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TOXICOLOGICAL REVIEW

OF

CHLOROPRENE

(CAS No. 126-99-8)

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

September 2009

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LIST OF ABBREVIATIONS AND ACRONYMS

ADAF	age dependent adjustment factor
AEH	alveolar epithelial hyperplasia
AIC	Akaike Information Criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
BMC	benchmark concentration
BMCL	lower bound on the benchmark concentration
BMD	benchmark dose
BMDL	lower confidence limit on the benchmark dose
BMDS	benchmark dose software
BMR	benchmark response
CASRN	Chemical Abstracts Service Registry Number
CI	confidence interval
CNS	central nervous system
CYP	cytochrome
DAF	dosimetric adjustment factor
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EH	epoxide hydralases
EPA	U.S. Environmental Protection Agency
GD	gestational day
GDH	glutamine dehydrogenase
GSH	glutathione
HEC	human equivalent concentration
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
LOAEL	lowest-observed-adverse-effect level
LOH	loss of heterozygosity
M	Molar
MLE	maximum likelihood estimate
MOA	mode of action
MV	minute volume
NCEA	National Center for Environmental Assessment
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NOAEL	no-observed-adverse-effect level
NPSH	nonprotein sulfhydryl
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OR	odds ratio
p	probability value
PBPK	physiologically based pharmacokinetic (model)
PCB	polychlorinated biphenyl

POD	point of departure
ppm	parts per million
PU	pulmonary
R	level of risk
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RGDR	regional gas dose ratio
RR	relative risk
SA	surface area
SD	standard deviation
SDH	sorbitol dehydrogenase
SIR	standard incidence ratio
SMR	standardized mortality ratio
UCL	upper confidence limit
UF	uncertainty factor
v/v	volume/volume
χ^2	chi squared

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 chloroprene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of chloroprene.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of 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 chloroprene. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

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

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

Development of these hazard identification and dose-response assessments for chloroprene has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC) (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996),

Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

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

2. CHEMICAL AND PHYSICAL INFORMATION

Beta-chloroprene (C_4H_5Cl) (hereafter referred to as chloroprene) is a volatile, flammable liquid used primarily in the manufacture of polychloroprene or neoprene rubber. The latter is used to make diverse products, such as tires, wire coatings, and tubing. While 90% of chloroprene is used to make neoprene solid, polychloroprene, about 10% is converted to polychloroprene latex, a colloidal suspension of polychloroprene in water (International Agency for Research on Cancer [IARC], 1999). In 1995, there was one commercial producer of chloroprene in the United States; other plants produced chloroprene for on-site use and processing, as a by-product of vinyl chloride production, or as a manufacturing impurity (National Toxicology Program [NTP], 2005). Used almost exclusively to produce polychloroprene, chloroprene is sold to only three U.S. companies for polychloroprene manufacture; less than 20 lb/yr is sold for research applications. The total estimated production of polychloroprene from 1986 to 1988 was approximately 250 to 300 million lb (113,000 to 136,000 metric tons), and the volume from 1995 to 1996 was approximately 200 to 250 million lb (90,700 to 113,000 metric tons).

There are no known natural occurrences of chloroprene in the environment. The main sources of releases to the environment are through effluent and emissions from facilities that use chloroprene to produce polychloroprene elastomers or transport of the product. In 1995, there were 14 facilities reporting releases of chloroprene to the atmosphere, eight of which reported individual atmospheric releases from 2 to 481,871 lbs (0.0009 to 218.6 Mg). Three plants in Kentucky, Texas, and Louisiana, each reporting atmospheric releases of > 100,000 lbs, accounted for most of the reported chloroprene releases. One of these sites produced chloroprene, while the other two converted chloroprene to polychloroprene (NTP, 2005). The chemical structure of chloroprene is shown in Figure 2-1.

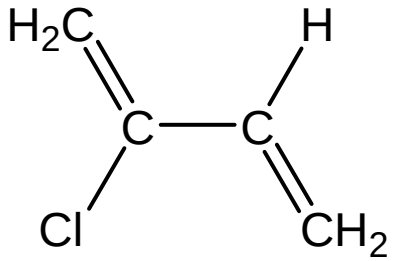

Figure 2-1. The chemical structure of chloroprene.

The starting material for the synthesis of chloroprene is 1,3-butadiene. Chloroprene is also a structural analogue of isoprene (2-methyl 1,3-butadiene) and resembles vinyl chloride as far as having a chlorine bound to a double-bonded carbon (alkene) backbone. However, chloroprene contains four carbons arranged with two double bonds. The odor of chloroprene is described as pungent and ether-like (National Library of Medicine [NLM], 2008). Chloroprene is volatile and highly reactive; it is not

expected to bioaccumulate or persist in the environment (Organisation for Economic Cooperation and Development [OECD], 1998). Because of its high vapor pressure (215 mm Hg at 25°C), chloroprene is expected to readily volatilize from water and solid surfaces (NTP, 2005). Chloroprene vapor has an estimated ionization potential of 8.95 ± 0.05 eV and an estimated half-life in the atmosphere of less than 20 hours (Grosjean, 1990). Reactions with $^{\bullet}OH$ (to produce formaldehyde), O_3, and NO_3 are the expected pathways of removal, although no experimental data exist (Grosjean, 1991).

Of particular relevance to any toxicological studies involving chloroprene is its propensity to spontaneously oxidize and form dimers and other oxygenated species unless stabilizers or inhibitors are added. Uninhibited chloroprene must be stored under nitrogen at temperatures below 0ºC (e.g., –20ºC) to prevent spontaneous polymerization. When bulk chloroprene with 5% n-octane added as an internal standard was stored at 55ºC for up to 6 hours, dimer content increased by 62% and chloroprene monomer decreased by 22% (NTP, 1996). Because these reaction products, if formed, may themselves account for any observed toxicity, toxicological studies that do not report storage or generation conditions may yield results that are questionable for their relevance to chloroprene monomer. A discussion of the polymerization process has been reported by Lynch (2001a), Kroshwitz and Howe-Grant (1993), Stewart (1971), and Nystrom (1948). Additional information on production and use has been reported by the International Agency for Research on Cancer (IARC, 1999). Structures have been proposed for some of the chloroprene dimers (Stewart, 1971); some dimers result upon reaction at room temperature while others result after prolonged heating.

In addition to volatilization, the potential fate of chloroprene that is released to soil is to leach into groundwater. Breakdown via hydrolysis is not likely, as it is only partially soluble in water (CambridgeSoft Corp., 2007). Chloroprene that is released to the water may only moderately adsorb to suspended sediments or particles, and there will be little bioaccumulation in aquatic organisms (K_{ow} = 2.2). The occupational exposure potential to chloroprene is limited to facilities in the U.S., Europe, and Asia where chloroprene is produced and converted to polychloroprene (Lynch, 2001b). The physical and chemical properties of chloroprene are shown in Table 2-1.

Table 2-1. Physical properties and chemical identity of chloroprene

CHLOROPRENE		REFERENCE
CASRN	126-99-8	NLM (2008)
Synonyms	2-chlorobuta-1,3-diene; 2-chlorobutadiene; 2-chloroprene; alpha-chloroprene; beta-chlorobutadiene; beta-chloroprene; chlorobutadiene; chloroprene	CambridgeSoft Corp. (2007)
Registered trade name	Neoprene	CambridgeSoft Corp. (2007)
Melting point	–130°C	NLM (2008)
Boiling point	59.4°C	NLM (2008)
Density	0.956 at 20°C (relative to the density of H_2O at 4°C)	NLM (2008)
Vapor pressure	215 mm Hg at 25°C	NLM (2008)
Vapor density	3.0 (air = 1)	NLM (2008)
Flashpoint (open cup)	–15.6°C	CambridgeSoft Corp. (2007)
Flammability limits	4–20% in air	NLM (2008)
Water solubility	2.115 g/L at 25°C	CambridgeSoft Corp. (2007)
Other solubilities	Miscible with ethyl ether, acetone, benzene; soluble in alcohol	NLM (2008)
Log K_{OW}	2.2	OECD (1998)
Henry's law constant	5.6×10^{-2} atm/m^3-mol at 25°C	NLM (2008)
Odor threshold	15 ppm (54 mg/m^3)	U.S. EPA (2000d)
Molecular weight	88.54	NLM (2008)
Conversion factors (in air)	1 mg/m^3 = 0.276 ppm; 1 ppm = 3.62 mg/m^3 at 25°C, 760 torr	NLM (2008)
Empirical formula	C_4H_5Cl	CambridgeSoft Corp. (2007)

3. TOXICOKINETICS

No reports are available that address the toxicokinetics of chloroprene in humans by any route of exposure. Limited information is available for animals regarding the absorption and in vivo metabolism of chloroprene. No information regarding tissue distribution of chloroprene from animal studies is available. In vitro studies have been conducted to evaluate the metabolism of chloroprene in lung and liver tissue fractions from rat, mouse, hamster, and humans (Munter et al., 2007a, b, 2003; Himmelstein et al., 2004a, 2001a, 2001b; Cottrell et al., 2001; Summer and Greim, 1980). Hurst and Ali (2007) evaluated the kinetics of R- and S-enantiomers of the chloroprene metabolite (1-chloroethenyl)oxirane in mouse erythrocytes. A physiologically based pharmacokinetic (PBPK) model has been developed to describe changes in chamber chloroprene concentrations during exposures with mice, rats, and hamsters (Himmelstein et al. 2004a, 2004b). No in vivo time-course data for blood or tissue concentration are available for model validation.

3.1. ABSORPTION

Quantitative data on the absorption of chloroprene from any route of exposure have not been reported. The Hazardous Substances Data Bank states that chloroprene is "rapidly absorbed by the skin" (Lefaux [1968] as cited in NLM [2008]). Chronic inhalation studies in B6C3F1 mice and F344/N rats suggest that chloroprene has multiple nonneoplastic and neoplastic targets (nose and lung, kidney, forestomach, Harderian gland, skin); therefore, the absorption and systemic distribution via the inhalation route can be inferred (NTP, 1998).

3.2. DISTRIBUTION

No quantitative in vivo data on the tissue distribution of chloroprene have been reported. As indicated above, the widespread distribution of chloroprene in vivo following absorption can be inferred from effects in several target organs (NTP, 1998). Himmelstein et al. (2004b) determined partition coefficients for chloroprene in mouse, F344 rat, Wistar rat, and hamster tissues by using the vial equilibration method described by Gargas et al. (1989), as given in Table 3-1. These tissue-to-air ratios suggest that chloroprene will be preferentially distributed in adipose tissue, followed by lung, kidney, liver, and muscle. The partition coefficient values suggest there are no significant species differences expected in tissue distribution of chloroprene.

Table 3-1. Tissue-to-air partition coefficients for chloroprene

TISSUE	TISSUE-TO-AIR PARTITION COEFFICIENTS[a]				
	Mouse	F344 rat	Wistar rat	Hamster	Human[b]
Blood	7.8 ± 0.1	7.3 ± 0.1	8.0 ± 0.5	9.3 ± 0.3	4.5 ± 0.1
Lung	18.6 ± 5.1	13.5 ± 1.6	11.2 ± 0.5	9.7 ± 0.6	13.3 ± 4.1
Liver	9.8 ± 0.9	11.5 ± 0.3	10.9 ± 0.2	10.5 ± 0.5	10.7 ± 1.1
Fat	135.3 ± 1.6	124.0 ± 1.5	126.3 ± 1.4	130.1 ± 0.9	128.9 ± 2.7
Muscle	4.6 ± 0.8	4.4 ± 0.4	4.0 ± 0.3	5.0 ± 0.2	4.5 ± 1.0
Kidney	13.7 ± 0.6	16.7 ± 0.6	9.4 ± 0.4	8.2 ± 0.3	12.0 ± 0.9

[a] Mean ± standard error for three replicates per rodent tissue.
[b] Human blood values determined for nine replicates (three subjects, three replicates/subject); human tissue partition coefficient values were derived from rodents with standard error adjusted to account for the proportion of variation from each set of rodent data.

Source: Himmelstein et al. (2004b).

