased incidence of peribronchial lymphoid hyperplasia in male and female CD rats and decreased mating performance in female CD rats. The endpoints were modeled using the dichotomous models (gamma, logistic, multi-stage, probit, and Weibull). Model eligibility was determined by assessing the goodness-of-fit using a value of $\alpha = 0.1$ (when appropriate), visual fit, and ranking by AIC. The decrease in mating performance in female CD rats was presented as the incidence of females that mated compared to total females, but when modeled the incidence data were modified to females that did not mate compared to total females. This was done because the dichotomous models can have difficulty modeling incidence data that decreases with increasing exposure.

Adequate model fits were not available for the increased incidence of peribronchial lymphoid hyperplasia in female rats. An adequate model fit was available for a single constrained model, log-logistic (slope ≥ 1), following BMD modeling of increased incidence of peribronchial lymphoid hyperplasia in male rats.

The BMD modeling of the decrease in mating performance in female CD rats resulted in adequate model fits from all of the dichotomous models. The log-probit model was selected to represent the decreased mating performance because it provided an adequate model fit and the lowest AIC.

Table C-1. BMD modeling used in the derivation of the RfC; final model selected for each endpoint

Endpoint	Species/sex	Model	Goodness-of-fit *p*-value	AIC	BMC	BMCL	BMR
Peribronchial lymphoid hyperplasia	Rat/male	Log-logistic (slope ≥ 1)	0.1081	69.7	1.6	0.84	10% extra risk
Decreased mating performance	Rat/female	Log-probit	0.3933	86.0	4.5	3.0	10% extra risk

Peribronchial lymphoid hyperplasia

Male CD rats

Model	Goodness-of-fit *p*-value	AIC	BMD	$BMDL_{10}$
Log-logistic (slope ≥ 1)	**0.1081**	**69.7**	**1.6**	**0.84**
Multistage (degree = 1), Weibull (power ≥ 1), gamma (power ≥ 1)	0.0065	73.7	2.5	1.8
log-probit (slope ≥ 1)	0.0012	72.3	3.3	2.4

Female CD rats

Model	Goodness-of-fit *p*-value	AIC	BMD	$BMDL_{10}$
Log-logistic (slope ≥ 1)	0.0697	77	5.7	2.9
Multistage (degree = 1)	0.0414	78.1	8.2	4.7
Weibull (power ≥ 1)	0.0414	78.1	8.2	4.7
Gamma (power ≥ 1)	0.0414	78.1	8.2	4.7
Log-probit (slope ≥ 1)	0.0133	81.4	16.8	8.9

```
================================================================
        Logistic Model. (Version: 2.10; Date: 09/23/2007)
        Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\PERI_LYMPHOID_HYPERPLASIA_MALES050608.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\PERI_LYMPHOID_HYPERPLASIA_MALES050608.plt
                                          Tue May 06 14:56:25 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = perilymphyper
   Independent variable = exposure
   Slope parameter is restricted as slope >= 1

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



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =            0
                     intercept =     -2.86665
                         slope =      1.10359


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified by
the user,
```

and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.87
slope	-0.87	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-2.69161	*	*	*
slope	1.08968	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.3416	6			
Fitted model	-32.8595	2	9.03583	4	0.06021
Reduced model	-54.9778	1	53.2723	5	<.0001

AIC: 69.7191

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	15	0.000
0.5000	0.0309	0.463	0	15	-0.691
1.5000	0.0954	1.431	0	15	-1.258
5.0000	0.2813	4.220	6	15	1.022
15.0000	0.5645	8.467	11	15	1.319
50.0000	0.8280	12.419	10	15	-1.655

Chi^2 = 7.58 d.f. = 4 P-value = 0.1081

Benchmark Dose Computation

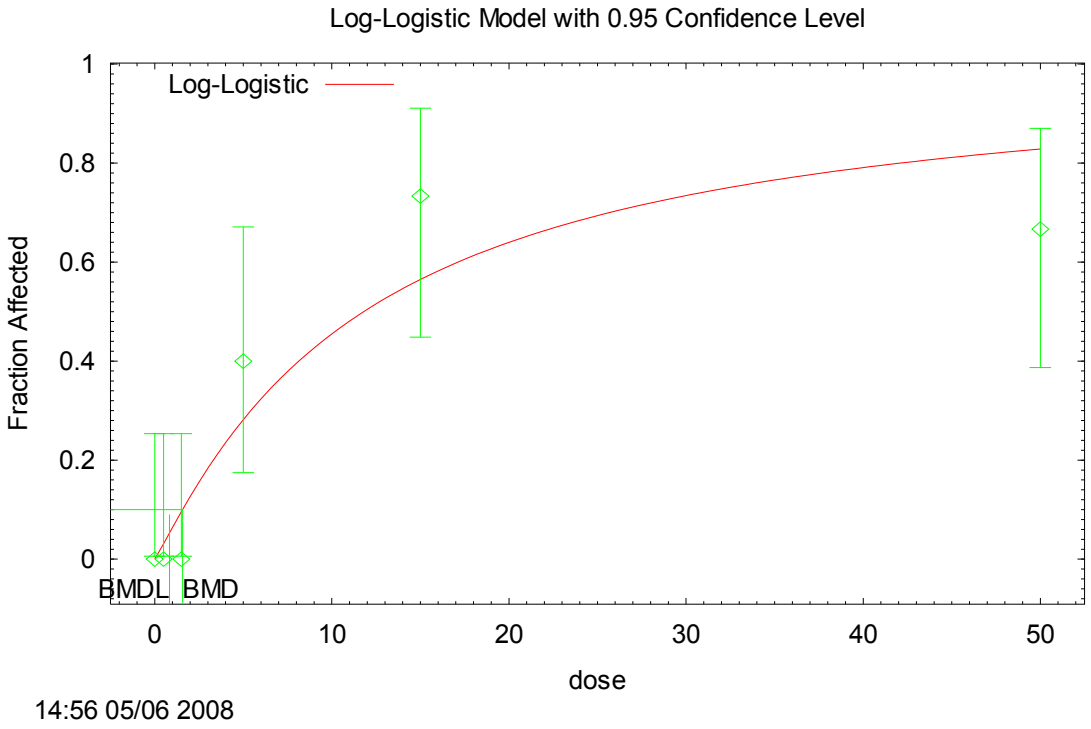
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.57411

BMDL = 0.836756


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
10
20
30
40
50
dose
14:56 05/06 2008

Decreased Mating Performance

Female CD rats

Model	Goodness-of-fit *p*-value	AIC	BMD	$BMDL_{10}$
Log-probit (slope ≥ 1)	**0.3933**	**86.0**	**4.5**	**3.0**
Log-logistic (slope ≥ 1)	0.3529	86.3	4.6	2.1
Gamma (power ≥ 1)	0.3462	86.4	4.8	2.1
Weibull (power ≥ 1)	0.3325	86.5	4.8	2.1
Multistage (degree = 2)	0.2955	86.7	5.3	2.1

```
=================================================================
        Probit Model. (Version: 2.9;  Date: 09/23/2007)
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                          Tue May 27 12:32:29 2008
 =================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   where CumNorm(.) is the cumulative normal distribution function


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

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



   User has chosen the log transformed model


                  Default Initial (and Specified) Parameter Values
                     background =        0.075
                      intercept =     -2.72992
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1        -0.18         0.11

 intercept        -0.18            1        -0.96

     slope         0.11        -0.96            1


                              Parameter Estimates

                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background        0.0521339        0.0252193         0.00270489           0.101563
     intercept         -2.84071         0.843497           -4.49394           -1.18749
         slope           1.0388         0.354414           0.344159            1.73344
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-38.4967	5			
Fitted model	-39.9962	3	2.99908	2	0.2232
Reduced model	-50.7251	1	24.4568	4	<.0001

AIC: 85.9924

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0521	2.085	3	40	0.651
0.5000	0.0523	1.046	1	20	-0.046
1.5000	0.0595	1.190	0	20	-1.125
5.0000	0.1670	3.341	4	20	0.395
15.0000	0.5156	10.313	10	20	-0.140

Chi^2 = 1.87 d.f. = 2 P-value = 0.3933

Benchmark Dose Computation

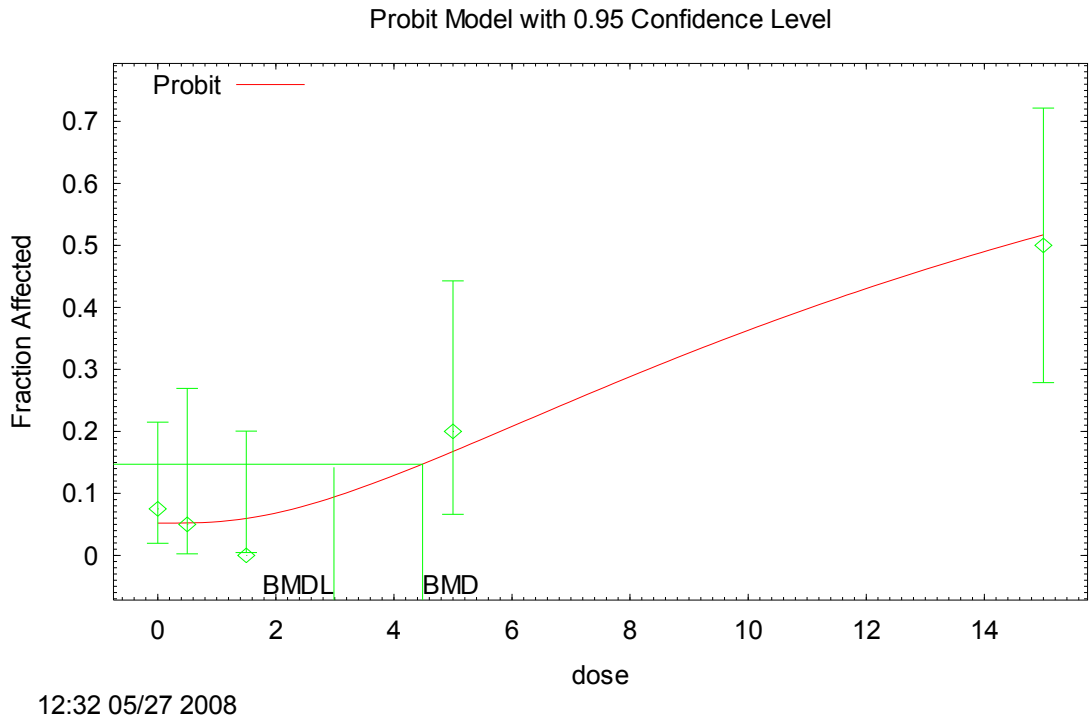
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.48585

BMDL = 2.98577



APPENDIX D: DERIVATION OF THE ORAL SLOPE FACTOR USING THE MULTISTAGE-WEIBULL MODEL

Table D-1. Tumor incidence data, with time to death with tumor; male rats exposed by gavage to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of death	Total examined	Number of animals with							
			Squamous cell neoplasia[a]		Pancreas tumors	Kidney adenomas	Preputial gland tumors	Zymbal's gland tumors	Liver tumors	Skin, squamous cell neoplasia
			Incidental	Fatal						
0	49	1	0	0	0	0	0	0	0	0
	64	10	0	0	0	0	0	0	0	0
	69	1	0	0	0	0	0	0	0	0
	72	1	0	0	0	0	1	0	0	0
	84	2	0	0	0	0	0	0	0	0
	86	1	0	0	0	0	0	0	0	0
	87	1	0	0	0	0	0	0	0	0
	88	3	0	0	0	0	0	0	0	0
	90	1	0	0	0	0	0	0	0	0
	93	1	0	0	0	0	0	0	0	0
	95	2	0	0	0	0	0	0	0	0
	97	1	0	0	0	0	0	0	0	0
	99	1	0	0	0	0	0	0	0	0
	104	11	0	1	3	0	2	0	0	0
	105	23	0	0	2	0	2	0	1	0
3	64	10	2	0	0	0	0	0	0	0
	82	1	0	0	0	0	0	0	0	0
	84	1	0	0	0	0	0	0	0	0
	86	3	0	1	0	0	0	0	0	0
	89	1	0	1	0	0	0	0	0	0
	93	1	1	0	0	0	1	0	0	0
	94	1	1	0	0	0	0	0	0	0
	95	1	0	0	0	0	0	0	0	0
	97	1	0	0	0	0	0	0	0	0
	98	3	1	2	2	0	1	0	0	1
	99	3	1	2	1	0	1	0	0	0
	100	1	1	0	0	0	1	0	0	0
	101	1	1	0	0	0	0	0	0	0
	104	32	25	0	17	2	2	0	1	1
10	4	1	0	0	0	0	0	0	0	0
	32	1	0	0	0	0	0	0	0	0
	58	2	1	1	0	0	1	0	0	0
	64	11	4	0	1	0	1	0	0	0
	67	1	0	1	1	0	0	0	0	0
	73	1	1	0	0	0	1	0	0	0
	74	1	0	0	0	0	0	0	0	0
	75	1	0	0	1	0	0	0	0	0
	77	3	2	1	1	0	0	0	0	0
	84	2	0	2	1	0	0	0	0	1
	87	1	0	1	1	0	0	0	0	0
	88	1	0	1	1	0	1	0	0	0
	91	1	1	0	1	0	0	0	0	0
	92	1	1	0	0	0	0	0	0	0
	93	2	0	2	1	0	1	0	0	0
	94	2	1	1	2	1	0	0	0	0
	95	2	0	2	1	1	0	0	0	0
	96	1	0	1	1	1	0	0	0	0
	97	1	1	0	1	0	0	0	0	0

Table D-1. Tumor incidence data, with time to death with tumor; male rats exposed by gavage to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of death	Total examined	Number of animals with							
			Squamous cell neoplasia[a]		Pancreas tumors	Kidney adenomas	Preputial gland tumors	Zymbal's gland tumors	Liver tumors	Skin, squamous cell neoplasia
			Incidental	Fatal						
	98	4	1	3	4	4	0	0	0	0
	100	2	1	1	1	2	0	0	0	0
	101	1	0	1	1	0	1	0	1	0
	103	1	0	1	1	0	0	0	0	0
	104	15	15	0	15	9	3	0	2	0
30	47	1	0	1	0	0	0	0	0	0
	48	1	1	0	0	0	0	0	0	0
	52	1	0	0	0	0	0	0	0	0
	53	3	0	3	0	0	0	0	0	0
	55	2	1	1	0	0	1	0	0	0
	56	1	0	0	0	0	0	1	0	0
	57	1	0	1	0	0	0	0	0	0
	60	1	0	1	1	1	0	0	0	0
	61	2	2	0	0	0	0	1	0	0
	62	2	0	2	1	0	1	1	0	0
	63	1	1	0	0	0	1	0	0	0
	64	9	0	1	2	5	1	0	0	0
	65	1	8	1	0	1	0	0	1	0
	66	1	0	1	0	0	1	0	0	3
	67	2	1	1	1	2	1	0	0	0
	68	3	1	2	3	1	2	0	0	0
	69	5	2	3	4	3	1	0	0	1
	70	5	2	3	3	4	2	0	0	0
	71	2	1	1	2	1	0	0	0	0
	72	1	0	1	1	0	1	0	0	0
	73	3	0	3	3	1	1	0	0	0
	74	2	0	2	1	1	1	0	1	0
	75	1	0	1	1	0	0	0	0	0
	76	9	9	0	8	6	3	0	1	2

[a]"Incidental" denotes incidence of papillomas, or of carcinomas observed at scheduled death. "Fatal" denotes incidence of carcinomas at unscheduled deaths. Some papillomas were present with carcinomas in both categories.

Source: NTP (1993).

Male Rat; Squamous Papillomas, Carcinomas

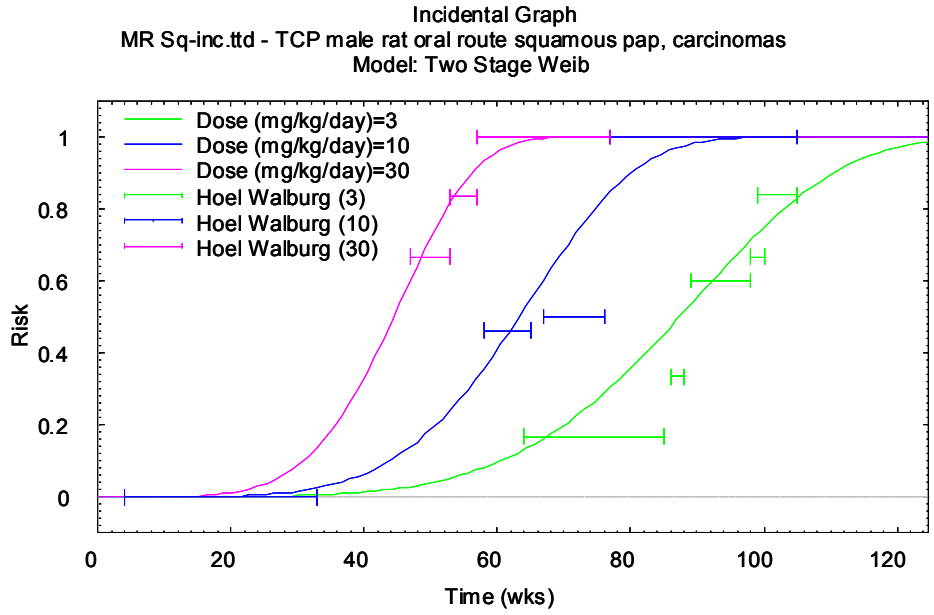
A. All tumors treated as incidental to death of animal

