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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)

October 2007

NOTICE

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
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMDL	Benchmark dose, 95% lower bound
BSO	1-Buthionine-(R,S)-sulfoximine
CASRN	Chemical Abstracts Service Registry Number
CBI	Covalent binding index
CPC	S-(3-chloro-2-hydroxypropyl)-L-cysteine
CYP450	Cytochrome P-450
DCA	1,3-Dichloroacetone
EPA	Environmental Protection Agency
GD	Gestation days
GMA	(S-glutathionyl)malonic acid
GSH	Reduced glutathione
HSDB	Hazardous Substances Data Bank
IRIS	Integrated Risk Information System
i.p.	Intraperitoneal
i.v.	Intravenous
K_{OW}	Oil/water partition coefficient
LDH	Lactate dehydrogenase
LOAEL	Lowest-observed-adverse-effect-level
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
NOAEL	No-observed-adverse-effect-level
NRC	National Research Council
NTP	National Toxicology Program
PBTK	Physiologically-based toxicokinetic
PD	Postnatal day
RACB	Reproductive Assessment by Continuous Breeding
RfC	Reference concentration
RfD	Reference dose
SD	Standard deviation
SDH	Sorbitol dehydrogenase
S-G	S-glutathionyl
WHO	World Health Organization

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to 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 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 subchronic exposure durations, and a carcinogenicity assessment.

The chronic RfD and chronic RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly 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 may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of average lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified.

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. 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 are derived from the application of a low-dose extrapolation procedure. Route-specific risk values are presented in some cases. The "oral slope factor" is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is an 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 Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA,

1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-life Exposures to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *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), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a, 2006), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) .

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 April 2007.

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 below (HSDB, 2005).

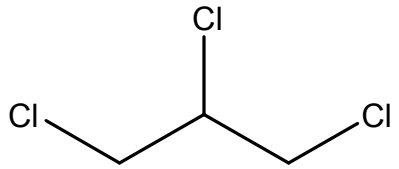


Figure 2-1. 1,2,3-Trichloropropane.

Chemical Formula:	$C_3H_5Cl_3$
Molecular Weight:	147.43
Melting Point:	-14.7° C
Boiling Point:	156.85° C
Density:	1.3889 g/mL (at 20° C)
Water Solubility:	1750 mg/L (at 25° C)
Log K_{OW}:	1.98 / 2.27
Vapor Pressure:	3.1 / 3.69 mm Hg at 25° C
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

Source: 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 by-product 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). 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 (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 ± 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. 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 of the radiolabel was sequestered in the liver, primarily as metabolites.

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 (CYP) or glutathione 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 glutathione creates a β-chlorothio ether, and internal displacement of another chlorine creates an episulfonium ion. This reactive ion could hydrolyze to a glutathione 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 glutathione conjugate and subsequent oxidation.

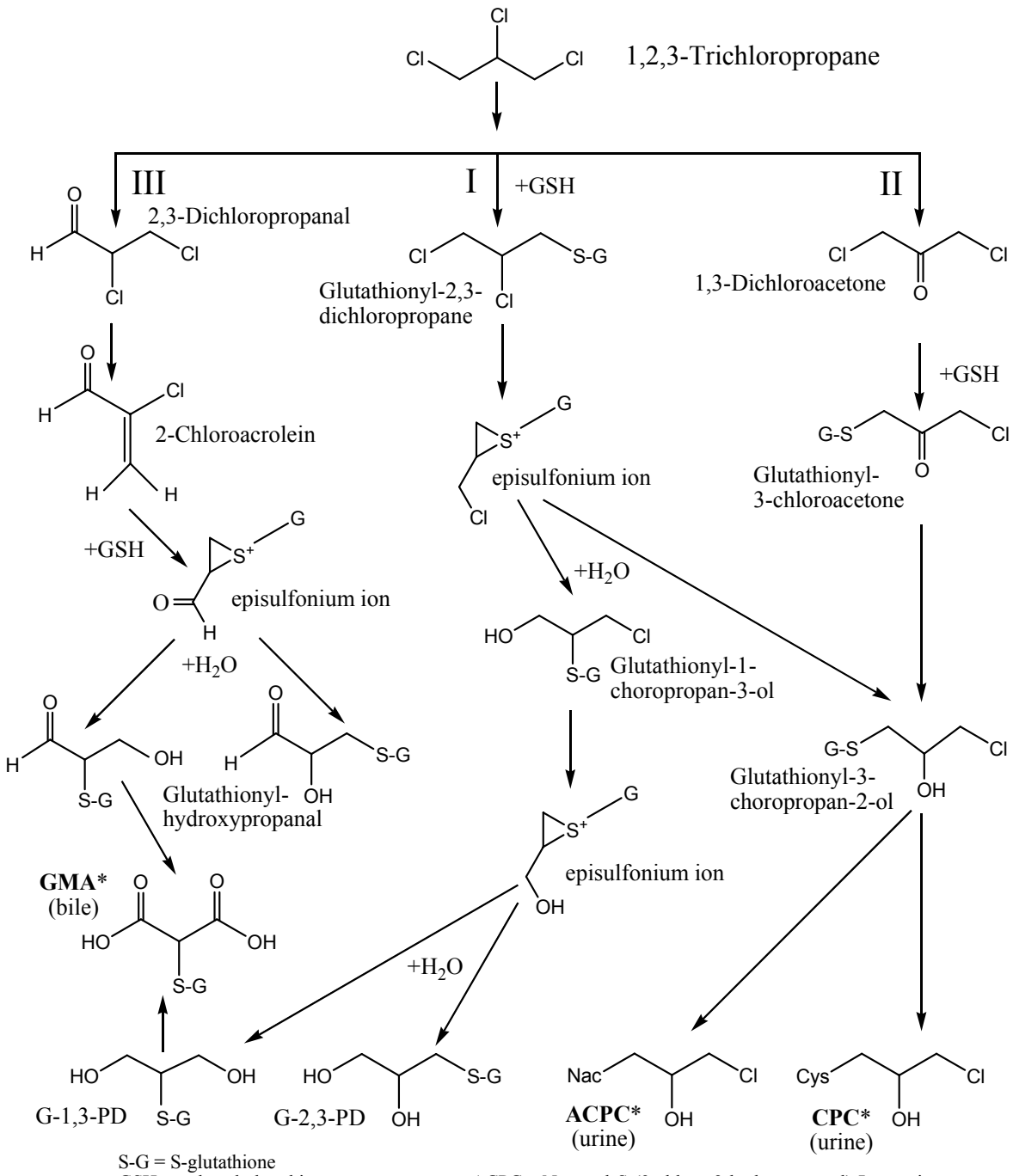


Figure 3-1. Possible metabolic pathways for 1,2,3-trichloropropane in rats

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

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

III) Oxidation, possibly by CYP 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 glutathione to form an episulfonium ion. This ion could then form 2-(S-glutathionyl)malonic acid (GMA) after the oxidation of the C2 and C3 carbon atoms to form carboxylic acids.

Evidence for the involvement of CYP 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 reduced nicotinamide adenine dinucleotide phosphate (NADPH) and was enhanced by the addition of such CYP inducers as phenobarbital and dexamethasone. Conversely, formation of 1,3-dichloroacetone was blocked by the CYP inhibitors SKF-525A and 1-aminobenzotriazol. In support of the Mahmood et al. (1991) scheme for the metabolic transformation of 1,2,3-trichloropropane, the findings of Weber and Sipes (1990) provide inferential evidence for the involvement of glutathione in 1,2,3-trichloropropane metabolism by the demonstration that experimental glutathione depletion was associated with increased 1,2,3-trichloropropane binding to hepatic protein and decreased binding to DNA.

3.4. ELIMINATION

Mahmood et al. (1991) and Volp et al. (1984) demonstrated that urine is the primary route of 1,2,3-trichloropropane excretion in rats and mice. Mahmood et al. (1991) analyzed the urine of F-344/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 radiolabel; after 24 hours 30% of the initial radiolabel had been exhaled, 40% had been released in the urine, and 18% 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 coadministration of [1,2,3-^{13}C]-trichloropropane and [2-^{14}C]-trichloropropane in soybean oil intraperitoneally to male F-344/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 contains seven compartments: blood, liver, kidney, adipose tissue, muscle, skin, and remaining distribution volume, and 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.

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 F-344/N rats and B6C3F1 mice. The findings of these subchronic studies were included in the National Toxicology Program 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, 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. 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,

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.

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% to 36% in males receiving 8 to 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; while absolute liver weights statistically significantly ($p \leq 0.05$) increased 17% to 61% in females receiving 16 to 125 mg/kg-day. 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; while absolute right kidney weights were statistically significantly ($p \leq 0.01$) increased 5 to 19% in males receiving 32, 63, or 125 mg/kg-day. 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; while absolute right kidney weights statistically significantly ($p \leq 0.01$) increased 11 to 25% in females receiving 63 to 125 mg/kg-day. NTP (1993) considered the changes in relative organ weights to be associated with the change in body weight, and not with organ toxicity. Absolute

heart weight was statistically significantly ($p \leq 0.01$) decreased 21% in male rats at 125 mg/kg-day.

Table 4-1. Relative organ weight changes in F-344/N rats receiving 1,2,3-trichloropropane by gavage for 120 days

Dose (mg/kg-day)	Change in mean relative liver weight		Change in mean relative right kidney weight	
	Male	Female	Male	Female
8	9%[a]	7%[a]	-1%[a]	7%[a]
16	12%	12%[d]	5%	7%
32	24%[d]	18%[d]	12%[c]	10%
63	47%[d]	37%[d]	26%[d]	32%[d]
125	78%[d]	105%[d]	54%[d]	43%[d]
250	NR[b]	NR	NR	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 ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[d] 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 organ weight changes in F-344/N rats receiving 1,2,3-trichloropropane by gavage for 120 days

Dose (mg/kg-day)	Change in mean absolute liver weight		Change in mean absolute right kidney weight	
	Male	Female	Male	Female
8	11% [a,d]	7% [a]	1% [a]	5% [a]
16	10%[d]	18%[d]	2%	11%
32	26%[d]	17%[c]	15%[d]	11%
63	23%[d]	32%[d]	5%[d]	25%[c]
125	19%[d]	61%[d]	19%[d]	11%[c]
250	NR	NR	NR	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 ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[d] showing statistically significant differences ($P \leq 0.01$) from the control group by Williams' or Dunnett's test

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

4-3). A time-dependent increase in the number of lesions was noted between the 8-week 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 one week.

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.

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.

Table 4-3. Incidence of liver, kidney, and nasal turbinate lesions in male and female F-344N rats in 17-week study

Endpoint	Dose (mg/kg-day)					
	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.

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, 1200%, 433%, and 1000%, 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 the 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 ($P \leq 0.05$).

In rats, hematocrit, hemoglobin, and erythrocyte counts were statistically significantly decreased by 1,2,3-trichloropropane treatment, but were not considered in this analysis to be biologically significant. At the 8-week sacrifice, hematocrit and red blood cell counts were significantly depressed, 13 to 23% and 10 to 18%, respectively, in males that received doses of 16 mg/kg-day or higher and in females that received doses of 8 mg/kg-day or higher. Hemoglobin was statistically significantly decreased 5-9% in male rats that received doses of 16 mg/kg-day or higher and female rats that received 63 mg/kg-day or higher.

The 17-week, less-than-lifetime rat study was conducted to determine appropriate doses for the two-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 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. 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 critical effect is hepatocellular necrosis in male rats, with a NOAEL of 16 mg/kg-day and a LOAEL of 32 mg/kg-day.

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 6 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, where as 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 relative and absolute liver weights was observed in males and females that received a dose of 125 mg/kg-day or higher. Mean relative liver weights were increased by 12% and 32% in males receiving 125 and 250 mg/kg-day, respectively, compared to controls (Table 4-4); 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 4-5). Mean relative liver weights were increased by 12% and 22% in females receiving 125 and 250 mg/kg-day, respectively, compared to controls; while absolute liver weights were statistically significantly ($p \leq 0.05$) increased at 125 and 250 mg/kg-day 24% at both doses. 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; while absolute right kidney weights were statistically significantly ($p \leq 0.05$) decreased 13% at 250 mg/kg-day. The changes in relative and absolute right kidney weights in male mice did not follow a clear dose-response pattern.

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 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 mg/kg-day to 125 mg/kg-day, with the decrease ranging from 6% to 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.

Table 4-4. Relative organ weight changes in B6C3F1 mice receiving 1,2,3-trichloropropane by gavage for 120 days

Dose (mg/kg-day)	Change in mean relative liver weight		Change in mean relative right kidney weight	
	Male	Female	Male	Female
8	1%[a]	1%[a]	3%[a]	-2%[a]
16	-3%	-8%	1%	-17%[c]
32	5%	3%	5%	-13%[c]
63	0%	4%	-10%	-11%[c]
125	10%[c]	12%[c]	-3%	-17%[c]
250	30%[c]	22%[c]	1%	-14%[c]

[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 4-5. Absolute organ weight changes in B6C3F1 mice receiving 1,2,3-trichloropropane by gavage for 120 days

Dose (mg/kg-day)	Change in mean absolute liver weight		Change in mean absolute right kidney weight	
	Male	Female	Male	Female
8	8%[a]	0%[a]	9%[a]	-2%[a]
16	3%	4%	7%	-2%
32	14%[b]	5%	14%	-10%
63	4%[b]	11%	-7%	-6%
125	22%[c]	24%[c]	6%	-7%
250	25%[c]	24%[c]	-3%	-13%[b]

[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.

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 4-6). 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.

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 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 4-6). 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.

Table 4-6. Incidence of liver, lung, and forestomach lesions in male and female B6C3F1 mice in 17-week study

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

[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.

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 two-year, 1,2,3-trichloropropane 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. The critical affect is liver necrosis in male and female mice, with a NOAEL of 63 mg/kg-day and a LOAEL of 125 mg/kg-day.

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 4-7). 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.

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 displaying an increased dose-response relationship in both male (2/10, 0/10, 1/10, 0/10, 9/10) and female rats (1/10, 1/10, 2/10, 3/10, 5/10).

Table 4-7. 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[a]	1/10[a]	2/10[a]	6/10[a]
	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.

Inflammation-associated myocardial necrosis was seen in all male dose groups, so the LOAEL for this affect is 1.5 mg/kg-day. The NOAEL and LOAEL for bile duct hyperplasia are 15 and 60 mg/kg-day. The NOAEL and LOAEL for plasma cell hyperplasia in the mandibular lymph node is 1.5 and 7.4 mg/kg-day for male rats, while the LOAEL is 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 1000 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 1000 mg/L exposure groups to calculate delivered doses of 18 and 149 mg/kg-day, respectively. The delivered dose for males in the 1000 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 1000 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 1000 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 1000 mg/L, respectively. Mean relative kidney weights in females were reportedly increased 14% and 34% in the 100 and 1000 mg/L treatment groups, respectively. Mean relative brain weights for the 1000 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 1000 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 1000 mg/L. Aniline hydroxylase activity was apparently significantly increased in males that received 1000 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 1000 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 1000 mg/L, but were still within the historical reference range for Sprague-Dawley rats from the laboratory. The critical effect for this investigation is the histological changes in the liver, kidney, and thymus, with a NOAEL of 15-20 mg/kg-day and a LOAEL of 113-149 mg/kg-day.

4.2.1.2. *Chronic Studies*

NTP (1993) conducted a 2-year study of the toxicity and carcinogenicity of 1,2,3-trichloropropane in F-344/N rats, the data of which was also published in Irwin et al. (1995). 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 to 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 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 4-8). 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 4-8. Survival rates and percent probability of survival for F-344/N rats exposed to 1,2,3-trichloropropane by gavage for two years

F-344/N Rats				
Dose (mg/kg-day)	**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 ($p\leq0.05$) 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 or higher. Absolute liver weights were significantly increased by 10%, 18%, and 38% in male rats and 14%, 16%, and 34% in female rats that received doses of 3, 10, and 30 mg/kg-day, respectively (Table 4-9b). 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 4-9a). Mean relative liver weights in female rats that received doses of 10 or 30 mg/kg-day were increased 12% and 40%, respectively (Table 4-9a).

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 or higher and female rats exposed to doses of 10 mg/kg-day or higher. 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 4-10b). Mean relative kidney weights in males from these treatment groups were increased by 4%, 10% and 29%, respectively (Table 4-10a). Mean relative kidney weights of females in the 10 and 30 mg/kg-day treatment groups were increased by 8% and 31% (Table 4-10a).

Table 4-9a. 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 Rats						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	31.2 ± 0.6[a]	–	10	30.8 ± 0.8[a]	–
3	10	33.1 ± 0.7	6%[b]	10	30.9 ± 0.6	0%
10	10	36.0 ± 0.7[c]	15%	8	34.6 ± 1.0[c]	12%[b]
30	8	39.8 ± 0.9[c]	28%	8	43.2 ± 0.7[c]	40%

[a] Mean ± standard error
[b] Percent change relative to control
[c] $p \leq 0.01$
[d] $p \leq 0.05$ by Williams' or Dunnett's test

Source: NTP, 1993.

Table 4-9b. Absolute liver 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 Rats						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	14.27 ± 0.37[a]	–	10	7.79 ± 0.13[a]	–
3	10	15.63 ± 0.37[d]	10%[b]	10	8.87 ± 0.31[c]	14%[b]
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] Mean ± standard error
[b] Percent change relative to control
[c] $p \leq 0.01$
[d] $p \leq 0.05$ by Williams' or Dunnett's test

Source: NTP, 1993.

Table 4-10a. Relative right kidney 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 Rats						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	2.96 ± 0.04[a]	–	10	3.08 ± 0.07[a]	–
3	10	3.09 ± 0.09	4%[b]	10	2.93 ± 0.07	-5%[b]
10	10	3.25 ± 0.05[c]	10%	8	3.34 ± 0.06[d]	8%
30	8	3.82 ± 0.05[c]	29%	8	4.04 ± 0.12[c]	31%

[a] Mean ± standard error
[b] Percent increase relative to control.
[c] $p \leq 0.01$
[d] $p \leq 0.05$ by Williams' or Dunnett's test

Source: NTP, 1993.

Table 4-10b. 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 Rats						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	1.35 ± 0.03[a]	–	10	0.786 ± 0.015[a]	–
3	10	1.46 ± 0.04[d]	8%[b]	10	0.839 ± 0.023	7%[b]
10	10	1.51 ± 0.03[c]	12%	8	0.869 ± 0.019[d]	11%
30	8	1.75 ± 0.05[c]	30%	8	0.971 ± 0.034[c]	24%

[a] Mean ± standard error
[b] Percent increase relative to control.
[c] $p \leq 0.01$
[d] $p \leq 0.05$ by Williams' or Dunnett's test

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 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 increased leukocytes was likely due to inflammation associated with the chemical-induced neoplasms (NTP, 1993).

An increase in the incidence of forestomach tumors was observed in all rat treatment groups (Table 4-11), 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 4-11). Male and female rats that received doses of 10 mg/kg-day 1,2,3-trichloropropane or higher had an increase in the incidence of oral cavity tumors (Table 4-11). In each male group that received doses of 10 mg/kg-day or higher, an increased incidence of renal tumors was observed. An increase was observed in females at both 10 mg/kg-day and 30 mg/kg-day for the clitoral gland tumors and at the 10 and 30 mg/kg-day for mammary gland tumors (Table 4-11). 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 4-11).

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.

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).

Table 4-11. Incidence of neoplasms in F-344/N rats chronically exposed to 1,2,3-trichloropropane by gavage

Tissue site/tumor type	Tumor incidence[a]							
	Males (mg/kg-day)				Females (mg/kg-day)			
	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.

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 rats is 3 mg/kg-day and in female rats the NOAEL is 3 mg/kg-day and the LOAEL is 10 mg/kg-day.

The NOAEL and LOAEL for relative right kidney weight in male and female rats is 3 and 10 mg/kg-day, respectively; while the LOAEL for absolute right kidney weight in male rats is 3 mg/kg-day and the NOAEL and LOAEL in female rats is 3 and 10 mg/kg-day, respectively. Tumors were evident in the oral cavity, forestomach, pancreas, kidney, Zymbal's gland of male and female rats, along with preputial gland tumors in males and clitoral gland and mammary gland tumors in females. The critical effect for non-cancer data is liver and right kidney weight change, while the critical effect for the cancer data is tumor development in the aforementioned organs.

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. Mice were treated with 0, 6, 20, or 60 mg/kg-day, and after 15 months (65–67 weeks), 8 to 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, 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, lactate dehydrogenase (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 or higher (Table 4-12). 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 4-12. Survival rates and percent probability of survival for B6C3F1 mice exposed to 1,2,3-trichloropropane by gavage for two year

B6C3F1 Mice				
Dose (mg/kg-day)	**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 4-13a). Other significant changes in organ weights among mice that received this dose included increased relative kidney weights in females (21%) (Table 4-14a), 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 (Table 4-13b, 4-14b).

Table 4-13a. Relative liver weights (mg organ weight/ g body weight) and percent change in B6C3F1 mice chronically exposed to 1,2,3-trichloropropane by gavage

B6C3F1 Mice						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	38.9 ± 1.9[a]	–	10	34.4 ± 0.8[a]	–
6	9	36.2 ± 1.5	-7%[b]	10	34.7 ± 1.1	1%[b]
20	8	44.6 ± 6.2	15%	9	35.7 ± 0.6	4%
60	5	51.2 ± 4.8[d]	32%	5	48.3 ± 2.8[c]	40%

[a] Mean ± standard error
[b] Percent change relative to control
[c] $p\leq0.01$
[d] $p\leq0.05$ by Williams' or Dunnett's test

Source: NTP, 1993.

Table 4-13b. Absolute liver weights (grams) and percent change in B6C3F1 mice chronically exposed to 1,2,3-trichloropropane by gavage

B6C3F1 Mice						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	1.72 ± 0.09[a]	–	10	1.49 ± 0.03[a]	–
6	9	1.63 ± 0.08	-5%[b]	10	1.33 ± 0.03[d]	-11%[b]
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] Mean ± standard error
[b] Percent change relative to control
[c] $p \leq 0.01$
[d] $p \leq 0.05$ by Williams' or Dunnett's test

Source: NTP, 1993.

Table 4-14a. Relative right kidney weights (mg organ weight/ g body weight) and percent change in B6C3F1 mice chronically exposed to 1,2,3-trichloropropane by gavage

B6C3F1 Mice						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	8.0 ± 0.25[a]	–	10	4.99 ± 0.09[a]	–
6	9	7.67 ± 0.41	-4%[b]	10	5.27 ± 0.14	6%[b]
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[c]	21%

[a] Mean ± standard error
[b] Percent increase relative to control.
[c] $p \leq 0.01$
[d] $p \leq 0.05$ by Williams' or Dunnett's test

Source: NTP, 1993.

Table 4-14b. Absolute right kidney weights (grams) and percent change in B6C3F1 mice chronically exposed to 1,2,3-trichloropropane by gavage

B6C3F1 Mice						
Dose (mg/kg-day)	**n**	**Males**		**n**	**Females**	
0	10	0.353 ± 0.011[a]	–	10	0.217 ± 0.006[a]	–
6	9	0.344 ± 0.019	-3%[b]	10	0.203 ± 0.006	-6%[b]
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] Mean ± standard error
[b] Percent increase relative to control.
[c] $p \leq 0.01$

[d] $p \leq 0.05$ by Williams' or Dunnett's test

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).

In mice, the sites of statistically ($p \leq 0.001$) significant neoplasm formation for both sexes were the forestomach and liver (Table 4-15). 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 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.

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.

Table 4-15. Incidence of neoplasms in B6C3F1 mice chronically exposed to 1,2,3-trichloropropane by gavage

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

The NOAEL and LOAEL for relative liver weight change in male and female mice is 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 is 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 lead 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 non-cancer data is weight change in the liver and right kidney, while the critical effect for the cancer data is 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 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. Brain and kidney weights and organ/body ratios were increased in the 300 and 600 ppm treatment groups. Ovary weights and organ/weight ratios were decreased in the 300 and 600 ppm groups, and spleen weights and organ/weight ratios and 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 4-16) 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 4-17) 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 4-16. 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

Male					
Dose (ppm)	**n**	**Absolute[a]**		**Relative[b]**	
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					
Dose (ppm)	**n**	**Absolute[a]**		**Relative[b]**	
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 ± standard deviation
[b] Percent increase relative to control.
[c] $p \leq 0.01$

Source: Biodynamics, Inc., 1979.

Table 4-17. 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

Male					
Dose (ppm)	**n**	**Absolute**		**Relative**	
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					
Dose (ppm)	**n**	**Absolute**		**Relative**	
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 ± standard deviation
[b] Percent increase relative to control.
[c] $p \leq 0.01$

Source: Biodynamics, Inc., 1979.