3.3. METABOLISM

The metabolism of chloroprene has been primarily evaluated in vitro with lung and liver tissue fractions from rat, mouse, hamster, and humans (Munter et al., 2007a, 2007b, 2003; Himmelstein et al., 2004a, 2001a, 2001b; Cottrell et al., 2001; Summer and Greim, 1980). In a 1978 review of the older literature, a number of reports suggested that chloroprene forms peroxides that interact with tissue thiol groups and that the disposition of chloroprene is likely similar to vinyl chloride and vinylidene chloride (Haley, 1978). This report was the first to postulate a metabolic profile of chloroprene, including formation of epoxides by cytochrome P450 (CYP450) enzymes that could give rise to aldehydes and eventually form mercapturic acid derivatives.

In studies using mouse and human liver microsomes, Bartsch et al. (1979) showed that 2-chloro-2-ethynyloxirane and/or (1-chloroethenyl)oxirane could be intermediates in the biotransformation of chloroprene. This was based on the finding that 4-(4-nitrobenzyl)pyridine trapped a volatile metabolite produced during reaction of mouse liver microsomes with chloroprene. Summer and Greim (1980) reported that in vitro incubation of chloroprene with hepatocytes isolated from male Wistar rats produced a concentration-dependent decrease in cellular glutathione (GSH), suggesting a GSH-dependent detoxification pathway. A report by Himmelstein et al. (2001b) was the first to quantitatively identify (1-chloroethenyl)oxirane as an epoxide metabolite of chloroprene and confirmed the identify of the volatile metabolite reported by Bartsch et al. (1979). Himmelstein et al. (2001b) reported that the oxidation of chloroprene to (1-chloroethenyl)oxirane was evident in rodent and human liver microsomes and most likely involved CYP 2E1, as evidenced by nearly complete in vitro inhibition with 4-methylpyrazole hydrochloride. A comparison across species suggested that a greater amount of (1-chloroethenyl)oxirane was present in B6C3F1 mice and F344 rat liver microsomes, followed by the Wistar rat, then humans and hamsters (Table 3-2).

Table 3-2. Liver microsomal metabolites as a percentage of 1-butanol internal standard

METABOLITE PEAK[a]	LIVER MICROSOMAL SUSPENSION				
	B6C3F1 mouse	F344 rat	Wistar rat	Hamster	Human
1	9.0	12.0	4.0	0.8	1.3
2	0.0	0.1	0.1	0.2	0.1
3	0.8	0.3	0.2	0.8	0.3
4	0.2	0.0	0.1	0.4	0.1
5	0.2	0.3	0.0	0.1	0.0
6	0.6	0.4	0.3	0.3	0.1

[a] Metabolite peak 1 = (1-chloroethenyl)oxirane. Metabolite peaks 2–5 had insufficient signal to obtain meaningful spectral data. A tentative spectral match for peak 6 was made as 3-chloro-2-butenal.

Source: Himmelstein et al. (2001b).

Further metabolism of (1-chloroethenyl)oxirane was observed in time-course evaluations with liver microsomes (Himmelstein et al., 2001b). In mouse liver microsomes, the (1-chloroethenyl)oxirane concentration showed an initial increase over 10 minutes that was followed by a decline, attributable to either epoxide hydrolase-mediated hydrolysis or further oxidative metabolism. Preliminary results indicated that the ranking of (1-chloroethenyl)oxirane hydrolysis in liver microsomes was as follows: hamsters ~ humans > Wistar rats > B6C3F1 mice and F344 rats.

Studies by Cottrell et al. (2001) are in agreement with reports from Himmelstein et al. (2001a, 2001b) and further define the structures and stereochemistry of chloroprene metabolites from rodent species and humans by comparison with synthetic reference standards. Based on these studies, the metabolic pathway illustrated in Figure 3-1 was proposed.

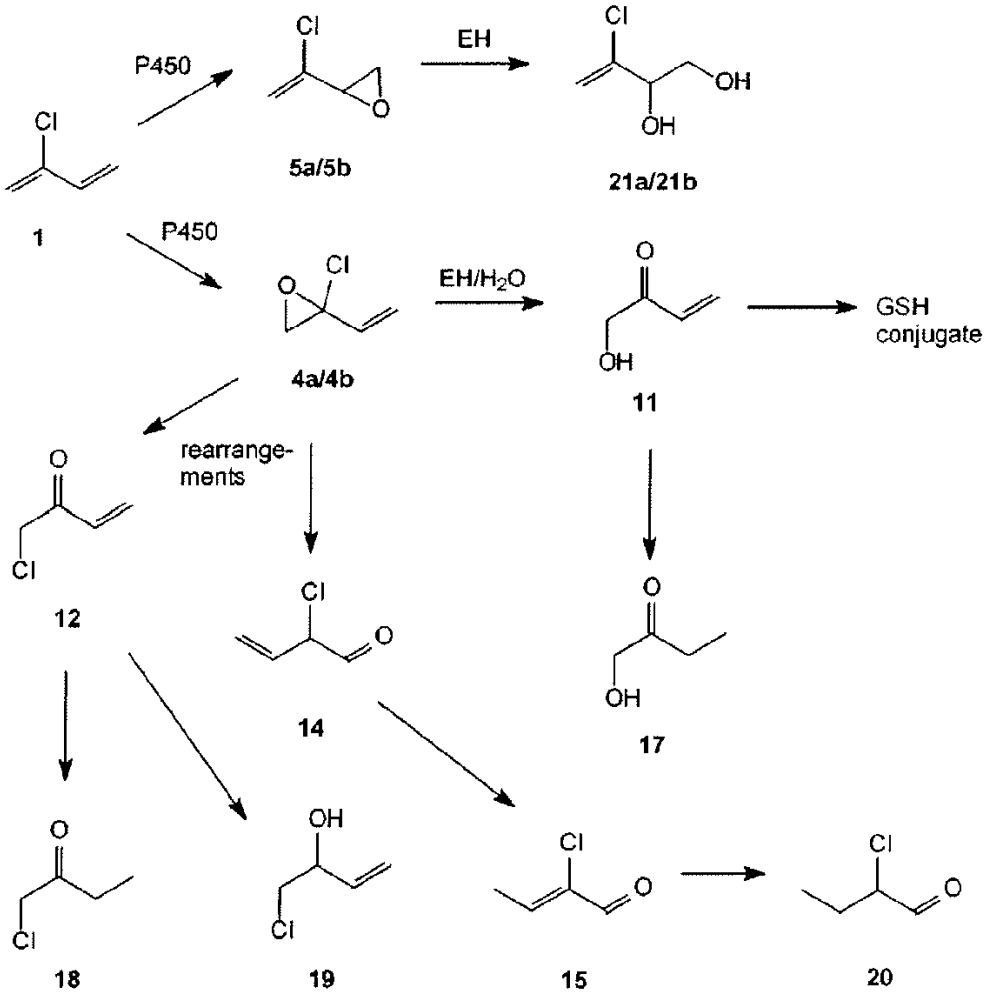

Figure 3-1. Proposed metabolism of chloroprene.

Key: **1** = chloroprene; **5a/5b** = R- and S-enantiomers of (1-chloroethenyl)oxirane; **21a/b** = R- and S-enantiomers of 3-chlorobut-3-ene-1,2-diol; **4a/4b** = R- and S-enantiomers of 2-chloro-2-ethenyloxirane; **11** = 1-hydroxybut-3-en-2-one; **17** = 1-hydroxybutan-2-one; **14** = 2-chlorobut-3-en-1-al; **15** = 2-chlorobut-2-en-1-al; **20** = 2-chloro-butanal; **12** = 1-chlorobut-3-en-2-one; **18** = 1-chlorobutan-2-one; and **19** = 1-chloro-2-hydroxy-but-3-ene.

Source: Adapted from Cottrell et al. (2001).

Comparing metabolism between species, Cottrell et al. (2001) observed that qualitative profiles of metabolites from liver microsomes obtained from B6C3F1 mice, Sprague-Dawley or F344 rats, and humans were similar. In all species and either gender, (1-chloroethenyl)oxirane was the major metabolite detected. An important difference among species was in the stereoselectivity of the formation of R- and S-enantiomers of (1-chloroethenyl)oxirane (Table 3-3). For liver microsomes from both male and female Sprague-Dawley and F344 rats, there was a distinct enantioselectivity in the mono-epoxidation of chloroprene to preferentially form the R-enantiomer of (1-chloroethenyl)oxirane. A further study by this group (Munter et al., 2003) verified significant

differences between species in the amounts of R- and S-enantiomers of (1-chloroethenyl)oxirane formed in microsomal liver incubations, the order being mouse > rat > human.

Table 3-3. Steriochemical comparison of relative amounts (percentages) of R- and S-enantiomers of the major chloroprene metabolite (1-chloroethenyl)oxirane from liver microsomes compared across species, strains, gender, and chloroprene concentration (mM)

MALE				FEMALE			
Chloroprene(mM)	**Species/strain[a]**	**% R**	**% S**	**Chloroprene(mM)**	**Species/strain[a]**	**% R**	**% S**
5	Sprague-Dawley rat	58	42		Sprague-Dawley rat		
10		62	38	10		56	44
20		61	39	20		56	44
30		60	40	30		55	45
40		64	36	40		59	41
5	F344 rat	62	38		F344 rat		
10		62	38	10		56	46
20		62	38	20		54	46
30		60	40	30		53	47
40		64	36	40		54	46
5	B6C3F1 mouse	48	52		B6C3F1 mouse		
10		47	53	10		47	53
20		46	54	20		45	55
30		47	53	30		47	53
40		47	53	40		46	54
10	Human	43	57	10	Human	43	57
20		43	57	20		44	56
30		43	57	30		42	58

[a]Average of three samples per species/strain.
[b]Percentage estimated error ± 1%.

Source: Cottrell et al. (2001)

Himmelstein et al. (2004a) developed a two-compartment closed vial model to describe both chloroprene and (1-chloroethenyl)oxirane metabolism in liver and lung fractions from rat (two strains, F344 and Wistar), mouse, hamster, and humans. Estimates for V_{max} and K_m for oxidation of chloroprene in liver microsomes ranged from 0.068–0.29 μmol/hour/mg protein and 0.53–1.33 μM, respectively. Oxidation (V_{max}/K_m) of chloroprene in the liver was slightly faster in the mouse and hamster than in rats or humans (Table 3-4). In lung microsomes, V_{max}/K_m was much greater for mice compared with the other species. Conversely, hydrolysis (V_{max}/K_m) of (1-chloroethenyl)oxirane in liver and lung microsomes was faster for the human and hamster, than for rat or mouse. Glutathione S-transferase-mediated metabolism of (1-chloroethenyl)oxirane in cytosolic tissue fractions was described as a pseudo second-order reaction, with rates ranging from 0.0016–0.0068 hour/mg cytosolic protein in liver and 0.00056–0.0022 hour/mg in lung.

Table 3-4. Kinetic parameters used to describe the microsomal oxidation of chloroprene

TISSUE	SPECIES	ACTIVITY OF MICROSOMAL OXIDATION		
		V_{max}	K_m	V_{max}/K_m
Liver	Mouse	0.23	1.03	224
	F344 rat	0.078	0.53	146
	Wistar rat	0.11	0.84	125
	Hamster	0.29	1.33	218
	Human	0.068	0.68	101
Lung	Mouse	0.10	1.5	66.7
	F344 rat	--	--	1.3[a]
	Wistar rat	--	--	1.3[a]
	Hamster	--	--	1.3[a]
	Human	--	--	1.3[a]

[a] The apparent rate of lung metabolism, over the range of biologically relevant concentrations tested, was linear and was estimated as V_{max}/K_m

Source: Himmelstein et al. (2004a).

Hurst and Ali (2007) evaluated the kinetics of R- and S-enantiomers of the chloroprene metabolite, (1-chloroethenyl)oxirane in mouse erythrocytes. These results implied that S-(1-chloroethenyl)oxirane was much more quickly detoxified than the R-enantiomer when incubated with mouse erythrocytes in vitro. The disappearance of S-(1-chloroethenyl)oxirane was blocked when erythrocytes were preincubated with diethyl maleate, indicating dependence on cellular glutathione for rapid removal. The study by Hurst and Ali (2007) suggested that the R-enantiomer of (1-chloroethenyl)oxirane is potentially more toxic because of slower detoxification.