```
Dataset: G:\_ToxRiskData\Trichloropropane\MR Sq-inc kh.ttd
Model: Two Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
         Maximum Log-Likelihood =  -5.624514e+001

       Parameter Estimates :  Q 0 = 1.087183E-012
                              Q 1 = 1.914937E-011
                              Q 2 = 2.116410E-012
                              Z   = 5.126149E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	1
3	60	0	39
10	59	0	48
30	60	0	58

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

		Human Equivalent Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	3.1642E-004	4.8863E-004	Not Reqstd
1.0000E-005	70.00	3.1642E-003	4.8864E-003	Not Reqstd
0.0001	70.00	3.1643E-002	4.8864E-002	Not Reqstd
0.0010	70.00	3.1656E-001	4.8874E-001	Not Reqstd
0.01	70.00	3.1784E+000	4.8973E+000	Not Reqstd
0.10	70.00	3.3146E+001	5.0061E+001	Not Reqstd



Male Rat; Squamous Cell Papillomas, Carcinomas

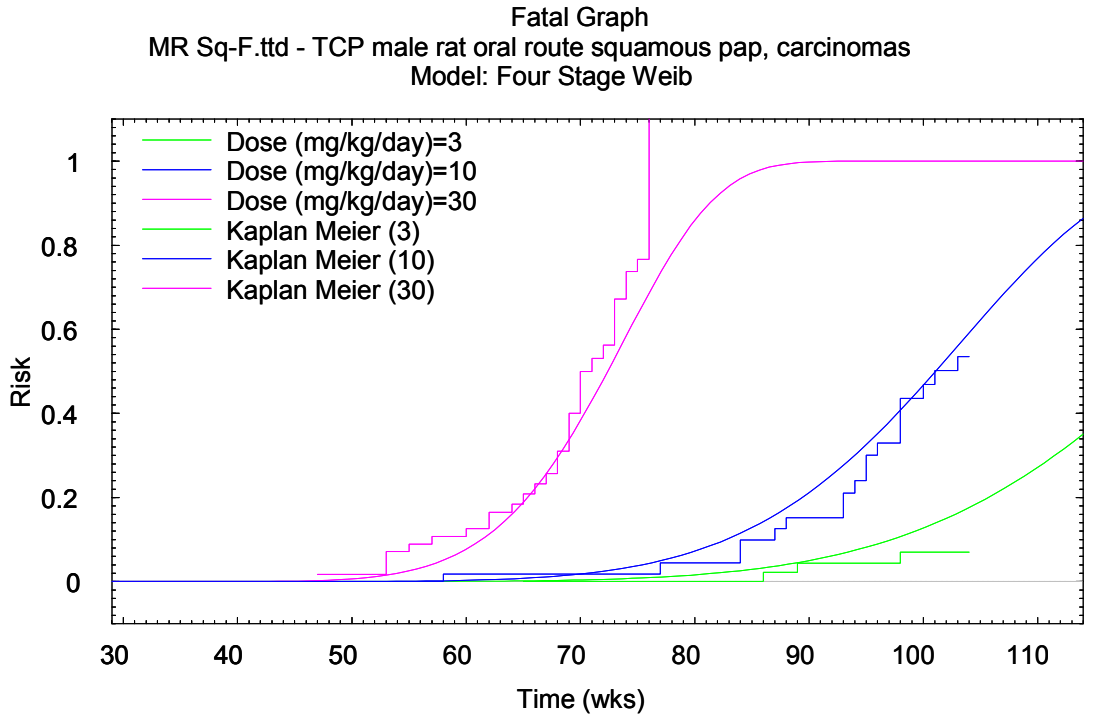
B. Carcinomas occurring at unscheduled deaths treated as cause of death:

```
Dataset: M:\_ToxRiskData\Trichloropropane\MR Sq-F.ttd
Model: Four Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2 ... - Q4 * D^4 )
                          * (T - T0)^Z]
         Maximum Log-Likelihood =   -3.000199e+002

       Parameter Estimates :
                              Q 0 = 2.864368E-015
                              Q 1 = 5.913534E-014
                              Q 2 = 0.000000E+000
                              Q 3 = 0.000000E+000
                              Q 4 = 2.482747E-017
                              Z   = 6.413765E+000
                              T0  = 2.901108E+001
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	1	0
3	60	3	36
10	59	18	30
30	60	32	26

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

		Human Equivalent Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time (yr)	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	3.0422E-004	3.9173E-004	Not Reqstd
1.0000E-005	70.00	3.0422E-003	3.9173E-003	Not Reqstd
0.0001	70.00	3.0423E-002	3.9175E-002	Not Reqstd
0.0010	70.00	3.0437E-001	3.9193E-001	Not Reqstd
0.01	70.00	3.0575E+000	3.9370E+000	Not Reqstd
0.10	70.00	3.2053E+001	4.1273E+001	Not Reqstd



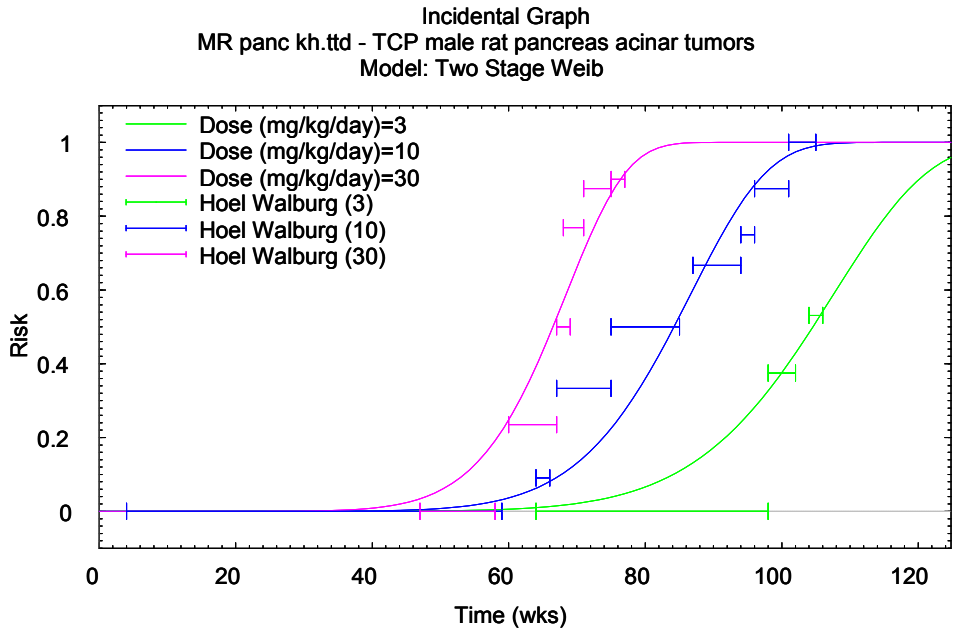
Male Rat; Pancreas Acinar Tumors

```
Dataset: G:\_ToxRiskData\Trichloropropane\MR panc kh.ttd
Model: Two Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
         Maximum Log-Likelihood =  -9.484725e+001

       Parameter Estimates :  Q 0 = 4.471590E-019
                              Q 1 = 2.430231E-019
                              Q 2 = 1.162004E-019
                              Z   = 8.663144E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	5
3	60	0	20
10	59	0	36
30	60	0	31

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

		Human Equivalent Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	9.9894E-004	2.8518E-003	Not Reqstd
1.0000E-005	70.00	9.9894E-003	2.8517E-002	Not Reqstd
0.0001	70.00	9.9894E-002	2.8500E-001	Not Reqstd
0.0010	70.00	9.9901E-001	2.8338E+000	Not Reqstd
0.01	70.00	9.9965E+000	2.6906E+001	Not Reqstd
0.10	70.00	1.0077E+002	2.0173E+002	Not Reqstd