A number of histopathologic lesions were observed (Table 4-18), 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 4-18. 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	Male rats (ppm via inhalation)					
	0	**0.5**	**1.5**	**5**	**15**	**50**
Peribronchial lymphoid hyperplasia	0/15	0/15	0/15	6/15	11/15	10/15
Hepatocellular hypertrophy	0/15	0/15	0/15	13/15	15/15	15/15
Hematopoiesis of the spleen	0/15	0/15	0/15	ND	ND	5/15
	Female rats (ppm via inhalation)					
	0	**0.5**	**1.5**	**5**	**15**	**50**
Peribronchial lymphoid hyperplasia	1/15	0/15	0/15	5/15	4/15	6/15
Hepatocellular hypertrophy	0/15	0/15	0/15	ND	ND	0/15
Hematopoiesis of the spleen	5/15	0/15	0/15	7/15	9/15	13/15

ND = no data.

Source: 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 are 5 and 15 ppm, respectively, while the NOAEL for decreased terminal body weight in male rats is 50 ppm. The LOAEL for increased absolute and relative liver weight in male rats is 5 ppm, while the NOAEL and LOAEL for increased absolute liver weight in female rats is 15 and 50 ppm, respectively, and the NOAEL and LOAEL for increased relative liver weight in females is 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-weeks study with doses up to 50 ppm. Treatment-related histopathological findings were not observed in any tissue examined (Table 4-18).

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 TCP exposure.

This second 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.

Miller et al. (1987a, b) conducted two inhalation studies of male and female F-344/N rats and B6C3F1 mice. These unpublished studies were submitted to the EPA. In the first rat and mouse study (Miller et al., 1987a), five animals/sex/group were exposed to a target concentration of 0, 10, 30, and 100 ppm 6 hours/day, 5 days/week, for 9 days, with a measured concentration of 0, 13 ± 0.5, 40 ± 0.4, or 132 ± 0.6 ppm (0, 78, 241, and 796 mg/m^3). Evaluation endpoints 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; and 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. At the highest exposure concentration, relative liver weights were increased by 27% and 42% in male and female rats, respectively, when compared with controls.

The concentrations of serum albumin and total protein were statistically significantly ($p \leq 0.05$) increased in both sexes of rats in the high exposure group, except for total protein in female rats, 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 in packed cell volume (hematocrit), 6% increase, and hemoglobin, 5% increase, was 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 treatment stress-related decrease in thymus size among rats. Very slight hepatocellular necrosis was observed in all male rats exposed to 132 ppm, with very slight depletion of lymphoid elements in the spleen 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 4-19). 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 4-20).

Table 4-19. 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

Severity[a]	Males (ppm)							Females (ppm)						
	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

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

Table 4-20. 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

Severity[a]	Males (ppm)							Females (ppm)						
	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

Source: 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; and 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 histopathologically-related changes were absent.

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, although the number of platelets in male and female mice at 132 ppm increased statistically significantly ($p \leq 0.05$) 25 and 42%, respectively.

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 noted in all mice that were exposed to this concentration. 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 noted (Table 4-21). There was a dose-related increase in inflammation in the olfactory epithelium of the nasal turbinates (Table 4-22) accompanied by the exudation of inflammatory cells into the nasal cavity lumen.

Table 4-21. Incidence and severity of decreased thickness and degeneration of the olfactory epithelium in the nasal turbinates in B6C3F1 mice exposed via inhalation to 1,2,3-trichloropropane

Severity[a]	Males (ppm)							Females (ppm)						
	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

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

Table 4-22. Incidence and severity of inflammation of the olfactory epithelium in the nasal turbinates of B6C3F1 rats exposed via inhalation to 1,2,3-trichloropropane

Severity[a]	Males (ppm)							Females (ppm)						
	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

[a] inflammation, bilateral and multifocal

Source: 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 adversely 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 4-19). Very slight inflammation in the olfactory epithelium was also evident in rats exposed to 0, 1, 3, and 10 ppm (Table 4-20). 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 4-21). Very slight inflammation in the olfactory epithelium was also observed in mice at 10 ppm (Table 4-22). 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.

4.2.2.2. 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 (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 4th to 5th 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.

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.

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

Litter	Dose group (mg/kg-day)							
	0		30		60		120	
	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 (% fertile).
[b] Significantly different ($p<0.05$) from the control group by Dunn's or Chi-square test.

Source: NTP, 1990.

Task 3, a one-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 ($p\leq0.05$) 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 ($p\leq0.05$) lower, 5 and 8%, respectively, than those of controls. The absolute kidney weights of treated F_0 females were statistically significantly ($p\leq0.05$) 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. 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 ($p\leq0.05$) decreases, 78 and 43% of controls, in the indices

for mating (# of females with plug/ # of cohabiting pairs) and fertility (# of fertile pairs/ # 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 to 11%, in the 60 and 120 mg/kg-day groups. There was a statistically significant ($p \leq 0.05$) increase, 17 to 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 to 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 NOEAL and LOAEL of 30 and 60 mg/kg-day, respectively, was 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 provides 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 is 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 was 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 (PD) 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 PDs 4 and 14, and when they were sacrificed on PD 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 mated out of 20 at 5 ppm and 10 mated out of 20 at 15 ppm, compared with 17 mated females out of 20; although fewer females in the high concentration group mated, no statistical significance was evident. 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, respectively, but not statistically significant mating indices. Fertility indices were unaffected by trichloropropane exposure. There was no treatment-related effect on litter and pup data. Histopathological evaluation of the testes, epididymis, and ovaries did not identify any treatment-related changes.

In the second study, adverse effects on mating performance and fertility indices due to 1,2,3-trichloropropane were not observed. 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 was 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 (MAK, 1998). A 4-hour LC_{50} of approximately 500 ppm (3000 mg/m^3) has been determined for rats and mice (MAK, 1998). 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, intraperitoneal injection to 5 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. Waterborne 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, 9.0, or 18.0 mg/L. The guppies were exposed for 16 months and the medaka for 13. 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 9.0 and 18.0 mg/L groups (NTP, 2005). At the 9 month evaluation, the incidence of choliangiocarcinomas was significantly increased in 9.0 and 18.0 mg/L males. The incidence of choliangiocarcinomas was significantly increased in all exposed males and females after 13 months, while the incidence of hepatocholangiocarcinomas was significantly increased only in the fish exposed to 18.0 mg/L 1,2,3-trichloropropane. The incidence of papillary adenomas of the gallbladder was significantly increased in the 9.0 and 18.0 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. It 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 18.0 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

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 F-344/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]TCP equivalents/mg, and RNA, 432 ± 74 pmol [^{14}C]TCP equivalents/mg, and approximately half as much was bound to DNA, 244 ± 29 pmol [^{14}C]TCP 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 glutathione (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 reduced glutathione (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 TCP 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. TCP or its metabolite(s) appear to conjugate with glutathione (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 (4 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 two 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 F-344/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 irrespective of the tissue type, and was determined to be S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione (La et al., 1995). A proposed formation pathway involves the biological activation, possibly by conjugation with glutathione, 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 F-344/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 4-24). For example, dose-dependent adduct formation was demonstrated in the forestomach of F-344/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 4-24. Comparison of tumor incidence and DNA-adduct formation in male F-344N rats and B6C3F1 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	ND[d]
	30	16/50	ND[d]
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., 1993.
[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.
[d] not detected.

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. This adduct is unusual in that it crosslinks a physiological oligopeptide, reduced glutathione, 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 glutathione 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.

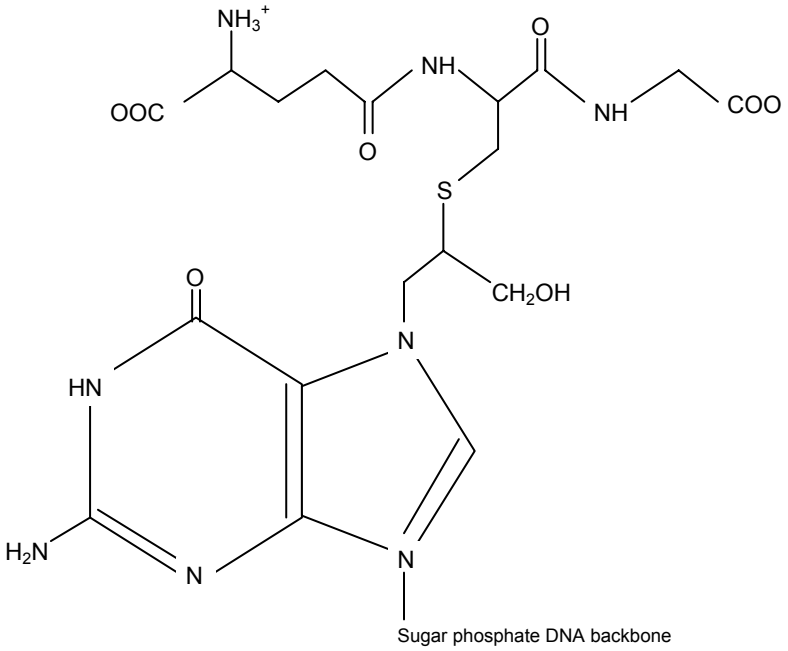


Figure 4-1. Structure of the DNA adduct S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione

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 4-25, 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. 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 4-25. Formation of DNA adducts by [14C]-1,2,3- trichloropropane (6 mg/kg-day) administered to B6C3F1 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]
Non-target 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 the presence of metabolic enzymes, also known as S9 fraction (Table 4-26). 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 a dose-dependent increase in revertant colonies in the presence of S9 fraction in tester stains: TA98, at 200 and 2000 µg/plate; TA100, at 20, 200, and 2000 µg/plate; and TA1537, at 200 and 2000 µg/plate. These investigators also detected revertants, at 200 and 2000 µg/plate in TA 1535 both in the presence and in the absence of an S9 fraction, with a greater number of revertants in the plates with microsomal activation.

NTP (1993) tested strains at doses of 3, 10, 33, 100, µg/plate or 333 and 10, 33, 100, 333, 666, 667, and 1000 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 a dose-dependent increase 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 non-activated 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 20-1000 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 2000 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 TCP 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).

Table 4-26. Genotoxicity bioassays of 1,2,3-trichloropropane

In Vitro Gene Mutation Assays					
Test System	**Cells/strain**	**Positive concentrations**	**Results**		**Reference**
			-S9	**+S9**	
(a) Bacterial Assays					
S. typhimurium (Ames test)	TA100, A1535	10, 33, 100, 333 µg/plate	–	+	Haworth et al., 1983
	TA1537, TA98		–	–	
	TA98	200, 2000 µg/plate	–	+	Shell Oil Co., 1979
	TA100	20, 200, 2000 µg/plate	–	+	
	TA1537	20, 200 µg/plate	–	+	
	TA1535	200, 2000 µg	–	+	
	TA1538		–	–	
	TA97, TA100, TA1535	10, 33, 100, 333 µg/plate	–	+	NTP, 1993
	TA98	100, 333 µg/plate	–	+	
	TA1537		–	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		–	–	von der Hude et al., 1988
E. coli (DNA-repair deficient strain)	WP2 uvrA	2000 µg/plate	–	+	Shell Oil Co., 1979
E. coli (DNA-repair-proficient strain)	WP2	N/A	–	–	

(b) Mammalian Cell assays					
Mouse Lymphoma	L5178Y	0.01, 0.02, 0.03, 0.04, 0.05, 0.06 µl/ml	–	+	NTP, 1993
	L5178Y	2.4, 3.2, 4.2, 5.6, 7.6, 10, 13, 18 µg/ml	NP	+	Shell Oil Co., 1982
In Vitro Chromosomal Damage Assays					
Test System	**Cells/strain**	**Positive concentrations**	**Results**		**Reference**
			-S9	**+S9**	
(a) Mammalian Cells					
Chromosomal Aberrations	CHO cells	59.5, 69.4, 79.2 µg/mL	–	+	NTP, 1993
	Rat liver epithelial	N/A	–	–	Shell Oil Co., 1979
Micronucleus	CHO	N/A	+ [a]		Douglas et al., 1985 (abstract)
	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 (UDS)	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
	CHO	N/A	+ [a]		Douglas et al., 1985 (abstract)
	V79	0.3, 1.0 mM	–	+	von der Hude et al., 1987
(b) Others: Cell transformation	Syrian Hamster embryo	N/A	+	NP	Hatch et al., 1983 (abstract)

In Vivo Bioassays				
Test System	**Cells/organs**	**Positive Doses**	**Results**	**Reference**
Chromosomal damage: mammalian				
Micronucleus	CD-1 mice, bone marrow erythrocytes	N/A	–	Crebelli et al., 1999
	Mouse bone marrow	N/A	–	Douglas et al., 1985 (abstract)
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
UDS	F344/N rats (male) hepatocytes	N/A	–	Mirsalis et al., 1983 (abstract)
DNA adducts	F344/N male rat, fore-stomach and liver	N/A	+	La and Swenberg, 1997 (abstract)
	F/344/N male rat (multiple organs)	3 or 30 mg/kg	+	La et al., 1995
	B6C3F1 male mice (multiple organs)	6 or 60 mg/kg	+	
Other in vivo assays				
Dominant lethal mutation	SD male rats, Implants and embryos	N/A	–	Saito-Suzuki et al., 1982
Wing spot test	Drosophila melanogaster	4.51μg/L (inhalation)	+	Chroust et al., 2007
High frequency of activating mutations in ras oncogenes	B6C3F1 mice Forestomach	N/A	+	Ito et al., 1996 (abstract)
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

N/A: Either chemical had no effect or information is not available (abstracts only)
NP: Assay is not performed
[a] Metabolic enzyme induction not specified

Mammalian cell assays

1,2,3-Trichloropropane has also been shown to induce genotoxic effects in cultured mammalian cells (Table 4-26). NTP (1993) conducted cytogenetic analysis in Chinese hamster ovary cells, and the results indicated that 1,2,3-trichloropropane induced both sister chromatid exchanges (SCEs), 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 1000 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). In a study by Douglas et al. (1985), sister chromatid exchanges and micronuclei were reported to be induced in CHO cells following 1,2,3-trichloropropane exposure, although the doses tested and induction levels were not specified. 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 SCE 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 8-fold, while the increase in MCL-5 was approximately 4-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). Hatch et al. (1983) reported that 1,2,3-trichloropropane enhanced DNA viral transformation in Syrian hamster embryo cells. 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 4-26). 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 one 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 one week, 0.08 mg/L for 2 weeks, and 0.002 mg/L for 3 months. After the one 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 F-344/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 F-344/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, and liver in male rats and the forestomach, liver, lung and kidney 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.

Ito et al. (1996) analyzed the forestomach tumors in the B6C3F1 mice (from the NTP, 1993 bioassay) for *ras* gene mutations. The DNA was isolated from the paraffin-embedded forestomach tumor sections and amplified by polymerase chain reaction. Ten of the 16 forestomach tumors contained highly specific H-*ras* or K-*ras* activating mutations, of which 6 tumors had H- *ras* mutations at codon 61 and 4 with K- *ras* mutations at codon 13. These mutations are not consistent with the miscoding properties of S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione, the major DNA adduct, and may indicate that another mechanism may be involved.

La and Swenberg (1997) analyzed forestomach and liver DNA from F344/N rats exposed to 1,2,3-trichloropropane for one week at 30 mg/kg-day by corn oil gavage, to examine whether 1,2,3-trichloropropane induces an increase in the concentration of endogenous DNA adducts. Following the exposure, the DNA adducts identified were 8-hydroxydeoxyguanosine, 1,N^6-ethenodeoxyadenosine, and 3,N^4-ethenodeoxycytidine. It was hypothesized from this

investigation that bolus doses of trichloropropane may saturate or deplete cellular defense mechanisms and increase the concentration of promutagenic lesions (La and Swenberg, 1997).

Male MOL:WIST rats were killed 1 hour after receiving 1,2,3-trichloropropane by ip 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). Douglas et al. (1985) also found negative results in the micronucleus test in mouse bone marrow in vivo, although the doses tested were not specified. 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). 1,2,3-Trichloropropane did not induce unscheduled DNA synthesis in hepatocytes from male Fischer-344 rats (Mirsalis et al., 1983)

4.5.3. Structure-Activity Relationships

Halogenated propanes as a class of compounds are generally found to be positive in assays which indicate mutagenicity (Lag et al., 1994; Ratpan and Plaumann, 1988), and there is evidence that at least one other member of this group, 1,2-dibromo-3-chloropropane (DBCP), is carcinogenic in whole animal models (NTP, 1982; NCI, 1978). In a study sponsored by the National Cancer Institute (NCI), DBCP was found to be carcinogenic to Osborne-Mendel rats and B6C3F1 mice when administered by gavage in corn oil at 15 or 29 mg/kg-day for up to 78 weeks and 114 or 219 mg/kg-day for up to 60 weeks, respectively (NCI, 1978). Neoplasm formation in the forestomach resulted in reduced survival in both species. These responses are qualitatively similar to those produced by 1,2,3-trichloropropane. A 2-year inhalation study of DBCP in F-344/N rats and B6C3F1 mice (NTP, 1982) found increased incidences of nasal, bronchial, and oral tumors in both sexes of the experimental animals.

The proposed metabolism of 1,2,3-trichloropropane is very similar to the metabolism of 1,2-dibromo-3-chloropropane (DBCP), involving mixed function oxidase catalysed oxidation and episulfonium ion formation (NTP, 1993). DBCP is a renal and testicular toxicant at acute doses, leads to tumor development in the forestomach, nasal turbinates and livers of rats and mice at chronic doses, and forms the same major DNA adduct, S-[1-(hydroxymethyl)-2-(N^7-

guanyl)ethyl]-glutathione, as 1,2,3-trichloropropane (Humphreys et al., 1991). DBCP also induced a dose-related increase in the incidence of aberrant cells in the spermatogonial and bone marrow cells of male rats after gavage administration of 0.73, 7.3 and 73 mg/kg-day for 5 days (Kapp, 1979).

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral Exposure

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.

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 metabolic products in urine, feces, or by exhalation (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 (Figure 3-1). Most of the metabolites are rapidly cleared from the body, although a fraction of the metabolites have been found to bind to intracellular proteins and nucleic acids (Weber, 1991; Weber and Sipes, 1991, 1990). It is not clear what impact this binding has on the etiology of noncancer effects, but the formation of DNA-adducts is thought to play an important role in the carcinogenic activity of the chemical (see Section 4.7.3).

The NTP (1993) toxicology and carcinogenesis studies conducted in F-344/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 Villeneuve et al. (1985), which provided 1,2,3-trichloropropane to rats via drinking water.

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, or greater, and in mice receiving a dose of 6 mg/kg-day or greater. Because the decrease in survival was associated with the increased incidence of tumors (NTP, 1993) it was determined that the decrease in survival should not be considered a noncancer effect. However, it is important to note that the non-neoplastic 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/kd-day.

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.

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. 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.

Similar effects to those outlined above following subchronic exposure were not observed in the chronic NTP (1993) studies which employed doses lower than those reported to produce these effects in the subchronic studies. This was most likely due to decreased survival attributable to the on-set of cancer in the chronic study. Histopathic lesions were 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. Travlos et al. (1996) point out in an investigation into treatment-related increases in clinical chemistry endpoints and histopathological findings, that treatment-related alteration in clinical chemistry is highly associated with histopathological changes.

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 studies (1993); 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 likely 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 Exposure

No inhalation studies of 1,2,3-trichloropropane in humans have been reported. 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 non-neoplastic 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 females was observed during gestation and lactation.

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, 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 non-neoplastic 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 high-dose group males, and hematopoiesis of the spleen in the highest dose group males and in the three high-dose group 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 4-17 and 4-18). Similar effects were also observed in mice that were exposed to 10, 13, 40 or 132 ppm concentrations (Tables 4-19 and 4-20).

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 4-18). 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-trichloropropane 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 Carcinogenic 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, and Harderian gland 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. US EPA's *Guidelines for Carcinogenic Risk Assessment* (2005) 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 non-neoplastic 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. This information suggests that 1,2,3-trichloropropane is likely to be carcinogenic by the inhalation route of exposure.

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 F-344/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 to 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.

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 carcinogenicity of a structural analogue of 1,2,3-trichloropropane, 1,2-dibromo-3-chloropropane

(NTP, 1982), which produces the same DNA adducts as 1,2,3-trichloropropane (Humphreys et al., 1991).

4.7.3. Mode of Action Information

4.7.3.1. Hypothesized Mode of Action

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. However, although there are in vitro data indicating that 1,2,3-trichloropropane may be genotoxic, there is a lack of in vivo information linking a mutagenic mode of action to the observed carcinogenicity in animal bioassays.

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 negative 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 lacking.

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 with DNA was presented in vivo (Weber and Sipes, 1990), in which the ability of 1,2,3-trichloropropane metabolites to form

covalent bonnds with hepatic DNA, RNA, and proteins in rats following intraperitoneal administration was apparent. However, the levels of radioactivity bound to DNA at 72 hours post administration, were below the level measured for one 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 F-344/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 glutathione in the activation and binding of a metabolite of 1,2,3-trichloropropane is supported by the pretreatment of Sprague-Dawley rats with buthionine sulfoximine (BSO) (Weber and Sipes, 1990). BSO pretreatment causes a decrease in hepatic glutathione in rats and a subsequent decrease in TCP- or reactive metabolite-binding to DNA. 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 F-344/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 4-24). The target organs of TCP-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 seen 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 glutathione, 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 (Tafazoli and Kirsch-Volders, 1996; Lag et al., 1994; NTP, 1993; Ratpan and Plaumann, 1988; von der Hude et al., 1987; Douglas et al., 1985; Hatch et al., 1983; Haworth et al., 1983; Kier, 1982; Stolzenburg and Hine, 1980; Shell Oil Co., 1979, 1982). In the absence of the enzyme-rich S9 fraction mutagenic activity is typically not observed. 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 Chinese Hamster ovary cell or V79 assays (NTP, 1993; von der Hude et al., 1987; Douglas et al., 1985), 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), 1,2,3-trichloropropane enhanced DNA viral transformation in Syrian hamster embryo cells (Hatch et al., 1983), and 1,2,3-trichloropropane induced micronucleus formation in the mammalian cell lines, AHH-1, MCL-5, and h2E1 (Doherty et al., 1996) and CHO cells (Douglas et al., 1985). 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 somatic mutation and recombination test (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. (1974, 1977) 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).

Ito et al. (1996) analyzed the forestomach tumors in the B6C3F1 mice from the NTP, 1993 bioassay for *ras* gene mutations. Ten of the 16 forestomach tumors contained highly specific H-*ras* or K-*ras* activating mutations, of which 6 tumors had H-*ras* mutations at codon 61 and 4 with K-*ras* mutations at codon 13. These mutations are not consistent with the miscoding properties of S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione, the major DNA adduct of 1,2,3-trichloropropane, and indicates that another mode of action may be involved. La and Swenberg (1997) observed an increase in the concentration of the endogenous DNA adducts, 8-hydroxydeoxyguanosine, 1,N^6-ethenodeoxyadenosine, and 3,N^4-ethenodeoxycytidine, in rats following 1,2,3-trichloropropane exposure for one week.

1,2,3-Trichloropropane tested negative in bacterial and mammalian cell 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 *Aspergillus nidulans* diploid strain P1 assay for aberrant mitotic segregation (Crebelli et al., 1992). Mammalian cell assays in which 1,2,3-trichloropropane tested negative 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 (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 negatively included the bone marrow micronucleus formation assay in CD-1 mice (Crebelli et al., 1999) and in an unspecified mouse strain (Douglas et al., 1985), the induction of unscheduled DNA synthesis in F344/N rats (Mirsalis et al., 1983), and the dominant lethal induction assay in male SD 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, 1,3-dichloroacetone (DCA), which the study authors characterized as a direct-acting mutagen. 1,3-Dichloroacetone, also referred to as 1,3-dichloropropanone or 1,3-dichloro-2-propanone, has shown mutagenicity in *Salmonella typhimurium* TA100 without microsomal activation (Meier et al., 1985). 1,3-Dichloroacetone 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).

1,3-Dichloroacetone 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 percentage of tumor-bearing mice after a single initiating dose of 37.5, 75, or 150 mg/kg 1,3-dichloroacetone was 47, 47 and 68%, respectively. The percentage of tumor-bearing mice after repeated doses of 300, 450, or 600 mg/kg 1,3-dichloroacetone 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 1000 μg/plate, and indicate that point mutations are the most consistent type of genetic alteration induced by 1,2,3-trichloropropane and occur 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 F-344/N rats, and found high concentrations of DNA adducts at 6 hours post-administration 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). Polyploidy was apparent in the hepatocytes of male albino rats dosed with 0.8 and 2.16 mg/L for two hours (Belyaeva et al., 1974), and a dose-dependent increase in DNA strand breaks was evident in hepatocytes from F344 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). Ito et al. (1996) observed a high frequency of activating mutations in *ras* oncogenes in the forestomach tumors from the NTP (1993) bioassay. A dose-dependent increase in the incidence of tumors was observed in rats from 3 to 30 mg/kg-day and in mice from 6 to 60 mg/kg-day (NTP, 1993). The in vivo data demonstrates 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 may be a key event in the mutagenic mode of action. 1,2,3-Trichloropropane metabolism follows three potential routes, each of which involves glutathione 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, 1,3-dichloroacetone (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, 1987; Douglas et al., 1985; Hatch et al., 1983; Haworth et al., 1983; Kier, 1982; Stolzenburg and Hine, 1980; Shell Oil Co., 1979, 1982).