The limited in vivo rodent studies support the postulated metabolic pathway for chloroprene. For example, male Wistar rats administered 100 or 200 mg/kg chloroprene by gavage demonstrated a rapid depletion of hepatic GSH and a dose-dependent increase in excreted urinary thioethers (presumably GSH-conjugates), which is consistent with in vitro studies using isolated liver hepatocytes (Summer and Greim, 1980). Pretreatment of rats or hepatocytes with phenobarbital or a polychlorinated biphenyl (PCB) mixture (Clophen A50) to induce the mixed-function oxidase enzymes enhanced the GSH depletion effect.

Himmelstein et al. (2004b) conducted closed-chamber gas uptake exposures with rats (Wistar and F344), mice, and hamsters to evaluate metabolism rates with and without metabolic inhibition by using pretreatment with 4-methyl pyrazole. Initial exposure concentrations ranged from 160–240 parts per million (ppm) chloroprene. A PBPK model was used to describe the decrease in chamber chloroprene concentrations over time by using metabolic parameters (Vmax, Km) scaled from in vitro studies (Himmelstein et al., 2004a). The in vitro scaling of total chloroprene metabolism (Table 3-5) was sufficient to explain the in vivo gas uptake data.

Table 3-5. Metabolic parameters of chloroprene

BIOCHEMICAL PARAMETERSa		SPECIES			
		Mouse	F344 rat	Wistar rat	Hamster
Liver	V_{max} (mg/kg-hour)	39.2	11.50	15.5	42.8
	K_m (mg/L)	0.091	0.047	0.075	0.118
	V_{max}/K_m (L/kg-hour)	431.0	244.0	208.0	363.0
Lung	V_{max} (mg/kg-hour)	1.02	---	---	---
	K_m (mg/L)	0.13	---	---	---
	V_{max}/K_m (L/kg-hour)	7.67	0.14	0.14	0.14

[a]Scaled from Himmelstein et al. (2004a) using microsomal protein content.

Source: Himmelstein et al. (2004b).

3.4. ELIMINATION

Limited information is available regarding the elimination of chloroprene in rodents. Summer and Greim (1980) administered male Wistar rats 100 or 200 mg/kg chloroprene by gavage and observed a dose-dependent, nonlinear increase in excreted urinary thioethers (presumably glutathione conjugates). The clearance of these thioethers reached a threshold at 24 hours after dosing, indicating that elimination was rapid.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Himmelstein et al. (2004b) published a physiologically based toxicokinetic model of chloroprene. Construction of the mathematical model was based on physicochemical, physiological, and metabolic parameters for chloroprene from mouse, rat, hamster, and humans (Table 3-6). The model consisted of distinct compartments for liver and lung, as well as lumped compartments for fat and slowly and rapidly perfused tissues. Metabolism of chloroprene was localized to the lung and liver compartments and described by Michaelis-Menten type saturable kinetics. Although the model was used to estimate the chloroprene concentration in each of the defined compartments, comparisons of model predictions were limited to experimental determinations of chloroprene vapor uptake in closed chambers. Inhibition of uptake was achieved with 4-methyl pyrazole pretreatment, indicating that the decline of chloroprene chamber concentration was due to CYP450 monooxygenase-mediated metabolism. The loss in chamber concentration in the presence of metabolic inhibition represented uptake due to chemical distribution within the animal. A satisfactory model description for inhibition was obtained by setting V_{max} to zero for both liver and lung metabolism. No blood or tissue time-course concentration data are available for model validation.

Table 3-6. Physiological parameters used for chloroprene PBPK modeling

PHYSIOLOGICAL PARAMETERS	SPECIES				
	Mouse	F344 rat	Wistar rat	Hamster	Human
Body weight (kg)	0.024–0.034	0.16–0.28	0.20–0.34	0.10–0.18	NA
Ventilation (L/kg-hour)	15	10.5	10.5	12	NA
Cardiac output (L/kg-hour)	15	9	9	12	NA
Tissue volumes (% body weight)					
Liver	5.5	4.0	4.0	4.0	2.6
Fat	5.0	7.0	7.0	7.0	21.4
Rapid perfused	3.5	5.0	5.0	5.0	7.7
Slow perfused	77.0	75.0	75.0	75.0	56.1
Lung	0.73	0.50	0.50	0.50	0.76
Blood flow (% cardiac output)					
Liver	16.1	18.3	18.3	18.3	22.7
Fat	7.0	7.0	7.0	7.0	5.2
Rapid perfused	51.0	51.0	51.0	51.0	47.2
Slow perfused	15.0	15.0	15.0	15.0	24.9

Source: Himmelstein et al. (2004b).

4. HAZARD IDENTIFICATION

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

Potential for human exposure to chloroprene primarily is via inhalation and perhaps by the dermal route. This section summarizes studies in occupationally-exposed populations from the period of 1978 to 2008.

4.1.1. Chloroprene Exposure and Cancer Effects

4.1.1.1. *Overview*

The NTP (1998, 2005) described chloroprene as *reasonably anticipated to be a human carcinogen* based on evidence of benign and malignant tumor formation at multiple sites in animals. Evidence in humans for the carcinogenicity was reported to be limited based on consideration of only two occupational epidemiological studies by Pell (1978) and Li et al. (1989). Rice and Boffetta (2001) and briefly examined evidence from five epidemiologic studies (Pell ,1978; Li et al., 1989; Bulbulyan et al., 1998; Bulbulyan et al., 1999; Colonna and Laydevant, 2001). Although several of these earlier epidemiological studies noted suggestive evidence of an association between chloroprene exposure and liver cancer risk, study limitations included possible bias from cohort enumeration, follow-up, and choice of reference population. Other study limitations noted included limited exposure assessment data, low statistical power and the possible confounding by unmeasured co-exposures (Rice and Boffetta, 2001). To date, there have been nine occupational epidemiological studies conducted covering 11 cohorts. This epidemiological database is reviewed in the following section.

4.1.1.2. *Individual Occupational Studies*

Pell (1978) conducted a cohort mortality study in two neoprene (polychloroprene) manufacturing plants of DuPont. The first cohort ("Louisville Works Cohort") consisted of 1,576 male workers identified from a roster of wage roll employees in 1957. All workers who were exposed to chloroprene were followed through December 31, 1974, accruing 26,939 person-years. Workers terminated before June 30, 1957 were excluded and 17 individuals were lost to follow-up. Causes of death were obtained from death certificates and coded according to the 7th and 8th revised editions of the "International Classification of Diseases" (ICD). Worker exposures to chloroprene were classified qualitatively as "high," "moderate," "low," and "varied" based on job description. Statistical analyses were performed using Poisson probability distribution with statistical significance level at $p < 0.05$. The general U.S. male population and all male DuPont wage roll employees were used as external and internal comparison populations, respectively. The study's primary objective was to examine respiratory system cancer mortality, but mortality from other site-specific cancers was also evaluated.

Among the 193 deaths detected in this cohort, 51 were due to cancer and 16 were due to cancer of the respiratory system. Compared to U.S. rates, the standardized mortality ratios (SMRs) for all-cause mortality, total cancer mortality and respiratory system cancer mortality were 69.0, 96.6, and 98.4, respectively. Based on the internal comparison SMRs of 114.0 were detected for total cancer mortality and 109.6 for respiratory system cancer mortality. The internal comparison yielded SMRs of 108.7 (15 cases) and 113.2 (12 cases) for respiratory cancer after 15- and 20-year latency periods, respectively. SMRs were lower for the same latency periods when compared with the U.S. general population. Thirteen of the 16 deaths due to respiratory system cancer occurred in smokers, while smoking history was unknown for the other three. Analyses by high exposure occupation did not show any significant change in SMRs or any statistically significant trend when analyzed by years since first exposure. Other cancer deaths that were detected included 19 of the digestive organs (SMR = 142.9 using an internal comparison) and seven of the lymphatic and hematopoietic tissues (SMR = 155.6 using an internal comparison). All the SMRs observed in this study were not statistically significant based on both internal (DuPont) and U.S. general population mortality rates.

These data were reanalyzed by the National Institute for Occupational Safety and Health (NIOSH) using a modified life-table analysis (Leet and Selevan, 1982). Workers were classified into high and low exposure categories based on a classification scheme developed by an industrial hygienist who worked at the plant. Eight hundred and fifty-one workers were allocated to the high exposure group and 823 to the low exposure group, with some workers contributing person-years in both categories when their exposures or job titles changed. A total of 26,304 person-years were accrued, with 13,606 person-years in the high-exposure and 12,644 in the low-exposure category. Compared to U.S. population rates, the overall SMR for the total cohort was 79. Excess deaths were observed for cancers of the digestive system (especially the biliary passages and liver), the lung, and the lymphatic/hematopoietic system. The only statistically significant SMR of biliary passage and liver was based on four cases, three from the high-exposure category (Table 4-1). Of these three deaths, one was due to liver cancer, and the other two to gall bladder cancer. Cancer mortality data were analyzed with respect to latency and duration of exposures stratified into 10-year intervals. Statistically significant trends were not observed in either the latency analysis or the years of presumed chloroprene exposure analysis, but these analyses were based on small numbers.

The main limitations of the Pell (1978) study and the NIOSH reanalysis (Leet and Selevan, 1982) are lack of smoking history data, lack of adjustment for other potential risk factors, and absence of quantitative exposure information. Exclusion of workers terminated prior to June 30, 1957, might have also resulted in some unidentified cancer deaths that could have been associated with earlier higher exposures. Moreover, as pointed out by Leet and Selevan (1982), the statistical power of the study to detect a significant excess in mortality was low when the sub-cohort analyses were conducted.

Table 4-1. Standardized mortality ratios (SMRs) for the DuPont Louisville Works cohort relative to general U.S. population rates.

CAUSE OF DEATH	TOTAL COHORT CASES, SMR (95% CI[a])	HIGH EXPOSURE CASES, SMR (95% CI)	LOW EXPOSURE CASES, SMR (95% CI)
All Causes	193, 79 (68–91)	91, 75 (61–92)	102, 82 (67–100)
All Cancers	51, 107 (80–141)	25, 107 (69–158)	26, 107 (70–157)
Digestive	19, 145 (87–227)	8, 125 (54–246)	11, 164 (82–294)
Biliary/liver	4, 571 (156–1463)	3, 750 (155–2192)	1, 250 (6–1393)
Trachea, bronchus, lung	17, 106 (62–170)	10, 128 (61–236)	7, 86 (35–178)
Lymphatic, hematopoietic	7, 140 (56–288)	4, 160 (44–410)	3, 120 (25–351)

[a]CI = confidence interval.

Source: Leet and Selevan (1982).

Pell (1978) also evaluated a second cohort that originally consisted of 270 males ("Chamber Works Cohort") believed to be exposed between 1931 and 1948 in a neoprene manufacturing facility and followed through December 31, 1974. Follow up was complete for 240 workers. Since historical records were not complete for this cohort, efforts were made to assess exposures for former employees based largely on the recall of other employees. The observation period, during which latency in tumor induction could be analyzed, was 30-40 years from date of first exposure. Examination of mortality following a long latency period was considered a strength of this study.

A total number of 55 deaths were observed in this cohort. Study exclusions included thirteen of these deaths occurring prior to 1957 (the starting point of observation assuming a 15-year latency period) and three deaths occurring due to heart disease and malignant melanoma among former laboratory personnel who had little or no exposure. The 39 observed deaths that occurred from 1957 to 1974 were slightly more than the 37.7 expected using the DuPont comparison population. The 12 observed cancer deaths were also elevated (SMR = 140) but were not statistically significant. There were three deaths due to digestive cancer compared to 2.7 expected and were four deaths due to lung cancer compared to 3.0 expected. The five observed cancers of the urinary system (3 bladder and 2 kidney) were significantly elevated (SMR = 300 compared to the DuPont population and SMR = 250 compared to the U.S. general population: $p < 0.01$ for both). The authors attributed the bladder cancers to beta-naphthylamine exposure. Biliary and liver cancers were not examined in this study. Small cohort size, low statistical power, and lack of quantitative exposure data were limitations of this analysis.