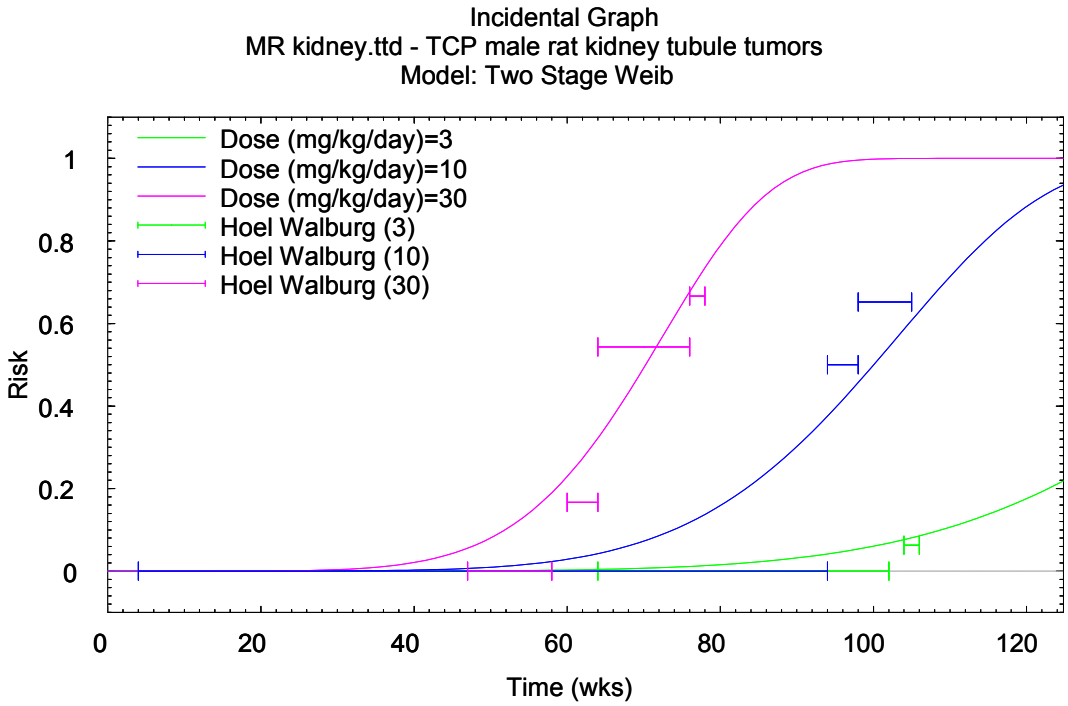
Male Rat; Kidney Tubule Adenomas

```
Dataset: G:\_ToxRiskData\Trichloropropane\MR kidney.ttd
Model: Two Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
         Maximum Log-Likelihood =  -6.953871e+001

       Parameter Estimates :  Q 0 = 0.000000E+000
                              Q 1 = 0.000000E+000
                              Q 2 = 2.539769E-015
                              Z   = 6.217551E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	0
3	60	0	2
10	59	0	18
30	60	0	26

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

		Human Equivalent Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	9.2297E-003	2.1530E+000	Not Reqstd
1.0000E-005	70.00	9.2286E-002	6.8083E+000	Not Reqstd
0.0001	70.00	9.2177E-001	2.1530E+001	Not Reqstd
0.01	70.00	8.2580E+001	2.1584E+002	Not Reqstd
0.05	70.00	3.1744E+002	4.8760E+002	Not Reqstd
0.10	70.00	5.2586E+002	6.9883E+002	Not Reqstd



Male Rat; Hepatocellular Adenomas and Carcinomas

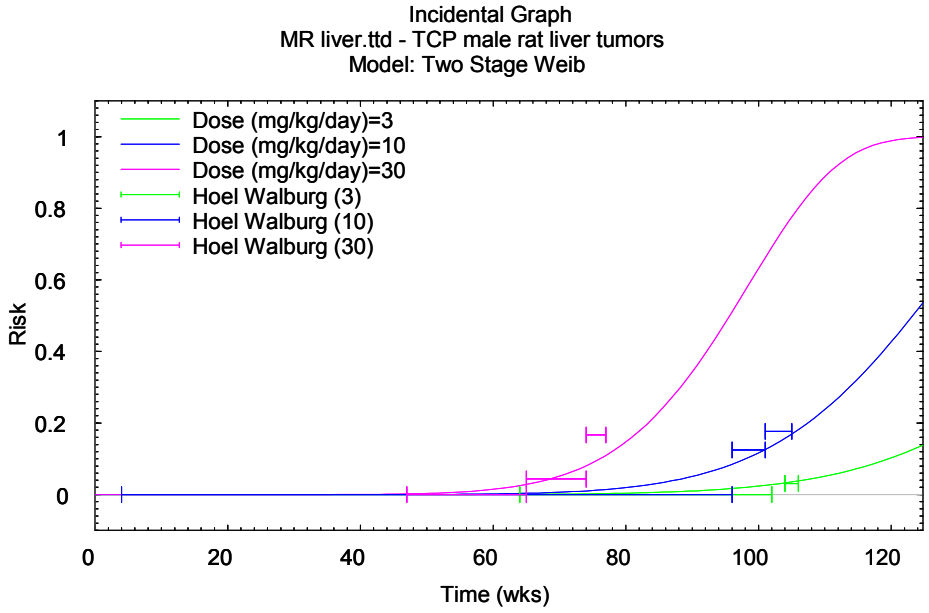
```
Dataset: M:\_ToxRiskData\Trichloropropane\MR liver.ttd
Model: Two Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
         Maximum Log-Likelihood =  -3.253334e+001

       Parameter Estimates :
                              Q 0 = 4.588266E-019
                              Q 1 = 0.000000E+000
                              Q 2 = 3.549482E-020
                              Z   = 8.244602E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	with fatal tumors	with incidental tumors
0	60	0	1
3	60	0	1
10	59	0	4
30	60	0	3

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-005	70.00	1.1502E-001	1.6447E+001	Not Reqstd
0.0001	70.00	1.1502E+000	5.2012E+001	Not Reqstd
0.0010	70.00	1.1501E+001	1.6451E+002	Not Reqstd
0.01	70.00	1.1483E+002	5.2141E+002	Not Reqstd
0.05	70.00	5.6302E+002	1.1779E+003	Not Reqstd
0.10	70.00	1.0536E+003	1.6882E+003	Not Reqstd



Male Rat; Squamous Cell Papillomas or Carcinomas, Skin

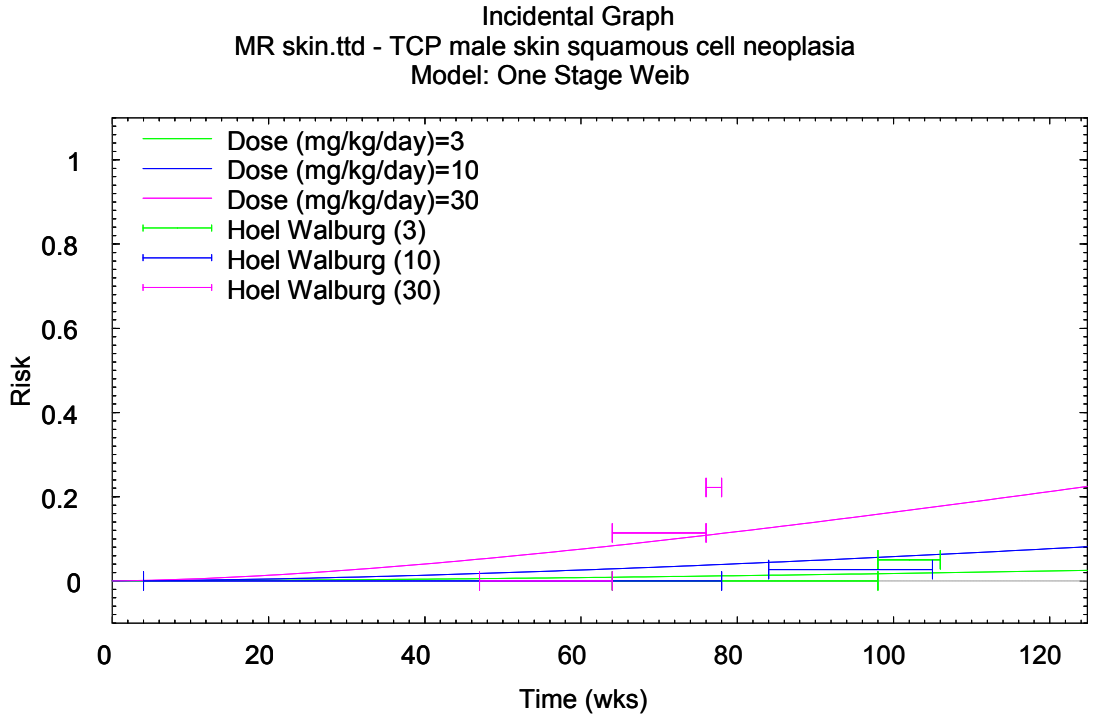
```
Dataset: M:\_ToxRiskData\Trichloropropane\MR skin.ttd
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -3.408636e+001

       Parameter Estimates :
                              Q 0 = 0.000000E+000
                              Q 1 = 3.644870E-006
                              Z   = 1.606192E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	with fatal tumors	with incidental tumors
0	60	0	0
3	60	0	2
10	59	0	1
30	60	0	6

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	1.3601E-002	3.1922E-002	Not Reqstd
1.0000E-005	70.00	1.3601E-001	3.1922E-001	Not Reqstd
0.0001	70.00	1.3602E+000	3.1924E+000	Not Reqstd
0.0010	70.00	1.3608E+001	3.1938E+001	Not Reqstd
0.01	70.00	1.3669E+002	3.2083E+002	Not Reqstd
0.10	70.00	1.4330E+003	3.3634E+003	Not Reqstd



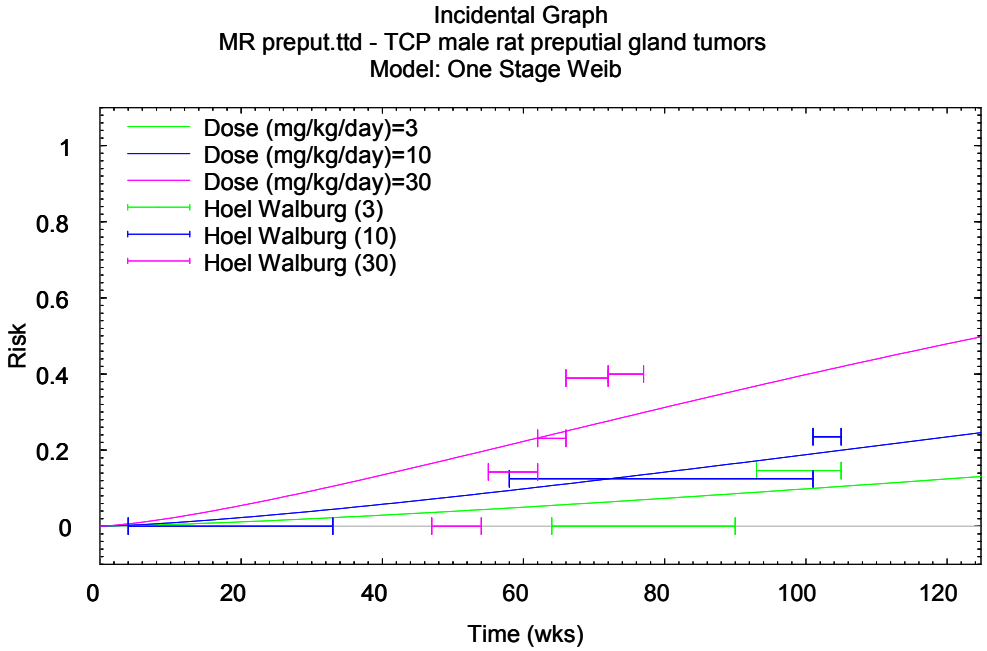
Male Rat; Preputial Gland Tumors

```
Dataset: G:\_ToxRiskData\Trichloropropane\MR preput.ttd
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -1.086836e+002

       Parameter Estimates :  Q 0 = 1.054336E-004
                              Q 1 = 2.704366E-005
                              Z   = 1.371929E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	5
3	60	0	6
10	59	0	9
30	60	0	17

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

		Human Equivalent Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	5.5682E-003	1.2735E-002	Not Reqstd
1.0000E-005	70.00	5.5682E-002	1.2735E-001	Not Reqstd
0.0001	70.00	5.5685E-001	1.2736E+000	Not Reqstd
0.0010	70.00	5.5710E+000	1.2741E+001	Not Reqstd
0.01	70.00	5.5962E+001	1.2799E+002	Not Reqstd
0.10	70.00	5.8667E+002	1.3418E+003	Not Reqstd