Evidence for the direct interaction of 1,2,3-trichloropropane and its metabolites with DNA, RNA, and hepatic proteins was observed 4 hours following intraperitoneal administration of 1,2,3-trichloropropane (Weber and Sipes, 1990). This investigation demonstrates the ability of 1,2,3-trichloropropane or a reactive metabolite 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 F-344/N rats administered 1,2,3-trichloropropane by ip injection, thus demonstrating that 1,2,3-trichloropropane, or a reactive metabolite, causes 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 F-344/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 4-24). Binding of 1,2,3-trichloropropane metabolites to DNA is currently the most likely theory for the mode of action of the 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 non-tumor 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 non-tumor forming organs.

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. Holme et al. (1989) found that 1,2-dibromo-3-chloropropane (DBCP), a structurally-related compound to 1,2,3-trichloropropane, induced DNA damage in liver cells at concentrations much lower than concentrations that resulted in cytotoxicity and bacterial (*S. typhimurium*) mutagenicity. CYP450 and glutathione transferase appeared to be involved in the damage to cellular DNA caused by DBCP, with the CYP450 dependent oxidation also resulting in bacterial mutagenicity. The activation of CYP450 may result in the in vitro mutagenicity, while the DNA damage and cytotoxicity of DBCP in vivo may be glutathione-dependent (La et al., 1995).

Data are available that indicate that the bolus exposure to 1,2,3-trichloropropane may overwhelm cellular glutathione levels in the forestomach and induce lipid peroxidation (La and Swenberg, 1997; Ito et al., 1996). This lipid peroxidation leads to an increase in the etheno DNA adducts 1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine and the hydroxyl radical-derived 8-hydroxydeoxyguanosine (La and Swenberg, 1997; Ito et al., 1996).

4.7.3.4. Conclusions About the Hypothesized Mode of Action

The mode of action for 1,2,3-trichloropropane tumorigenicity may involve mutagenicity via reactive metabolites. However, there is uncertainty regarding this mode of action that is outlined later in this section. 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 somatic mutation and recombination test 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 F-344/N rats, with DNA adducts present in tumor-forming organs of male rats and mice
- dose-dependent increase in the formation of the intermediate metabolite, and reported mutagen and tumor initiator, 1,3-dichloroacetone, 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 nucleic acids and a capacity to form DNA adducts; however, the available database lacks in vivo assays which measure mutagenicity, such as mutations or chromosomal damage in target organs, as well as dose-response and temporal data that would provide additional evidence that 1,2,3-trichloropropane mutagenicity leads to carcinogenesis.

A number of assays have tested negative for DNA reactivity and mutagenicity of 1,2,3-trichloropropane. The assays in which 1,2,3-trichloropropane tested negative include:

- Ames assays without activation by S9 fraction, in the SOS chromotest in *E. coli*, in the DNA-repair proficient *E. coli* WP2,
- the induction of trifluorothymidine resistance in mouse lymphoma cells not activated with S9 fraction,

- the micronucleus formation assay in human lymphocytes,
- the unscheduled DNA synthesis assay in rat hepatocytes,
- the induction of chromosomal damage in cultured rat liver cells;
- in vivo studies measuring dominant lethal induction or micronucleus formation. However, 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).

In vivo data supporting a mutagenic mode of action for carcinogenicity are limited and areas of uncertainty exist. For example, regular test batteries for different genetic end points 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 or missing from the database. Evidence of gene mutations would provide substantial support for a mutagenic mode of action, but these studies have not been conducted. Also, evidence of cytogenetic effects in humans would be useful to better characterize the mode of action for 1,2,3-trichloropropane. While the most plausible scenario is that bioactivated 1,2,3-trichloropropane acts by inducing mutations in cancer-related genes, data demonstrating these events are largely unavailable.

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 F-344/N rats, with approximately half the amount bound to DNA as the amount bound separately to RNA or protein (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, with the amount bound to hepatic protein increasing linearly with increasing doses over time.

Weber and Sipes (1991) administered i.p. injections to male F-344/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 F-344/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 F-344/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 TCP-toxicity, liver, kidney, forestomach, and intestine, also contain the highest concentration of covalently-bound 1,2,3-trichloropropane and related metabolites (Mahmood et al., 1991which supports a role for metabolic activation and binding in the early stages of carcinogenesis.

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. The toxicokinetic and toxicodynamic processes that would enable reactive metabolites to produce mutations in animal models are biologically plausible in humans.

1,2,3-trichloropropane in the presence of S9 fraction is positive in in vitro *S. typhimurium* assays that detect base pair mutations (TA1535), frame shift mutations (TA1537) and primary DNA damage (TA98, TA100) (Lag et al., 1994; NTP, 1993; Ratpan and Plaumann, 1988; Haworth et al., 1983; Kier, 1982; Stolzenberg and Hine, 1980; Shell Oil Co., 1979) When tested in mammalian cells, 1,2,3-trichloropropane has induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells (NTP, 1993; Doulgas et al., 1985), induced forward mutations in the mouse lymphoma assay (NTP, 1993; Shell Oil Co., 1979), enhanced DNA viral transformation in Syrian hamster embryo cells (Hatch et al., 1983), induced micronucleus formation in the mammalian cell lines, AHH-1, MCL-5, and h2E1 (Doherty et al., 1996), and induced DNA strand breaks in human lymphocytes (Tafazoli and Kirsch-Volders, 1996).

The in vivo data supporting a mutagenic mode of action include the covalent binding of 1,2,3-trichloropropane or a metabolite to hepatic proteins, DNA, and RNA, the formation of DNA strand breaks in the hepatocytes of F344/N rats (Weber and Sipes, 1991, 1990), the induction of DNA strand breaks in the kidney DNA of rats (Lag et al., 1991), the formation of DNA adducts in various organs in both B6C3F1 mice and F-344/N rats with high concentrations of DNA adducts in the tumor-forming organ tissues (La et al., 1995), an increase in the number of mononuclear hepatocytes with a nucleus of high ploidy (Belyaeva et al., 1974, 1977), and the induction of wing spots in *Drosophila melanogaster* following 1,2,3-trichloropropane treatment (Chroust et al., 2006).

Ito et al. (1996) observed highly specific H-*ras* or K-*ras* activating mutations in the forestomach tumors of the B6C3F1 mice from the NTP (1993) study, with 6 of these tumors containing H- *ras* mutations at codon 61 and 4 with K- *ras* mutations at codon 13. La and Swenberg (1997) observed an increase in the concentration of the endogenous DNA adducts, 8-hydroxydeoxyguanosine, $1,N^6$-ethenodeoxyadenosine, and $3,N^4$-ethenodeoxycytidine, in rats following 1,2,3-trichloropropane exposure for one week.

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).

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* (*Supplemental Guidance*) (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* (US EPA, 2005b) recommends the application of age-dependent adjustment factors (ADAFs) for carcinogens that act through a mutagenic mode of action and are assumed to convey early-life susceptibility. Given the weight of the available evidence, 1,2,3-trichloropropane may be acting through a mutagenic mode of carcinogenic action; however, the database is lacking in vivo evidence that mutagenic events occur following 1,2,3-trichloropropane exposure. For these reasons, the application of ADAFs when assessing risks associated with early-life exposure is not recommended.

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. This 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

Glutathione appears to be necessary for the formation of the DNA adduct S-[1-(hydroxymethyl)-2-(N^7-guanyl)ethyl]glutathione. Individuals with a glutathione deficiency may be less susceptible to the carcinogenic effects of 1,2,3-trichloropropane. Conversely, individuals with increased expression of glutathione may have an increased susceptibility to the genotoxic effects of 1,2,3-trichloropropane. CYP450 may also influence the toxicity of 1,2,3-trichloropropane in a similar manner, with altered CYP450 activity increasing the availability of hepatotoxic but not DNA-reactive metabolites.

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 animals studies includes a 2-year gavage study in F-344 rats and B6C3F1 mice (NTP, 1993; Irwin et al., 1995), 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 F-344 rats (Hazleton Laboratories, 1983a; NTP, 1993), a 17-week gavage study in B6C3F1 mice (Hazleton Laboratories, 1983b; NTP 1993), 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 are, however, used to corroborate the findings of the chronic studies.

The dose-dependent, non-cancer 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, reduction in number of live pups/litter, and decreased proportion of male pups born alive (NTP, 1993; Merrick et al., 1991; NTP, 1990; Villeneuve et al., 1985).

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

pseudocholinesterase, from the subchronic NTP (1993) studies, and increased serum concentrations of hepatocellular enzymes, decreased concentration of 5'-nucleotidase, and increase incidence of histopathologic liver lesions, from the chronic NTP (1993) studies, thus, increased liver weight may be on a continuum of adverse liver effects associated with oral exposure to 1,2,3-trichloropropane. The designation of the liver as a target organ for noncancer effects is consistent with the findings from the mechanistic data (Weber and Sipes, 1990) which demonstrate 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. The increase in kidney weights after both subchronic and chronic exposure is accompanied by renal tubular necrosis in the subchronic NTP (1993) study. The subchronic NTP (1993) study demonstrated epithelial necrosis in the nasal turbinates of rats and regenerative lung lesions in mice. 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, reference doses for the changes in kidney weight, fertility, and pups/liter were quantified for comparison purposes .

5.1.2. Methods of Analysis - Including Models

Benchmark dose (BMD) modeling was conducted using EPA BMD software version 1.4.1. to analyze the changes in liver and kidney weight, fertility, and 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 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* (US 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 BMR. For this analysis of absolute and relative liver and kidney weight changes in both rats and mice, a BMR of 10% is analogous to the 10% change in body weight used to identify maximum tolerated doses (MTDs) (A BMR of 1 SD was also included for comparison with other chemicals affecting absolute and relative liver weight changes). In the developmental study, 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 the reproductive toxicity 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) when a BMR representing a minimal level of change is selected as the primary BMR for the analysis.

Table 5-1 presents BMDs and the 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 outputs from best fitting models for each of the four data sets: male rats, female rats, male mice, and female mice. Model fit was determined by assessing the goodness-of-fit using a significance value of $\alpha = 0.1$ for eligibility, visual fit, and ranking by Akaike Information Criterion (AIC) (see Appendix B).

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

End Point	Species/ Sex	Model	AIC	BMD[a]	BMDL[a]	BMR
Absolute liver weight	Rat/male	Hill	63.9	3.8	1.6	10 % change in mean organ weight
Absolute liver weight	Rat/ male	Hill	63.9	3.2	1.4	1 SD
Relative liver weight	Rat/male	Hill	98.8	5.5	3.1	10 % change in mean organ weight
Relative liver weight	Rat/male	Hill	98.8	3.2	1.8	1 SD
Absolute kidney weight	Rat/female	Hill	-151.8	9.0	3.4	10 % change in mean organ weight
Relative kidney weight	Rat/male	Hill	-84.1	10.5	6.4	10 % change in mean organ weight
Fertility generating 4th litter	mice	log Probit (slope $\geq$ 1)	46.5	52.6	37.3	10 % change in fertility rate
Fertility generating 5th litter	mice	Probit	102.20	31.2	23.3	10 % change in fertility rate
Live pups/litter-4th litter	mice	polynomial	295.6	13.8	3.2	1% change in mean live pups/litter
Live pups/litter-5th litter	mice	polynomial	193	13.6	5.6	1% change in mean live pups/litter

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

Both increased absolute and relative liver weights in male rats were fitted adequately. The increase in liver weight was chosen as the critical effect and absolute liver weight was selected to represent the increase in liver weight because it is a more direct measure of liver weight change, as opposed to relative liver weight, which is the ratio of liver-to-body weight and can be affected by decreased body weight with an increase in dose. The change in liver weight is the first effect evident, and the increases in liver enzymes associated with liver damage and

increased rate of hepatic necrosis, as well as the hepatocellular damage-related decrease in pseudocholinesterase, supports the liver as the critical target organ.

The increase in absolute liver weight is a more sensitive endpoint than the decrease in the number of live pups/litter in the fourth and fifth litters. Statistically significant reductions in the number of live pups/litter were observed in mice compared to 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. When comparing the benchmark dose modeling results, the lower point of departure for the increase in absolute liver weight ($BMDL_{10\%}$ of 1.6 mg/kg-day) is thought to be more sensitive 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 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 has led to the selection of the two-year oral gavage study in Fischer rats (NTP, 1993) and increased liver weight in males as the principal study and critical effect for deriving the chronic RfD for 1,2,3-trichloropropane. The dose-response relationships for oral exposure to 1,2,3-trichloropropane and impaired fertility in CD-1 mice are also suitable for deriving a chronic RfD, but are associated with higher BMDLs that would be protected by the selected critical effect and corresponding BMDL.

The BMDL analysis corresponds to the lower bound on the dose associated with a 10% increase in mean liver weight. The BMD calculated from the Hill model for absolute liver weight change in male F-344 rats based on a BMD_{10} is 3.8 mg/kg-day and the $BMDL_{10}$ is 1.6 mg/kg-day. The benchmark response (BMR) of 10% change in mean value was used for absolute and relative liver and kidney weight modeling because the *Benchmark Dose Technical Guidance Document* (US EPA, 2000) recommends using a minimal amount of change in the endpoint that is considered to be biologically significant to define the BMR. Duration-adjustment of the point of departures was done to approximate daily exposure 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 point of departure 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 for consideration of intraspecies variation (UF_H: human variability), and 3 for database deficiencies (UF_D: database deficiency). The rationale for application of these UFs is provided described below.

A 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). No information was available to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans.

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

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

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 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.

The database of chronic and subchronic animal studies includes a 2-year gavage study in F-344 rats and B6C3F1 mice (NTP, 1993; Irwin et al., 1995), 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 F-344 rats (Hazleton Laboratories, 1983a; NTP, 1993), a 17-week gavage study in B6C3F1 mice (Hazleton Laboratories, 1983b; NTP 1993), and a two-generation reproductive/fertility assessment in Swiss CD-1 mice (NTP, 1990). A 3-fold UF 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 beyond two generations and a developmental toxicity 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.

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

$$RfD = BMDL_{ADJ} \div UF$$
$$= 1.1 \text{ mg/kg-day} \div 300$$

$= 4 \times 10^{-3}$ mg/kg-day (rounded to one significant figure)

5.1.4. Chronic RfD Comparison Information

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

Figures 5-2 presents the point of departure, applied uncertainty factors, and derived chronic RfD for additional effect endpoints that were modeled using EPA BMD software version 1.4.1. and which appear in Table 5-1. This comparison is intended to provide information on additional health effects associated with 1,2,3-trichloropropane exposure.

Points of departures (PODs) and chronic reference doses (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 weight, decreased fertility generating the 4^{th} and 5^{th} 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 3-fold UF for database deficiencies.

The change in liver weight is the first effect evident, and the increases in liver enzymes associated with liver damage and increased rate of hepatic necrosis, as well as the hepatocellular damage-related decrease in pseudocholinesterase, supports the liver as the critical target organ. The dose-response relationships for oral exposure to 1,2,3-trichloropropane and impaired fertility in CD-1 mice are also suitable for deriving a chronic RfD, but are associated with higher BMDLs that could be protected by the selected critical effect and corresponding BMDL. 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 has led to the selection of the two-year oral gavage study in Fischer rats (NTP, 1993) and increased liver

weight in males as the principal study and critical effect for deriving the chronic RfD for 1,2,3-trichloropropane.

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

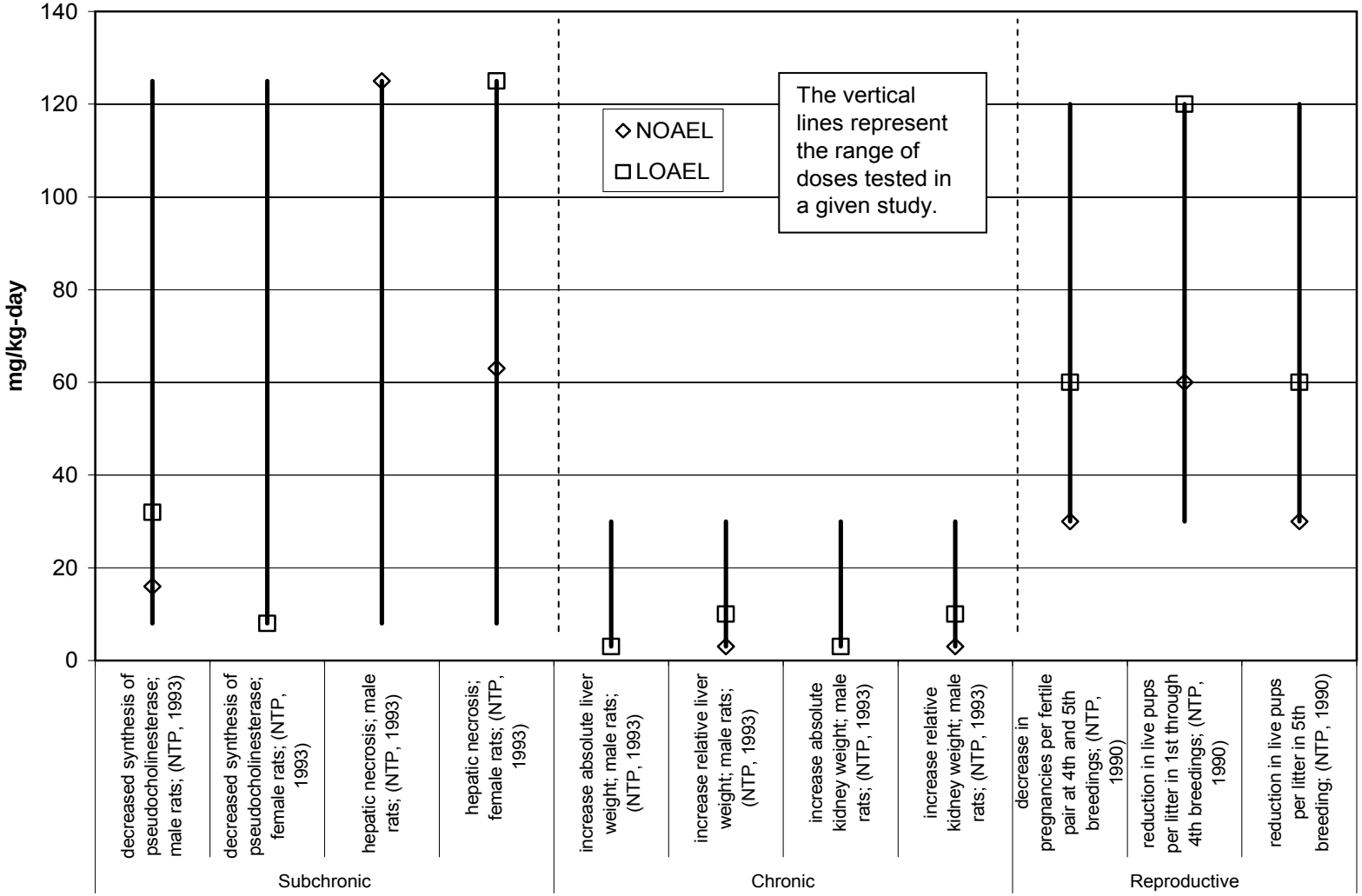
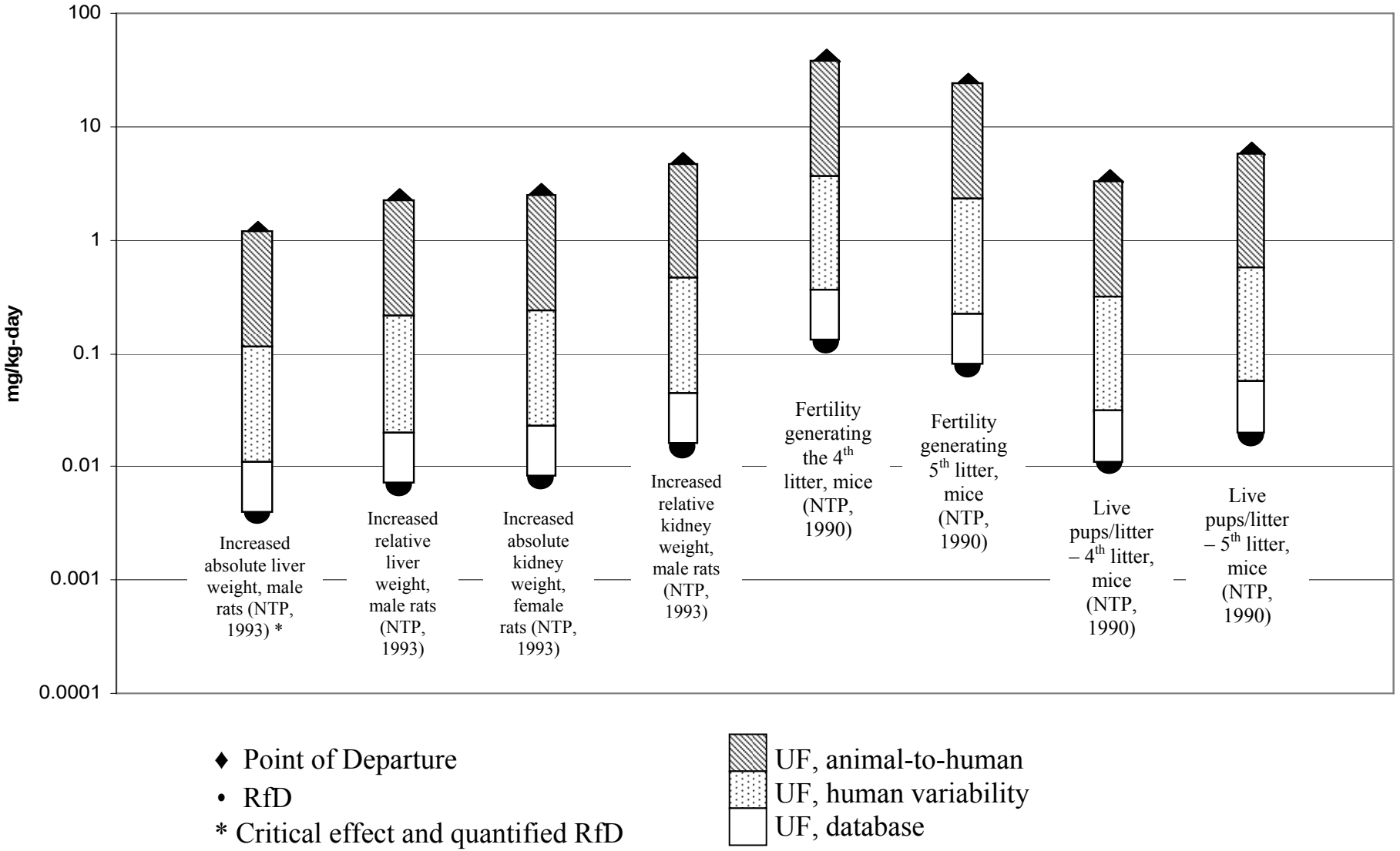


Figure 5-2. Points of Departure for endpoints from Table 5-2 with corresponding applied uncertainty factors and derived chronic RfD.



5.1.5. Previous Oral Assessment

The previous IRIS assessment for 1,2,3-trichloropropane was entered on the database on 03/31/1987 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 F-344 rats following a 17-week oral gavage exposure (Hazleton Laboratories, 1983a; NTP, 1983). 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) by 15 minute exposures to 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 at al. 1987a; Biodynamics, Inc., 1979). An increase in relative lung weight was also observed in female rats (Biodynamics, Inc., 1979). The histology data demonstrate that 1,2,3-trichloropropane is both a local irritant affecting the nasal epithelium (Miller at 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). No significant effects were observed in the reproductive toxicity studies (Johannsen et al., 1988; Biodynamics, Inc., 1979).

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. The increase in lung weight had a NOAEL of 5 ppm and a LOAEL of 15 ppm. 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 hepatocellular hypertrophy evident in male rats was considered potentially adaptive in the absence of additional overt toxicity in the liver, and the hematopoiesis of the spleen in female rats was not considered adverse, as there was no change in the clinical chemistry and hematology parameters.

The NOAEL of 1.5 ppm for peribronchial lymphoid hyperplasia is not necessarily a 0% response level. Rather, the NOAEL of 1.5 ppm in 15 male rats has a 95% confidence limit for 0% response of 0 to 22%; or, in other words, there is 95% confidence that the "true" response of peribronchial lymphoid hyperplasia at 1.5 ppm is no higher than 22%. 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).

5.2.2. Methods of Analysis - Including Models

A NOAEL/LOAEL approach was used to derive the chronic RfC for 1,2,3-trichloropropane. The chronic RfC was based on the NOAEL of 1.5 ppm 1,2,3-trichloropropane for peribronchial lymphoid hyperplasia in the lungs of male rats identified in Johannsen et al. (1988). Benchmark dose modeling was not utilized because the peribronchial lymphoid hyperplasia incidences were not amenable to modeling due to the inconsistent dose response at the three highest doses in both males and females, with model outputs that did not adequately fit the data.