Li et al. (1989) conducted a cohort mortality study of Chinese employees who worked in one of three shops with chloroprene exposure (a chloroprene monomer workshop, a neoprene workshop, and a laboratory) within a larger chemical plant. A cohort of 1,258 employees who had worked for at least one year of chloroprene-related work prior to June 30, 1980 were identified from an employee roster. The follow-up period for cancer deaths was from July 1, 1969 through June 30, 1983. Cancer mortality was assessed by searching the death registries at the plant's hospital and the police substation; cancer diagnoses were verified by review of medical records at the city general hospitals and cancer hospitals.

Exposures were assigned to occupations based upon measured concentrations in air at work sites and duration of exposure at different sites. When these levels were not available, exposures were determined through interviews with workers and administrators. Exposure assignments took into account movement between exposure areas and were designed to roughly represent time-weighted average exposure values. Follow up was achieved for 1,213 (96%) cohort members (955 males and 258 females) and SMRs were calculated using sex- and age-specific mortality in the local area. A total of 721 (75%) males and 131 (51%) females were exposed for more than 15 years, while 131 (14%) males and 9 (3%) females were exposed for more than 25 years. Statistically significant differences ($p < 0.005$) in exposure to chloroprene were detected in males compared to females based on > 15 years and > 25 years of exposure.

Person-years were computed by 5-year categories for the total cohort and for the subgroup starting from July 1, 1969 or when the individual first started working with chloroprene through June 30, 1983 for live individuals or until their date of death.[1] SMRs were calculated using sex- and age-specific local area rates in 1973-1975. The results presented below are for male workers only as all sixteen cancer deaths were reported among male workers (Table 4-2). The all-cancer SMR for the male workers was 271 ($p < 0.01$). Of 955 males, 464 (49%) were employed in occupations with high exposures such as maintenance mechanics and monomer/polymer operators. The SMRs for male workers in several high exposure areas were statistically significant for liver and lung cancer mortality. An increased SMR for liver cancer was observed, with four deaths occurring among monomer workers and two deaths occurring in polymer mechanics. Half of the cancers in the monomer shop were primary liver cancers (4 observed, SMR = 482, $p < 0.01$), with two occurring among the maintenance mechanics (SMR = 1667, $p < 0.05$).

[1] Person-years accrued were not reported in the paper.

Table 4-2. Standardized mortality ratios (SMRs) for all cancers, liver and lung cancer among males exposed to chloroprene relative to general Chinese population rates

	NUMBER OF DEATHS/SMR		
EXPOSURE AREA	**ALL CAUSE**	**LIVER CANCER**	**LUNG CANCER**
Total cohort	16/271*	6/242	2/513
Monomer workshop	8/377	4/482**	1/714
Vinylacetylene operator[a]	0/---	0/---	0/---
Monomer operator	4/450**	2/465	0/---
Maintenance mechanic[a]	4/1,290**	2/1667**	1/5000**
Neoprene workshop	5/176	2/165	1/556
Polymer operator[a]	5/394**	0/---	
Final treatment	0/---	0/---	
Maintenance[a] mechanic	0/---	2/357	1/1250
Laboratory	3/319	0/---	0/---
Quality monitor[a]	1/129	0/---	0/---
Researcher	21,176**	0/---	0/---

Statistical significance: * $p < 0.01$; ** $p < 0.05$.
[a]: High Exposure Area

Source: Li et al. (1989)

One of the limitations of the Li et al. (1989) study include insufficient mortality data especially since only three years of local area data were available to calculate SMRs. If these years are not representative of the entire study period, then the SMRs could be biased. For example, if the general population experienced higher mortality during the times periods not examined (i.e., 1969-1972 & 1976-1983) then the SMRs would be underestimated due to a lower expected number of deaths. If the mortality was lower during the other time periods, the SMRs would be overestimated. Lack of quantitative exposure information precluded conducting internal analyses by latency or duration of exposure. Additionally, there was no data on alcohol use or smoking history and limited information was available on other potential confounders such as co-exposures to chloroprene oligomers. The authors did consider potential confounding exposures due to benzene and anti-ager D (N-phenyl-Z-naphthylamine) but determined that these exposures were limited and not likely to influence the results.

Li et al. (1989) also conducted a case-control study for the entire plant. Of 55 observed cancer deaths, 54 were matched with the same number of non-cancer deaths among plant workers based upon gender, age (± 2 years) and date of death (± 2 years). The authors observed that 16 of the cancer deaths (30%) were among workers exposed to chloroprene compared to only four of the non-cancer deaths (7%), yielding an odds ratio of 13 ($p < 0.005$). Although the average age at death was 12.7 years less for the exposed cancer cases relative to the unexposed cancer cases ($p < 0.001$), these findings are limited by lack of data on co-exposures and other potential confounders.

Bulbulyan et al. (1998) examined cancer mortality at a Moscow shoe factory with exposures to chloroprene from glue and from polychloroprene latex (a colloidal suspension of polychloroprene in water). The cohort consisted of 5,185 workers (4,569 women and 616 men) employed for at least two

years during 1960–1976 at specific production departments (i.e., cutting, fitting, lasting and making, and finishing). Auxiliary departments and management employees were excluded. Work histories were obtained from the personnel department, and subjects were assigned exposure levels based on department and job; industrial hygiene measurements of exposure levels were conducted in the 1970s. The authors provided detailed exposure data by job and department, ranging from a high of 20 mg/m^3 (gluers in the finishing department) to an intermediate level of 0.4–1 mg/m^3 (all other jobs in the finishing department and all jobs in the lasting and making department) to the unexposed (all jobs in the cutting and fitting departments).

The authors concluded that the industrial hygiene data were not systematic enough to assign quantitative exposures to each worker since the collection of samples varied by locations and by different years. They, therefore, devised a relative exposure system, where workers in the high-exposure assignment was assigned a level of 10, intermediate-exposure - a level of 1, and unexposed - a level of 0. Cumulative exposures for individual workers were calculated by multiplying years of exposure by the level of exposure, taking into account changes in job and department. In addition, workers were classified by their highest exposure category. The authors considered confounding exposures, including benzene exposures (6-20 ppm) in the high polychloroprene exposure group during the 1950s, but did not adjust for those exposures in their analysis.

Mortality follow up was conducted from 1979-1993 which included 70,328 (62,492 in females and 7,836 in males) person-years of observation. Thirty-seven percent of cohort members contributing 26,063 (female and male distribution was not provided) person-years were unexposed. Death certificates were acquired from the National Registry Office Card Index and causes of deaths were classified using ICD-9. Mortality rates of general population of Moscow were used for comparison. For the general population, mortality data for five cancers (liver, kidney, bladder, pancreas, and malignant neoplasm of mediastinum and rhabdomyosarcoma of the heart) were only available for 1992-1993. Therefore, the number of expected deaths among these sites during 1992-1993 was applied to the entire cohort for the entire period of observation. A Poisson distribution was used to calculate the 95% CIs. One hundred thirty-one (2.5%) workers were lost to follow up. SMRs were calculated for the entire cohort and separately for females and males. Among the total cohort, SMRs were statistically significant for all cancers, liver cancer and leukemia (Table 4-3). Cancer-specific SMRs for liver cancer and leukemia were statistically significant in females but not in males, while the SMR for lung cancer was significant in males only.

Table 4-3. Standardized mortality ratios (SMRs) for selected cancer risks relative to general population rates of Moscow, Russia.

CAUSE OF DEATH	TOTAL COHORT CASES, SMR (95% CI)	MEN CASES, SMR (95% CI)	WOMEN CASES, SMR (95% CI)
All causes	900; 103 (97–110)	181; 121 (104–140)	719; 100 (93–107)
All cancers	265; 122[a] (107–137)	56; 158 (119–205)	209; 115 (100–131)
Liver cancer	10; 240[a] (110–430)	2; 240 (30–860)	8; 230[a] (100–460)
Lung cancer	31; 140 (90–200)	17; 170[a] (100–270)	14; 110 (60–190)
Leukemia	13; 190[a] (100–330)	2; 190 (20–700)	11; 190[a] (100–350)

[a] Statistical significance $p < 0.05$.

Source: Bulbulyan et al. (1998).

Internal relative risk (RR) analyses (controlling for gender, age, and calendar period) were conducted for selected cancers by using multivariate Poisson regression models, with trends evaluated with the Mantel-extension test. Estimates for liver cancer were relatively imprecise since only one liver cancer death was observed in the no-exposure category (a low number since this category included 29% of all observed deaths). Stratified analyses by gender were not reported. Internal analyses comparing the high exposure group to the unexposed resulted in statistically significant RRs for all causes of death (Table 4-4). Although they were not statistically significant largely due to a small number of cases, elevated RRs ranging from 2.2-4.9 were detected for leukemia, and cancers of the liver, kidney and colon.

Table 4-4. Selected relative risk (RRs) estimates for the high exposure group relative to unexposed factory workers

CAUSE OF DEATH	CASES	HIGH-EXPOSURE RR (95% CI)[a]
All causes	194	1.23[b] (1.02–1.49)
Liver cancer	3	4.9 (0.5–47)
Colon cancer	8	2.6 (0.8–7.9)
Kidney cancer	2	3.3 (0.3–37)
Leukemia	5	2.2 (0.6–8.4)

[a] Reference group is defined as workers with no chloroprene exposure.
[b] Statistical significance $p < 0.05$.

Source: Bulbulyan et al., (1998).

Although there were only a few deaths in each group, RR analysis by duration of employment in the highest exposure categories (1–9 years, 10–19 years, 20+ years) relative to no exposure showed a significant trend for liver cancer but not for leukemia mortality (Table 4-5). The cumulative exposure analysis indicated an increased risk of liver cancer mortality based upon six deaths in the intermediate exposure category (RR = 7.1, 95% CI: 0.8-61) and three deaths in the highest exposure category (RR = 4.4, 95% CI: 0.4-44). Kidney cancer was increased in all categories but none of the RRs were statistically significant and no overall trend was observed.

Table 4-5. Internal relative risks (RRs) by duration of employment in the high-exposure category.

CAUSE OF DEATH	1–9 YEARS CASES; RR (95% CI)	10–19 YEARS CASES; RR (95% CI)	20+ YEARS CASES; RR (95% CI)	TREND
Liver cancer	1; 2.7 (0.2–45)	1; 8.3 (0.5–141)	1; 45.0 (2.2–903)	p = 0.02
Leukemia	2; 1.3 (0.2–7.3)	2; 3.4 (0.6–19)	1; 8.8 (0.7–66)	p = 0.07

Source: Bulbulyan et al. (1998).

The most prominent finding in the Bulbulyan et al. (1998) cohort was 10 deaths occurring from liver cancer. The authors detected 11 deaths (3 in males and 8 in females) due to cirrhosis, a precursor of primary liver cancer, but did not adjust for this as a potential confounder. Increased mortality due to leukemia was observed in all categories for both cumulative exposure and duration of employment (with high exposure) but neither trend was statistically significant. The authors suspected a causal role of chloroprene in the leukemia deaths but could not rule out a possible role of exposure to benzene. A significant increase in lung cancer was observed among males only, which may have been due to confounding by smoking. Potential confounding by smoking could not be examined due to lack of data for this cohort. Pancreatic cancer, which may be smoking related, was also observed in males only. No excess risk for lung cancer was observed in females or in the total cohort. Lack of precise quantitative exposure information, no adjustment for confounding risk factors, and exclusion of deaths prior to 1979 resulting in relatively low statistical power were some of the limitations of this study. Similar to Li et al study, the minimal data on observed deaths for some cancers among the general population may have also resulted in biased SMR values if these years were not representative of mortality during the entire study period.