Male Rat; Zymbal's Gland Carcinomas

```
Dataset: G:\_ToxRiskData\Trichloropropane\MR Zymbal gl.ttd
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -1.360128e+001

       Parameter Estimates :  Q 0 = 0.000000E+000
                              Q 1 = 1.632672E-005
                              Z   = 1.000000E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	0
3	60	0	0
10	59	0	0
30	60	0	3

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	4.8346E-002	1.1804E-001	Not Reqstd
1.0000E-005	70.00	4.8347E-001	1.1804E+000	Not Reqstd
0.0001	70.00	4.8349E+000	1.1805E+001	Not Reqstd
0.0010	70.00	4.8371E+001	1.1810E+002	Not Reqstd
0.01	70.00	4.8590E+002	1.1864E+003	Not Reqstd
0.10	70.00	5.0938E+003	1.2437E+004	Not Reqstd

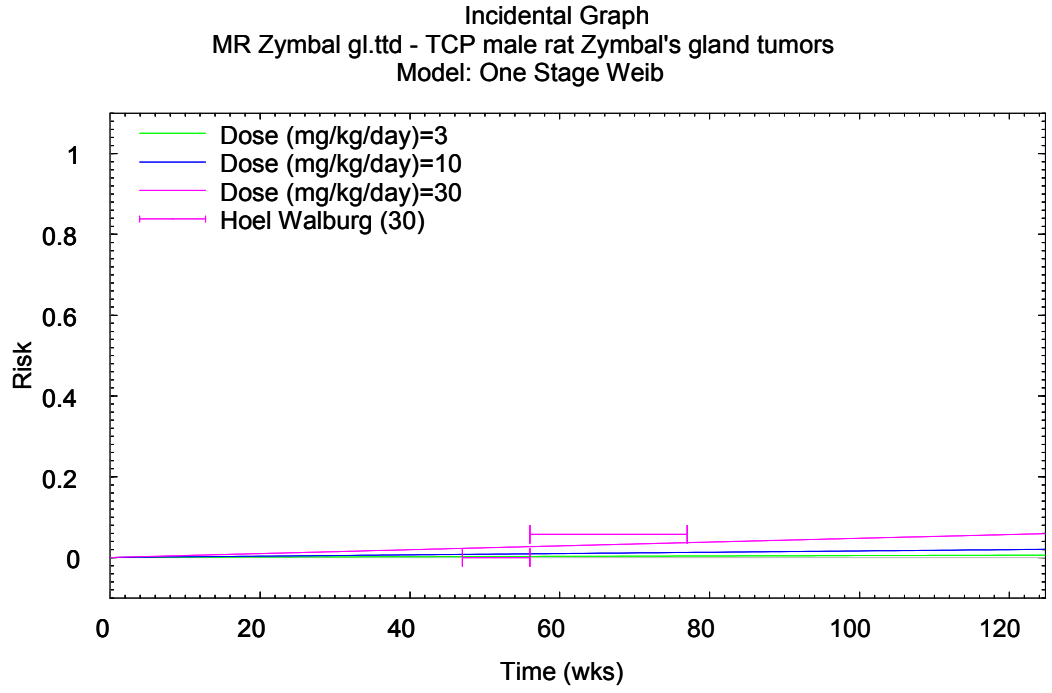


Table D-2. Tumor incidence data, with time to death with tumor; female rats exposed to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of observation	Total examined	Number of animals with					
			Squamous cell neoplasia		Mammary gland tumors		Clitoral gland tumors	Zymbal's gland tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal		
0	61	1	0	0	0	0	0	0
	64	1	0	0	0	1	0	0
	66	10	0	0	0	0	0	0
	68	1	0	0	0	0	0	0
	75	1	0	0	0	0	0	0
	78	1	0	0	0	0	0	0
	79	2	0	0	0	0	0	0
	85	1	0	0	0	0	0	0
	86	1	0	0	0	0	0	0
	89	1	0	0	0	0	0	0
	92	2	0	0	0	0	0	0
	93	1	0	0	0	0	0	0
	96	1	0	0	0	0	0	0
	98	1	0	0	0	0	0	0
	100	1	0	0	0	0	0	0
	101	1	0	0	0	0	0	0
	102	2	0	0	1	0	1	0
	105	18	0	1	0	0	2	0
	106	13	0	0	0	0	2	0
3	62	1	0	0	0	0	0	0
	66	11	1	0	0	0	1	0
	67	1	0	0	0	1	0	0
	73	1	1	0	0	0	0	0
	78	1	0	0	0	0	0	0
	83	1	0	0	0	0	0	0
	84	1	0	0	0	0	0	0
	86	1	0	0	0	0	0	0
	95	1	1	0	0	0	1	0
	96	1	0	0	0	0	0	0
	97	1	1	0	0	0	1	0
	99	3	0	0	0	0	0	0
	101	1	1	0	0	0	0	0
	102	2	1	1	0	1	0	1
	104	2	1	0	0	0	1	0
	105	30	14	0	4	0	7	0

Table D-2. Tumor incidence data, with time to death with tumor; female rats exposed to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of observation	Total examined	Number of animals with					
			Squamous cell neoplasia		Mammary gland tumors		Clitoral gland tumors	Zymbal's gland tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal		
10	36	1	0	0	0	0	0	0
	58	2	1	0	0	0	0	0
	61	1	0	0	0	1	0	0
	62	1	0	0	0	0	1	0
	64	2	1	0	0	0	2	0
	66	8	5	0	0	0	1	0
	68	1	1	0	0	1	0	0
	72	1	1	0	0	0	1	0
	73	3	3	1	0	1	1	0
	74	2	1	1	0	1	0	0
	77	2	2	2	0	1	1	0
	79	1	1	0	0	1	0	0
	80	1	1	1	0	0	0	0
	81	2	2	2	0	0	0	0
	82	1	1	0	0	1	0	0
	83	2	2	1	1	0	0	0
	85	1	1	1	0	0	0	0
	86	2	2	1	0	0	1	0
	87	3	2	1	0	2	2	0
	90	1	1	1	0	0	0	0
	91	2	2	1	0	0	0	0
	92	3	2	2	0	0	0	0
	96	1	1	0	0	0	0	0
	97	1	1	1	0	0	1	0
	98	1	1	0	0	0	0	0
	100	2	2	1	0	0	1	0
	101	1	1	0	0	0	0	0
	103	1	1	1	0	0	0	0
	104	2	2	1	1	0	0	0
	105	8	8	0	3	0	6	0
30	12	2	0	0	0	0	0	0
	26	1	0	0	0	0	0	0
	33	1	1	0	0	0	0	0
	34	1	0	0	0	1	0	0
	36	1	0	0	0	1	0	0
	42	2	2	2	0	0	0	0
	44	3	2	1	0	1	1	0
	46	1	1	1	0	0	1	0
	47	3	1	1	0	1	2	0
	48	3	2	0	0	2	1	1
	49	5	2	1	0	2	3	0
	50	1	1	1	0	0	0	0
	51	1	1	1	0	1	1	0
	52	3	2	1	0	1	1	0
	53	4	2	0	0	3	0	0
	54	1	1	1	0	0	1	0
	55	2	2	1	0	2	0	0
	57	4	4	3	0	3	1	0
	58	2	2	2	0	0	1	0
	59	3	3	3	0	0	1	0
	60	3	3	3	0	0	0	0
	62	1	1	0	0	1	0	0
	63	2	2	0	0	1	0	1

Table D-2. Tumor incidence data, with time to death with tumor; female rats exposed to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of observation	Total examined	Number of animals with					
			Squamous cell neoplasia		Mammary gland tumors		Clitoral gland tumors	Zymbal's gland tumors
			Incidental[a]	Fatal[a]	Incidental	Fatal		
30	64	1	1	0	0	1	1	0
	66	9	9	1	0	2	2	2

[a]"Incidental" denotes incidence of papillomas only, or of carcinomas observed at scheduled sacrifice. "Fatal" denotes incidence of carcinomas at unscheduled deaths. Some papillomas were present with carcinomas in both categories.

Source: NTP (1993).

Female Rat; Alimentary System Squamous Papillomas, Carcinomas

A. All tumors treated as incidental to death of animal:

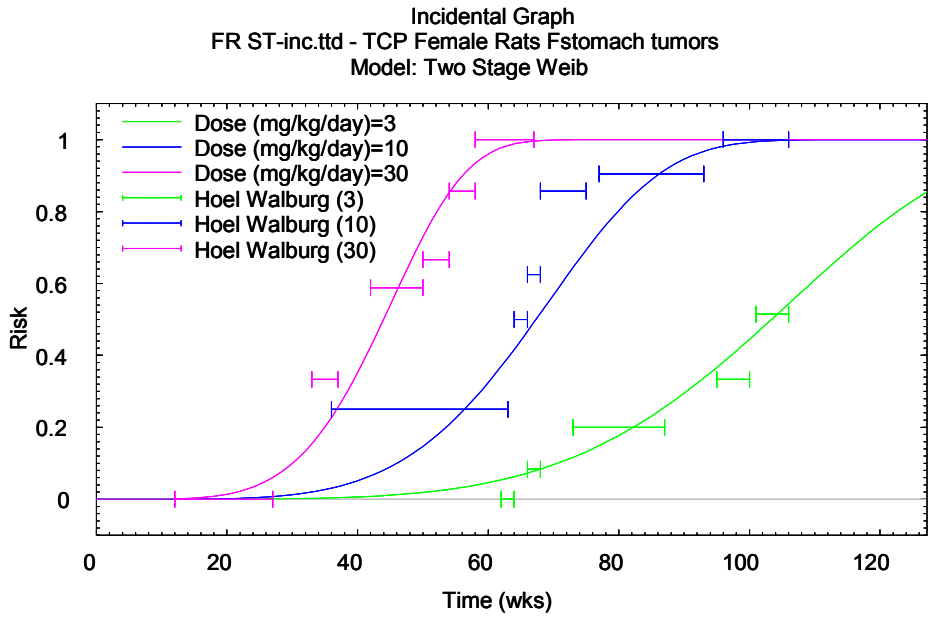
```
Dataset: G:\_ToxRiskData\Trichloropropane\FR ST-inc kh.ttd
Model: Two Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
         Maximum Log-Likelihood =  -9.100477e+001

       Parameter Estimates :
                            Q 0 = 2.485425E-012
                            Q 1 = 8.109448E-012
                            Q 2 = 5.601264E-012
                            Z   = 4.940580E+000
                            T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	1
3	59	0	22
10	60	0	49
30	60	0	44

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	5.2769E-004	2.2831E-003	Not Reqstd
1.0000E-005	70.00	5.2769E-003	2.2829E-002	Not Reqstd
0.0001	70.00	5.2771E-002	2.2810E-001	Not Reqstd
0.0010	70.00	5.2793E-001	2.2622E+000	Not Reqstd
0.01	70.00	5.3009E+000	2.1036E+001	Not Reqstd
0.10	70.00	5.5310E+001	1.4711E+002	Not Reqstd



Female Rat; Alimentary System Squamous Papillomas, Carcinomas (cont.)

B. Carcinomas occurring at unscheduled deaths treated as cause of death:

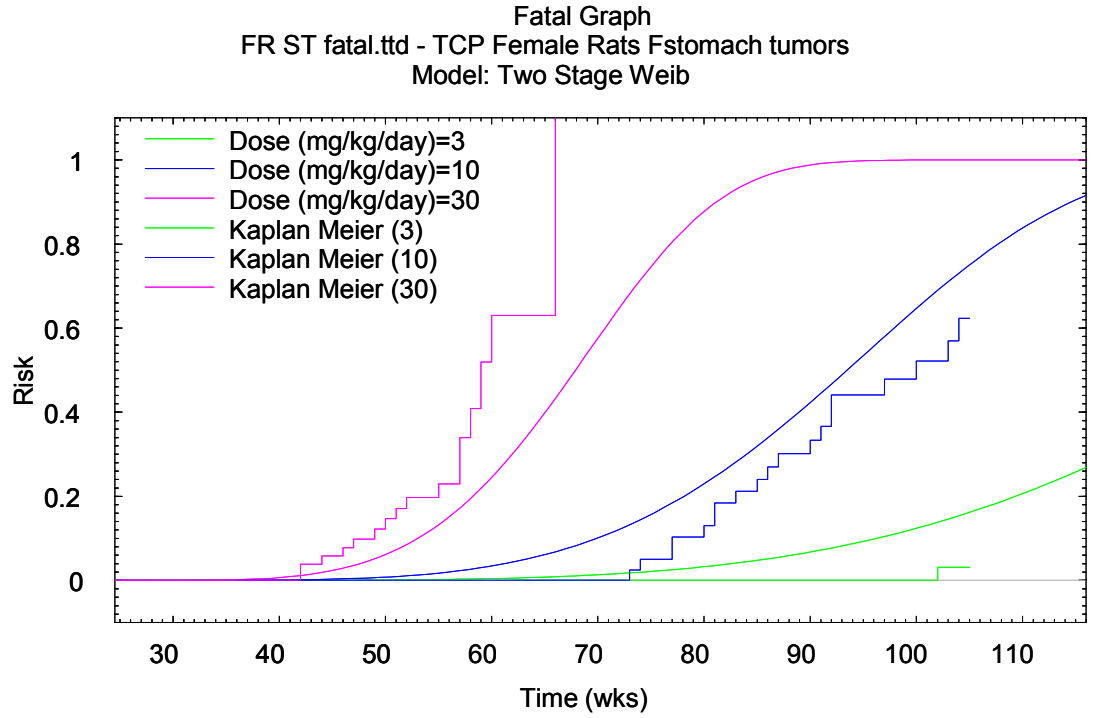
```
Model: Two Stage Weib
Dataset: M:\_ToxRiskData\Trichloropropane\FR ST fatal.ttd
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
         Maximum Log-Likelihood =  -2.626680e+002

       Parameter Estimates :
                            Q 0 = 1.708057E-011
                            Q 1 = 5.693671E-011
                            Q 2 = 2.666721E-011
                            Z   = 4.532096E+000
                            T0  = 2.708322E+001
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	1
3	59	1	21
10	60	18	31
30	60	16	28

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	9.7449E-004	2.3918E-003	Not Reqstd
1.0000E-005	70.00	9.7449E-003	2.3917E-002	Not Reqstd
0.0001	70.00	9.7446E-002	2.3902E-001	Not Reqstd
0.0010	70.00	9.7417E-001	2.3752E+000	Not Reqstd
0.01	70.00	9.7131E+000	2.2449E+001	Not Reqstd
0.10	70.00	9.4856E+001	1.6553E+002	Not Reqstd



Female Rat; Mammary Adenomas, Adenocarcinomas[1]

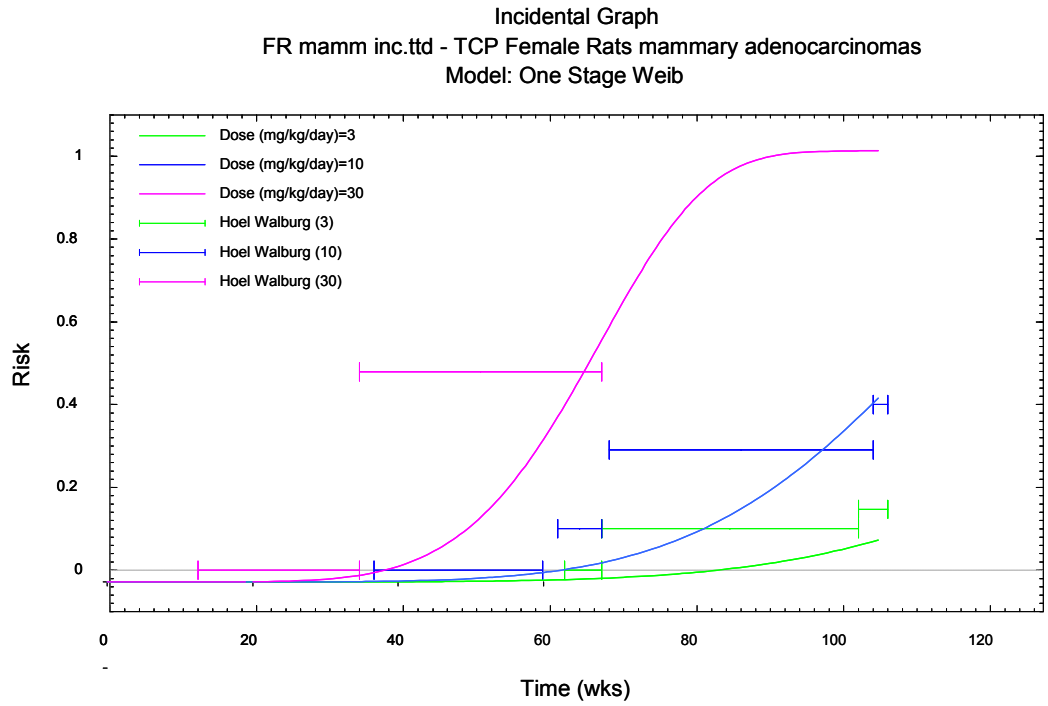
A. All tumors treated as incidental to death of animal:

```
=====================================================================
         Multistage Weibull Model. (Version: 1.3;  Date: 08/30/2007)
         Input Data File: FRmamm_M=U_msw1I.msw
                                              Mon Sep 14 16:27:55 2009
 =====================================================================

 TCP: NTP Female Rats mammary adenocarcinomas, M=U

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive


   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 125
 Total number of records with missing values = 0

               Data Summary (N Totals)
                        CLASS
               C      F       I      U  Total
 DOSE
        0     55      0       2      3     60
        3     50      0       6      3     59
       10     39      0      14      7     60
       30     28      0      23      9     60

 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1


   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         c      =      1.11111
                         t_0    =            0   Specified
                         beta_0 =  0.000201751
                         beta_1 =  0.000184682


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -c          -t_0
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                 beta_0       beta_1

    beta_0            1        -0.35

    beta_1        -0.35            1


                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c                     1               NA
         beta_0      0.000345237      0.000318783        -0.000279565          0.00097004
```

[1] Note: 22 female rats across the dose groups had missing tissues. While the multistage Weibull model can take advantage of the length of time that the animals were on study without developing tumors, the version of ToxRisk used in this assessment did not provide correct estimates. The program used in this case (MSW, currently under development by EPA) provides correct estimates.