Human equivalent concentrations (HECs) were calculated from the candidate point of departure. PODs were converted to mg/m^3, adjusted to continuous exposure (7 days a week, 24 hours a 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, 1994) 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 category 1 gases for portal-of-entry effects and category 3 methods for systemic effects (U.S. EPA, 1994). 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 peribronchial lymphoid hyperplasia in rats exposed to 1,2,3-trichloropropane (category 3) 6 hours/day, 5 days/week for 13 weeks was calculated from a NOAEL of 1.5 ppm (1.5 ppm × MW[147.43] / 24.45 = 9.04 mg/m^3). Adjustment to a continuous exposure was calculated as follows:

$$\begin{aligned} NOAEL_{ADJ} &= NOAEL \times (6 \text{ hours})/(24 \text{ hours}) \times (5 \text{ days})/(7 \text{ days}) \\ &= 9.0 \text{ mg/m}^3 \times 0.25 \times 0.71 \\ &= 1.6 \text{ mg/m}^3 \end{aligned}$$

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, 1994) when the partition coefficients are unknown a ratio of 1 is used. This allows a $NOAEL_{HEC}$ to be derived as follows:

$$\begin{aligned} NOAEL_{HEC} &= NOAEL_{ADJ} \text{ (mg/m}^3) \times (H_{b/g})_A/(H_{b/g})_H \\ &= NOAEL_{ADJ} \text{ (mg/m}^3) \times 1 \\ &= 1.6 \text{ mg/m}^3 \end{aligned}$$

Application of the inhalation dosimetry methods to peribronchial lymphoid hyperplasia in the lung resulted in a $NOAEL_{HEC}$ of 1.6 mg/m^3.

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

The $NOAEL_{HEC}$ value of 1.6 mg/m^3 for increased incidence of peribronchial lymphoid hyperplasia in the lungs of male CD rats (Johannsen et al., 1988) was used as the point of departure to derive the chronic RfC for 1,2,3-trichloropropane. A total UF of 3000 was applied to this point of departure: 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 study (UF_S), and 10 for database deficiencies. The rationale for application of the UFs is described below. A graphical representation of the point of departure, applied uncertainty factors, and quantified chronic RfC for the critical effect selected, an increased incidence of peribronchial lymphoid hyperplasia in the lungs of male rats, was not included in this assessment.

A factor 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 $NOAEL_{ADJ}$ to a $NOAEL_{HEC}$ has been incorporated. Application of a full uncertainty factor of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a human equivalent concentration 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 and an UF of 3 is retained to fully address this component.

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

A 10-fold UF was used to account for uncertainty in extrapolating from a subchronic to chronic exposure duration. The critical effect, peribronchial lymphoid hyperplasia, may be more severe at lower doses with a prolonged exposure, and additional critical effects not observed following subchronic exposure may arise following chronic exposure.

A 10-fold UF was used to account for deficiencies in the database. The database of 1,2,3-trichloropropane inhalation studies, 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 a single-generation reproductive toxicity study (Johannsen et al., 1988; Biodynamics, Inc., 1979), provides reliable dose-response data from subchronic studies of two species and a single-generation reproductive toxicity study. However, the database is lacking a multigenerational reproductive study and a developmental toxicity study. The lack of the multigenerational study and a developmental toxicity 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.

An UF for LOAEL-to-NOAEL extrapolation was not used since a NOAEL was used to derive the chronic RfC.

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

$$\begin{aligned} RfC &= NOAEL_{(HEC)} \div UF \\ &= 1.6 \text{ mg/m}^3 \div 3000 \\ &= 5 \times 10^{-4} \text{ mg/m}^3 \text{ (rounded to one significant figure)} \end{aligned}$$

5.2.4. Chronic RfC Comparison Information

Similar to the oral toxicity database, the inhalation studies found statistically significant increases in organ weights. A dose-dependent increase absolute and relative liver weights were observed in male rats and female rats following subchronic exposure and in male and female mice following a two-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 study investigators did not examine epithelial tissue in their investigation. 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.

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 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 hepatocellular hypertrophy evident in male rats was considered potentially adaptive in the absence of additional overt toxicity in the liver, and the hematopoiesis of the spleen in female rats was not considered adverse, as there was no change in the clinical chemistry and hematology parameters.

5.2.5. Previous Inhalation Assessment

A reference concentration is not available from the current IRIS assessment, which was first on-line on March 31, 1987 (U.S. EPA, 2007).

5.3. UNCERTAINTIES IN CHRONIC ORAL REFERENCE DOSE (RfD) AND INHALATION REFERENCE CONCENTRATION (RfC)

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 (5.1.2 and 5.1.3; 5.2.2 and 5.2.3), the uncertainty factor approach, following EPA practices and RfC and RfD guidance (U.S. EPA, 1993, 1994), was applied to a point of departure (POD), a $BMDL_{HEC}$ for the RfD and a $NOAEL_{HEC}$ 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 to account for 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 are 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 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 are numerous absorption, distribution, metabolism, and excretion references and genotoxicty studies. 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

has led to the selection of the two-year oral gavage study in Fischer rats (NTP, 1993) and increased liver weight in males as the principal study and critical effect for deriving the chronic RfD for 1,2,3-trichloropropane. The dose-response relationships for oral exposure to 1,2,3-trichloropropane and impaired fertility in CD-1 mice are also suitable for deriving a chronic RfD, but are associated with higher BMDLs that would be protected by the selected critical effect and corresponding BMDL.

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, 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 hepatocellular hypertrophy evident in male rats was considered potentially adaptive in the absence of additional overt toxicity in the liver, and the hematopoiesis of the spleen in female rats was not considered adverse, as there was no change in the clinical chemistry and hematology parameters.

The selection of the benchmark dose model for the quantitation of the chronic RfD does not lead to significant uncertainty in estimating the POD since benchmark effect levels were within the range of experimental data. However, the selected model, the 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.

The derived chronic RfC was quantified using a NOAEL for the point of departure. A POD based on a NOAEL or LOAEL is, in part, a reflection of the particular exposure concentration or dose at which a study was conducted. It lacks characterization of the dose-response curve and for this reason is less informative than a POD obtained from benchmark dose-response modeling. In addition, the NOAEL of 1.5 ppm for peribronchial lymphoid hyperplasia should not be assumed to be a 0% response level. The NOAEL of 1.5 ppm for peribronchial lymphoid hyperplasia is consistent with a 95% confidence limit for 0% response of 0 to 22% (assuming a binomial distribution and using tabled confidence limit values). In other words, there is 95% confidence that the response rate is no higher than 22%, and in which case the derived RfC would overestimate the inhalation exposure likely to be without an appreciable risk of adverse health effects over a lifetime.

Extrapolating from animals to humans embodies further issues and uncertainties. The effect and the magnitude associated with the concentration at the point of departure 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 $NOAEL_{ADJ}$ to a $NOAEL_{HEC}$ has been incorporated. Application of a full uncertainty factor of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a human equivalent concentration 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 and an 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, also, in extrapolating dose from a subset or smaller sized population, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population. In the absence of 1,2,3-trichloropropane-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation 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 is unavailable.

Data gaps have been identified with uncertainties associated with database deficiencies on developmental toxicity associated with 1,2,3-trichloropropane oral exposure. The two-generation reproductive assessment toxicity study indicates that the developing fetus may be a target of toxicity. In addition, the lack of the multigenerational study, beyond two generations, 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. Thus, the absence of a study specifically evaluating developmental toxicity represents an area of uncertainty or gap in the database. The database of inhalation studies is of particular concern due to the lack of a multigenerational reproductive study and a developmental toxicity study. The lack of the multigenerational study is of particular concern due to the issue described previously in this paragraph.

5.4. CANCER ASSESSMENT

There are no available studies on cancer in humans associated with exposure to 1,2,3-trichloropropane. NTP (1993) provided evidence of 1,2,3-trichloropropane-induced forestomach and other benign and malignant tumors in male and female Fischer 344 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 Chinese Hamster ovary cell assays (NTP, 1993; Douglas et al., 1985), 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), enhanced DNA viral transformation in Syrian hamster embryo cells (Hatch et al., 1983), and the induction of micronucleus formation in the mammalian cell lines, AHH-1, MCL-5, and h2E1 (Doherty et al., 1996) and CHO cells (Douglas et al., 1985).

Under the *Guidelines for Carcinogenic 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, and Harderian gland in mice, were reported.

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 with an adequate number of animals per dose group. The number of test animals allocated among three dose levels and an untreated control group was adequate, with 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).

5.4.2. Dose-Response Data

In the NTP (1993) study, groups of 60 male and female F344 rats and $B6C3F_1$ mice were administered 3, 10 or 30 and 6, 20, or 60 mg/kg-day 1,2,3-trichloropropane, respectively, by gavage, 5 days/week, for two 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 two 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/ 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 (by life table test or logistic regression, $p \leq 0.001$). These data are summarized in Tables 5-2 (male rats), 5-3 (female rats), 5-4 (male mice), 5-5 (female mice). Data are not available to indicate whether the malignant tumors developed specifically from progression of the benign tumors. However, as a default approach 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 5-2. Tumor incidence, (percent), and time of first occurrence in male rats following oral gavage exposure to 1,2,3-trichloropropane (NTP, 1993)

Site	0 mg/kg-day	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day	Trend test *p*-value
Alimentary system, total squamous neoplasms[b]	1/59[a] (2%) 104[c]	39/60 (65%) 64	48/57 (84%) 58	58/60 (97%) 47	<0.001
Pancreas: acinar cell, adenoma or adenocarcinoma	5/59 (8%) 104	20/60[d] (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[d] (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
Zymbal's gland, carcinoma	0/59 (0%) -	0/60 (0%) -	0/57 (0%) -	3/58 (5%) 56	<0.001

[a] 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 Week 52, whichever was earlier.

[b] Squamous papillomas or squamous cell carcinomas of the pharynx/palate, tongue, or forestomach.

[c] Week first observed.

[d] 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 the individual animal histopathology tables. Table 5-6 reflects the incidence in the individual animal histopathology tables.

Table 5-3. Tumor incidence, (percent), and time of first occurrence in female rats following oral gavage exposure to 1,2,3-trichloropropane (NTP, 1993)

Site	0 mg/kg-day	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day	Trend test *p*-value
Alimentary system, total squamous neoplasms[b]	1/60[a] (2%) 104[c]	22/59[a] (37%) 73	49/59[a] (83%) 58	44/58[a] (76%) 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	6/57 (10%) 67	14/52 (27%) 61	23/48 (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] 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 Week 52, whichever was earlier.
[b] Squamous papillomas or squamous cell carcinomas of the pharynx/palate, tongue, or forestomach.
[c] Week first observed.

Table 5-4. Tumor incidence in male mice following oral gavage exposure to 1,2,3-trichloropropane (NTP, 1993)

Site	0 mg/kg-day	6 mg/kg-day	20 mg/kg-day	60 mg/kg-day	Trend test *p*-value
Alimentary system, total squamous neoplasms[b]	3/59[a] (5%) 69[c]	57/59[a] (97%) 61	57/60[a] (95%) 55	59/60[a] (98%) 46	<0.001
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

[a] 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 Week 52, whichever was earlier.
[b] Squamous papillomas or squamous cell carcinomas of the pharynx/palate, tongue, or forestomach.
[c] Week first observed.

Table 5-5. Tumor incidence in female mice following oral gavage exposure to 1,2,3-trichloropropane (NTP, 1993)

Site	0 mg/kg-day	6 mg/kg-day	20 mg/kg-day	60 mg/kg-day	Trend test *p*-value
Alimentary system, total squamous neoplasms[b]	0/59[a] (0%) -[c]	54/60[a] (90%) 59	59/60[a] (98%) 45	59/60[a] (98%) 42	<0.001
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	0.04
Uterus/cervix: adenoma or adenocarcinoma	0/59 (0%) -	5/59 (8%) 100	3/59 (5%) 83	11/57 (19%) 66	<0.001

[a] 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 Week 52, whichever was earlier.
[b] Squamous papillomas or squamous cell carcinomas of the pharynx/palate, tongue, or forestomach.
[c] Week first observed.

NTP noted additional tumor sites with apparent dose-related increases, squamous cell papillomas and carcinomas and hepatocellular adenomas and carcinomas, in male rats. These tumors displayed a dose-related increase, but their incidences were not individually statistically significantly greater than controls. NTP concluded that because the incidence in no one group was statistically significantly higher than control, the overall trends were not dose-related.

The male and female mice tumor incidence data, while clearly demonstrating carcinogenicity, were not suitable for deriving low-dose quantitative risk estimates. The NTP study design 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 the comparable exposure level, suggesting greater sensitivity of the mice, there is no information concerning the dose-response relationships at lower exposure levels that could be compared with the rat data. In other words, the high dose behavior of 1,2,3-TCP in mice does not inform the mouse tumor response to lower exposures of 1,2,3-TCP. Extrapolation from high response levels is not justified when other more suitable data, here in rats, are available. Consequently, dose-response modeling was not carried out with the mouse tumor data.

5.4.3. Dose Adjustments and Extrapolation Methods

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) stipulate 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. The linear approach is used as a default option if the mode-of-action of carcinogenicity is not understood (U.S. EPA, 2005). In the case of 1,2,3-trichloropropane, there are data available that suggest that bioactivated 1,2,3-trichloropropane may bind directly to DNA resulting in a mutagenic event that may lead to tumorigenicity in animals. However, the database contains limited in vivo evidence that mutagenic events occur following 1,2,3-trichloropropane exposure. A linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,2,3-trichloropropane exposure as the default option. This approach is supported by the positive evidence of genotoxicity and a potential mutagenic mode of action.

It is possible that the squamous neoplasms may result primarily as a portal of entry effect and may not have a low-dose response pattern that extends linearly from the observed responses. The tumors in other organs demonstrate absorption of 1,2,3-trichloropropane, although it is not clear whether absorption was impacted by adverse effects in the forestomach.

Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and early termination of at least one dose group, 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, because it incorporates the time at which death-with-tumor occurred. The multistage-Weibull model 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 estimated in fitting the model, 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 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 5.3 (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 5-2 and 5-3 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. Although the 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, it was not clear that a determination could be made for each animal with multiple tumors. Therefore, all tumors were treated as incidental.

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 chi-square 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. 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.

Points of departure 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, $[P(d) - P(0)]/[1 - P(0)]$. 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 point of departure. 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 (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 male and female rat tumor incidence data are provided in Table 5-6. Note that while identifying a point of departure (POD) near the lower end of the observed data for linear extrapolation to lower doses is consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S.EPA, 2005a), TOX_RISK does not provide the exposure levels corresponding to BMRs greater than 10%. Consequently, an oral slope factor for each of the tumor sites was calculated by dividing the BMR level (usually 10%) by its corresponding BMDL to obtain points of departure, for comparison across tumor sites and with other chemicals. 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.

Human equivalent oral slope factors estimated from the tumor sites with statistically significant increases ranged from 0.020 to 3.0 per mg/kg-day, a range of about two orders of magnitude, with both extremes coming from the male rat data. The highest slope factor in rats corresponded to squamous neoplasms of the alimentary system, and the lowest slope factor corresponded to Zymbal's gland tumors. The slope factor corresponding to pancreatic acinar cell tumors in male rats was close to the maximum, at 1.0 per mg/kg/day. The slope factors corresponding to the female rat tumors fell between these two extremes, with squamous neoplasms of the alimentary system, at 1.3 per mg/kg/day, the highest slope factor in that set.

The oral slope factor corresponding to alimentary system squamous neoplasms in male F344 rats was the highest oral slope factor obtained. Given the multiplicity of tumor sites, however, basing the oral slope factor on one tumor site may underestimate the carcinogenic potential of 1,2,3-trichloropropane. An approach suggested in the cancer guidelines 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 5-6), from 1.0 (male rat Zymbal's gland tumors), indicating a slight tendency toward earlier tumor occurrence with increasing exposure level, to 8.7 (male rat pancreatic tumors), indicating a clearly earlier 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, 2005), 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 rats (Table 5-7). Although the inclusion of a central tendency estimate would improve the transparency of this document, the calculation of a central tendency estimate by TOX_RISK was not feasible. Note that the 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. The estimate involved the following steps:

I) It was assumed that the tumor types associated with 1,2,3-trichloropropane exposure were statistically independent - that is, that the occurrence of a pancreatic acinar cell 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. 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.

II) 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 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.

III) 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,

pancreas, preputial gland, kidney, and Zymbal's gland tumors for male F344 rats. Similarly, for female rats the MLEs of unit potency were summed across squamous cell neoplasms, mammary gland adenocarcinomas, clitoral gland tumors and Zymbal's gland carcinomas.

IV) 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:

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

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 were summed across tumor sites to obtain the variance of the sum of the MLE. The 95% UCL on the sum of the individual MLEs was calculated from the variance of the sum of the MLE.

The resulting combined upper bound slope factor for male rats was 3.8 per mg/kg/day, compared with 3.0 per mg/kg/day for only alimentary system tumors and 0.020 per mg/kg-day for only Zymbal's gland tumors. Overall, the consideration of the other tumor sites increased the slope factor by about 20%. The increase was due largely to the pancreatic tumors, with very little contribution from the other three tumor sites. A sensitivity analysis (not included in this document) showed that the summed risk was essentially the same (to 2 significant digits) whether or not the individual risks were estimated in the region of 10^{-3} risk or near the PODs.

For female rats the combined upper bound slope factor was 1.9 per mg/kg-day, a 50% increase compared with 1.3 per mg/kg-day for alimentary system tumors only. Both the clitoral and mammary gland tumors contributed to the increased risk estimate. As with the summed risk for male rats, there was little difference when the individual risks were estimated in the region of 10^{-3} risk or near the PODs.

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 **4 per mg/kg-day**, rounding the summed risk for male rats above to one significant digit. The value based on male rats was recommended because male rats are the most sensitive to tumor induction following exposure to 1,2,3-trichloropropane. This slope factor should not be used

with exposures greater than 0.05 mg/kg/day, the point of departure for the male rat 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. The recommended estimate reflects the time-to-tumor dimension of the responses as well as the exposure-response relationships for the multiple tumor sites.

Table 5-6. Dose-response modeling summary for rat tumor sites associated with oral exposure to 1,2,3-trichloropropane; tumor incidence data from NTP (1993)

Tumor type	MLE coefficients[a]	Human Equivalent Continuous Point of departure[b], mg/kg-day		Slope factor[c], $(mg/kg\text{-}day)^{-1}$
		BMD_{10}	$BMDL_{10}$	
Male rats				
Alimentary system, total squamous neoplasms	$q_0 = 1.1 \times 10^{-12}$ $q_1 = 1.9 \times 10^{-11}$ $q_2 = 2.1 \times 10^{-12}$ $z = 5.1$	0.050	0.033	3.0
Pancreatic acinar cell 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[d]	0.32[d]	0.16
Zymbal's gland carcinoma	$q_1 = 1.6 \times 10^{-5}$ $z = 1.0$	1.2[e]	0.49[e]	0.020
Female rats				
Alimentary system, total squamous neoplasms	$q_0 = 2.5 \times 10^{-12}$ $q_1 = 8.1 \times 10^{-12}$ $q_2 = 5.6 \times 10^{-12}$ $z = 4.9$	0.17	0.075	1.3
Clitoral gland adenoma or carcinoma	$q_0 = 3.1 \times 10^{-7}$ $q_1 = 6.5 \times 10^{-7}$ $z = 2.4$	0.32	0.24	0.42
Mammary gland adenocarcinoma[f]	$q_0 = 0.034$ $q_1 = 0.027$	0.64	0.43	0.23
Zymbal's gland carcinoma[c]	$q_1 = 1.4 \times 10^{-5}$ $z = 1.2$	0.40[e]	0.15[e]	0.067

[a] Model: multistage-Weibull, extra risk: $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t \pm t_0)^z]$, coefficients estimated in terms of mg/kg-day as administered in bioassay; lower 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% effect (extra risk) level; $BMDL_{10}$ = 95% lower bound on concentration at 10% effect (extra risk) level.

[c] Slope factors estimated by dividing the BMR by the BMDL.

[d] BMD and BMDL correspond to BMR=5%.

[e] BMD and BMDL correspond to BMR=1%.

[f] Multistage-Weibull model did not fit adequately (see Appendix B). Multistage model (extra risk without adjustment for time) was used: $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, omitting the high-dose group.

Table 5-7. Summary of cancer risk values estimated by R/BMD_R and summed across tumor sites for male and female rats.

Male Rat	BMD (mg/kg-day)	BMDL (mg/kg-day)	Cancer risk value at BMD[a] $(mg/kg\text{-}day)^{-1}$	Oral slope factor $(mg/kg\text{-}day)^{-1}$
oral route squamous papillomas, carcinomas; $R=10^{-2}$	4.9×10^{-3}	3.2×10^{-3}	2.0	3.1
pancreas acinar tumors	2.8×10^{-3}	1.0×10^{-3}	3.5×10^{-1}	1.0
kidney tubule adenomas	6.8×10^{-2}	9.1×10^{-3}	1.5×10^{-2}	1.1×10^{-1}
preputial gland tumors	1.3×10^{-2}	5.6×10^{-3}	7.8×10^{-2}	1.8×10^{-1}
Zymbal's gland carcinomas	1.2×10^{-1}	4.8×10^{-2}	8.5×10^{-3}	2.1×10^{-2}
		Sum	2.5	
			Upper bound on summed risk	3.8
Female Rat	BMD (mg/kg-day)	BMDL (mg/kg-day)	Cancer risk value at BMD[a] $(mg/kg\text{-}day)^{-1}$	Oral slope factor $(mg/kg\text{-}day)^{-1}$
oral route squamous papillomas, carcinomas.	3.0×10^{-3}	7.4×10^{-3}	3.4×10^{-1}	1.4
clitoral gland adenomas, carcinomas $R=10^{-2}$	3.0×10^{-1}	2.3×10^{-1}	3.3×10^{-2}	4.4×10^{-1}
mammary adenocarcinomas $R=10^{-2}$	7.1×10^{-2}	4.1×10^{-2}	1.4×10^{-2}	2.4×10^{-2}
Zymbal's gland carcinomas $R=10^{-2}$	4.0×10^{-1}	1.5×10^{-1}	2.7×10^{-2}	4.2×10^{-2}
		Sum	0.8	
			Upper bound on summed risk	1.9

[a] The MLE slope factor = R/BMD_R, where $R = 1 \times 10^{-3}$ except where specified.

5.4.5. 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. Several types of uncertainty may be considered quantitatively, but other important uncertainties cannot be considered quantitatively. Thus an overall integrated quantitative uncertainty analysis is not presented. Section 5.4.5.1 and Table 5-8 summarize principal uncertainties.

5.4.5.1. Sources of Uncertainty

Choice of low-dose extrapolation approach. The MOA 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 as the default option. 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, 2005).

The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the MOA for 1,2,3-trichloropropane were known with a higher degree of confidence is of interest, but additional supporting data on the MOA of 1,2,3-trichloropropane is not available. Even if it were, incorporation of MOA into dose-response modeling might not be straightforward and might not significantly reduce the uncertainty about low-dose extrapolation.

Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and early termination of at least one dose group, 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; 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

0.020 to 3.0 per mg/kg-day, a range of about two orders of magnitude, with both extremes coming from the male rat data.

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. 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. Following the recommendations of the NRC (1994) and the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), 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 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 estimate of the 95% upper bound on the summed oral slope factor is 4 per mg/kg-day, about 20% higher than the slope factor for alimentary system tumors only.

Dose metric. 1,2,3-Trichloropropane is metabolized to intermediates with carcinogenic potential. However, it is unknown whether a metabolite or some combination of parent compound and metabolites is responsible for the observed toxicity. If the actual carcinogenic moiety is proportional to administered exposure, then use of administered exposure as the dose metric is the least biased choice. On the other hand, if this is not the correct dose metric, then the impact on the slope factor is unknown.

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

Statistical uncertainty at the point of departure. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the multistage-Weibull model applied to the male rat data, there is a reasonably small degree of uncertainty at the 10% excess incidence level (the point of departure for linear low-dose extrapolation). The upper bound on the summed risk for male rats is approximately 1.5-fold higher than the summed risk.

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 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 adequate, 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.

Choice of species/gender. The oral slope factor for 1,2,3-trichloropropane was quantified using the tumor incidence data for male rats, which were thought to be more sensitive than female rats to the carcinogenicity of 1,2,3-trichloroporpane. The male and female mice tumor incidence data, while clearly demonstrating carcinogenicity, were not suitable for deriving low-dose quantitative risk estimates. The NTP study design 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 the comparable exposure level, suggesting greater sensitivity of the mice, there is no information concerning the dose-response relationships at lower exposure levels that could be compared with the rat data. In other words, the high dose behavior of 1,2,3-TCP in mice does not inform the mouse tumor response to 1,2,3-TCP at lower exposures. Extrapolation from high response levels is not justified when other more suitable data, here in rats, are available. Consequently, dose-response modeling was not carried out with the mouse tumor data.