Bulbulyan et al. (1999) conducted a retrospective cohort study of 2,314 workers (1,897 males, 417 females) who had been employed in production departments of a chloroprene monomer production plant in Yerevan, Armenia, for at least two months between 1940 and 1988 and were alive as of 1979. Mortality was followed from 1979–1988, and vital status was accessed through the Yerevan Address Bureau. Death certificates were coded by using ICD-9 revision. Sixty-three (3%) individuals were lost to follow-up. Industrial hygiene exposure measurements of chloroprene were available both before and after 1980, when production changes led to a dramatic decrease in exposures. Before 1980, exposures averaged 5.59–69.80 mg/m^3 (1.54–19.3 ppm) during the summer and 2.30–249.5 mg/m^3 (0.63–68.9 ppm) during the winter. After 1980, for the same seasons, these averages were 0.80–3.60 mg/m^3 (0.22–0.99 [summer] ppm) and 0.55–2.10 mg/m^3 (0.15–0.58 [winter] ppm), respectively. Work histories were obtained from the personnel department, including the start and end of each job, and from the departments of employment. Relative exposure values were assigned based on either high exposure (production operators: six units before 1980, three units after 1980) or low exposure (other production workers: two units before 1980 and one unit after 1980). Unexposed workers were assigned a relative exposure value score of zero. SMRs and standardized incidence ratios (SIRs) were calculated based on comparison rates for the entire Armenian population, and 95%

CIs were also calculated by using a Poisson distribution assumption. Internal RR estimates were calculated by using multivariate Poisson regression models and adjusting for age, calendar period, and gender.

A total of 21,107 person-years were contributed by the study population. There were 20 deaths during the observation period with four due to stomach cancers and three each resulting from liver and lung cancers. The SMR was statistically significant for liver cancer only (SMR = 339, 95% CI 109–1050). Two liver and two lung cancer deaths were identified among males, while one liver cancer death and one lung cancer death were identified in females. No internal comparisons were included in the SMR analysis. Cancer incidence data was examined from 1979–1990 through the Armenian Cancer Registry. Several types of cancers (37 cases) were identified with six liver and six lung cancers (five each in males) being the most prevalent (Table 4-6). The SIRs for liver cancer were statistically significant for the total cohort (SIR = 327, 95% CI 147–727) and for males (SIR = 303, 95% CI 126–727) when stratified by gender. SIRs below 100 were observed for lung cancer among both the total cohort as well as among males only.

Table 4-6. Selected standardized incidence ratios (SIRs) for chloroprene monomer cohort relative to the general Armenian population.

CANCER TYPE	OBSERVED	SIR (95% CI)
All cancers	37	0.68 (0.49–0.94)
Lung cancer	6	0.53 (0.24–1.19)
Liver cancer	6	3.27[a] (1.47–7.27)

[a] Statistical significance $p < 0.05$.

Source: Bulbulyan et al. (1999).

Internal trend analyses of plant workers only showed increasing incidence of liver cancer by duration of employment with a statistically significant relative risk among chloroprene production workers who were employed for more than 20 years (4 cases, SIR =3.45, 95% CI: 1.29-9.20). Evaluation of liver cancer incidence by duration of employment (<1 year, 1-9 years and 10+ years) in the high chloroprene exposure groups resulted in a statistically significant SIR in the 10+ years category (SIR = 6.1; 95% CI: 2.3-16.3). Similar findings were noted when analyzed using cumulative exposure with a statistically significant SIR of 4.9 (95% CI: 2.0-11.7) among the five cases in highest cumulative exposure of 40+ units. All six cases of liver cancer in this study occurred among highly exposed operators. These internal analyses suggested a possible dose-response relationship between chloroprene exposure and liver cancer incidence.

The authors discussed the strong healthy worker effect observed in this study. In particular, they suggested that the low SMRs might be due, in part, to potential loss of early cases resulting from not beginning the follow-up period until 1979. In addition to the incomplete enumeration of outcomes among the workers, the authors acknowledged that there might be misclassification as well as incomplete registration of liver cancers in the Armenian registry. Furthermore, although measurements

of chloroprene levels were available, investigators were unable to develop quantitative estimates and assigned exposure units to the workers depending upon their job description. The role of potential confounding by alcohol use and smoking could not be examined due to lack of data. The high incidence (27 in males and 5 in females) of liver cirrhosis, a precursor for liver cancer, is an unlikely confounder as it is likely an intermediate in the causal pathway precluding statistical adjustment. There was also little evidence that several other co-exposures (i.e., vinyl acetate, toluidine, talc, and mercaptans) that were not adjusted for either in mortality or incidence analyses are liver carcinogens.

Romazini et al. (1992) investigated cancer mortality in a retrospective cohort study of 660. French chloroprene polymer manufacturing workers (599 males, 61 females) employed for at least two years at a polychloroprene plant. The follow-up period was from 1966-1989 with 32 observed deaths included in the study; an additional 18 potential study subjects were lost to follow up. No excess mortality was observed compared to regional rates. In a nested case-control study comparing time of employment, the authors found that workers exposed to conditions prior to 1977 had a much higher risk of death compared to those exposed to chloroprene after 1977. Similar to other studies, the small size of this cohort and inability to control for smoking and other potential confounders limited the conclusions that could be drawn from this study.

Colonna and Laydevant (2001) conducted a cohort cancer incidence study among 533 males who worked a chloroprene production plant in Isère, France, for at least two years between January 1966 (when the plant opened) and December, 1997. Cancer incidence cases were traced through the Isère cancer registry from 1979 (when the registry was founded) through 1997. Workers who died before 1979 or who left the area were not traced (the number of untraced incident cancers was not estimated). Work histories were collected and jobs were classified into low, intermediate, and high chloroprene exposure groups based on estimated exposure of < 2 ppm, 2-5 ppm, and > 5 ppm respectively. Exposure duration was divided into three groups of $\leq$ 10 years, 11-20 years and > 20 years. The cohort was divided into two groups, workers employed prior to 1977 and those employed in 1977 or later, based on lower anticipated exposures following significant changes in worker protection. SIRs were calculated using the general population rates of Isère as a reference and confidence intervals were calculated using a Poisson distribution.

A total of 7,950 person-years were accrued. Of the 34 incident cancers, 32 occurred prior to 1977. There were nine lung cancers, nine cancers of the head and neck (including three laryngeal cancers), and one liver cancer. SIRs were calculated for various cancers including those occurring in the head and neck, larynx, lung, liver and colon/rectum (Table 4-7). With the exception of colon/rectum, all of the SIRs exceeded 100 with most of the cases and higher SIRs noted for earlier periods of first employment (i.e., before 1977).

Table 4-7. Standardized incidence ratios (SIRs) for elevated cancer risks for plant workers relative to general population rates of Isère, France.

CANCER TYPE	TOTAL COHORT CASES, SIR (95% CI)	BEFORE 1977 CASES, SIR (95% CI)
All Cancers	34, 1.26 (0.88-1.77)	32, 1.46 (1.00-2.06)
Head and Neck	9, 1.89 (0.87-3.59)	8, 2.09 (0.90-4.11)
Larynx	3, 2.43 (0.50-7.13)	3, 2.97 (0.61-8.68)
Lung	9, 1.84 (0.84-3.49)	8, 1.99 (0.86-3.91)
Liver	1, 1.36 (0.04-7.63)	1, 1.64 (0.05-9.13)
Colon/Rectum	2, 0.66 (0.08-2.39)	2, 0.79 (0.10-2.87)

Source: Colonna and Laydevant, (2001).

Although none of the SIRs were statistically significant, a significant trend was observed when the data were analyzed by duration of exposure. Five lung cancers were found in workers with > 20 years of exposure (SIR = 2.57), 3 in 11-20 years exposure (SIR = 1.49) and 1 in $\leq$ 10 years exposure (SIR = 1.06). No significant excesses were observed in head and neck cancer by duration of exposure. No trend was detected for lung cancer incidence in relation to intensity of exposure with SIRs of 4.63 (95% CI: 1.27-11.91), 1.25 (95% CI: 0.15-4.51), and 1.23 (95% CI: 0.26-3.61) reported for the low, intermediate and high exposure categories, respectively.

Increased lung cancer and laryngeal cancer were observed in this study. Given that smoking is strongly associated with lung cancer, and since seven of the eight lung cancer cases were smokers, the investigators concluded that the lung cancers excess was unlikely to be due to chloroprene exposure. Although smoking and alcohol consumption was discussed as strongly associated with laryngeal cancer, no additional information was provided in the paper. This study found only one incident liver cancer but noted that liver cancer incidence was likely under-estimated due to difficulties in case enumeration. Study limitations included lack of precise quantitative exposure information, low cancer incidence, and reduced power because of elimination of workers who had died or left the area prior to 1979.

More recently, Marsh et al. (2007a) evaluated mortality patterns of four chloroprene production facilities by using external regional rates and using internal comparisons (Marsh et al., 2007b). This study attempted to address the problems identified with earlier studies by conducting a detailed exposure assessment for both the chloroprene and a potential confounding co-exposure, vinyl chloride monomer (Esmen et al., 2007a, 2007b, 2007c; Hall et al., 2007). As described in detail by Esmen et al. (2007c), a historical review of processes at all four plants led to the assignment of exposures to 257 unique tasks. Taking into account shared tasks or rotation between tasks, job title-based exposures to chloroprene were assigned to one of seven categories, including unexposed (< 0.0005 ppm). Vinyl

chloride exposures were assigned to one of five categories, including unexposed (< 0.01 ppm) (Esmen et al., 2007b).

Two of the facilities evaluated were in the U.S. - DuPont/Dow plants at Louisville (L), Kentucky and Pontchartrain (P), Louisiana. The third one was in Northern Ireland - the Maydown (M) plant, and the fourth facility was in Grenoble (G), France - the Enichem Elastomer plant. These plant cohorts included 5,507 workers (L), 1,357 workers (P), 4,849 workers (M), and 717 workers (G). Median cumulative exposures to chloroprene at these plants were 18.35, 0.13, 0.084, and 1.01 ppm-years, respectively. Vinyl chloride exposures existed in only two plants, Louisville and Maydown. Their median cumulative vinyl chloride exposures were 1.54 and 0.094 ppm-years, respectively.

The study period for the cohorts encompassed 52 (L), 41(M), 39 (P), and 34 (G) years resulting in 197,919, 127,036, 30,660, and 17,057 person-years, respectively (Marsh et al., 2007a). Vital status was assessed using several different sources. A trained nosologist using the ICD codes in effect at the time of death coded the underlying cause of death. A total of 3,002 deaths had occurred during the follow-up period in the chloroprene cohort and cause of death was ascertained for 2,850 individuals (95%). A modified Occupational Cohort Mortality Program was used to conduct statistical analyses. Independent analyses were conducted for the four facilities for total cancer deaths and certain site-specific deaths. Person-years at risk were computed for each individual by race, sex, age group, calendar time, duration of employment, and the time since first employment. SMRs and 95% CIs were calculated for the total cohort and selected sub-cohorts for each plant.

All cause combined mortality was significantly reduced (compared to local county rates) for each of the four cohorts (Table 4-8). In addition, each cohort had significantly reduced mortality for all cancers, and the largest cohort, Louisville, had significantly reduced mortality from respiratory cancers. The total number of cancer deaths observed at each of the four plants was 652 (L), 128 (M), 34 (P), and 20 (G). Reported lung cancer deaths were 266, 48, 12, and 10, while liver cancer deaths were 17, 1, 0, and 1 for L, M, P, and G, respectively. Compared to the local population rates, fewer deaths from liver cancer were observed in the Louisville (SMR = 90, 95% CI: 52-144) cohort than expected. All other sites had no more than one death due to liver cancer. Similar to the healthy worker effect observed in other studies, fewer cancer deaths were were reported in the occupational cohorts when compared to general population estimates. When chloroprene exposed and unexposed workers were analyzed separately, the SMRs for all cancers were all significantly reduced for exposed workers at each plant, while they were generally higher (at or above expected levels for all plants except at Grenoble) for unexposed workers. Given that there were few unexposed workers in these cohorts, these values are unstable and are also difficult to interpret given the healthy worker effect bias.