```
         beta_1      0.000292263     7.43911e-005         0.000146459         0.000438067

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


                      Analysis of Deviance Table
      Model      Log(likelihood)   # Param's Deviance  Test d.f.    P-value
     Full Model                0         6
   Fitted Model         -100.346         4             0      2         <.0001
  Reduced Model                0         3             0      3         <.0001

           AIC:          204.693


   Benchmark Dose Computation
Specified effect =            0.1

Time             =            104

Risk Response    =     Incidental

Risk Type        =          Extra

Confidence level =            0.9

             BMD =        3.46634

            BMDL =        2.58643

            BMDU =        4.64231
```

The BMD and BMD above were then adjusted to estimate human equivalent continuous exposure using $BW^{3/4}$ cross-species scaling and by multiplying by (5 days)/(7 days): $BMD_{HED} = 0.605$, $BMD_{HED} = 0.426$ mg/kg-day.



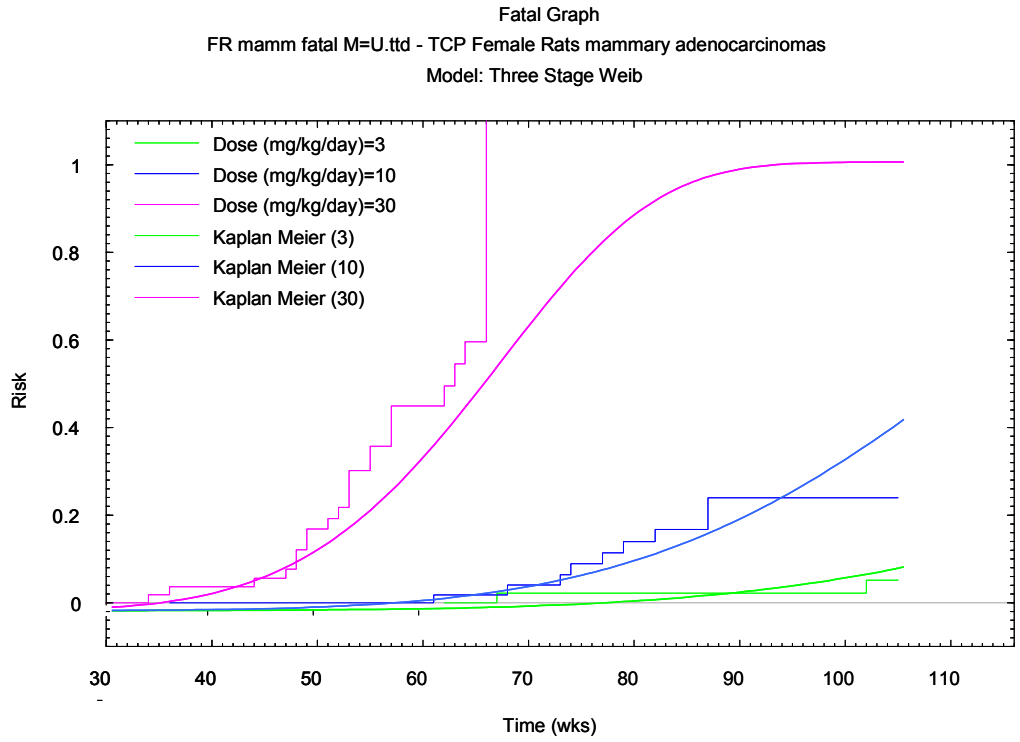
Female Rat; Mammary Adenomas, Adenocarcinomas

B. Carcinomas occurring at unscheduled deaths treated as cause of death:

```
=====================================================================
         Multistage Weibull Model. (Version: 1.3;  Date: 08/30/2007)
         Input Data File: FRmamm_M=U_msw3f.msw
                                          Mon Sep 14 17:03:49 2009
 =====================================================================

 TCP: NTP Female Rats mammary adenocarcinomas, M=U

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive


   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 125
 Total number of records with missing values = 0

               Data Summary (N Totals)
                        CLASS
               C      F      I      U  Total
 DOSE
        0     55      1      1      3     60
        3     50      2      4      3     59
       10     39      9      5      7     60
       30     28     23      0      9     60

 Minimum observation time for F tumor context =         34

 Total number of parameters in model = 6
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         c      =      3.33333
                         t_0    =          6.8
                         beta_0 = 8.44695e-009
                         beta_1 = 5.32978e-009
                         beta_2 = 7.59254e-011
                         beta_3 = 2.12067e-011


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

                 c            t_0          beta_0       beta_1       beta_3

    c                 1        -0.52        -0.98        -0.97           -1

    t_0           -0.52            1         0.51          0.5         0.53

    beta_0        -0.98         0.51            1         0.92         0.98

    beta_1        -0.97          0.5         0.92            1         0.96

    beta_3           -1         0.53         0.98         0.96            1


                                Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              5.32444         0.954401             3.45384             7.19503
         t_0            4.70501           2.2168             0.36016             9.04986
         beta_0     9.83773e-013     4.32656e-012       -7.49612e-012        9.46367e-012
         beta_1     3.34357e-013       1.699e-012       -2.99562e-012        3.66433e-012
```

```
         beta_2                 0                NA
         beta_3      7.55424e-015       2.9788e-014       -5.08292e-014        6.59377e-014

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


                      Analysis of Deviance Table
       Model      Log(likelihood)   # Param's Deviance  Test d.f.    P-value
     Full Model         -230.308         6
   Fitted Model         -230.308         6              0       0         NA
  Reduced Model                0         3              0       3         <.0001

           AIC:          468.616


   Benchmark Dose Computation
Specified effect =             0.1

Time             =             104

Risk Response    =      Incidental

Risk Type        =           Extra

Confidence level =            0.95

             BMD =         4.13847

            BMDL =         1.95917

            BMDU =         7.01446
```

The BMD and BMD above were then adjusted to estimate human equivalent continuous exposure using $BW^{3/4}$ cross-species scaling and by multiplying by (5 days)/(7 days): $BMD_{HED} = 0.723$, $BMD_{HED} = 0.342$ mg/kg-day.



Female Rat; Clitoral Gland Adenomas, Carcinomas

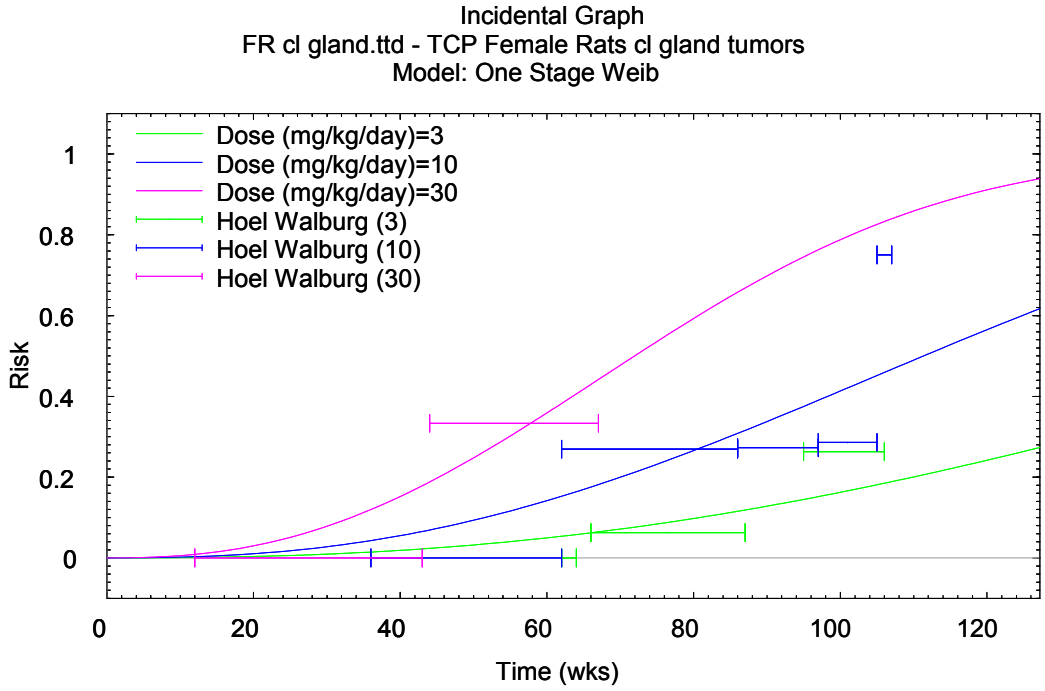
```
Model: One Stage Weib
Dataset: G:\_ToxRiskData\Trichloropropane\FR cl gland.ttd
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -1.422177e+002

       Parameter Estimates :
                              Q 0 = 3.143833E-007
                              Q 1 = 6.526662E-007
                              Z   = 2.445897E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	5
3	58	0	11
10	58	0	18
30	60	0	17

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	2.2971E-003	2.9484E-003	Not Reqstd
1.0000E-005	70.00	2.2971E-002	2.9484E-002	Not Reqstd
0.0001	70.00	2.2973E-001	2.9485E-001	Not Reqstd
0.0010	70.00	2.2983E+000	2.9499E+000	Not Reqstd
0.01	70.00	2.3087E+001	2.9632E+001	Not Reqstd
0.10	70.00	2.4203E+002	3.1064E+002	Not Reqstd