Relevance to humans. The derivation of the oral slope factor is derived using the tumor incidence in the alimentary system, pancreas, kidney, preputial gland, and Zymbal's gland in male rats. The human relevance of the forestomach tumors, as included in the tumor incidence in the alimentary system, is of concern because humans lack a forestomach, which serves as a food storage organ (Proctor et al., 2007). The oral cavity, pharynx, and glandular stomach are histologically similar to the rat forestomach, but the tissue dose in these human organs is different than the tissue dose in the rodent forestomach (Proctor et al., 2007). Chemicals that are genotoxic and cause tumors at multiple sites in the absence of forestomach irritation are lilely relevant to human carcinogenesis (Proctor et al., 2007). 1,2,3-Trichloropropane may be carcinogenic through a mutagenic mode of action and is a multi-site carcinogen in rodents. In addition, hyperplasia of the forestomach epithelium, a proliferative response often associated with gavage chemical administration (Proctor et al., 2007), was not observed during the 120-day subchronic study conducted by NTP (1993). Therefore, the carcinogenicity observed in the rodent studies is relevant to human exposure. Also, the concordance of the alimentary system tumors across rats and mice lends strength to the concern for human carcinogenic potential.

Human population variability. The extent of inter-individual variability in 1,2,3-trichloropropane metabolism has not been characterized. A separate issue is that the human

variability in response to 1,2,3-trichloropropane is also unknown. Although a mutagenic MOA 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. In addition, due to the lack of information linking the mode of action for 1,2,3-trichloropropane to the observed carcinogenicity, the application of ADAFs for estimating risks associated with early-life exposure is not recommended.

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	Application of default linear-low-dose extrapolation may overestimate risk; alternatives could ↓ slope factor by an unknown extent	Multistage-Weibull model to determine POD, linear low-dose extrapolation from POD (default approach)	A linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,2,3-trichloropropane exposure as the default option. 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, 2005).
Dose metric	Alternatives could ↑ or ↓ slope factor by an unknown extent	Used administered exposure	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not clearly identified.
Cross-species scaling	Alternatives could ↓ or ↑ slope factor [e.g., 3.5-fold ↓ (scaling by BW) or ↑ 2-fold (scaling by $BW^{2/3}$)]	$BW^{3/4}$ (default approach)	There are no data to support alternatives. Because the dose metric was not an AUC, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks.
Statistical uncertainty at POD	↓ slope factor 1.5-fold if MLE used rather than lower bound on POD	LEC (default approach for calculating reasonable upper bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on administered exposure.
Bioassay	Alternatives could ↑ or ↓ slope factor by an unknown extent	NTP study	Alternative bioassays were unavailable.
Species /gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Male rat MCL	There are no MOA data to guide extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across species. Generally, direct site concordance is not assumed; consistent with this view, some human tumor types are not found in rodents and rat and mouse tumor types also differ.
Human relevance of rat tumor data	Human relevance of rat tumor tumor data could ↓ slope factor	Forestomach tumors in rats are relevant to human exposure	1,2,3-Trichloropropane may be carcinogenic through a mutagenic mode of action and is a multi-site carcinogen in rodents; therefore, the carcinogenicity observed in the rodent studies is relevant to human exposure. In addition, hyperplasia of the forestomach epithelium, a proliferative response often associated with gavage chemical administration, was not observed during the 120-day subchronic study conducted by NTP (1993).
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity, including whether children are more sensitive. Mutagenic MOA (if fully established) would indicate increased early-life susceptibility.

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 by-products 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, 1990, 1991).

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 F-344/N rats and B6C3F1 mice (NTP, 1993) and several subchronic studies (NTP 1993; Merrick et al. 1991; and 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 continuum of hepatic effects has been reported, ranging from cellular necrosis at high doses to significantly increased organ weights at lower doses. 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 non-neoplastic 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 or greater (Table 4-11). Similar effects were also observed in mice that were exposed to concentrations of 10 ppm 1,2,3-trichloropropane or greater (Table 4-12).

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, or greater, for 13 weeks (Johannsen et al., 1988). Increased liver weights were observed following 2-week exposures to 40 ppm, or greater, in rats and 132 ppm in mice (Miller et al., 1986a). Other organ weight changes included increased relative lung weights in female rats that were exposed to concentrations of 15 ppm or greater 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;
- 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.

In vitro bacterial mutation assays have generally 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; and to induce wing spots (caused by genotoxic effects such as somatic mutation, chromosomal rearrangement, or nondisjunction) in *D. melanogaster*. In vivo studies measuring dominant lethal induction or micronucleus formation were negative and although this does not necessarily negate the positive mutagenicity studies, these data do limit the confidence in the hypothesized mode of action.

The data supporting a mutagenic mode of action for carcinogenicity are limited and areas of uncertainty exist. For example, regular test batteries for different genetic end points in vitro and, especially, in vivo, are limited or missing from the database. Evidence of gene mutations in in vivo systems would provide substantial support for a mutagenic mode of action, but these studies have not been conducted. In addition, evidence of cytogenetic effects in humans would be useful to better characterize the mode of action for 1,2,3-trichloropropane. Therefore, the available data indicate that a mutagenic mode of action is possible, but the database is limited by a lack of evidence that mutagenic events occur following 1,2,3-trichloropropane exposure.

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 pseudocholinesterase, 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 appears to be on a continuum of adverse liver effects 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 in the subchronic NTP (1993) study. In addition, 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, reference doses for the changes in kidney weight, fertility, and pups/liter were quantified for comparison purposes.

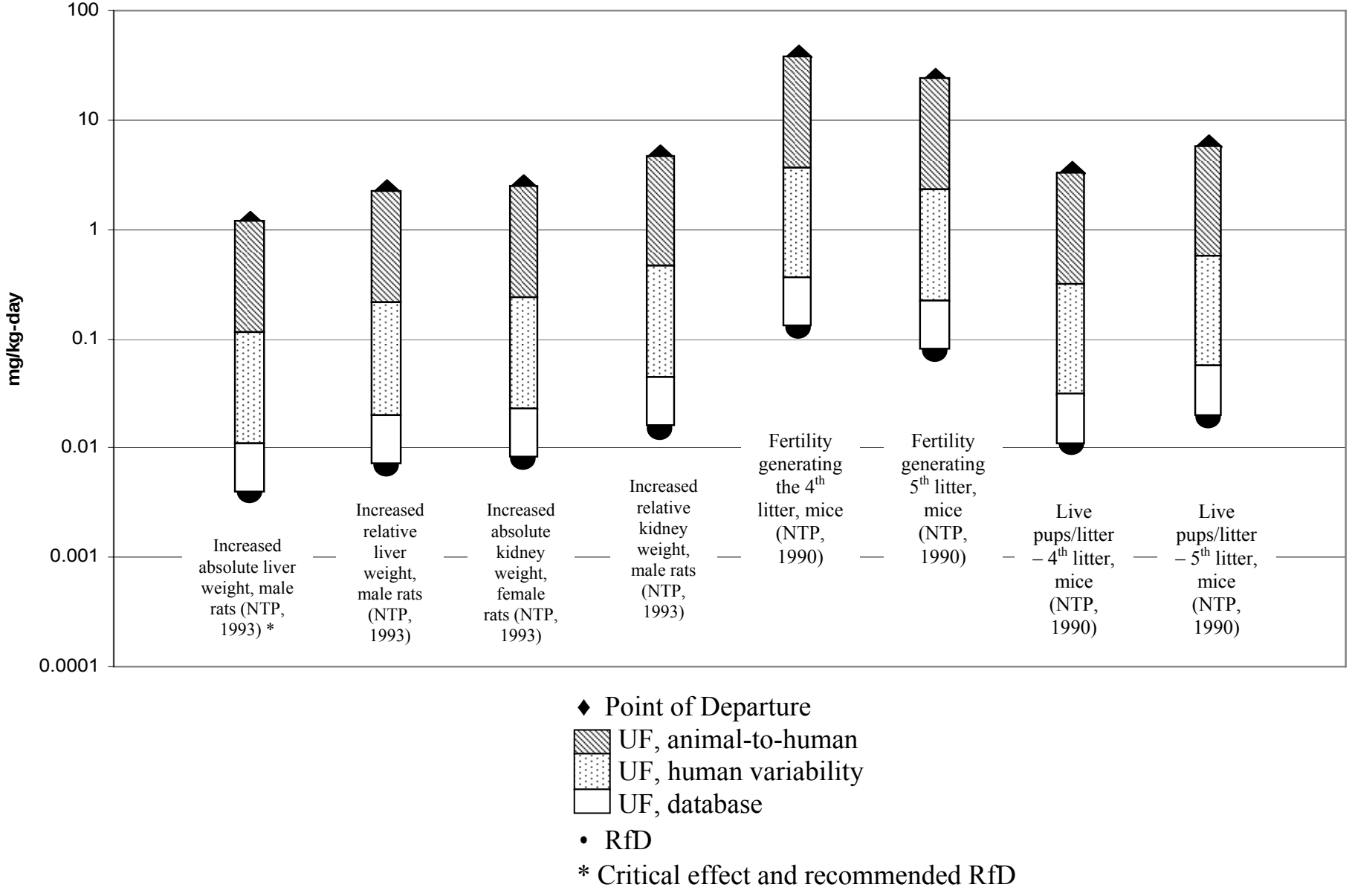
Benchmark dose (BMD) modeling was conducted to calculate potential points of departure for deriving the chronic RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$) for the changes in liver and kidney weight, fertility, and live pups/litter 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 1SD 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 BMR 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 1.14 mg/kg-day for increased absolute liver weight in male rats chronically exposed to 1,2,3-trichloropropane by gavage (NTP, 1994). 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 uncertainty factors of 10 were applied. In addition, a 3-fold database uncertainty factor was applied due to the lack of information addressing the potential developmental toxicity associated with 1,2,3-trichloropropane. The RfD Comparison Figure below presents the potential points of departure, applied uncertainty factors, and derived chronic RfD and

comparison RfDs for the critical effect and additional endpoints, respectively, from Table 5-1 in Section 5.

The overall confidence in this chronic RfD assessment 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. Points of Departure for endpoints from Table 5-1 with corresponding applied uncertainty factors and derived chronic RfD.



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. The increase in lung weight had a NOAEL of 5 ppm and a LOAEL of 15 ppm. Increased liver weights were also apparent, however, the hepatocellular hypertrophy observed in males at 5, 15, and 50 ppm appeared to be at mild centrilobular to midzonal levels and was not observed in the highest dose group females, and was considered potentially adaptive in the absence of additional overt toxicity in the liver.

There is uncertainty in the POD not captured by the NOAEL/LOAEL approach, because it lacks characterization of the dose-response curve and is less informative than a POD obtained from benchmark dose-response modeling. A NOAEL or LOAEL reflects the particular exposure concentration or dose at which a study was conducted, and the number of study subjects or test animals and typically are dissimilar in detection ability and statistical power. The NOAEL of 1.5 ppm for peribronchial lymphoid hyperplasia may not represent a 0% response level. Rather, the NOAEL of 1.5 ppm in 15 male rats has a 95% confidence limit for 0% response of 0 to 24%; or, in other words, there is a 95% chance that the "true" response of peribronchial lymphoid hyperplasia at 1.5 ppm would be as high as 24%. The associated uncertainty in the POD cannot be characterized quantitatively.

Human equivalent concentrations (HECs) were calculated from the candidate point of departure. HECs were converted to mg/m^3, adjusted to continuous exposure (7 days a week, 24 hours a 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, 1994) 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 5×10^{-4} mg/m^3 was calculated from a $NOAEL_{HEC}$ of 1.6 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 3000 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 uncertainty factor 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.

The overall confidence in this chronic RfC assessment is low. Confidence in the principal study (Johannsen et al.; 1988) is low. 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 chronic inhalation bioassay is of concern because the critical effect, peribronchial lymphoid hyperplasia, may be more severe at lower doses with a prolonged exposure, and additional critical effects not observed following subchronic exposure may arise following chronic exposure. 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-to-medium confidence in the database, confidence in the chronic RfC is low.

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 study 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 (US EPA, 2005a). This assumption, if incorrect, has some limited potential to over-estimate the carcinogenic potential of 1,2,3-trichloropropane, and is an accepted practice (McConnell et al., 1986).

The male and female mouse tumor incidence data, while clearly demonstrating carcinogenicity, were not suitable for deriving low-dose quantitative risk estimates. The NTP study design 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. Consequently, dose-response modeling was not carried out with the mouse tumor data. The elimination of the mouse data from the dose-response modeling has the potential to under-estimate the carcinogenic risk of 1,2,3-trichloropropane if mice are in fact more sensitive than the rats. Unfortunately, the high dose behavior of 1,2,3-trichloropropane in mice does not inform whether mice would be more, the same, or less sensitive than rats to 1,2,3-TCP carcinogenity at lower exposures.

The MOA 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 as the default option. This approach is supported by the positive evidence of genotoxicity and a potential mutagenic mode of action.

The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the MOA for 1,2,3-trichloropropane were known with a high degree of confidence is of interest, but additional supporting data on the MOA of 1,2,3-trichloropropane are not available. Even if it were, incorporation of MOA into dose-response modeling might not be straightforward and might not significantly reduce the uncertainty about low-dose extrapolation.

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.

Points of departure 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 point of departure. 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 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 0.020 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, 2005), 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 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 derived from male animal data is 4 per mg/kg-day, compared with 3 per mg/kg/day for only alimentary system tumors and 0.020 per mg/kg-day for only Zymbal's gland tumors. This estimate reflects the time-to-tumor response as well as the exposure-response relationships for the multiple tumor sites in male rats. The value based on male rats is recommended because male 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 exposures greater than 0.05 mg/kg/day, since the observed dose-response does not continue linearly above this level. For female rats, the combined slope factor was about two-fold lower, at 2.0 per mg/kg-day, a 50% increase compared with 1.3 per mg/kg-day for alimentary system tumors only.

The uncertainties associated with the quantitation of the oral slope factor are described below:

Choice of low-dose extrapolation approach: The MOA 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 as the default option. 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, 2005).

The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the MOA for 1,2,3-trichloropropane were known with a higher degree of confidence is of interest, but additional supporting data on the MOA of 1,2,3-trichloropropane is not available.

Without data sufficient to drive a more biologically-based model, a dose-response model with the capacity to accommodate some of the observed data, specifically the times to death-with-tumor, was used. It is unknown, however, how well the multistage-Weibull model or the linear low-dose extrapolation predicts low-dose risks for 1,2,3-trichloropropane.

Dose metric. 1,2,3-Trichloropropane is metabolized to intermediates with carcinogenic potential. However, it is unknown whether a metabolite or some combination of parent compound and metabolites is responsible for the observed toxicity. If the actual carcinogenic moiety is proportional to administered exposure, then use of administered exposure as the dose metric is the least biased choice. On the other hand, if this is not the correct dose metric, then the impact on the slope factor is unknown.

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

Statistical uncertainty at the point of departure. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the multistage-Weibull model applied to the male rat data, there is a reasonably small degree of uncertainty at the 10% excess incidence level (the point of departure for linear low-dose extrapolation). The upper bound on the summed risk for male rats is approximately 1.5-fold higher than the summed risk.

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 with an adequate number of animals per dose group. The number of test animals allocated among three dose levels and an untreated control group was adequate, 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.

Choice of species/gender. The oral slope factor for 1,2,3-trichloropropane was quantified using the tumor incidence data for male rats, which were thought to be more sensitive than female rats to the carcinogenicity of 1,2,3-trichloroporpane. The male and female mice tumor incidence data, while clearly demonstrating carcinogenicity, were not suitable for deriving low-dose quantitative risk estimates. The NTP study design 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 the comparable exposure level, suggesting greater sensitivity of the mice, there is no information concerning the dose-response relationships at lower exposure levels that could be compared with the rat data. In other words, the high dose behavior of 1,2,3-TCP in mice does not inform the mouse tumor response to 1,2,3-TCP at lower exposures. Extrapolation from high response levels is not justified when other more suitable data, here in rats, are available. Consequently, dose-response modeling was not carried out with the mouse tumor data.

Relevance to humans. The derivation of the oral slope factor is derived using the tumor incidence in the alimentary system, pancreas, kidney, preputial gland, and Zymbal's gland in male rats. The human relevance of the forestomach tumors, as included in the tumor incidence in the alimentary system, is of concern because humans lack a forestomach, which serves as a food storage organ (Proctor et al., 2007). The oral cavity, pharynx, and glandular stomach are histologically similar to the rat forestomach, but the tissue dose in these human organs is different than the tissue dose in the rodent forestomach (Proctor et al., 2007). 1,2,3-Trichloropropane may be carcinogenic through a mutagenic mode of action and is a multi-site carcinogen in rodents; therefore, the carcinogenicity observed in the rodent studies is relevant to human exposure. In addition, hyperplasia of the forestomach epithelium, a proliferative response often associated with gavage chemical administration (Proctor et al., 2007), was not observed during the 120-day subchronic study conducted by NTP (1993). The concordance of the alimentary system tumors across rats and mice lends strength to the concern for human carcinogenic potential.

Human population variability. The extent of inter-individual variability in 1,2,3-trichloropropane metabolism has not been characterized. A separate issue is that the human variability in response to 1,2,3-trichloropropane is also unknown. Although a mutagenic MOA 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. In addition, due to the lack of information linking the mode of action for 1,2,3-trichloropropane to the observed carcinogenicity, the application of ADAFs for estimating cancer risks associated with early-life exposure is not recommended.

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

7. REFERENCES

Anderson, EL; McGaughy, R; Bayard, S; et al. (1983) Quantitative approaches in use to assess cancer risk. Risk Anal 4: 277-295.

ATSDR (Agency for Toxic Substances and Disease Registry). (1992) Toxicological profile for 1,2,3-trichloropropane. Public Health Service, U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA. Available online at <http://www.atsdr.cdc.gov/toxprofiles/tp57.html>.

Bailer, AJ; Portier, CJ. (1986) Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples.

Belyaeva, NN; Tsulaya, VR; Marshak, TL; et al. (1974) Effect of 1,2,3-trichloropropane on the ploidy of rat liver cells. Bull Exp Biol Med (USSR) 78(12):74-77.

Belyaeva, NN: Bonashevskaya, TI; Marshak, TL; et al. (1977) Investigation of the effect of certain chlorinated hydrocarbons on the composition of the hepatocyte population of the rat liver. Bull Exp Biol Med (USSR) 83(3):345-348.

Biodynamics, Inc. (1979) A 13-week inhalation toxicity study of 1,2,3-trichloropropane in rats with attachments and cover letter dated 053092. Submitted under TSCA 8E. EPA Document No. 88920003764. NTIS No. OTS0542010.

Chroust, K; Pavlová, M; Zbyněk, P; Mendel, J; Božková, K; Kubát, Z; Zajíčková, V; Damborský, J. (2006) Quantitative structure-activity relationships for toxicity and genotoxicity of halogenated aliphatic compounds: wing spot test of *Drosophila melanogaster*. Chemosphere, doi:10.1016j.chemosphere.2006.09.020.

Crebelli, R; Andreoli, C; Carere, A; et al. (1992) The induction of mitotic chromosome malsegregation in Aspergillus nidulans. quantitative structure activity relationship (QSAR) analysis with chlorinated aliphatic hydrocarbons. Mutat Res 266:117-134.

Crebelli, R; Carere, A; Leopardi, P; et al. (1999) Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test. Mutagenesis 14:207-215.

Dearfield, KL; Moore, MM. (2005) Use of genetic toxicology information for risk assessment. Environ Molecul Mutagen 46:236-245.

Doherty, AT; Ellard, S; Parry, EM; et al. (1996) An investigation into the activation and deacativation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells. Mutagenesis 11(3):247-274.

Douglas, GR; Nestmann, ER; Lee, E; et al. (1985) How well do in vitro tests predict in vivo genotoxicity? Environ Mutagen 7(Suppl 3): 31.

Eriksson, L; Jonsson, J; Hellberg, S; et al. (1991) A strategy for ranking environmentally occurring chemicals. Part V: the development of two genotoxicity QSARs for halogenated aliphatics. Environ Toxicol Chem 10:585-596.

Ezaz-Nikpay, K; Verdine, GL. (1984) The effects of N7-methylguanine on duplex DNA structure. Chem Biol 1:235-240.

Fortoul, TI; Cano-Valle, F; Oliva, et al. (1985) Follicular bronchiolitis in association with connective tissue diseases. Lung 163:305-314.

Gart, JJ; Krewski, D; Lee, PN; et al. (1986) Statistical Methods in Cancer Research. Lyon: International Agency for Research on Cancer.

Gasparutto, D; Michel, T; Ramirez-Fuentes, T; et al. (2005) Epoxide adducts at the guanine residue within single-stranded DNA chains: reactivity and stability studies. Nucleosides Nucleotides Nucleic Acids 24(5-7):545-552.

Hatch, G; Anderson, T; Elmore, E; et al. (1983) Status of enhancement of DNA viral transformation for determination of mutagenic and carcinogenic potential of gaseous and volatile compounds. Environ Mutagen 5:422.

Haworth, S; Lawlor, T; Mortelmans, K; et al. (1983) Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen 5:3-142.

Hazelton Laboratories. (1983a) Initial submission: 120-day gavage toxicity study with 1,2,3-trichloropropane in Fischer 344 rats (final report) with attachments and cover letter dated 050692. Submitted under TSCA 8E. EPA Document No. 88920002189. NTIS No. OTS0536324.

Hazelton Laboratories. (1983b) Initial submission: 120-day gavage toxicity study in B6C3F1 mice with 1,2,3-trichloropropane (final report) with attachments and cover letter dated 050692. Submitted under TSCA Section 8E. EPA Document No. 88920002188. NTIS No. OTS0536323.

Holme, JA; Soderlund, EJ; Brunborg, G; Omichinski, JG; Bekkedal, K; Trygg, B; Nelson, SD; Dybing, E. (1989) Different mechanisms are involved in DNA damage, bacterial mutagenicity, and cytotoxicity induced by 1,2-dibromo-3-chloropropane in suspensions of rat liver cells. Carcinog 10(1):49-54.

Holme, JA; Soderlund, EJ; Brunborg, G; et al. (1991) DNA damage and cell death induced by 1,2-dibromo-3-chloropropane (DBCP) and structural analogs in monolayer culture of rat hepatocytes: 3-aminobenzamide inhibits the toxicity of DBCP. Cell Biol Toxicol 7:413-432.

Howling, SJ; Hansell, DM; Wells, AU; et al. (1999) Follicular bronchiolitis: thin-section CT and histologic findings. Radiology 212:637-642.

HSDB (Hazardous Substances Data Bank). (2005) 1,2,3-Trichloropropane. National Library of Medicine, Bethesda, MD. Available online at <http://toxnet.nlm.nih.gov>.

Humphreys, WG; Kim, D-H; Guengerich, FP. (1991) Isolation and characterization of N^7-guanyl adducts derived from 1,2-dibromo-3-chloropropane. Chem Res Toxicol 4: 445-453.

Irwin, RD; Haseman, JK; Eustis, SL. (1995) 1,2,3-Trichloropropane: a multisite carcinogen in rats and mice. Fundam Appl Toxicol 25:241-252.

Ito, N; La, DK; Holt, S; et al. (1996) Analysis of ras mutations in forestomach tumors from B6C3F1 mice exposed to 1,2,3-trichloropropane. Proc Amer Assoc Cancer Res 37:137.

Johannsen, FR; Levinskas, GJ; Rusch, GM; et al. (1988) Evaluation of the subchronic and reproductive effects of a series of chlorinated propanes in the rat. I. Toxicity of 1,2,3-trichloropropane. J Toxicol Environ Health 25:299-315.

Kapp, RW. (1979) Mutagenicity of 1,2-dibromo-3-chloropropane (DBCP): in vivo cytogenetics study in the rat. Toxicol Appl Pharmacol 48(1):A46.

Kier, LD. (1982) Ames/salmonella mutagenicity assays of 1,2,3-trichloropropane, 1,2,2,3-tetrachloropropane, and 1,1,2,2,3-pentachloropropane. Prepared by Environmental Health Laboratory, Monsanto Company, St. Louis, MO, (NTIS/OTS 0000815, 19 August, 1982):1-19.

Krewski, D; Crump, KS; Farmer, J; et al. (1983) A comparison of statistical methods for low dose extrapolation utilizing time-to-tumour data. Fundam Appl Toxicol 3:140-160.

La, DK; Lilly, PD; Anderegg, RJ; et al. (1995) DNA adduct formation in B6C3F1 mice and Fischer 344 rats exposed to 1,2,3-trichloropropane. Carcinogenesis 16:1419-1424.

La, DK; Schoonhoven, R; Ito, N; et al. (1996) The effects of exposure route on DNA adduct formation and cellular proliferation by 1,2,3-trichloropropane. Toxicol Appl Pharmacol 140:108-114.