Table 4-8. Standardized mortality ratios (SMRs) at each of four chloroprene production facilities

CAUSE OF DEATH	LOUISVILLE (L) CASES, SMR (95% CI)	MAYDOWN (M) CASES, SMR (95% CI)	PONTCHARTRAIN (P) CASES, SMR (95% CI)	GRENOBLE (G) CASES, SMR (95% CI)	TOTAL CASES, SMR (95% CI)
All Causes	2403 74 (71-77)	435 60 (55-67)	102 53 (43-65)	62 65 (50-83)	3002 70 (67-73)
All Cancers	652 75 (69-80)	128 68 (56-80)	34 68 (47-95)	20 59 (36-91)	834 73 (68-78)
Respiratory Cancers	266 75 (66-85)	48 79 (58-105)	12, 62 (32-109)	10 85 (41-156)	336 75 (68-74)
All Cancers:					
Exposed	651 74 (69-80)	114 62 (51-75)	26 57 (37-84)	15 59 (33-97)	806 71 (66-76)
Unexposed	1 99 (3-551)	14 126 (69-212)	8 144 (62-285)	5 61 (20-142)	28 108 (72-156)

Source: Marsh et al. (2007a).

In their companion paper (Marsh et al., 2007b), the authors conducted internal RR analyses at each of these four plants. Exposure-response trends across categories of exposures (based on quartiles) were examined using a forward stepwise regression modeling approach to adjust for potential confounding. Analyses were conducted by considering 5- and 15-year lagged exposures and using white/blue collar as a surrogate for lifetime smoking. Absolute mortality rates were estimated by calculating exposure category-specific SMRs using external mortality rates. The internal analyses for all cancers showed increasing RRs with duration of exposure (< 10, 10-19, 20+ years) to chloroprene in plants L and M, but a statistically significant trend ($p < 0.007$) was only noted for Plant M. Relative to less than 10 years of exposure, increased RRs were noted for 10-19 years (RR = 1.53; 95% CI = 1.00-2.34) and 20+ years (RR = 1.78; 95% CI = 1.11-2.84) of exposure. The external comparison consistently showed SMRs less than the internal analysis (and mostly below 1) for both the plants suggestive of bias due to the healthy worker effect.

The internal analysis for liver cancer could only be conducted in the Louisville cohort, which contained 17 of the 19 observed deaths and also had the highest chloroprene levels. Despite the limited number of deaths, these data show some evidence of a dose-response effect across the four exposure levels ($p = 0.09$). Although the individual RRs were not statistically significant, the range for the highest three exposure levels was from 1.9-5.1.

The results of the internal analyses for respiratory cancers at the three plants (M, P, G) without worker status adjustment showed higher RRs with increasing cumulative exposure (Table 4-9). The observed trends were not statistically significant but were based on a small number of respiratory cancers. In contrast, the plant with the most cases (L) showed little evidence of an exposure-response relationship. The investigators adjusted for the potential confounding by smoking status in the analyses of lung cancer mortality at Louisville only (due to small numbers at the other plants) using the

employment status as a surrogate of blue versus white collar workers. This decision was justified by the authors based upon this variable being a surrogate for variables associated with smoking such as education and socio-economic status. It is impossible, however, to discern whether this surrogate resulted in control for smoking or resulted in an over-adjustment since work status is so highly correlated with chloroprene exposures.

Table 4-9. Relative risks (RRs) for respiratory cancers by cumulative chloroprene exposure

PLANT	LEVEL 1[a] (LOWEST) N	LEVEL 2 N, RR (95% CI)	LEVEL 3 N, RR (95% CI)	LEVEL 4 N, RR (95% CI)	TREND
Louisville (L)	62, Reference	67, 1.00 (0.71-1.43)	77, 1.32 (0.94-1.88)	60, 0.85 (0.58-1.23)	p = 0.71
Maydown (M)	14, Reference	9, 1.65 (0.66-4.15)	12, 1.89 (0.72-4.96)	13, 2.28 (0.86-6.01)	p = 0.10
Pontchartrain (P)	3, Reference	3, 1.60 (0.20-12.8)	2, 2.90 (0.20-34.1)	4, 2.32 (0.30-21.8)	p = 0.34
Grenoble (G)	2, Reference	1, 0.61 (0.05-6.76)	4, 2.87 (0.35-39.7)	3, 3.14 (0.30-48.0)	p = 0.17

[a]Chloroprene exposure (in ppm years) levels varied by plant: L (<4.7->164.1); M (<0.04->24.5); P (<0.02->16.2); G (<0.05->23.9).

Source: Marsh et al. (2007b)

The authors also conducted internal analyses of cancer mortality and vinyl chloride exposure (the primary co-exposure in this study) at the Louisville plant. They found an inverse association (many of them statistically significant) between risk of both respiratory and liver cancer in relation to vinyl chloride exposures. In fact, the vast majority of respiratory and liver cancers occurred among workers who were unexposed to vinyl chloride. If vinyl chloride is a negative confounder of the association between chloroprene and liver cancer, then the reported association between chloroprene and liver cancer would be an underestimate of the association adjusted for vinyl chloride. Given this, it is highly unlikely that confounding by vinyl chloride could explain the associations observed between chloroprene and these cancers. In addition, the authors reported that there was no correlation between cumulative exposures to vinyl chloride and chloroprene among these workers.

The Marsh et al. (2007a, 2007b) study is one of the more comprehensive studies to date, largely due to exposure assessment data which allowed for internal comparisons. Although the authors concluded that their study provided no evidence of cancer risk associated with chloroprene exposures, the data on chloroprene exposure and liver cancer risk was not inconsistent with previous findings. While the authors stated that "chance alone does not appear to be an explanation for the cancer deficits observed among unexposed workers," they rejected the healthy worker effect as a plausible explanation. Instead, they postulated "some heretofore unknown selection factors for low cancer incidence or mortality were operating on the unexposed subjects" (Marsh et al., 2007b). This is inconsistent with the data that Marsh et al. (2007b) presented on cancer mortality among exposed and

unexposed workers (Table 4-9). It can be concluded that the explanations for observed associations between chloroprene exposure and cancer, especially liver and respiratory cancers, given by Marsh et al. (2007b) are not entirely consistent with the data presented. Despite the study limitations including small numbers, this study is informative given the findings of the internal analysis including the data on cumulative exposure and liver cancer risk.

4.1.1.3. *Summary and Discussion of Relevant Methodological Issues*

Nine studies covering 11 cohorts were reviewed to assess the relationship between exposure to chloroprene and cancer incidence and mortality. Four cohorts had less than 1000 workers, while the remaining cohorts had sample sizes less than 6000. The most consistent finding was excess liver (Bulbulyan et al. 1999, 1998; Li et al., 1989; Leet and Selevan, 1982) and lung/respiratory system (Marsh et al., 2007b; Colonna and Laydevant, 2001; Bulbulyan et al. 1999, 1998; Leet and Selevan, 1982; Pell et al., 1978) cancer incidence or mortality (Tables 4-10 and 4-11). The limitations of each of the aforementioned studies are discussed in this section. Most occupational cohort studies are historical in nature gathering human subject information from existing records and going back many years. In general, the constructed databases do not include detailed information on the workers' individual habits (e.g., tobacco use, alcohol consumption) and usually only have limited exposure information. These limitations often limit the ability to control for bias due to confounding variables and to assess the potential for misclassification of exposure.

One of the limitations of the occupational epidemiologic studies examining chloroprene exposure is the potential for the healthy worker effect to influence the results. Since occupational studies involve workers who are healthier than the general population, a reduced mortality risk is often observed among these populations when compared to external populations. This potential bias was likely reduced in some studies by using internal comparisons or other study designs such as a nested case-control study.

Another concern in these occupational studies is the reliance on death certificates for outcome ascertainment especially in the mortality studies. Although misclassification of cause of death can be minimized by the review of medical records or by histological confirmation, this was not done in any of the studies. Incomplete enumeration of incident cases was another limitation of several of the studies. This may limit the ability to detect associations as it directly reduces statistical power through reduced sample sizes. Outcome misclassification can also bias the measures of associations that were examined, but it is difficult to gauge the potential impact of this bias on the reported findings.

Finally, the lack of quantitative exposure assessment is clearly a limiting factor of most occupational studies; however, they still are able to contribute to the overall qualitative weight of evidence considerations. In many cases where exposure data were missing or insufficient to provide quantitative assessments, exposure levels were differentiated based upon job titles and industrial hygiene knowledge of the processes involved. Although measurement error is present in all studies to

varying degrees, there is no evidence that this error differed by outcome (i.e., was non-differential) in these studies. Although there are rare exceptions, non-differential misclassification of workers' exposures due to lack of information usually results in an underestimate of the association between exposure and outcome.

Table 4-10. Epidemiologic summary results of respiratory system cancers: Overall standardized mortality ratios (SMRs) and SMRs for intermediate and high chloroprene exposures relative to external population comparison

STUDY	TOTAL COHORT SMR (95% CI)	HIGH EXPOSURE[A] SMR (95% CI)	INTERMEDIATE EXPOSURE[A] SMR (95% CI)
Bulbulyan et al., 1998	140 (90-200)	0.8 (0.3–2.4)[c,d]	1.0 (0.4–2.5)[c,d]
Bulbulyan et al., 1999	50 (16-155)	-----	-----
Colonna and Laydevant, 2001	184 (84-349)[e]	123 (26-361)[e]	125 (15–451)[e]
Leet and Sullivan, 1982	106 (62-170)	128 (61-236)	86 (35-178)[b]
Marsh et al., 2007a,b-Louisville	75 (66-85)	65 (50-85)[d,e]	92 (73-115)[d,e]
Marsh et al., 2007a,b-Maydown	79 (58-105)	113 (60-192)[d,e]	97 (50-169)[d,e]
Marsh et al., 2007a,b-Pontchartrain	62 (32-109)	85 (23-218)[d,e]	96 (12-348)[d,e]
Marsh et al., 2007a,b-Grenoble	85 (41-156)	128 (26-373)[d,e]	119 (32-304)[d,e]

[a]Relative to Low or Unexposed Groups
[b]Low Exposure Group
[c]Relative Risk of Death from Lung Cancer
[d]Cumulative Chloroprene Exposures
[e]Standardized Incidence Ratios

Table 4-11. Epidemiologic summary results of liver/biliary passage cancers: Total cohort standardized mortality ratios (SMRs) and relative risk (RRs) for intermediate and high chloroprene exposures

STUDY	TOTAL COHORT SMR[A] (95% CI)	HIGH EXPOSURE[B] RR (95% CI)	INTERMEDIATE EXPOSURE[B] RR (95% CI)
Bulbulyan et al., 1998	240 (110-430)	4.4 (0.4–44)[c,d]	7.1 (0.8–61)[c,d]
Bulbulyan et al., 1999	339 (109-1050)	4.9 (2.02-11.7)[d,e]	2.9 (0.41-20.8)[d,e]
Colonna and Laydevant, 2001	136 (4–763)[e]	------	------
Leet and Sullivan, 1982	571 (156-1463)	750 (155-2192)[f]	250 (6-1393)[f]
Li et al, 1989	482 (N/R, p < 0.01)	------	-----
Marsh et al., 2007a,b-Louisville	90 (52-144)	3.3 (0.5, 39.3)[d]	5.1 (0.9, 54.5)[d]
Marsh et al., 2007a,b-Maydown	24 (1-134)	------	------
Marsh et al., 2007a,b-Pontchartrain	------	------	------
Marsh et al., 2007a,b-Grenoble	56 (1-312)	------	------

N/R: Not Reported
[a]Relative to External Population Rates
[b]Relative to Low or Unexposed Groups
[c]Relative Risk of Death from Liver Cancer
[d]Cumulative Chloroprene Exposures
[e]Standardized Incidence Ratio
[f] Standardized Mortality Ratio

Lung Cancer Summary

An increased risk of lung cancer incidence and mortality was observed in a few studies (Colonna and Laydevant, 2001; Bulbulyan et al., 1998; Pell et al., 1978; Li et al., 1989), although few statistically significant associations were reported. None of the studies adjusted for smoking because the investigators either did not have this information available or because the majority of their lung cancer cases were observed in smokers. Marsh et al. (2007b) used white/blue collar as a surrogate for smoking habits assuming that blue collar workers smoked more than white collar workers. But due to small number of deaths in white collar workers the authors reportedly only adjusted the lung cancer risk for worker type in the Louisville, Kentucky plant. Since worker pay type is a crude surrogate of smoking status, it is difficult to rule out the potential confounding effects of smoking. Worker pay status is also a marker of chloroprene exposure. Therefore, inclusion of this variable in regression models may result in over-adjustment distorting the relationship between cancer mortality and chloroprene exposure. A few studies noted higher SMRs for lung cancer among workers exposed to chloroprene; however, there was no evidence of an exposure-response relationship across various chloroprene exposure categories.