Female Rat; Zymbal's Gland Carcinomas

```
Dataset: G:\_ToxRiskData\Trichloropropane\FR Zymbal gl.ttd
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -2.101568e+001

       Parameter Estimates :
                              Q 0 = 0.000000E+000
                              Q 1 = 1.393807E-005
                              Z   = 1.198267E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	with fatal tumors	with incidental tumors
0	60	0	0
3	59	0	1
10	60	0	0
30	60	0	4

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	1.4957E-002	4.6884E-002	Not Reqstd
1.0000E-005	70.00	1.4958E-001	4.6884E-001	Not Reqstd
0.0001	70.00	1.4958E+000	4.6886E+000	Not Reqstd
0.0010	70.00	1.4965E+001	4.6907E+001	Not Reqstd
0.01	70.00	1.5033E+002	4.7120E+002	Not Reqstd
0.10	70.00	1.5759E+003	4.9397E+003	Not Reqstd

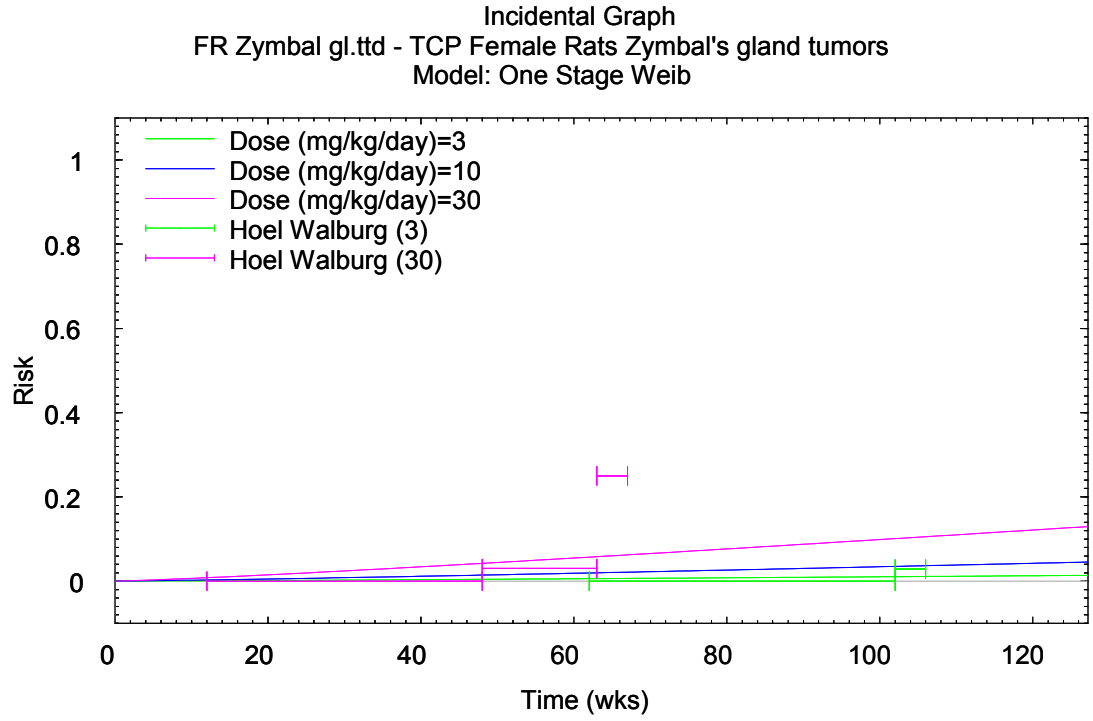


Table D-3. Tumor incidence data, with time to death with tumor; male mice exposed by gavage to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of death	Total examined	Number of animals with			
			Squamous cell neoplasia[a]		Liver tumors	Harderian gland adenomas
			Incidental	Fatal		
0	3	1	0	0	0	0
	65	2	0	0	0	0
	66	8	0	0	1	0
	70	1	1	0	1	0
	71	1	0	0	0	0
	77	1	0	0	0	0
	85	1	0	0	1	0
	88	1	0	0	0	0
	94	1	0	0	1	0
	98	1	0	0	1	0
	105	42	2	0	9	1
6	58	1	0	0	0	0
	61	1	0	1	0	0
	65	1	0	1	0	0
	66	9	8	0	0	0
	75	2	0	2	1	0
	77	1	0	1	0	0
	78	2	0	2	0	0
	81	2	0	2	2	0
	82	1	0	1	0	0
	83	2	0	2	2	0
	84	2	0	2	0	0
	85	1	0	1	0	0
	89	1	1	0	1	0
	91	1	0	1	0	0
	92	3	0	3	1	1
	93	1	1	0	0	1
	94	1	0	1	0	0
	95	1	0	1	1	0
	97	3	1	2	3	0
	98	1	0	1	1	0
	99	2	0	2	1	0
	101	2	0	2	1	0
	105	18	18	0	10	0
20	55	2	0	2	0	0
	59	1	0	1	1	0
	63	1	0	1	1	0
	64	2	0	2	0	0
	65	1	1	0	0	0
	66	8	4	2	1	0
	67	1	0	1	0	0
	68	2	0	1	1	0
	69	4	0	4	2	0
	72	2	0	2	0	0
	73	2	0	2	2	1
	74	1	0	1	1	0
	76	2	0	1	0	0
	77	1	0	1	1	1
	78	3	0	2	0	1
	80	1	0	1	0	0
	81	4	0	4	0	1
	83	2	0	2	2	0
	84	2	0	2	1	1
	85	3	0	3	2	2

Table D-3. Tumor incidence data, with time to death with tumor; male mice exposed by gavage to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of death	Total examined	Number of animals with			
			Squamous cell neoplasia[a]		Liver tumors	Harderian gland adenomas
			Incidental	Fatal		
	86	1	0	1	0	0
	88	3	0	3	3	1
	89	11	11	0	7	2
60	46	1	1	0	1	0
	50	1	0	1	1	0
	53	2	0	2	0	0
	55	1	0	1	0	0
	56	3	3	2	1	0
	57	1	0	1	0	0
	58	1	0	1	0	0
	59	1	0	1	0	0
	60	1	0	1	0	0
	61	1	0	1	1	0
	62	2	2	1	0	0
	63	3	0	3	0	0
	64	3	0	3	1	0
	65	3	0	3	1	2
	66	6	6	2	3	2
	67	1	0	1	0	1
	68	4	0	4	3	1
	69	1	0	1	0	0
	70	3	0	3	3	0
	71	1	0	1	0	0
	72	2	0	2	1	1
	73	1	0	1	0	0
	74	1	0	1	1	0
	75	1	0	1	1	0
	76	1	0	1	1	0
	77	2	0	2	2	1
	78	2	0	2	2	0
	79	1	0	1	1	1
	80	9	9	0	9	2

[a]"Incidental" denotes incidence of papillomas or of carcinomas observed at scheduled death. "Fatal" denotes incidence of carcinomas at unscheduled deaths. Papillomas present with carcinomas in both categories are not indicated.

Source: NTP (1993).

Male Mouse; Alimentary System Squamous Cell Papillomas, Carcinomas

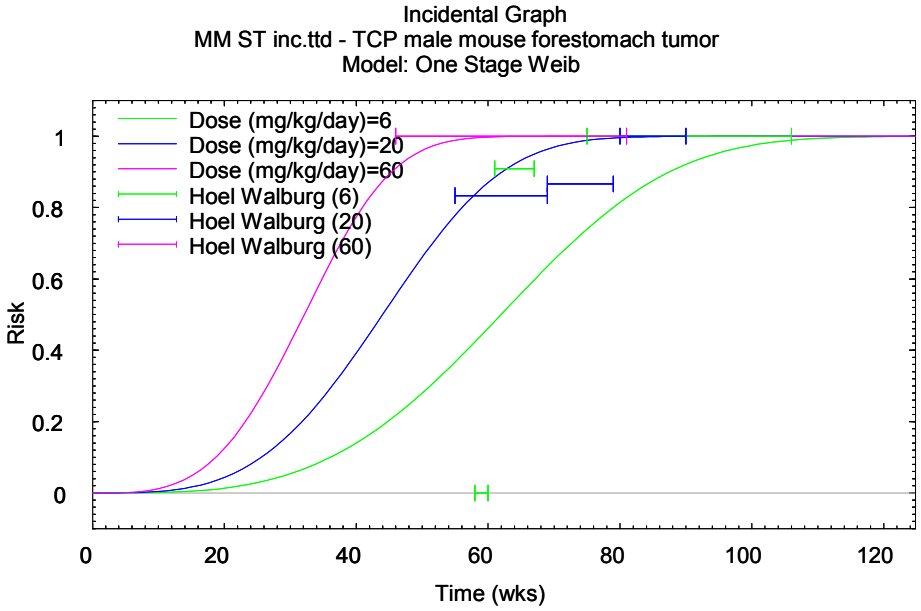
A. All tumors treated as incidental to death of animal:

```
Model: One Stage Weib         Dataset: M:\_ToxRiskData\Trichloropropane\MM ST inc.ttd
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -4.395030e+001

       Parameter Estimates :
                            Q 0 = 6.451129E-009
                            Q 1 = 6.539700E-008
                            Z   = 3.481792E+000
                            T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	3
6	59	0	57
20	60	0	55
60	60	0	60

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

		Human Equivalent Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time (yr)	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	1.6127E-004	2.8611E-004	Not Reqstd
1.0000E-005	70.00	1.6127E-003	2.8611E-003	Not Reqstd
0.0001	70.00	1.6127E-002	2.8612E-002	Not Reqstd
0.0010	70.00	1.6135E-001	2.8625E-001	Not Reqstd
0.01	70.00	1.6208E+000	2.8755E+000	Not Reqstd
0.10	70.00	1.6991E+001	3.0145E+001	Not Reqstd



Male Mouse; Alimentary System Squamous Cell Papillomas, Carcinomas (cont.)

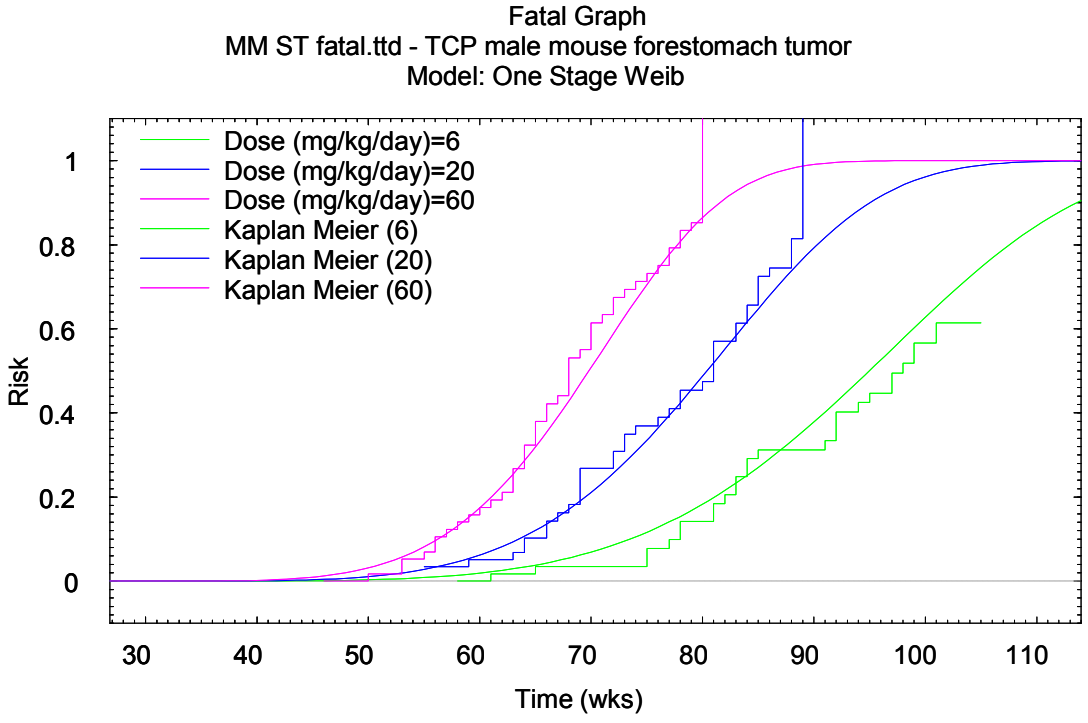
B. Carcinomas occurring at unscheduled deaths treated as cause of death:

```
Dataset: M:\_ToxRiskData\Trichloropropane\MM ST fatal.ttd
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -5.550902e+002

       Parameter Estimates :
                              Q 0 = 6.129572E-012
                              Q 1 = 8.676377E-011
                              Z   = 4.972905E+000
                              T0  = 2.678381E+001
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	3
6	59	28	29
20	60	50	5
60	60	51	9

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

		Human Equivalent Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time (yr)	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	1.6037E-004	2.1199E-004	Not Reqstd
1.0000E-005	70.00	1.6037E-003	2.1200E-003	Not Reqstd
0.0001	70.00	1.6038E-002	2.1200E-002	Not Reqstd
0.0010	70.00	1.6045E-001	2.1210E-001	Not Reqstd
0.01	70.00	1.6118E+000	2.1306E+000	Not Reqstd
0.10	70.00	1.6897E+001	2.2336E+001	Not Reqstd



Male Mouse; Liver Tumors

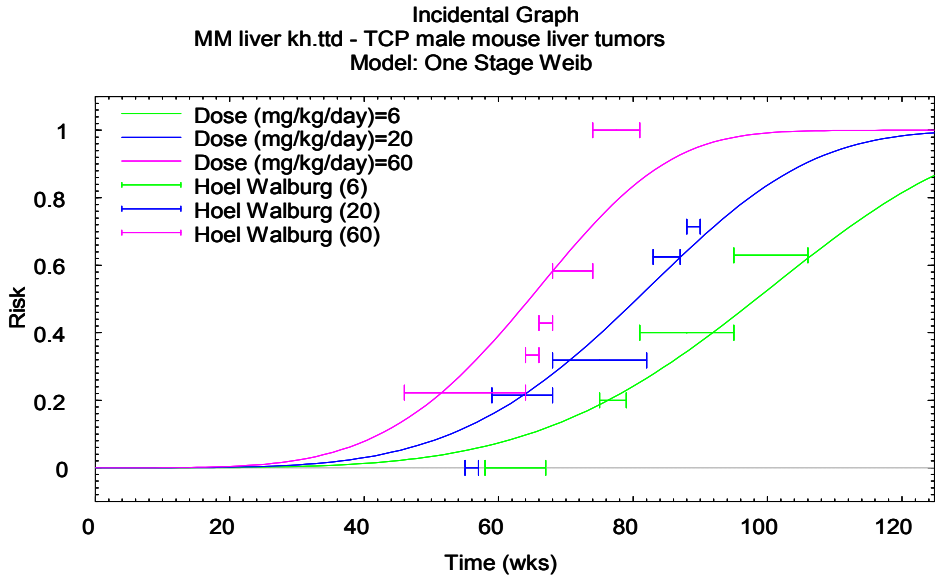
```
Dataset: M:\_ToxRiskData\Trichloropropane\MM liver kh.ttd
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -1.400104e+002

       Parameter Estimates :
                            Q 0 = 3.361501E-010
                            Q 1 = 8.911625E-011
                            Z   = 4.466842E+000
                            T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	with fatal tumors	with incidental tumors
0	60	0	14
6	59	0	24
20	60	0	25
60	60	0	33

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	1.2990E-003	2.0840E-003	Not Reqstd
1.0000E-005	70.00	1.2990E-002	2.0840E-002	Not Reqstd
0.0001	70.00	1.2991E-001	2.0841E-001	Not Reqstd
0.0010	70.00	1.2997E+000	2.0850E+000	Not Reqstd
0.01	70.00	1.3055E+001	2.0944E+001	Not Reqstd
0.10	70.00	1.3686E+002	2.1957E+002	Not Reqstd