La, DK; Swenberg, JA. (1997) The induction of endogenous DNA adducts in tissues of Fischer-344 rats following gavage administration of 1,2,3-trichloropropane. Proc Amer Assoc Cancer Res 38:131.

Lag, ML; Soderlund, EJ; Omichinski, JG; et al. (1991) Effect of bromine and chlorine positioning in the induction of renal and testicular toxicity by halogenated propanes. Chem Res Toxicol 4: 528-534.

Lag, M; Omichinski, JG; Dybing, E; et al. (1994) Mutagenic activity of halogenated propanes and propenes: Effect of bromine and chlorine positioning. Chem Biol Interact 93:73-84.

Lutz, WK. (1979) In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat Res 65:289-356.

McConnell EE, Solleveld HE, Swenberg JA, Boorman GA. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst. 76(2):283-9.

McOmie, WA; Barnes, TR. (1949) Acute and subacute toxicity of 1,2,3-trichloropropane in mice and rabbits. Fed Proc Pharm Exp Therap 8:319.

Mahmood, NA; Overstreet, D; Burka, LT. (1991) Comparative disposition and metabolism of 1,2,3-trichloropropane in rats and mice. Drug Metab Dispos 19:411-418.

Meier, JR; Ringhand, HP; Coleman, WE; Munch, JW; Streicher, RP; Kaylor, WH; Schenck, KM. (1985) Identification of mutagenic compounds formed during chlorination of humic acid. Mutat Res 157:11-122.

Merrick, BA; Smallwood, CL; Meier, JR; McKean, DL; Kaylor, WH; Condie, LW. (1987) Chemical reactivity, cytotoxicity, and mutagenicity of chloropropanes. Toxicol Appl Pharmacol 91:46-54.

Merrick, BA; Robinson, M; Condie, LW. (1991) Cardiopathic effect of 1,2,3-trichloropropane after subacute and subchronic exposure in rats. J Appl Toxicol 11:179-187

Miller, RR; Quast, JF; Gushow, TS. (1987a) 1,2,3-Trichloropropane: 2-week vapor inhalation study in rats and mice. Dow Chemical Co., submitted to EPA under TSCA Sect. 8D. NTIS Nr. OTS 86-870002260.

Miller, RR; Quast, JF; Momany-Pfruender, JJ. (1987b) 1,2,3-Trichloropropane: 2-week vapor inhalation study to determine the no-adverse-effect level in rats and mice. Dow Chemical Co., submitted to EPA under TSCA Sect. 8D. NTIS Nr. OTS 86-8700.

Mirsalis, J; Tyson, K; Beck, J; et al. (1983) Induction of unscheduled DNA synthesis (UDS) in hepatocytes following in vitro and in vivo treatment. Environ Mutagen 5:482.

Myers, JL; Kurtin, PJ. (1995) Lymphoid proliferative disorders of the lung. In: Thurlbeck, WM; Churg, AM, eds. Pathology of the lung. 2nd ed. New York, NY:Thieme, 1995:535-588.

NCI (National Cancer Institute). (1978) Bioassay of dibromochloropropane for possible carcinogenicity. Prepared for the National Cancer Institute, U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. DHEW No. 78-828. (as cited in NTP, 1993)

NRC (National Research Council). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NRC (National Research Council). (1994) Science and Judgment in Risk Assessment. National Academy Press, Washington, DC.

NTP (National Toxicology Program). (1978)) Bioassay of 1,2-dichloroethane for possible carcinogenicity (CAS No. 107-06-2). Public Health Service, U.S. Department of Health and Human Services; NTP TR 55. NIH Publication No. 78-1361. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP. (1982) Carcinogenesis bioassay of 1,2-dibromo-3-chloropropane (CAS NO. 96-12-8) in F344/N rats and B6C3F1 mice (inhalation studies) [review draft]. Public Health Service, U.S. Department of Health and Human Services; NTP TR 206. NIH Publication No. 82-1762. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC. (as cited in NTP, 1993).

NTP. (1986) Toxicology and carcinogenesis studies of 1,2-dichloropropane (propylene dichloride) (CAS No. 78-87-5) in F344/N rats and B6C3F1 mice (gavage studies). Public Health Service, U.S. Department of Health and

Human Services; NTP TR 263. NIH Publication No. 86-2519. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP. (1989) Toxicology and carcinogenesis studies of hexachloroethane (CAS No. 67-72-1) in F344/N rats (gavage studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 361. NIH Publication No. 89-2816. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP. (1990) 1,2,3-Trichloropropane reproduction and fertility assessment in Swiss CD-1 mice when administered via gavage (final report). Public Health Service, U.S. Department of Health and Human Services; NTP-90-209. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP. (1993) Toxicology and carcinogenesis studies of 1,2,3-trichloropropane (CAS No. 96-18-4) in F344/N rats and B6C3F1 mice (gavage studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 384. NIH Publication No. 94-2839. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP. (2005) Carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol, nitromethane, and 1,2,3-trichloropropane (CAS Nos. 3296-90-0, 75-52-5, and 96-18-4) in guppies (Poecilia reticulate) and medaka (Oryzias latipes) (waterborne studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 528. NIH Publication No. 06-4464. Available from: National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Ozawa, N; Guengerich, FP. (1983) Evidence for formation of an S-[2-(N^7-guanyl)ethyl]glutathione adduct in glutathione-mediated binding of the carcinogen 1,2-dibromoethane to DNA. Proc Natl Acad Sci 80:5266-5270.

Proctor, DM; Gatto, NM; Hong, SJ; et al. (2007) Mode-of-action framework for evaluating the relevance of rodent forestomach tumors in cancer risk assessment. Toxicol Sciences April 10.

Ratpan, F; Plaumann, H. (1988) Mutagenicity of halogenated propanes and their methylated derivatives. Environ Mutagen 12:253-259.

Reyna, MS. (1987) Acute inhalation of trichloropropane, NTIS/OTS 0000815, US Department of Commerce, Springfield, VA.

Robinson, M; Bull, RJ; Olson, GR; Stober, J. (1989) Carcinogenic activity associated with halogenated acetones and acroleins in the mouse skin assay. Cancer Letters 48:197-203.

Rusyn, I; Bradham, CA; Cohn, L; et al. (1999) Corn oil rapidly activates nuclear factor-κB in hepatic Kupffer cells by oxidant-dependent mechanisms. Carcinogenesis 20(11):2095-2100.

Saito-Suzuki, R; Teramoto, S; Shirasu, Y. (1982) Dominant lethal studies in rats with 1,2-dibromo-3-chloropropane and its structurally related compounds. Mutat Res 101:321-327.

Shell Oil Co. (1979) In vitro mutation studies with 1,2,3-trichloropropane. Submitted under TSCA 8D. EPA Document No. 86870001651. NTIS No. OTS0515727.

Shell Oil Co. (1982) Initial submission: assay of 1,2,3-trichloropropane for gene mutation in mouse lymphoma cells (final report) with cover letter dated 120391. Submitted under TSCA 8E. EPA Document No. 88920000535. NTIS No. OTS0534986.

Silverman, L.; Schulte, HF.; First, MW. (1946) Further Studies on Sensory Response to Certain Industrial Solvent Vapors. J Ind Hyg Toxicol 28:262-266.

Stolzenberg, SJ; Hine, CH. (1980) Mutagenicity of 2- and 3-carbon halogenated compounds in the Salmonella/mammalian microsome test. Environ Mutagen 2:59-66.

Tafazoli, M; Kirsch-Volders, M. (1996) In vitro mutagenicity and genotoxicity study of 1,2-dichloroethylene, 1,1,2 trichloroethylene, 1,3-dichloropropane, 1,2,3-trichloropropane and 1,1,3-trichloropropene, using the micronucleus test and the alkaline single cell gel electrophoresis technique (comet assay) in human lymphocytes. Mutat Res 371:185-202.

Travlos, GS; Morris, RW; Elwell, MR; et al. (1996) Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13-week toxicity studies in rats. Toxicology 107: 17-29.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014-34025.

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006-34012.

U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk assessment. EPA 600/6-87/008. Available from: National Technical Information Service, Springfield, VA; PB88-179874/AS.

U.S. EPA. (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798-63826.

U.S. EPA. (1992) Draft report: a cross-species scaling factor for carcinogen risk assessment based on equivalence of $mg/kg^{3/4}$/day. Fed Reg 57(109):24152-24174.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F. Available from: National Technical Information Service, Springfield, VA; PB2000-500023, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. U.S. Environmental Protection Agency. EPA/630/R-94/007. Available from: National Technical Information Service (NTIS) , Springfield, VA; PB95-213765, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274-56322.

U.S. EPA. (1998a) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926-26954.

U.S. EPA. (1998b) Science policy council handbook: peer review. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-98-001. Available from: National Technical Information Service, Springfield, VA; PB98-140726, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000a) Science policy council handbook: peer review. 2nd edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000b) Science policy council handbook: risk characterization. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000c) Benchmark dose technical guidance document [external review draft]. EPA/630/R-00/001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000d) Supplemental guidance for conducting for health risk assessment of chemical mixtures. EPA/630/R-00/002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2005a) Guidelines for carcinogen risk assessment. EPA/630/P-03/001B. Risk Assessment Forum, Washington, DC. Available from: <http://www.epa.gov/cancerguidelines>.

U.S. EPA. (2005b) Supplemental guidance for assessing susceptibility from early-life exposures to carcinogens. EPA/630/R-03/003F. Risk Assessment Forum, Washington, DC. Available from: <http://www.epa.gov/cancerguidelines>.

U.S. EPA. (2006) Science policy council handbook: peer review. 3rd edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-06-002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2007) Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC; < http://epa.gov/iris/>.

Villeneuve, DC; Chu, I; Secours, VE; et al. (1985) Results of a 90-day toxicity study on 1,2,3- and 1,1,2-trichloropropane administered via the drinking water. Sci Total Environ 47:421-426.

Volp, RF; Sipes, IG; Falcoz, C; et al. (1984) Disposition of 1,2,3-trichloropropane in the Fischer 344 rat: Conventional and physiological pharmacokinetics. Toxicol Appl Pharmacol 75:8-17.

von der Hude, W; Scheutwinkel, M; Gramlich, U; et al. (1987) Genotoxicity of three-carbon compounds evaluated in the SCE test in vitro. Environ Mutagen 9:401-410.

von der Hude, W; Behm, C; Guertler, R; et al. (1988) Evaluation of the SOS chromotest. Mutat Res 203:81-94.

Weber, GL. (1991) Metabolism and bioactivation of 1,2,3-trichloropropane (TCP) [dissertation: The University of Arizona]. Ann Arbor, MI: University Microfilms International:3-142.

Weber, GL; Sipes, IG. (1990) Covalent interactions of 1,2,3-trichloropropane with hepatic macromolecules: studies in the male F-344 rat. Toxicol Appl Pharmacol 104:395-402.

Weber, GL; Sipes, IG. (1991) Rat hepatic DNA damage induced by 1,2,3-trichloropropane. Adv Exp Med Biol 283:853-855.

Weber, GL; Sipes, IG. (1992) In vitro metabolism and bioactivation of 1,2,3-trichloropropane. Toxicol Appl Pharmacol 113:152-158.

WHO (World Health Organization). (2003) Concise International Chemical Assessment Document 56: 1,2,3-Trichloropropane. WHO, Geneva. Available from: <http://www.inchem.org/documents/cicads/cicads/cicad56.htm>

Williams, GM; Mori, H; McQueen, CA. (1989) Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. Mutation Research 221:263-286.

Yousem, SA; Colby, TV; Carrington, CB. (1985) Follicular bronchitis/bronchiolitis. Hum Pathol 16:700-706.

Appendix B-1: Benchmark Dose Modeling Results for the Derivation of the RfD

Benchmark dose (BMD) modeling was performed to identify the point of departure 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, 2000) using Benchmark Dose Software Version 1.4.1. The BMD modeling results for the derivation of the chronic RfD are summarized in Table B-1, and the model outputs are attached. A brief discussion of the modeling results is presented below.

The following critical effects were modeled using the Benchmark Dose Software 1.4.1: absolute and relative liver weight, absolute and relative kidney weight, decreased fertility inthe 4^{th} litter, decreased fertility in the 5^{th} litter, pups/litter in the 4^{th} litter, and pups/litter in the 5^{th} litter. The endpoint being modeled specified which set of models, continuous (liner, polynomial, power, and Hill) or dichotomous (gamma, logistic, multi-stage, probit, quantal-linear, quantal-quadratic, 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 Akaike Information Criterion (AIC).

For absolute liver weight, the male rat data using the Hill model and a benchmark response of 10% change in mean organ weight was selected. The male rat data using the Hill model and a benchmark response 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 (US EPA, 2000) when a BMR representing a minimal level of change is selected as the primary BMR for the analysis. For absolute kidney weight, the female rat data using the Hill model and a benchmark response of 10% change in mean organ weight was the best fit. The male rat data using the Hill model and a benchmark response 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 4^{th} litter was the log Probit model (slope ≥ 1) with a benchmark response of 10% extra risk. The best model fit for decreased fertility in the 5^{th} litter was the Probit model with a benchmark response of 10% extra risk. The best model fit for the number of pups/litter in the 4^{th} litter, as well as for the number of live pups/litter in the 5^{th} litter, was the polynomial model with a benchmark response of 1% change in mean live pups/litter. The benchmark dose 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. Benchmark Dose modeling used in the derivation of the RfD

End Point	Species/ Sex	Model	Goodness-of-fit p-value	Chi-squared p-value	AIC	BMD	BMDL	BMR
Absolute liver weight	Rat/ male	Hill	0.677	0.28	63.9	3.8	1.6	10 % extra risk
Absolute liver weight	Rat/ male	Hill	0.677	0.28	63.9	3.2	1.4	1 SD
Relative liver weight	Rat/ male	Hill	0.986	0.01	98.8	5.5	3.1	10 % extra risk
Relative liver weight	Rat/ male	Hill	0.986	0.01	98.8	3.2	1.8	1 SD
Absolute kidney weight	Rat/ female	Hill	0.359	0.697	-151.8	9.0	3.4	10 % extra risk
Relative kidney weight	Rat/ male	Hill	0.549	0.478	-84.1	10.5	6.4	10 % extra risk
Decreased fertility in the 4th litter	mice	log Probit (slope ≥ 1)	0.9458	0.548	46.5	52.6	37.3	10 % extra risk
Decreased fertility in the 5^{th} litter	mice	Probit	0.9953	0.071	102.2	31.2	23.3	10 % extra risk
Pups/litter-4th litter	mice	polynomial	0.8157	-0.122	295.6	13.8	3.2	1% change in mean live pups/litter
Pups/litter-5th litter	mice	polynomial	0.337	-0.361	193	13.6	5.6	1% change in mean live pups/litter

Absolute liver weight change – male rats

```
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MOVED\M_R_ABLIVWT.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\M_R_ABLIVWT.plt
                                                Mon Apr 16 12:20:13 2007
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 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = Dose
   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

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     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

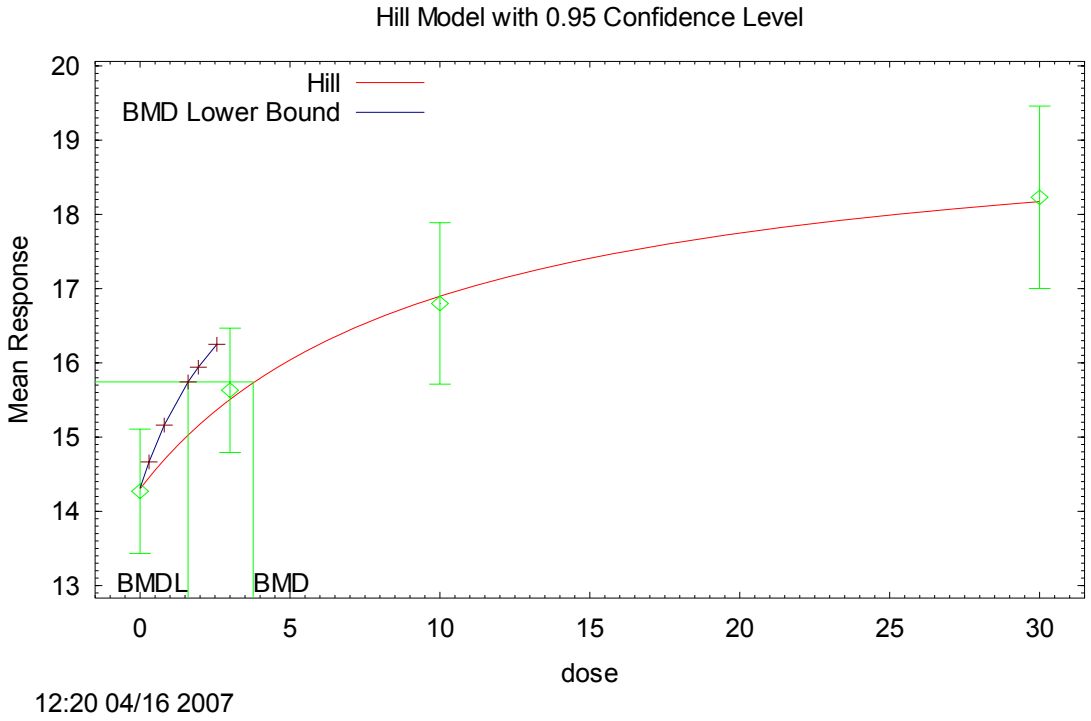
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 3.77203

BMDL = 1.60397



Absolute liver weight change – male rats (BMR of 1 SD)

```
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        Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\M_R_ABLIVWT.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND
SETTINGS\MGEHLHAU\DESKTOP\BMDS MOVED\M_R_ABLIVWT.plt
                                        Mon May 07 14:18:51 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = Dose
   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

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     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.


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Res.
------     ---   --------     --------   -----------  -----------   ----------

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    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

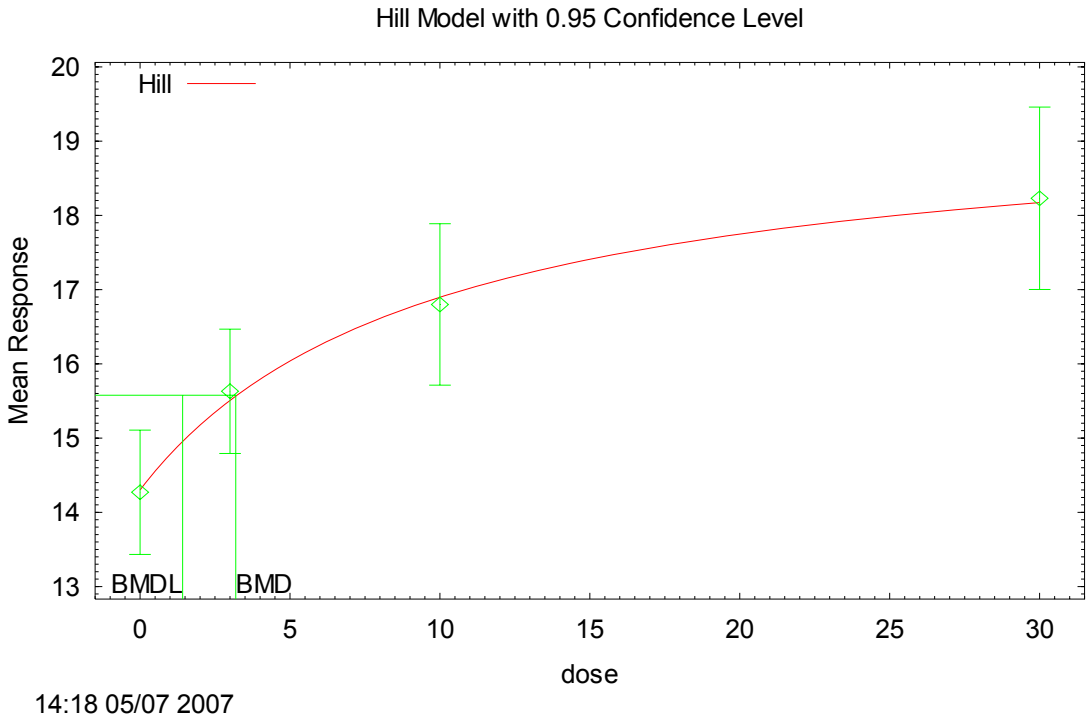
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3.19188

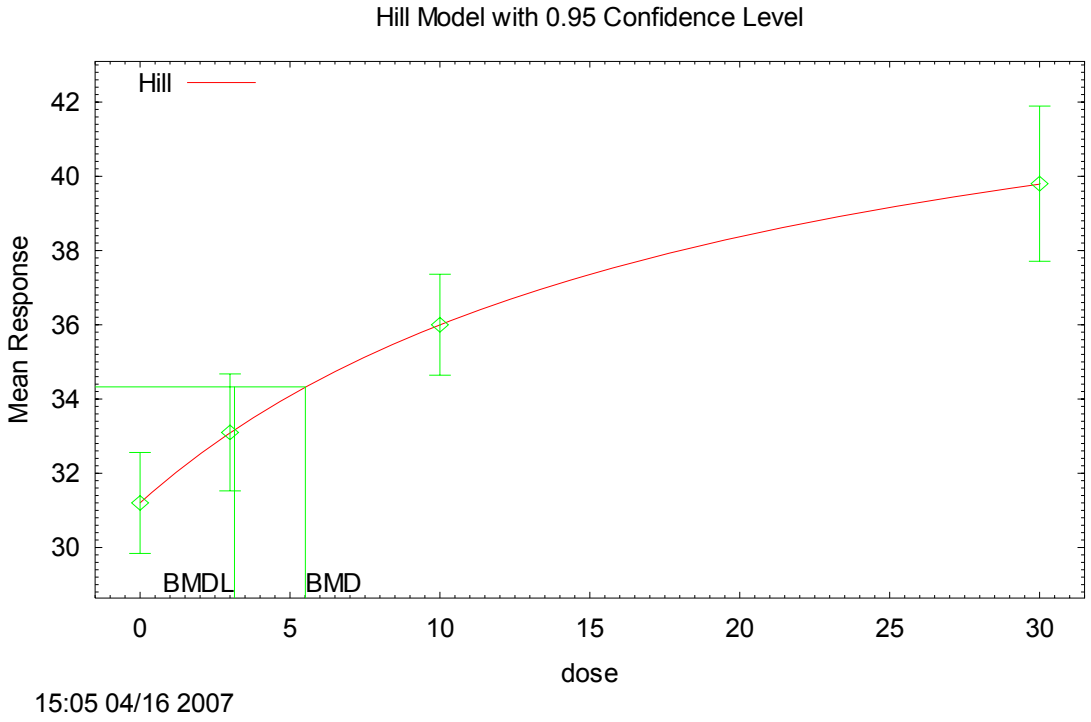
BMDL = 1.42159


Hill Model with 0.95 Confidence Level
Hill
Mean Response
20
19
18
17
16
15
14
13
BMDL
BMD
0
5
10
15
20
25
30
dose
14:18 05/07 2007

```
================================================================
         Hill Model. (Version: 2.12;  Date: 02/20/2007)
         Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\M_R_REL_LIVERWT.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\M_R_REL_LIVERWT.plt
                                              Mon Apr 16 15:05:35 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     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
```



Relative liver weight change – male rats (BMR of 1 SD)

```
 ================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\M_R_REL_LIVERWT.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND
SETTINGS\MGEHLHAU\DESKTOP\BMDS MOVED\M_R_REL_LIVERWT.plt
                             Mon May 07 14:55:25 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 =      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

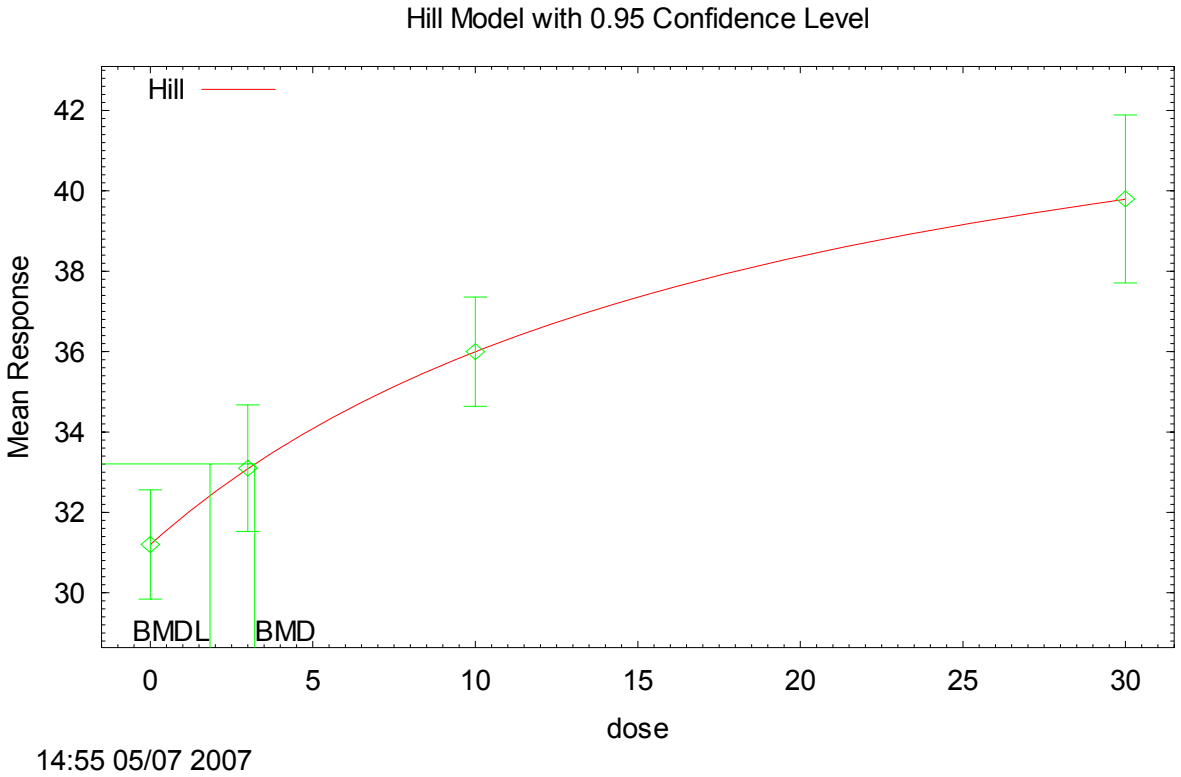
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3.21217

BMDL = 1.83718