Liver Cancer Summary

Statistically significant excesses of liver cancers were detected in four of the cohorts that were examined (Bulbulyan et al. 1999, 1998; Li et al., 1989; Leet and Selevan, 1982). Although no statistically significant increase in risk of liver cancer was detected in the Louisville plant (Marsh et al., 2007b), the relative risk increased with increasing cumulative exposures indicating a dose-response trend. In three of the cohorts, there was only one case of liver cancer or mortality from liver cancer (Marsh et al., 2007a, 2007b and Colonna and Laydevant, 2001) detected, while the Pontchartrain cohort study had no reported liver cancer deaths (Marsh et al., 2007b). This precluded meaningful examination especially in the latter studies with more detailed exposure information.

Confounding by occupational co-exposures is addressed in some studies but few of these included direct adjustments for the possible confounders. Some studies have selected workers from several different processes where the co-exposures might have been different or non-existent in some processes to help address the potential for confounding. Bulbulyan et al. (1999, 1998) discussed other possible exposures and concluded that confounding was unlikely, since none of the known co-exposure chemicals were known to be associated with liver cancer. Marsh et al. (2007b) conducted a separate analysis with vinyl chloride in the Louisville plant and found that 15 out of 17 liver cancer cases were found in workers that were not exposed to vinyl chloride. The authors also reported that there was no correlation between cumulative exposures to vinyl chloride and chloroprene among these workers. Given these data, it is highly unlikely that confounding by vinyl chloride could explain the association observed between chloroprene and these cancers. No adjustments for other risk factors for liver cancer, such as alcohol consumption, were performed in any of the cohorts observing statistically significant increases in liver cancer mortality. If alcohol consumption was associated with chloroprene exposure this might be a source of residual confounding. Further limitations in these cohorts include the lack of precise quantitative exposure information, limited statistical power to detect effects due to insufficient general population mortality data, and incomplete ascertainment of health outcomes. Studies that relied upon comparisons to external population mortality rates are also susceptible to the healthy worker effect although the potential impact on cancer mortality in these populations is unclear (see above).

Primary liver cancer is relatively rare in the U.S. It accounts for approximately 1.3% of new cancer cases and 2.6% of cancer deaths (Jemal et al., 2003). There are few identified chemicals that have been associated with primary liver cancer. The observation of an increased risk of liver cancer mortality is reasonably consistent and there is some evidence of an exposure-response relationship among workers exposed to chloroprene in different cohorts in different continents (i.e. U.S., China, Russia, and Armenia).

4.1.2. Chloroprene Exposure and Noncancer Effects

4.1.2.1. *Acute-, Short-, and Subchronic-Duration Noncancer Effects*

Nystrom (1948) reported effects associated with the levels (not specified) of chloroprene exposure experienced during the start-up of chloroprene production in Sweden. The author noted a high level of symptoms among workers in two departments, chloroprene distillation and polymerization, in both the pilot plant and early period of regular production. Over the time period from 1944–1997, the author conducted a series of employee medical examinations. In the distillation department of the production plant, 19 of 21 workers (90%) complained of fatigue and pressure or pains over the chest, with much fewer numbers (3–6 employees) complaining of palpitations, giddiness, irritability, and dermatitis. No workers experienced loss of hair.

In the polymerization department of the production plant, temporary hair loss affected 11 of 12 workers or 90%. The author attributed this to systemic rather than direct skin exposure (which was carefully controlled). Dermatitis was present in four workers (30%), and all other symptoms evaluated were limited to no more than one worker.

Guided by animal studies and reports from other companies, Nystrom (1948) evaluated employees for impaired renal and liver function, basal metabolism, and pulmonary and cardiovascular abnormalities by conducting general body examination, clinical chemistry of the urine and blood, and other tests referred to as "special investigations" (including X-rays, electrocardiograms, and hypoxemia and stress tests). The results of these evaluations were reported in an anecdotal manner with no qualitative or quantitative (e.g., statistical significance of results) details. Except for increased symptoms with exercise right after exposure (among distillation department workers), no clear pathologies were observed. In the pilot plant, where exposures were less controlled, Nystrom (1948) noted anemia among exposed workers. The author also observed that, when the workers were educated about the dangers and safety precautions were enforced, the symptoms decreased.

In a Russian review of the effects of chloroprene, Sanotskii (1976) noted that medical examinations of chloroprene production workers had found changes in the nervous system, hepatic and renal function, cardiovascular system, and hematology. Assessment of exposures in Russian latex and rubber manufacturing plants showed that chloroprene was the main hazard and that exposures ranged from 1–7 mg/m^3 in exposed work areas.

One of the studies reported in this review included medical exams of 12 men and 53 women, of whom two-thirds had been employed in a chloroprene production plant for less than 5 years. Cardiovascular examinations found muffled heart sounds in 30 workers, reduced arterial pressure in 14, and tachycardia in 9. There was also a reduction in RBC counts, with hemoglobin substantially below the limit of physiological variation. Erythrocytopenia, leucopenia, and thrombocytopenia were observed. Increases in vestibular function disturbance were associated with duration of work.

In another study reviewed by Sanotskii (1976), women aged 19–23 and employed in jobs with chloroprene exposure for 2–4 years had abnormal diurnal variation in arterial pressure, with reduced systolic and diastolic components at the end of the workday when compared with controls. Their pulse rates were considerably higher than those of controls ($p < 0.01$). Central nervous system (CNS) function was also affected with lengthening of sensorimotor response to visual cues compared with controls. Olfactory thresholds increased with duration of employment.

4.1.2.2. *Chronic Noncancer Effects*

Chronic effects in exposed workers at an electrical engineering plant were also reported in the review by Sanotskii (1976). When compared to 118 unexposed controls, the chloroprene-exposed cohort (143 workers) exhibited an increased incidence of disturbances of spermatogenesis after 6–10 years of work and morphological disturbances after 11 years or more. A questionnaire showed that cases of spontaneous abortion in the wives of chloroprene workers occurred more than three times as frequently as in the control group. This study presents interpretational difficulties concerning the level of participation of the exposed workers and their wives, the quantitative interpretation of the reported sperm abnormalities, and the appropriate matching of exposed and control populations. In an earlier evaluation of this study, U.S. EPA (1985) concluded that recall bias associated with a retrospective questionnaire, such as was used in the study reviewed by Sanotskii (1976), was likely, and the likelihood that the study would have discovered a real increase in the rate of spontaneous abortions was remote, as embryos with chromosomal abnormalities are spontaneously aborted early in pregnancy. Many pregnancies are lost spontaneously often before a woman recognizes that she is pregnant, and the clinical signs of miscarriage are often mistaken for a heavy or late menses (Griebel et al., 2005). Thus, U.S. EPA (1985) concluded that it was not reasonable to draw conclusions on the possible effect of chloroprene on early fetal losses based on the Sanotskii (1976) review. In addition, the EPA suggested that the low participation of male volunteers available for sperm analysis (9.5% participation, 15/143 workers) indicated that a large degree of selection bias may have been present. If males with reproductive deficits self-selected themselves for participation, the meaningful interpretation of the study results may be limited.

The final conclusion of the EPA analysis was that it is not possible to interpret the results in the Sanotskii (1976) review with any degree of reliability (U.S. EPA, 1985). Savitz et al. (1994) and Schrag and Dixon (1985) separately reviewed the study and also concluded that insufficient methodological details were available to critically evaluate the observation reported by Sanotskii (1976).

Sanotskii (1976) also reported a study of chromosome aberrations in leukocyte culture cells of chloroprene production employees. The occurrence of chromosomal aberrations were significantly higher ($p <0.001$) in the exposed group compared to the control group, as well as elevated compared to reported levels among healthy persons. Similar results were reported for a different study of two sets

of female employees: (1) 20 women aged 19–23 and exposed to 3–7 mg/m^3 (0.83–1.93 ppm) chloroprene for 1–4 years; and (2) 8 women aged 19–50 and exposed to 1–4 mg/m^3 (0.28–1.1 ppm) for 1–20 years. The results of these two studies are shown in Table 4-12.

Table 4-12. Frequency of chromosomal aberrations in lymphocyte culture cells from chloroprene production workers

CHLOROPRENE EXPOSURE	# EXAMINED	YEARS EXPOSED	AGE RANGE	# CELLS ANALYZED	PERCENT ABERRANT (+/-)	PERCENT TYPE ABERRANT	
						Chromatid	Chromosome
Chloroprene Workers	18	----	----	1,666	4.77 (0.57)[a]	74.4	25.6
Control	9	----	----	572	0.65 (0.56)	100	0
1–4 mg/m^3	8	1–20	19–50	648	2.5 (0.49)[b]	----	----
3–7 mg/m^3	20	1–4	19–23	1,748	3.49 (0.51)[a]	----	----
Population Control	181	----	----	28,386	1.19 (0.06)	50.3	49.7

[a] $p < 0.001$. All values means ± SE
[b] $p < 0.05$

Source: Sanotskii (1976).

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

4.2.1. Oral Exposure

The only available long-term animal study using the oral route of administration was part of a developmental/reproductive study. Ponomarkov and Tomatis (1980) administered chloroprene dissolved in olive oil by stomach tube to 17 female BD IV rats at a single dose (100 mg/kg body weight) on gestational day (GD) 17. Progeny from treated females (81 males and 64 females) were treated weekly with 50 mg/kg body weight by stomach tube from the time of weaning for life (120 weeks). A control group of 14 female rats was treated with 0.3 mL olive oil. The purity of the chloroprene was reported as 99% with 0.8% 1-chlorobutadiene; storage conditions were not reported. All survivors were sacrificed at 120 weeks or when moribund and autopsied. Major organs, as well as those that showed gross abnormalities, were examined histologically.

Litter sizes and preweaning mortality, survival rates, and body weights did not differ between chloroprene-treated animals and controls. Animals treated with chloroprene that died within the first 23–35 weeks of treatment showed severe congestion of the lungs and kidneys. Some animals (number not specified) autopsied 80–90 weeks after the onset of treatment showed multiple liver necroses. Animals that died after 90 weeks and some survivors euthanized at 120 weeks showed degenerative lesions of the liver parenchymal cells (large cells with clear cytoplasm in nodular arrangements).

Tumor incidences and distribution reported in this study are summarized in Tables 4-13 and 4-14. No statistically significant differences were reported between treated and control rats. However, several tumors observed in males (intestinal leiomyosarcoma, osteoma, kidney mesenchymal tumor, bone hemangioma, neurinoma of the optic nerve, transition-cell carcinoma of urinary bladder, and forestomach papilloma.) and females (ovarian and mammary tumors) treated weekly with chloroprene were not seen in the vehicle control group. Subcutaneous fibromas were more numerous in chloroprene-treated male rats than in controls.

Table 4-13. Tumor incidence in female BD IV rats treated orally with chloroprene (100 mg/kg) on GD17 and in their progeny treated (50 mg/kg) weekly for life (120 weeks)

GROUP	NUMBER[a]	TUMOR BEARING RATS		NUMBER OF TUMORS		ANIMALS WITH MORE THAN ONE TUMOR	
		n	%	Total	Per rat	n	%
Treated females	16	9	56.2	14	0.9	5	31.3
Treated progeny							
Males	54	15	27.8	18	0.3	3	5.6
Females	62	33	53.2	37	0.6	4	6.5
Control females	14	5	35.7	7	0.5	2	14.3
Control progeny							
Males	49	16	32.7	16	0.3	---	---
Females	47	24	51.1	29	0.6	5	10.6

[a]Survivors at the time the first tumors were observed.

Source: Ponomarkov and Tomatis (1980).