Male Mouse; Harderian Gland Tumors

```
Dataset: M:\_ToxRiskData\Trichloropropane\MM harderian.ttd
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -6.696811e+001

       Parameter Estimates :
                              Q 0 = 1.980258E-010
                              Q 1 = 2.197164E-010
                              Z   = 3.927133E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	1
6	59	0	2
20	60	0	10
60	60	0	11

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	5.4492E-003	1.0720E-002	Not Reqstd
1.0000E-005	70.00	5.4492E-002	1.0720E-001	Not Reqstd
0.0001	70.00	5.4495E-001	1.0720E+000	Not Reqstd
0.0010	70.00	5.4519E+000	1.0725E+001	Not Reqstd
0.01	70.00	5.4766E+001	1.0774E+002	Not Reqstd
0.10	70.00	5.7413E+002	1.1294E+003	Not Reqstd

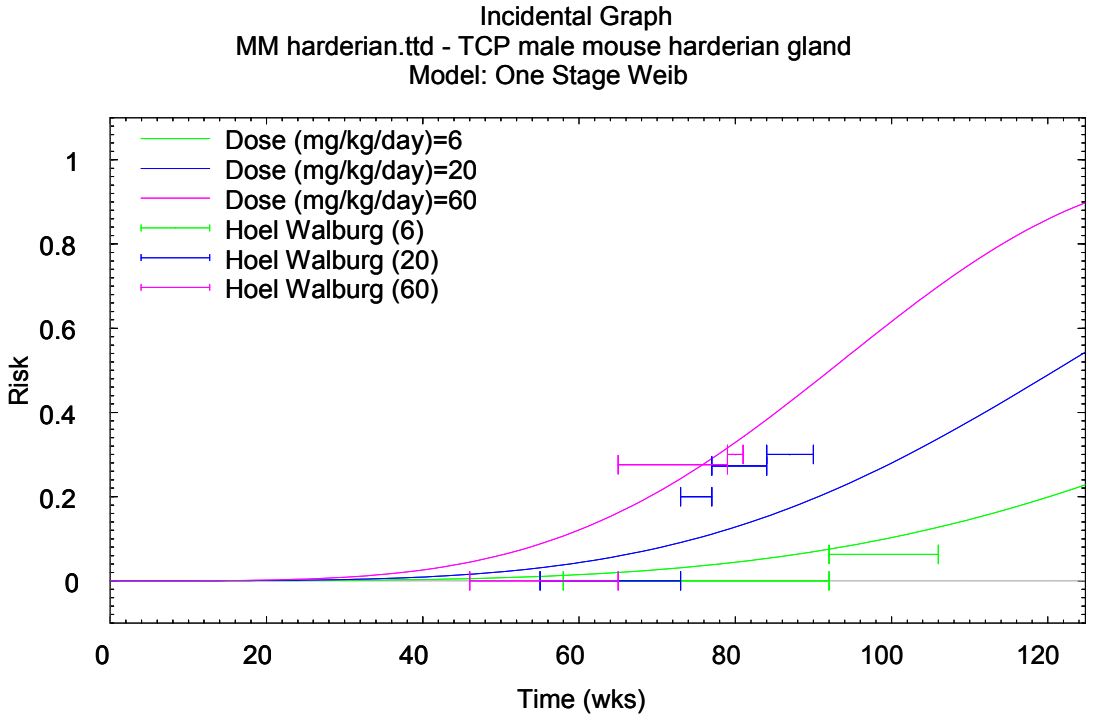


Table D-4. Tumor incidence data, with time to death with tumor; female mice exposed by gavage to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of death	Total examined	Number of animals with				
			Squamous cell neoplasia[a]		Liver tumors	Harderian gland adenomas	Uterine adenomas or carcinomas
			Incidental	Fatal			
0	10	1	0	0	0	0	0
	66	10	0	0	1	1	0
	69	2	0	0	0	0	0
	73	1	0	0	0	0	0
	90	1	0	0	0	0	0
	99	1	0	0	0	0	0
	100	2	0	0	0	0	0
	104	1	0	0	0	0	0
	105	41	0	0	7	2	0
6	23	1	0	0	0	0	0
	30	1	0	0	0	0	0
	59	1	0	1	0	0	0
	64	1	0	1	0	0	0
	66	10	6	0	0	0	0
	74	1	0	1	0	0	0
	77	1	0	1	1	0	0
	80	2	0	2	0	1	0
	81	1	0	1	0	0	0
	82	2	0	2	0	0	0
	83	3	0	3	1	0	0
	84	1	0	1	0	0	0
	86	3	0	3	0	0	0
	87	2	0	2	0	1	0
	89	2	0	2	1	0	0
	90	1	0	1	0	0	0
	91	1	0	1	0	0	0
	92	1	0	1	0	0	0
	93	1	0	1	0	1	0
	96	1	0	1	0	0	0
	97	2	0	2	0	0	0
	98	2	0	2	1	1	0
	99	1	0	1	0	0	0
	100	1	0	1	0	0	1
	101	1	0	1	0	0	0
	103	2	0	2	1	0	1
	104	1	0	1	0	0	0
	105	13	13	0	6	2	3
20	2	1	0	0	0	0	0
	45	1	0	1	0	0	0
	55	1	0	1	0	0	0
	63	2	0	2	0	0	0
	64	1	0	1	0	0	0
	65	2	0	2	1	0	0
	66	12	10	2	1	0	0
	67	2	0	2	0	0	0
	68	1	0	1	0	0	0
	71	2	0	2	0	0	0
	72	4	0	4	0	0	0
	73	2	0	2	0	0	0
	75	2	0	2	0	0	0
	76	1	0	1	0	0	0
	77	1	0	1	0	0	0
	78	1	0	1	0	1	0

Table D-4. Tumor incidence data, with time to death with tumor; female mice exposed by gavage to 1,2,3-trichloropropane

Dose group, mg/kg-d	Wk of death	Total examined	Number of animals with				
			Squamous cell neoplasia[a]		Liver tumors	Harderian gland adenomas	Uterine adenomas or carcinomas
			Incidental	Fatal			
	79	1	0	1	0	0	0
	80	2	0	2	0	1	0
	81	2	0	2	0	0	0
	82	2	0	2	0	2	0
	83	3	0	3	0	0	1
	84	1	0	1	0	1	0
	86	1	0	1	0	0	0
	87	1	0	1	0	0	0
	88	1	0	1	1	0	0
	89	10	0	10	6	2	2
60	42	1	0	1	0	0	0
	49	1	0	0	0	0	0
	54	4	0	4	0	0	0
	55	2	0	2	0	0	0
	56	1	1	0	0	0	0
	57	1	0	1	0	0	0
	58	2	0	2	0	0	0
	59	1	0	1	0	0	0
	60	2	1	1	1	0	0
	61	2	0	2	1	0	0
	62	2	0	2	1	0	0
	63	2	1	1	1	0	0
	64	3	0	3	1	1	0
	66	6	5	1	5	0	2
	67	6	0	6	4	1	0
	68	3	0	3	1	0	0
	69	2	0	2	1	1	0
	70	4	1	3	4	1	2
	71	3	0	3	3	2	1
	72	4	0	4	4	2	2
	73	8	1	7	7	2	4

[a]"Incidental" denotes incidence of papillomas or of carcinomas observed at scheduled death (interim sacrifice, or terminal sacrifice). "Fatal" denotes incidence of carcinomas at unscheduled deaths or groups terminated early. Papillomas present with carcinomas in both categories are not indicated.

Source: NTP (1993).

Female Mouse; Alimentary System Squamous Cell Papillomas, Carcinomas

A. All tumors treated as incidental to death of animal:

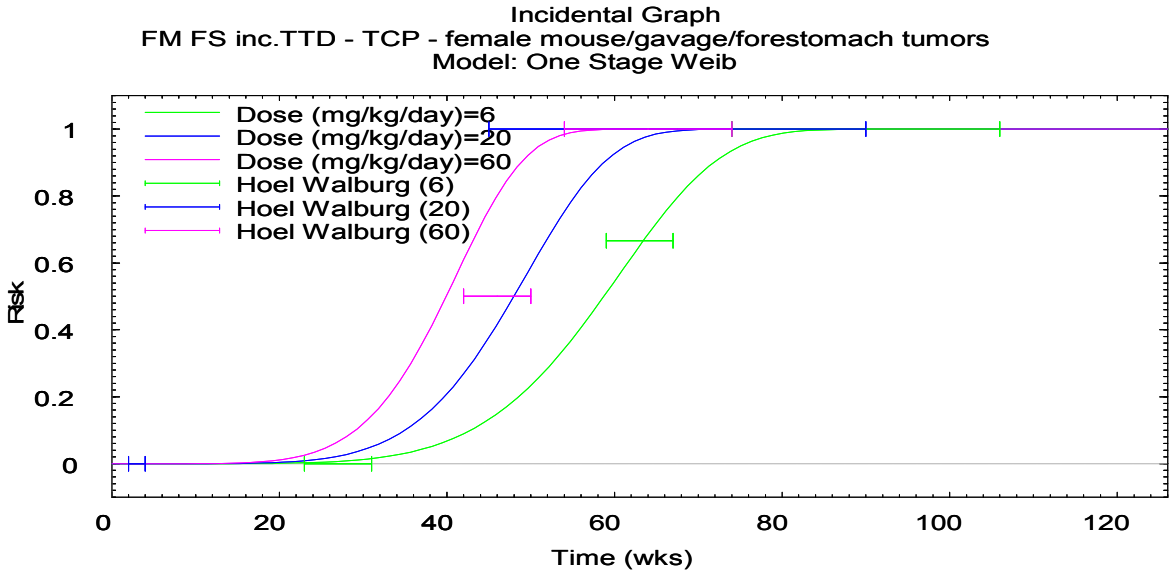
```
TITLE: TCP - female mouse/gavage/forestomach tumors

Dataset: M:\_ToxRiskData\Trichloropropane\FM FS inc.TTD
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -1.291538e+001

       Parameter Estimates :
                              Q 0 = 0.000000E+000
                              Q 1 = 3.331328E-012
                              Z   = 5.958858E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	0
6	60	0	54
20	60	0	59
60	60	0	59

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	6.1978E-006	2.9932E-005	Not Reqstd
1.0000E-005	70.00	6.1978E-005	2.9932E-004	Not Reqstd
0.0001	70.00	6.1981E-004	2.9934E-003	Not Reqstd
0.0010	70.00	6.2009E-003	2.9947E-002	Not Reqstd
0.01	70.00	6.2290E-002	3.0083E-001	Not Reqstd
0.10	70.00	6.5300E-001	3.1537E+000	Not Reqstd



Female Mouse; Alimentary System Squamous Cell Papillomas, Carcinomas (cont.)

B. Carcinomas occurring at unscheduled deaths treated as cause of death:

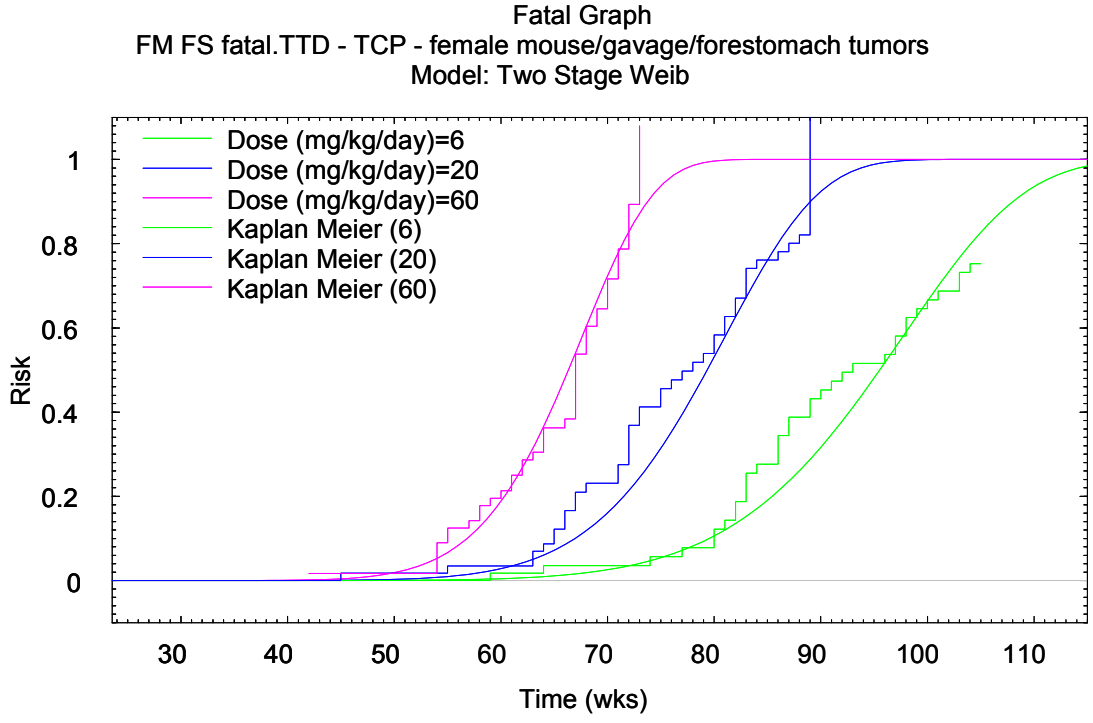
```
Dataset: M:\_ToxRiskData\Trichloropropane\FM FS fatal.TTD
Model: Two Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
         Maximum Log-Likelihood =  -5.423304e+002

       Parameter Estimates :
                            Q 0 = 0.000000E+000
                            Q 1 = 7.259298E-016
                            Q 2 = 9.569303E-017
                            Z   = 7.510793E+000
                            T0  = 2.356030E+001
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	0
6	60	35	19
20	60	49	10
60	60	49	10

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	3.7108E-005	9.2044E-005	Not Reqstd
1.0000E-005	70.00	3.7108E-004	9.2044E-004	Not Reqstd
0.0001	70.00	3.7110E-003	9.2047E-003	Not Reqstd
0.0010	70.00	3.7126E-002	9.2076E-002	Not Reqstd
0.01	70.00	3.9005E-001	9.2366E-001	Not Reqstd
0.10	70.00	3.9064E+000	9.5469E+000	Not Reqstd



Female Mouse; Liver Tumors

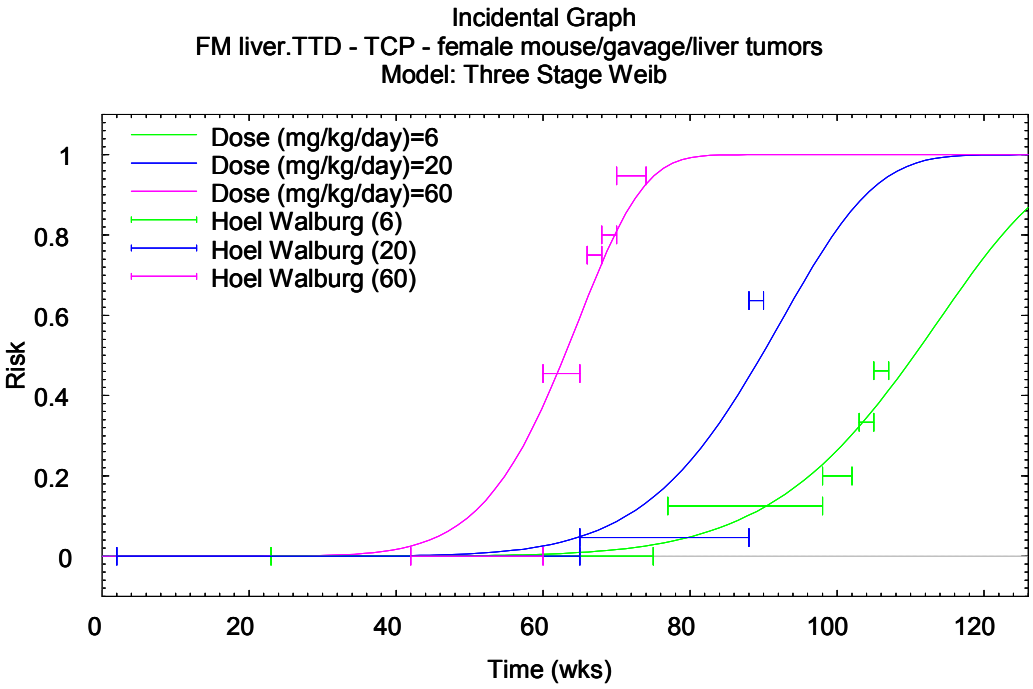
```
Dataset: M:\_ToxRiskData\Trichloropropane\FM liver.TTD
Model: Three Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3 )
                 * (T - T0)^Z]
         Maximum Log-Likelihood =  -9.623458e+001