```
================================================================
         Hill Model. (Version: 2.12;  Date: 02/20/2007)
         Input Data File: C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\F_R_ABSKIDNEYWT.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\MGEHLHAU\DESKTOP\BMDS
MOVED\F_R_ABSKIDNEYWT.plt
                                              Thu Apr 19 13:10:47 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 =   0.00477394
                            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

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     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

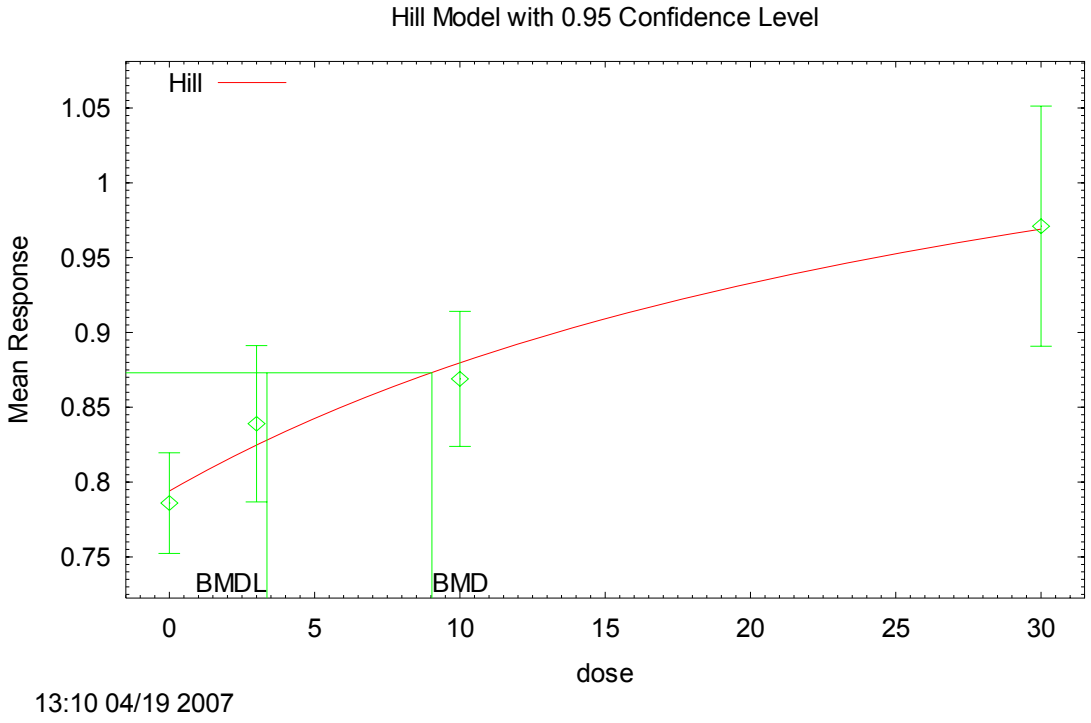
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 9.03706

BMDL = 3.3571



```
================================================================
         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

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     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
```
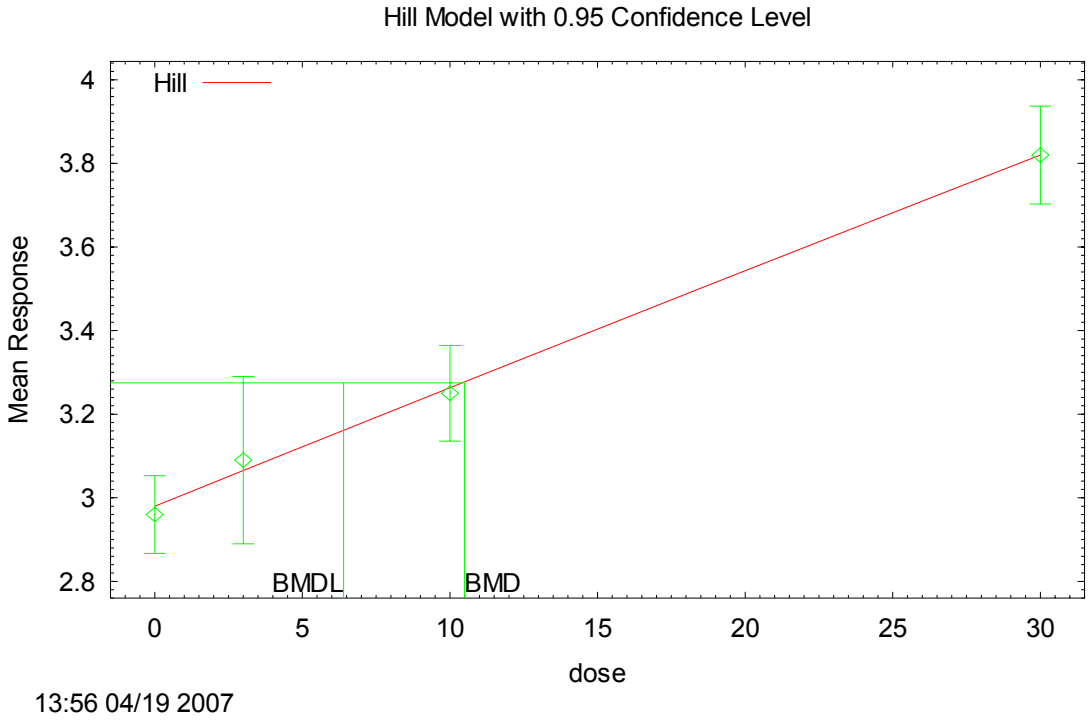

```
             n               1              NA
             k         1717.72           42099          -80794.8
84230.2

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
```



```
================================================================
         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

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     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

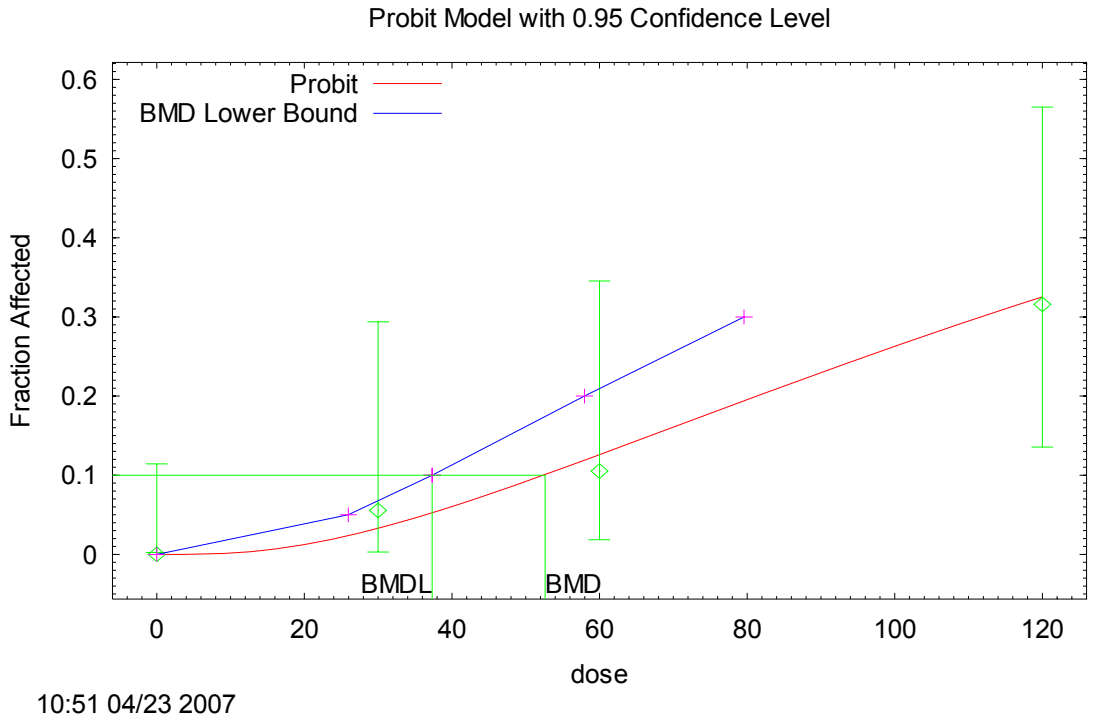
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 52.6244

BMDL = 37.3271



```
================================================================
         Probit Model. (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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

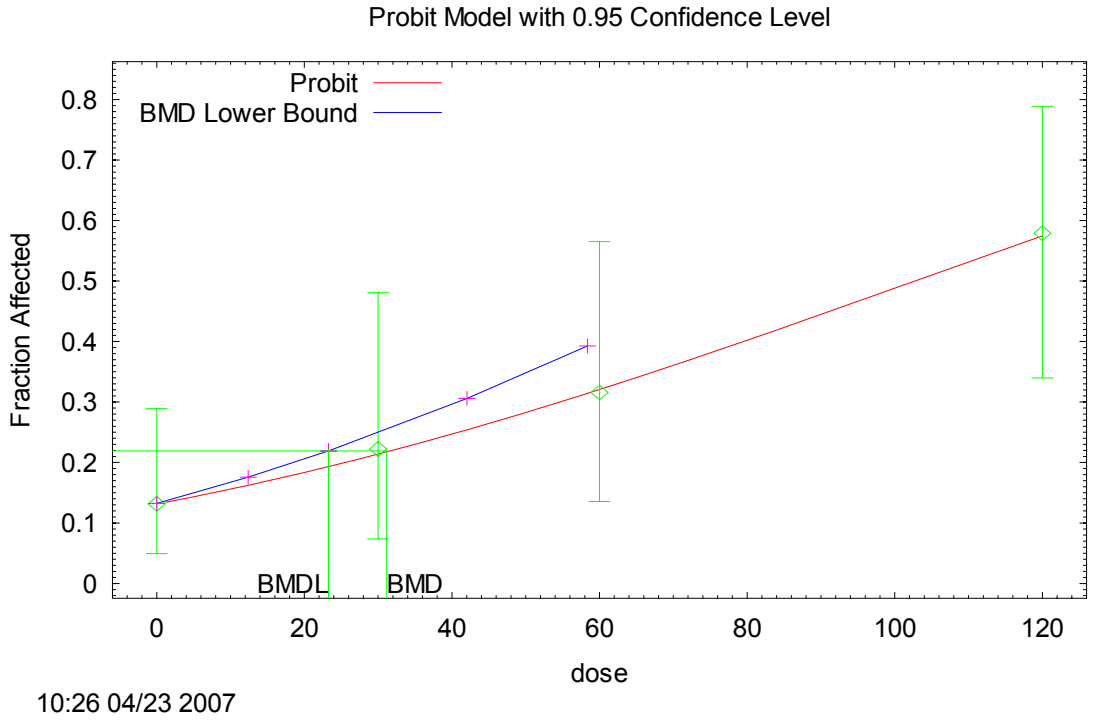
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 31.1591

BMDL = 23.2749

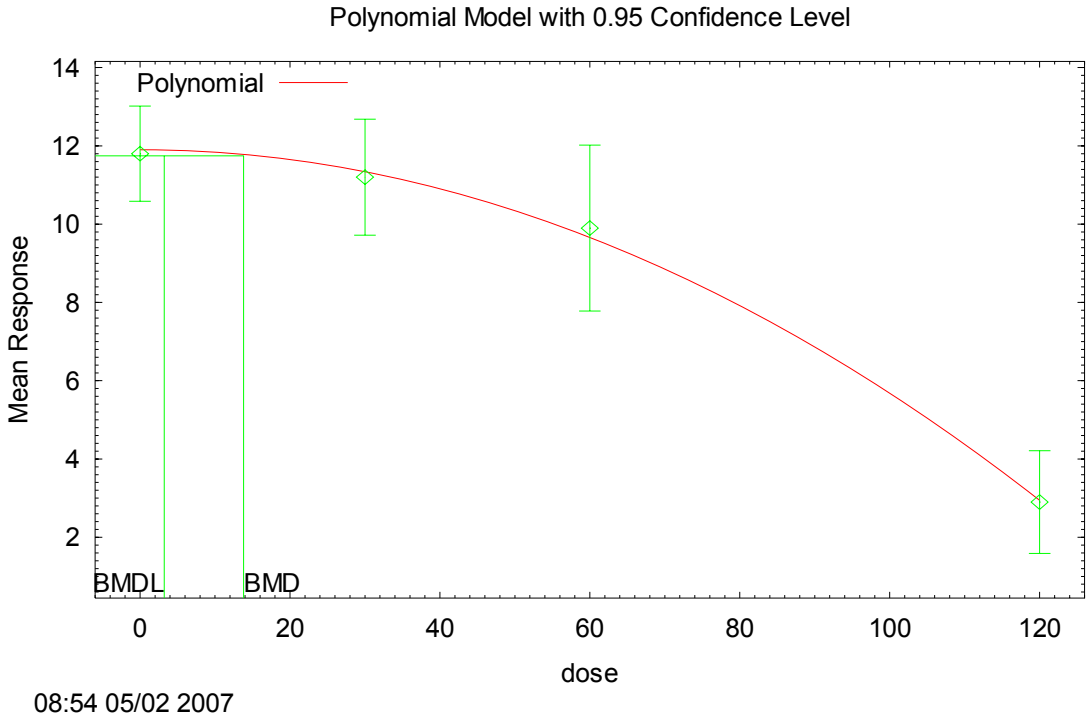


Live pups per litter in the 4th litter

```
================================================================
        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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

```
================================================================
        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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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

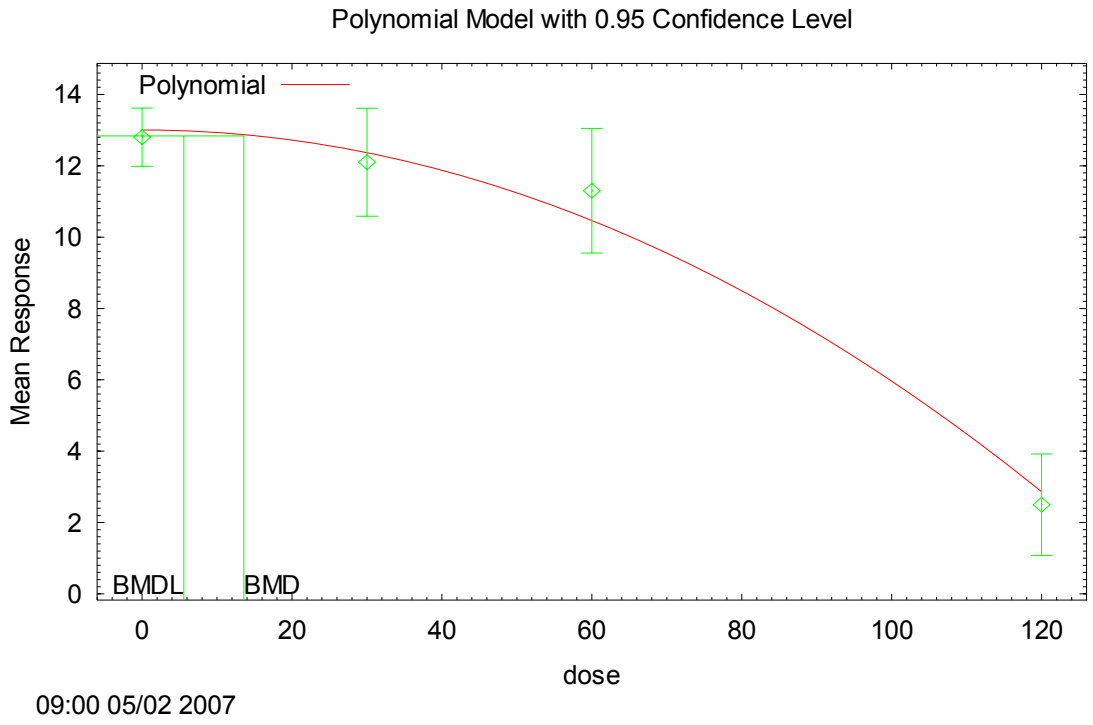
Specified effect = 0.01

Risk Type = Relative risk

Confidence level = 0.95

BMD = 13.571

BMDL = 5.5772



Appendix C-1: Derivation of the oral slope factor using TOX_RISK software

Table C-1: Tumor incidence data, with time to death with tumor; male rats exposed by gavage to 1,2,3-trichloropropane

Dose group (mg/kg-day)	Week of death	Total examined	Number of animals with				
			Squamous cell neoplasia	Pancreas tumors	Kidney adenomas	Preputial gland tumors	Zymbal's gland tumors
0	49	1	0	0	0	0	0
	64	10	0	0	0	0	0
	69	1	0	0	0	0	0
	72	1	0	0	0	1	0
	84	2	0	0	0	0	0
	86	1	0	0	0	0	0
	87	1	0	0	0	0[a]	0
	88	3	0	0	0	0	0
	90	1	0	0	0	0	0
	93	1	0	0	0	0	0
	95	2	0	0	0	0	0
	97	1	0	0	0	0	0
	99	1	0	0	0	0	0
	104	11	1	3	0	2	0
	105	23	0	2	0	2	0
3	64	10	2	0	0	0	0
	82	1	0	0	0	0	0
	84	1	0	0	0	0	0
	86	3	1	0	0	0	0
	89	1	1	0	0	0	0
	93	1	1	0	0	1	0
	94	1	1	0	0	0	0
	95	1	0	0	0	0	0
	97	1	0	0	0	0	0
	98	3	2	2	0	1	0
	99	3	3	1	0	1	0
	100	1	1	0	0	1	0
	101	1	1	0	0	0	0
	104	32	26	17	2	2[a]	0
10	4	1	0	0	0	0	0
	32	1	0	0	0	0	0
	58	2	2	0	0	1	0
	64	11	4	1	0	1	0
	67	1	1	1	0	0	0
	73	1	1	0	0	1	0
	74	1	0	0	0	0	0
	75	1	0	1	0	0	0
	77	3	3	1	0	0	0
	84	2	2	1	0	0	0

Table C-1: Tumor incidence data, with time to death with tumor; male rats exposed by gavage to 1,2,3-trichloropropane

Dose group (mg/kg-day)	Week of death	Total examined	Number of animals with				
			Squamous cell neoplasia	Pancreas tumors	Kidney adenomas	Preputial gland tumors	Zymbal's gland tumors
	87	1	1	1	0	0	0
	88	1	1	1	0	1	0
	91	1	1	1	0	0	0
	92	1	1	0	0	0	0
	93	2	2	1	0	1	0
	94	2	2	2	1	0	0
	95	2	2	1	1	0	0
	96	1	1	1	1	0	0
	97	1	1	1	0	0	0
	98	4	4	4	4	0	0
	100	2	2	1	2	0	0
	101	1	1	1	0	1	0
	103	1	1	1	0	0	0
	104	15	15	15	9	3	0
30	47	1	1	0	0	0	0
	48	1	1	0	0	0	0
	52	1	0	0	0	0	0
	53	3	3	0	0	0[a]	0
	55	2	2	0	0	1	0
	56	1	0	0	0	0	1
	57	1	1	0	0	0	0
	60	1	1	1	1	0	0
	61	2	2	0	0	0	1
	62	2	2	1	0	1	1
	63	1	1	0	0	1	0
	64	9	9	2	5	1	0
	65	1	1	0	1	0	0
	66	1	1	0	0	1	0
	67	2	2	1	2	1	0
	68	3	3	3	1	2	0
	69	5	5	4	3	1	0
	70	5	5	3	4	2	0
	71	2	2	2	1	0	0
	72	1	1	1	0	1	0
	73	3	3	3	1	1[a]	0
	74	2	2	1	1	1	0
	75	1	1	1	0	0	0
	76	9	9	8	6	3	0

[a]Tissue from one animal was missing at this time point.

Source: NTP (1993).

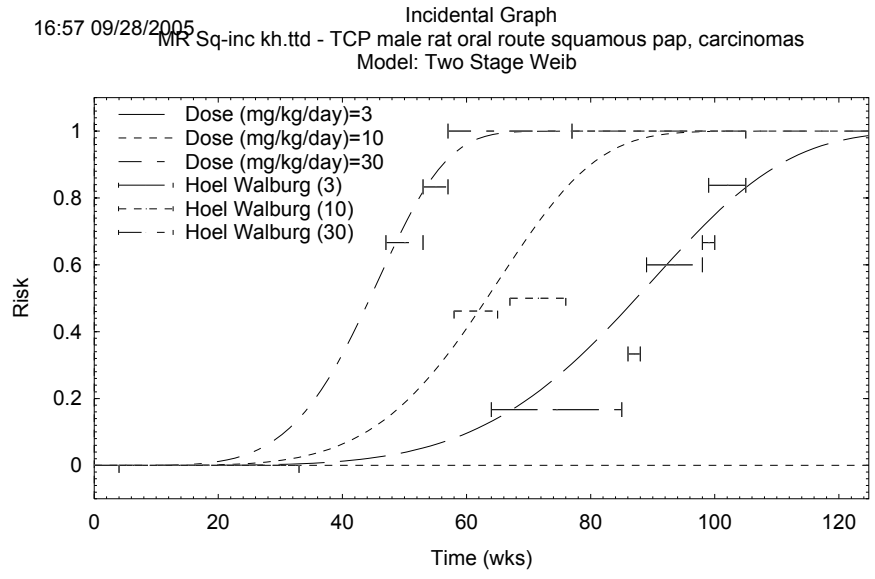
Male Rat Squamous Papillomas, Carcinomas

```
Model: Two Stage Weib         Dataset: G:\_ToxRiskData\Trichloropropane\MR Sq-inc kh.ttd
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

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 2.0465E+000   Upper Bound(q1*) = 3.1604E+000

 Induction Time (T0) Set by User to 0
```

		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



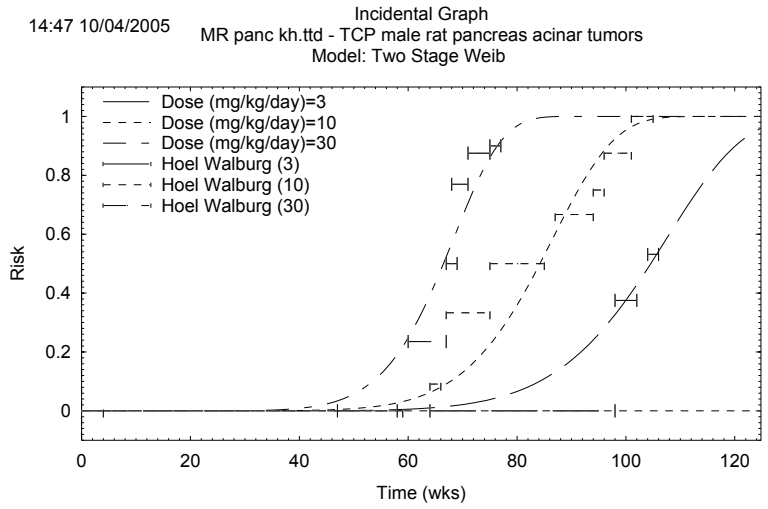
Male Rat Pancreas Acinar Tumors

```
Model: Two Stage Weib         Dataset: G:\_ToxRiskData\Trichloropropane\MR panc kh.ttd
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

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 3.5065E-001   Upper Bound(q1*) = 1.0011E+000

 Induction Time (T0) Set by User to 0
```