       Parameter Estimates :
                              Q 0 = 5.629531E-018
                              Q 1 = 9.202992E-019
                              Q 2 = 0.000000E+000
                              Q 3 = 5.479834E-021
                              Z   = 8.197183E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	8
6	60	0	11
20	60	0	9
60	60	0	36

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	1.3081E-003	3.0192E-003	Not Reqstd
1.0000E-005	70.00	1.3081E-002	3.0192E-002	Not Reqstd
0.0001	70.00	1.3082E-001	3.0193E-001	Not Reqstd
0.0010	70.00	1.3088E+000	3.0207E+000	Not Reqstd
0.01	70.00	1.3146E+001	3.0328E+001	Not Reqstd
0.10	70.00	1.3711E+002	3.0250E+002	Not Reqstd



Female Mouse; Harderian Gland Tumors

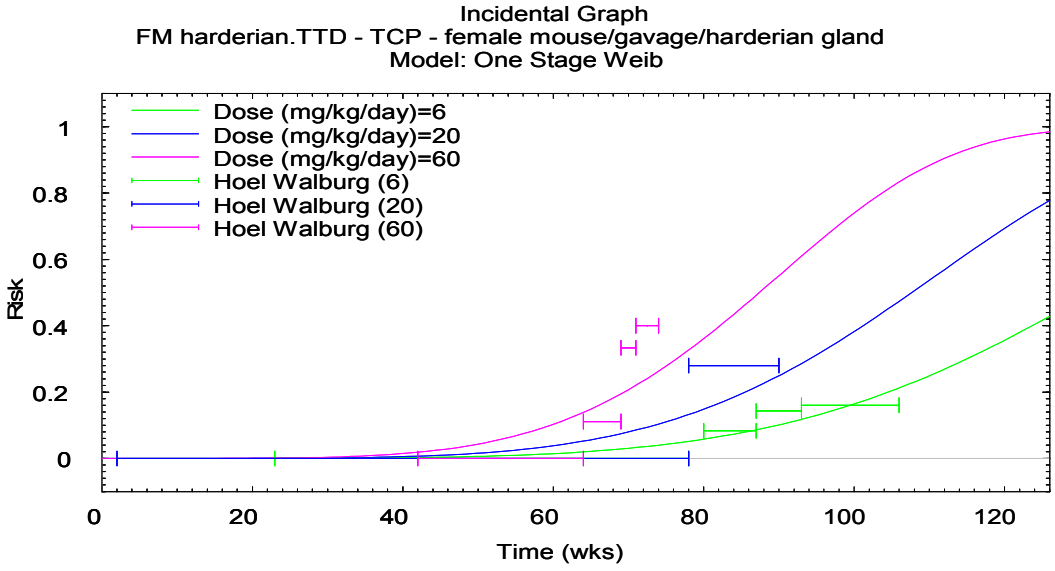
```
Dataset: M:\_ToxRiskData\Trichloropropane\FM harderian.TTD
Model: One Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -7.460252e+001

       Parameter Estimates :
                              Q 0 = 6.904433E-012
                              Q 1 = 3.003880E-012
                              Z   = 4.928402E+000
                              T0  = 0.000000E+000   Set by User
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	3
6	60	0	6
20	60	0	7
60	60	0	10

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	1.8987E-003	3.9648E-003	Not Reqstd
1.0000E-005	70.00	1.8987E-002	3.9648E-002	Not Reqstd
0.0001	70.00	1.8987E-001	3.9650E-001	Not Reqstd
0.0010	70.00	1.8996E+000	3.9668E+000	Not Reqstd
0.01	70.00	1.9082E+001	3.9848E+001	Not Reqstd
0.10	70.00	2.0004E+002	4.1774E+002	Not Reqstd



Female Mouse; Uterus Adenomas or Carcinomas

```
Dataset: M:\_ToxRiskData\Trichloropropane\FM uterus.TTD
Model: Two Stage Weib
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
Maximum Log-Likelihood =  -4.567713e+001

       Parameter Estimates :
                            Q 0 = 0.000000E+000
                            Q 1 = 5.955970E-023
                            Q 2 = 2.395006E-023
                            Z   = 1.000000E+001
                            T0  = 0.000000E+000
```

Avg. Doses (mg/kg/day)	Number of animals	Number with fatal tumors	Number with incidental tumors
0	60	0	0
6	60	0	5
20	60	0	3
60	60	0	11

Animal to human conversion method: MG/KG BODY WEIGHT(3/4)/DAY

Human Equivalent Dose Estimates (ug/kg/day)

Incid Extra Risk	Time (yr)	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	2.2635E-003	1.0593E-002	Not Reqstd
1.0000E-005	70.00	2.2635E-002	1.0589E-001	Not Reqstd
0.0001	70.00	2.2634E-001	1.0550E+000	Not Reqstd
0.0010	70.00	2.2617E+000	1.0192E+001	Not Reqstd
0.01	70.00	2.2459E+001	8.0875E+001	Not Reqstd
0.10	70.00	2.1205E+002	4.2138E+002	Not Reqstd

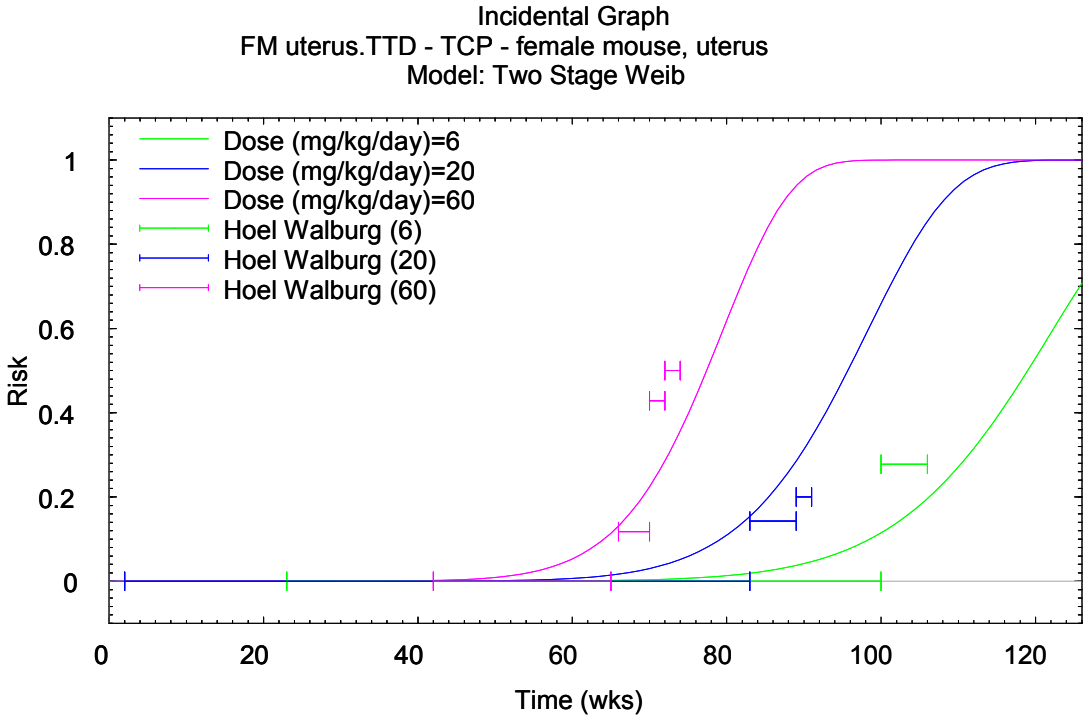


Table D-5. Summary of human equivalent overall cancer risk values estimated by R/BMD_R, based on male and female rat and mouse tumor incidence

Tumor site	Tumor context	Risk, R	BMD_R, mg/kg-day	$BMDL_R$, mg/kg-d	Risk value at BMD_R[a], per mg/kg-d	Oral slope factor[b], per mg/kg-d	SD	SD^2	Proportion of total variance	
Male rats										
Oral route squamous papillomas, carcinomas	Incidental	0.001	0.00049	0.00032	2.046	3.16	0.6765	0.4577		0.73
	Fatal	**0.001**	**0.00039**	**0.00030**	**2.551**	**3.29**	**0.4462**	**0.1991**	**0.55**	
Pancreas acinar tumors	Incidental	**0.001**	**0.0028**	**0.0010**	**0.353**	**1.00**	**0.3940**	**0.1552**	**0.43**	0.25
Preputial gland tumors	Incidental	**0.001**	**0.0127**	**0.0056**	**0.078**	**0.18**	**0.0614**	**0.0038**	**0.01**	0.01
Kidney tubule adenomas	Incidental	**0.001**	**0.6988**	**0.5259**	**0.015**	**0.11**	**0.0578**	**0.0033**	**0.01**	0.01
Skin: squamous papillomas, carcinomas	Incidental	**0.001**	**0.0319**	**0.0136**	**0.031**	**0.07**	**0.0256**	**0.0007**	**0.00**	0.00
Zymbal's gland carcinomas	Incidental	**0.001**	**0.1181**	**0.0484**	**0.008**	**0.02**	**0.0074**	**0.00006**	**0.00**	0.00
Hepatocellular tumors	Incidental	**0.001**	**0.1645**	**0.0115**	**0.006**	**0.09**	**0.0491**	**0.0024**	**0.00**	0.00
Sum, MLE cancer risks calculated at R=0.001[c]:					**3.04[c]** (2.54)		Sum, SD^2:	**0.365** (0.623)		
Upper bound on sum of risk estimates[e]:						**4.04** (3.84)	Overall SD[d]:	**0.603** (0.789)		
Female rats										
Oral route squamous papillomas, carcinomas	Incidental	0.001	0.00226	0.00053	0.442	1.894	0.8828	0.7793		0.992
	Fatal	**0.001**	**0.00238**	**0.00097**	**0.421**	**1.027**	**0.3681**	**0.1355**	**0.880**	
Mammary adenocarcinomas	Incidental	0.001	0.00575	0.00405	0.174	0.247	0.0442	0.0020		0.003
	Fatal	**0.001**	**0.00952**	**0.00332**	**0.105**	**0.302**	**0.1194**	**0.0143**	**0.093**	
Clitoral gland adenomas, carcinomas	Incidental	**0.001**	**0.00295**	**0.00230**	**0.339**	**0.435**	**0.0584**	**0.0034**	**0.022**	0.004
Zymbal's gland carcinomas	Incidental	**0.001**	**0.04691**	**0.01497**	**0.021**	**0.067**	**0.0277**	**0.0008**	**0.005**	0.001
Sum, MLE cancer risks calculated at R=0.001[c]:					**0.91[c]** (1.01)	**0.001**	Sum, SD^2:	**0.155** (0.786)		
Upper bound on sum of risk estimates[e]:						**1.53** (2.47)	Overall SD[d]:	**0.394** (0.886)		

Table D-5. Summary of human equivalent overall cancer risk values estimated by R/BMD_R, based on male and female rat and mouse tumor incidence

Tumor site	Tumor context	Risk, R	BMD_R, mg/kg-d	$BMDL_R$, mg/kg-d	Risk value at BMD_R[a], per mg/kg-d	Oral slope factor[b], per mg/kg-d	SD	SD^2	Proportion of total variance	
Male mice										
Oral route squamous papillomas, carcinomas.	Incidental	0.001	0.00029	0.00016	3.49	6.20	1.6439	2.703		0.99
	Fatal	**0.001**	**0.0030**	**0.0013**	**4.71**	**6.23**	**0.9226**	**0.851**	**0.96**	
Liver adenoma or carcinoma	Incidental	**0.001**	**0.0021**	**0.0013**	**0.48**	**0.77**	**0.1762**	**0.031**	**0.04**	0.01
Harderian gland	Incidental	**0.001**	**0.0107**	**0.0055**	**0.09**	**0.18**	**0.0548**	**0.003**	**0.00**	0.00
Sum, MLE cancer risks calculated at R=0.001[c]:					**5.29**[c] (4.07)		Sum, SD^2:	**0.885** (2.737)		
Upper bound on sum of risk estimates[e]:						**6.84** (6.79)	Overall SD[d]:	**0.94** (1.65)		
Female mice										
Oral route squamous papillomas, carcinomas.	Incidental	0.001	0.000030	0.000006	33.392	161.267	77.7353	6042.78		1.00
	Fatal	**0.001**	**0.000092**	**0.000037**	**10.861**	**26.935**	**9.7719**	**95.49**	**1.00**	
Liver adenoma or carcinoma	Incidental	**0.001**	**0.0030**	**0.0013**	**0.331**	**0.764**	**0.2632**	**0.07**	**0.00**	0.00
Harderian gland	Incidental	**0.001**	**0.0040**	**0.0019**	**0.252**	**0.526**	**0.1668**	**0.03**	**0.00**	0.00
Uterus	Incidental	**0.001**	**0.0102**	**0.0023**	**0.098**	**0.442**	**0.2091**	**0.04**	**0.00**	0.00
Sum, MLE cancer risks calculated at R=0.001[c]:					**11.5**[c] (34.1)		Sum, SD^2:	**95.63** (6042.9)		
Upper bound on sum of risk estimates[e]:						**27.6** (162)	Overall SD[d]:	**9.8** (77.7)		

[a]R/BMD_R
[b]$R/BMDL_R$
[c]Summary statistics in bold were calculated using the bolded table entries, including the "fatal" entries for the oral route (and mammary gland for female rats). The summary statistics in parentheses were calculated using the "incidental" entries for the oral route and the bolded entries for the other tumor sites.
[d]Overall SD = $(\text{Sum, } SD^2)^{0.5}$
[e]Upper bound on the overall risk estimate = sum of MLE cancer risks + 1.645 × overall SD

Source: NTP (1993).