		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



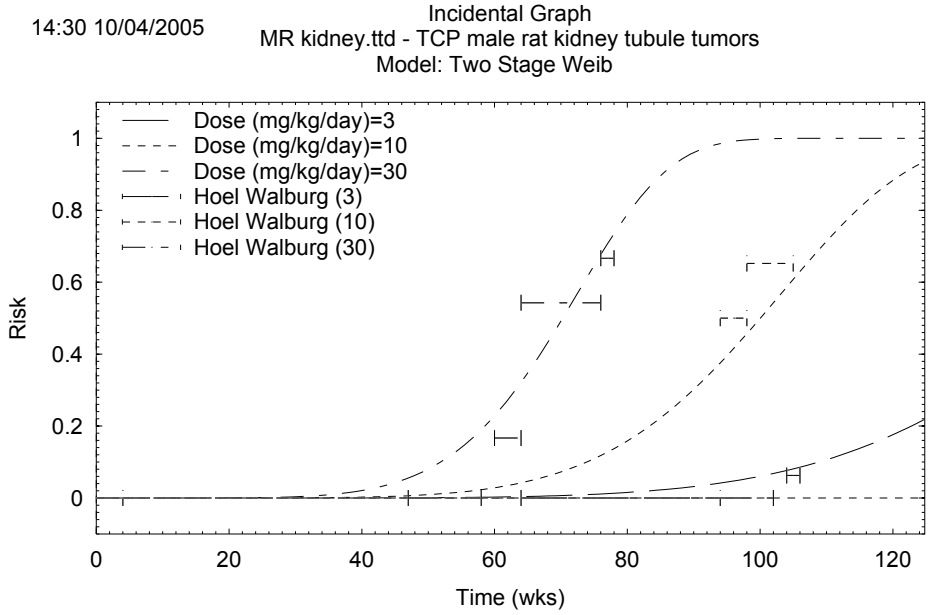
Male Rat Kidney Tubule Adenomas

```
Model: Two Stage Weib         Dataset: G:\_ToxRiskData\Trichloropropane\MR kidney.ttd
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	with fatal tumors	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

         Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 4.6448E-004   Upper Bound(q1*) = 1.0835E-001

 Induction Time (T0) Set by User to 0
```

		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



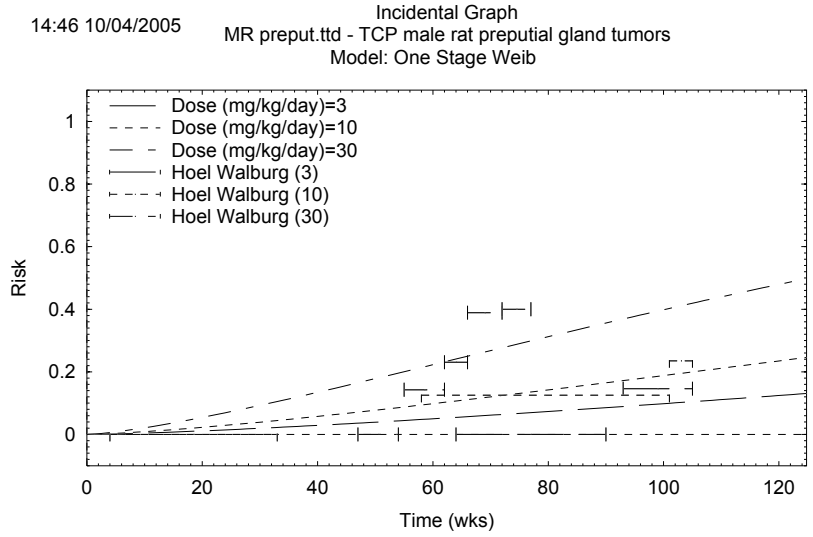
Male Rat Preputial Gland Tumors

```
Model: One Stage Weib         Dataset: G:\_ToxRiskData\Trichloropropane\MR preput.ttd
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	with fatal tumors	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

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 7.8523E-002   Upper Bound(q1*) = 1.7959E-001

 Induction Time (T0) Set by User to 0
```

		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

```
Model: One Stage Weib         Dataset: G:\_ToxRiskData\Trichloropropane\MR Zymbal gl.ttd
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	with fatal tumors	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

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 8.4715E-003   Upper Bound(q1*) = 2.0684E-002

 Induction Time (T0) Set by User to 0
```

		Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	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

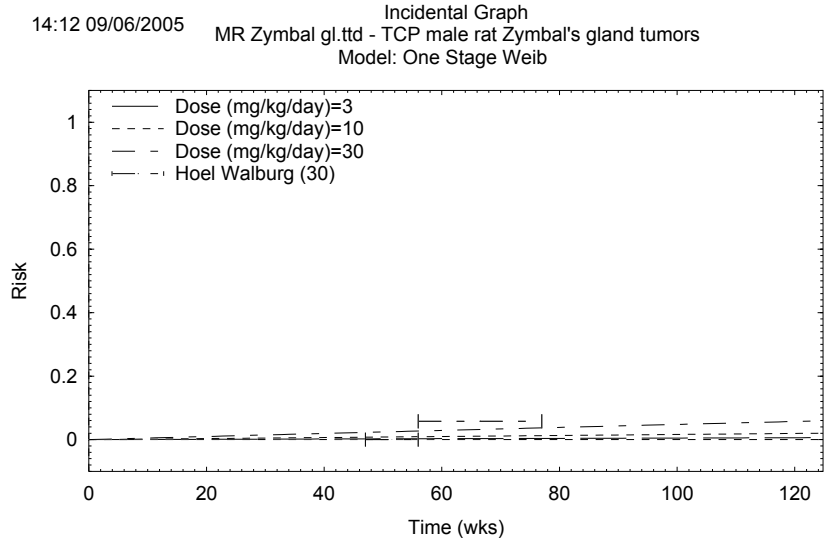


Table C-2. Tumor incidence data, with time to death with tumor; female rats exposed to 1,2,3-trichloropropane

Dose group (mg/kg-day)	Week of observation	Total examined	Number of animals with			
			Any squamous cell neoplasia	Mammary gland tumors	Clitoral gland tumors	Zymbal's gland tumors
0	61	1	0	0[a]	0[a]	0
	64	1	0	1	0	0
	66	10	0	0	0	0
	68	1	0	0	0	0
	75	1	0	0	0	0
	78	1	0	0	0	0
	79	2	0	0	0	0
	85	1	0	0	0	0
	86	1	0	0	0	0
	89	1	0	0	0	0
	92	2	0	0	0	0
	93	1	0	0	0	0
	96	1	0	0	0	0
	98	1	0	0	0	0
	100	1	0	0	0	0
	101	1	0	0	0	0
	102	2	0	1	1	0
	105	18	1	0[a]	2[c]	0
	106	13	0	0[a]	2	0
3	62	1	0	0	0	0
	66	11	1	0	1	0
	67	1	0	1	0	0
	73	1	1	0[a]	0[a]	0
	78	1	0	0	0	0
	83	1	0	0	0	0
	84	1	0	0	0	0
	86	1	0	0	0	0
	95	1	1	0	1	0
	96	1	0	0	0	0
	97	1	1	0	1	0
	99	3	0	0[a]	0	0
	101	1	1	0	0[a]	0
	102	2	2	1	0	1
	104	2	1	0	1	0
	105	30	14	4[a]	7[a]	0
10	36	1	0	0	0	0
	58	2	1	0[a]	0	0
	61	1	0	1	0	0
	62	1	0	0[a]	1	0
	64	2	1	0[a]	2	0
	66	8	5	0	1	0
	68	1	1	1	0	0
	72	1	1	0	1	0
	73	3	3	1	1	0
	74	2	1	1[a]	0	0
	77	2	2	1	1	0
	79	1	1	1	0	0
	80	1	1	0	0	0
	81	2	2	0	0	0

Table C-2. Tumor incidence data, with time to death with tumor; female rats exposed to 1,2,3-trichloropropane

Dose group (mg/kg-day)	Week of observation	Total examined	Number of animals with			
			Any squamous cell neoplasia	Mammary gland tumors	Clitoral gland tumors	Zymbal's gland tumors
	82	1	1	1	0[a]	0
	83	2	2	1	0[a]	0
	85	1	1	0	0	0
	86	2	2	0	1	0
	87	3	2	2[a]	2	0
	90	1	1	0[a]	0	0
	91	2	2	0	0	0
	92	3	2	0[a]	0	0
	96	1	1	0	0	0
	97	1	1	0	1	0
	98	1	1	0	0	0
	100	2	2	0	1	0
	101	1	1	0	0	0
	103	1	1	0	0	0
	104	2	2	1	0	0
	105	8	8	3	6	0
30	12	2	0	0[a]	0	0
	26	1	0	0	0	0
	33	1	1	0	0	0
	34	1	0	1	0	0
	36	1	0	1	0	0
	42	2	2	0[a]	0	0
	44	3	2	1	1	0
	46	1	1	0	1	0
	47	3	1	1[b]	2	0
	48	3	2	2[a]	1	1
	49	5	2	2[a]	3	0
	50	1	1	0	0	0
	51	1	1	1	1	0
	52	3	2	1[a]	1	0
	53	4	2	3	0[a]	0
	54	1	1	0	1	0
	55	2	2	2	0	0
	57	4	4	3	1	0
	58	2	2	0	1	0
	59	3	3	0	1	0
	60	3	3	0[a]	0	0
	62	1	1	1	0	0
	63	2	2	1	0	1
	64	1	1	1	1	0
	66	9	9	2	2	2

[a]Tissue from one animal was missing at this time point.
[b]Tissues from two animals were missing at this time point.
[c]Tissues from three animals were missing at this time point.

Source: NTP (1993)

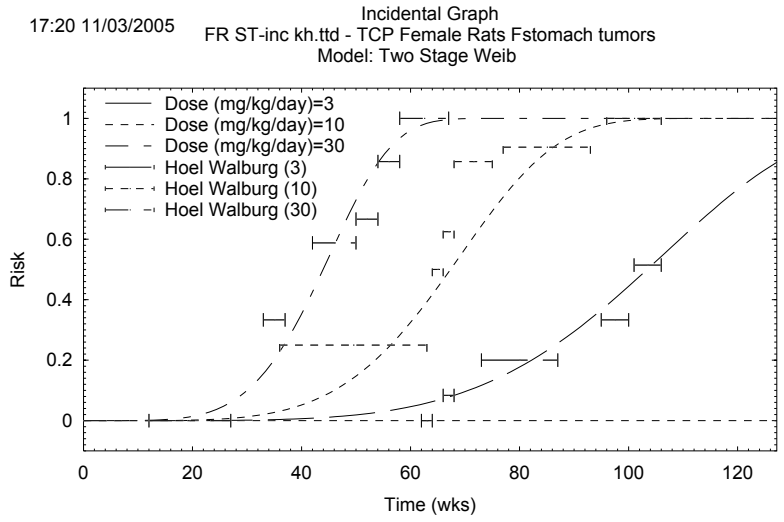
Female Rat Squamous Papillomas, Carcinomas

```
Model: Two Stage Weib         Dataset: G:\_ToxRiskData\Trichloropropane\FR ST-inc kh.ttd
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	with fatal tumors	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

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 3.3093E-001   Upper Bound(q1*) = 1.3576E+000

 Induction Time (T0) Set by User to 0
```

		Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	7.3658E-004	3.0218E-003	Not Reqstd
1.0000E-005	70.00	7.3658E-003	3.0214E-002	Not Reqstd
0.0001	70.00	7.3659E-002	3.0171E-001	Not Reqstd
0.0010	70.00	7.3670E-001	2.9752E+000	Not Reqstd
0.01	70.00	7.3777E+000	2.6537E+001	Not Reqstd
0.10	70.00	7.4958E+001	1.6683E+002	Not Reqstd



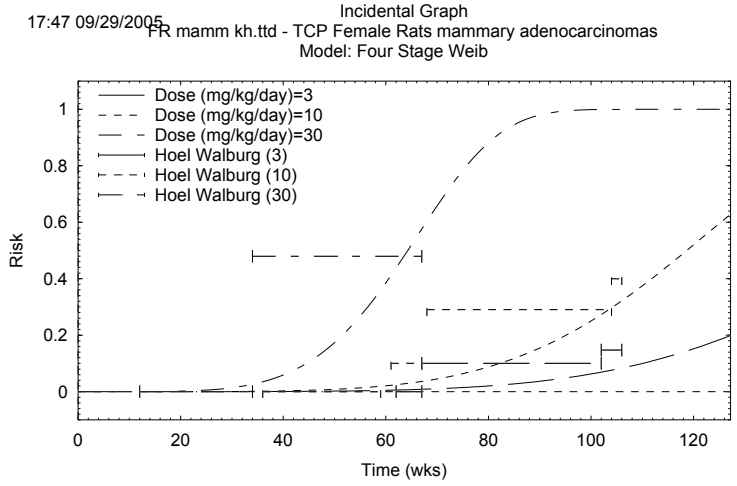
Female Rat, Mammary Adenocarcinomas

```
Model: Four Stage Weib        Dataset: G:\_ToxRiskData\Trichloropropane\FR mamm kh.ttd
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2 ... - Q4 * D^4 )
                            * (T - T0)^Z]
         Maximum Log-Likelihood =  -8.051389e+002

       Parameter Estimates :
                            Q 0 = 0.000000E+000
                            Q 1 = 1.134556E-012
                            Q 2 = 0.000000E+000
                            Q 3 = 0.000000E+000
                            Q 4 = 3.995933E-016
                            Z   = 5.136630E+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	2
3	59	0	6
10	60	0	14
30	60	0	23

```
    Animal to human conversion method: MG/KG  BODY WEIGHT(3/4)/DAY

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 1.0766E-001   Upper Bound(q1*) = 1.9378E+000

 Induction Time (T0) Set by User to 0
```

		Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	5.1606E-004	9.2888E-003	Not Reqstd
1.0000E-005	70.00	5.1606E-003	9.2888E-002	Not Reqstd
0.0001	70.00	5.1608E-002	9.2892E-001	Not Reqstd
0.0010	70.00	5.1632E-001	9.2934E+000	Not Reqstd
0.01	70.00	5.1866E+000	9.3337E+001	Not Reqstd
0.10	70.00	5.4371E+001	8.4981E+002	Not Reqstd



Female Rat, Mammary Adenocarcinomas (cont.)

Although the 4-stage multistage Weibull provided the most parsimonious fit of the mammary adenocarcinoma data, the uncertainty in the benchmark doses was relatively high; the risk-specific BMDs (MLEs) were approximately 15-fold higher than the BMDLs. There was some underestimation of the terminal tumor incidence in the mid- and low-dose groups. Consequently, some modifications to the modeling were considered.

First, the high dose was dropped. The most parsimonious multistage Weibull fit had 2 stages, but the BMDs and BMDLs were still approximately 15-fold apart, and the fit was similar to the earlier fit (results not shown).

Second, the simpler multistage model was considered, using the adjusted incidences in Table 5-3. The high dose was adjusted for early termination of that group by multiplying by the default adjustment of (experiment length/usual lifespan length)^3, $(66/104)^3 = 0.26$ (Anderson et al., 1983; Bailer and Portier, 1986), reducing the high dose from 30 mg/kg-day to 7.7 mg/kg-day, less than the mid-dose of 10 mg/kg-day. Because the tumor response monotonically increased with increasing administered dose, this adjustment led to a non-monotonic response pattern not handled well by the multistage model (overall goodness-of-fit p-value<0.01; results not shown). Because the mammary adenocarcinomas in the control and lower two dose groups started occurring around the same time, roughly week 65, and those in the high dose group started occurring in Week 34, it seemed reasonable to treat the tumors in the lower dose groups as involving more comparable processes that are also more relevant to low dose extrapolation, and model those without the high dose group. This led to a one-stage multistage model, with goodness of fit p=0.89, and a BMD_{10} and $BMDL_{10}$ less than 2-fold apart (see print-out below).

The resulting BMD_{10} and $BMDL_{10}$ must be converted to human equivalents; unlike the multistage-Weibull model, this conversion is not included in BMDS. Application of the interspecies scaling factor $(BW_a/BW_h)^{1/4}$= $(0.25 \text{ kg}/70 \text{ kg})^{1/4} = 0.24$, and the continuous exposure adjustment of 5 days/7 days = 0.71, yields a $BMD_{10} = 3.8 \times 0.24 \times 0.71 = 0.64$ mg/kg-day, and a $BMDL_{10} = 2.5 \times 0.24 \times 0.71 = 0.43$ mg/kg-day. Note that the BMD_{10} is very similar to that obtained from the multistage-Weibull fit, 0.85 mg/kg-day. The agreement between the two MLEs provides some confidence that the models converge on useful estimates of low dose risk. While a biologically based model that could accommodate the high dose behavior would be preferred, the one-stage multistage model appears to provide an adequate fit of the mammary adenocarcinoma data for estimating low dose risk.

Female Rat, Mammary Adenocarcinomas (cont.)

```
=====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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


                 Default Initial Parameter Values
                    Background =    0.0317156
                       Beta(1) =    0.0279933


          Asymptotic Correlation Matrix of Parameter Estimates

            Background      Beta(1)

Background            1        -0.68

   Beta(1)        -0.68            1


                         Parameter Estimates

       Variable           Estimate             Std. Err.
     Background            0.034025            0.109277
        Beta(1)           0.0273727           0.0206733


                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -58.1342
   Fitted model         -58.1443     0.0202092      1           0.887
  Reduced model         -64.9339       13.5995      2        0.001114

          AIC:          120.289
                    Goodness  of  Fit

     Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
  ----------------------------------------------------------------------
```

```
i: 1
    0.0000     0.0340         1.939         2          57       0.032
i: 2
    3.0000     0.1102         6.280         6          57      -0.050
i: 3
   10.0000     0.2653        13.798        14          52       0.020

 Chi-square =       0.02     DF = 1        P-value = 0.8874
```

Benchmark Dose Computation

Specified effect = 0.1 Risk Type = Extra risk Confidence level = 0.95 BMD = 3.84911 BMDL = 2.51405	Specified effect = 0.0001 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.00415893 BMDL = 0.00238964
Specified effect = 0.01 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.415303 BMDL = 0.240155	Specified effect = 1e-005 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.00036533 BMDL = 0.000238615
Specified effect = 0.001 Risk Type = Extra risk Confidence level = 0.95 BMD = 0.0415838 BMDL = 0.0239071	

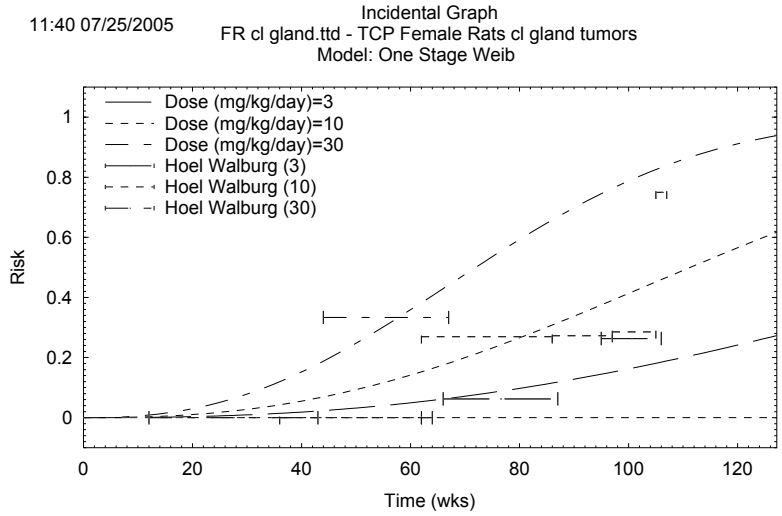
Female Rat, Clitoral Gland Adenomas, Carcinomas

```
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

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 3.3152E-001   Upper Bound(q1*) = 4.4070E-001

 Induction Time (T0) Set by User to 0
```

		Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	2.2691E-003	3.0164E-003	Not Reqstd
1.0000E-005	70.00	2.2691E-002	3.0164E-002	Not Reqstd
0.0001	70.00	2.2692E-001	3.0166E-001	Not Reqstd
0.0010	70.00	2.2702E+000	3.0179E+000	Not Reqstd
0.01	70.00	2.2805E+001	3.0316E+001	Not Reqstd
0.10	70.00	2.3907E+002	3.1781E+002	Not Reqstd



Female Rat, Zymbal's Gland Carcinomas

```
Model: One Stage Weib         Dataset: G:\_ToxRiskData\Trichloropropane\FR Zymbal gl.ttd
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

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 2.4968E-002   Upper Bound(q1*) = 6.5997E-002

 Induction Time (T0) Set by User to 0
```

		Dose Estimates (ug/kg/day)		
		95.00 %		95.00 %
Incid Extra Risk	Time	Lower Bound	MLE	Upper Bound
1.0000E-006	70.00	1.5152E-002	4.0052E-002	Not Reqstd
1.0000E-005	70.00	1.5152E-001	4.0052E-001	Not Reqstd
0.0001	70.00	1.5153E+000	4.0054E+000	Not Reqstd
0.0010	70.00	1.5160E+001	4.0072E+001	Not Reqstd
0.01	70.00	1.5228E+002	4.0253E+002	Not Reqstd
0.10	70.00	1.5964E+003	4.2198E+003	Not Reqstd

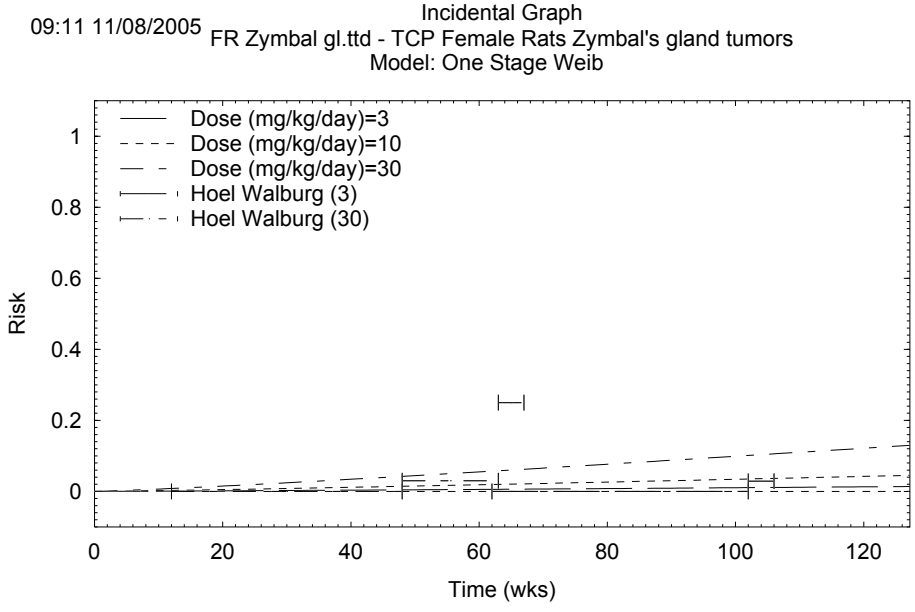


Table C-3. Summary of cancer risk values estimated by R/BMDR and summed across tumor sites for male and female rats.

Tumor site	Risk, R	BMD_R, mg/kg-day	$BMDL_R$, mg/kg-day	Cancer risk value at BMD_R[a], $(mg/kg\text{-}day)^{-1}$	Oral slope factor[b] $(mg/kg\text{-}day)^{-1}$	SD	SD^2	Proportion of total variance
Male Rats								
Oral route squamous papillomas, carcinomas.	0.01	4.90E-03	3.18E-03	2.04E+00	3.14E+00	6.71E-01	4.50E-01	0.73
Pancreas acinar tumors	0.001	2.83E-03	9.99E-04	3.53E-01	1.00E+00	3.94E-01	1.55E-01	0.25
Kidney tubule adenomas	0.001	6.81E-02	9.11E-03	1.47E-02	1.10E-01	5.786E-02	3.30E-03	0.01
Preputial gland tumors	0.001	1.28E-02	5.57E-03	7.81E-02	1.80E-01	6.16E-02	3.76E-03	0.01
Zymbal's gland carcinomas	0.001	1.18E-01	4.84E-02	8.48E-03	2.07E-02	7.42E-03	5.51E-05	<0.01
Sum of MLE risk estimates:				2.495		Total variance:	0.617	
95% Upper bound on sum of central tendence risk estimates:					3.783	SD = $(variance)^{1/2}$:	0.783	
Female Rats								
Oral route squamous papillomas, carcinomas.	0.001	2.98E-03	7.37E-04	3.36E-01	1.36E+00	6.21E-01	3.85E-01	0.98
Mammary adenocarcinomas	0.01	7.12E-02	4.12E-02	1.40E-01	2.43E-01	6.23E-02	3.88E-03	0.01
Clitoral gland adenomas, carcinomas	0.01	3.03E-02	2.28E-02	3.30E-01	4.39E-01	6.60E-02	4.36E-03	0.01
Zymbal's gland carcinomas	0.01	4.00E-01	1.50E-01	2.50E-02	6.67E-02	2.53E-02	6.42E-04	<0.01
Sum of MLE risk estimates:				0.831		Total variance:	0.394	
95% Upper bound on sum of MLE risk estimates:					1.864	SD = $(variance)^{1/2}$:	0.628	

[a] The MLE risk estimate = R/BMD_R

[b] **The oral slope factor = $R/BMDL_R$**