Table 4-14. Distribution of tumors in female BD IV rats treated orally with chloroprene (100 mg/kg) on GD17 and their progeny treated (50mg/kg) weekly for life (120 weeks)

GROUP	ORAL CAVITY		MAMMARY		OVARY		THYROID		SOFT TISSUE		PITUITARY		OTHER	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Treated	1	6.3	6	37.5	2	12.5	---	---	---	---	1	6.3	4[a]	25.0
Treated														
Males	---	---	---	---	---	---	1	1.9	7	13.0	2	3.7	8[b]	14.8
Females	---	---	25	40.3	9	14.5	1	1.6	---	---	2	3.2	---	---
Control	1	7.1	4	28.6	---	---	---	---	1	7.1	---	---	1[c]	7.1
Control														
Males	2	4.1	---	---	---	---	---	---	4	8.2	2	4.1	8[d]	16.3
Females	1	2.1	22	46.8	3	6.4	---	---	---	---	1	---	3[e]	6.4

[a] 1 each: uterine squamous cell carcinoma; lung reticulosarcoma; forestomach papilloma; sebaceous basal cell carcinoma.
[b] 1each: intestinal leiomyosarcoma; osteoma; kidney mesenchymal tumor; bone hemangioma; neurinoma of the optic nerve; adrenal cortical adenoma; transition-cell carcinoma of urinary bladder; forestomach papilloma.
[c] Adrenal cortical adenoma.
[d] 2 lymphomas; 1 each: lung epidermoid carcinoma; spleen hemangioma; osteosarcoma; mediastinal sarcoma; meningioma; adrenal cortical adenoma.
[e] 1 each: stomach fibrosarcoma; lymphoma; uterine adenoma.

Source: Ponomarkov and Tomatis (1980).

4.2.2. Inhalation Exposure

The NTP conducted 16-day, 13-week, and 2-year inhalation exposure studies with chloroprene in F344/N rats and B6C3F1 mice (NTP, 1998). Results of the 13-week study were reported by Melnick et al. (1996), while the cancer results of the 2-year study were discussed separately by Melnick et al. (1999) in relation to observations noted with 1,3-butadiene in mice. All experimental regimes consisted of 6 hours per day, 5 days per week whole-body exposures. Group sizes were 10 animals/sex/group in the 16-day and 13-week studies and 50 animals/sex/group in the 2-year study. Overall purity of the bulk chloroprene was determined to be approximately 96% by gas chromatography. Vapor was generated in the 13-week and 2-year studies from chloroprene in an evaporation flask kept at 66°C (72ºC in the 16-day studies) followed by a temperature-controlled condenser column (to remove less volatile impurities such as chloroprene dimers); the chloroprene reservoir was kept at dry ice temperature (16-day study) or under nitrogen (13-week and 2-year studies). The actual concentrations generated from the evaporator flask were within 99% of target concentrations at the beginning of the exposures and were 95% pure at the end of the exposure period. Chloroprene was dragged from the evaporator by a metered flow of nitrogen before being injected into the mixer column, where it was diluted with HEPA- and charcoal-filtered air. Impurities more volatile than chloroprene, such as chlorobutene, never exceeded more than 0.6% of the desired chloroprene concentration when sampled from the distribution line, the last sampling point upstream from the actual exposure chambers. Histopathology was performed by a study pathologist and reviewed by a quality assurance pathologist and the Pathology Working Group.

In the 16-day study, rats were exposed to 0, 32, 80, 200, or 500 ppm chloroprene (NTP, 1998). On day 4, rats were placed in metabolism cages for 16-hour urine collection. A necropsy was performed on all animals, and histopathological examinations were performed on controls, 80 ppm female rats, and 200 and 500 ppm male and female rats. Tissues and organs examined included brain, liver, kidney, lung, bone marrow, thymus, spleen, and testes. Sperm morphology and vaginal cytology were not evaluated.

Survival and body weights of rats are given in Table 4-15. Only one male in the high-exposure group (500 ppm) survived. Females in the high-exposure group had a higher survival (7/10) with a significantly decreased body weight (–6% compared with controls). Significantly decreased body weight gain was also observed in males and females at 200 ppm, and in females at 500 ppm.

Table 4-15. Survival and body weights of rats in the 16-day inhalation study of chloroprene

SEX	EXPOSURE (ppm)	SURVIVAL	MEAN BODY WEIGHT (g)		
			Initial	Final	Change
Male	0	7/10	115 ± 4	139 ± 5	+ 20 ± 2
	32	10/10	113 ± 4	134 ± 6	+ 20 ± 2
	80	10/10	118 ± 5	136 ± 5	+ 18 ± 1
	200	9/10	114 ± 4	127 ± 5	+ 11 ± 2**
	500	1/10	114 ± 4	104	4[a]
Female	0	9/10	100 ± 2	110 ± 3	+ 9 ± 1
	32	9/10	100 ± 2	109 ± 3	+ 8 ± 1
	80	9/10	103 ± 2	112 ± 2	+ 9 ± 1
	200	3/10	101 ± 2	101 ± 4	+ 4 ± 1**
	500	7/10	102 ± 2	103 ± 3	- 1 ± 1**

[a] No standard error calculated due to high mortality
** Significantly different ($p \leq 0.01$) from the chamber control group by Williams' or Dunnett's test

Source: NTP (1998)

The incidences of minimal to mild olfactory epithelial degeneration in all exposed groups of males and females were significantly greater than those in the chamber control groups (Table 4-16). Mild to moderate centrilobular hepatocellular necrosis was observed in male and female rats exposed to 200 or 500 ppm. Hematological and clinical chemistry parameters indicated increased serum alanine aminotransaminase (ALT), glutamine dehydrogenase (GDH), and sorbitol dehydrogenase (SDH) activities, as well as anemia and thrombocytopenia (decreased platelet count) in the 200 and 500 ppm groups, on day 4 only. In females, significant increases in kidney weights (right kidney only) were seen at 80 and 500 ppm, and significantly increased liver weights were seen at 200 and 500 ppm.

Table 4-16. Incidences of selected nonneoplastic lesions in rats in the 16-day inhalation study of chloroprene

	CONTROL	32 ppm	80 ppm	200 ppm	500 ppm
Male					
Nose[a]	10/10	10/10	10/10	10/10	10/10
Degeneration, olfactory epithelium	1/10	10/10[c]	10/10[c]	10/10[c]	10/10[c]
Metaplasia, squamous, olfactory epithelium	0/10	0/10	0/10	1/10	4/10[b]
Metaplasia, respiratory, olfactory epithelium	0/10	2/10	5/10[b]	6/10[b]	1/10
Metaplasia, squamous, olfactory epithelium	1/10	1/10	0/10	0/10	7/10
Liver[a]	10/10	1/10	10/10	10/10	10/10
Necrosis, centrilobular	0/10	0/10	0/10	1/10	9/10[c]
Inflammation, chronic	0/10	0/10	0/10	0/10	1/10
Female					
Nose[a]	10/10	10/10	10/10	10/10	10/10
Degeneration, olfactory epithelium	0/10	9/10[c]	10/10[c]	10/10[c]	10/10[c]
Metaplasia, squamous, olfactory epithelium	0/10	1/10	1/10	4/10[b]	0/10
Metaplasia, respiratory, olfactory epithelium	0/10	7/10[c]	8/10[c]	3/10	7/10[c]
Metaplasia, squamous, olfactory epithelium	1/10	1/10	0/10	0/10	4/10
Liver[a]	10/10	3/10	10/10	10/10	10/10
Necrosis, centrilobular	0/10	0/10	0/10	7/10[c]	3/10
Inflammation, chronic	0/10	0/10	0/10	2/10	5/10[b]

[a] Number of animals with tissue examined microscopically.
[b] $p \leq 0.05$.
[c] Significantly different ($p \leq 0.01$) from the chamber control group by the Fisher's exact test.

Source: NTP (1998)

In the mouse portion of the 16-day NTP (1998) study, exposure levels were 0, 12, 32, 80, and 200 ppm. Additional groups of 10 male and 10 female mice designated for day 5 hematology and clinical chemistry analyses were exposed to the same chloroprene concentrations. Histopathology examinations were performed on chamber controls and 80 and 200 male and female mice as well as on selected target organs in other groups. Tissues and organs examined were identical to those described for the rat. Survival and body weights for mice are given in Table 4-17. All male and female animals in the high-concentration group died, exhibiting signs of narcosis, hepatocellular and thymic necrosis, and hypertrophy of the myocardium. Significantly decreased body weight gain (compared with controls) was seen in males at 32 and 80 ppm. There were no other clinical findings related to chloroprene exposure in the mouse.

Table 4-17. Survival and body weights of mice in the 16-day inhalation study of chloroprene

EXPOSURE (ppm)	SURVIVAL	MEAN BODY WEIGHT (g)		
		Initial	Final	Change
Male				
0	10/10	24.7 ± 0.5	27.0 ± 0.5	+ 2.3 ± 0.1
12	10/10	24.8 ± 0.5	27.1 ± 0.6	+ 2.3 ± 0.3
32	10/10	25.3 ± 0.3	26.5 ± 0.3	+ 1.2 ± 0.3[a]
80	10/10	24.8 ± 0.5	26.1 ± 0.6	+ 1.3 ± 0.2[a]
200	0/10	24.2 ± 0.4	---	---
Female				
0	10/10	19.5 ± 0.7	22.6 ± 0.5	+ 2.3 ± 0.3
12	10/10	20.4 ± 0.8	23.1 ± 0.4	+ 2.6 ± 0.3
32	10/10	19.9 ± 1.0	22.1 ± 0.2	+ 1.8 ± 0.3
80	10/10	20.1 ± 0.8	22.5 ± 0.3	+ 2.7 ± 0.3
200	0/10	20.0 ± 0.6	---	---

[a]Significantly different ($p \leq 0.01$) from the chamber control group by Williams' or Dunnett's test.

Source: NTP (1998)

A range-finding 13-week inhalation study was conducted by NTP (1998) (reported by Melnick et al., 1996), using both mice and rats. In the rat, exposure groups were 0, 5, 12, 32, 80, and 200 ppm. Separate groups of 10 male and 10 female rats designated for coagulation studies were exposed to these concentrations for 2 days. Rats designated for hematology and clinical chemistry tests were first placed in metabolism cages for 16-hour urine collections. Sperm samples were collected from male rats at the end of the studies. Samples of vaginal fluid and cells were collected for up to 7 consecutive days prior to the end of the studies for cytology evaluations. Five male and five female rats were exposed to 0, 5, 32 or 200 ppm for glutathione evaluations. At week 11, all male and female core study rats were administered neurobehavioral tests measuring the following parameters: forelimb/hind-limb grip strength, horizontal activity, rearing activity, total activity, tail-flick latency, startle response latency, and startle response amplitude. Survival and body weights of rats are given in Table 4-18. No effects on final mean body weights were seen.

Table 4-18. Survival and body weights of rats in the 13-week inhalation study of chloroprene

EXPOSURE (ppm)	SURVIVAL	MEAN BODY WEIGHT (g)		
		Initial	Final	Change
Male				
0	10/10	109 ± 4	311 ± 9	+ 202 ± 8
5	10/10	119 ± 2*	323 ± 11	+ 204 ± 10
12	10/10	116 ± 1	306 ± 9	+ 190 ± 8
32	10/10	117 ± 2	327 ± 11	+ 209 ± 10
80	10/10	116 ± 1	301 ± 8	+ 184 ± 7
200	9/10	116 ± 3	304 ± 8	+ 185 ± 7

EXPOSURE (ppm)	SURVIVAL	MEAN BODY WEIGHT (g)		
		Initial	Final	Change
Female				
0	10/10	102 ± 2	191 ± 4	+ 89 ± 3
5	10/10	101 ± 1	193 ± 4	+ 92 ± 3
12	10/10	102 ± 2	199 ± 5	+ 97 ± 4
32	10/10	101 ± 2	195 ± 4	+ 94 ± 4
80	10/10	103 ± 1	192 ± 3	+ 90 ± 3
200	10/10	102 ± 1	183 ± 3	+ 81 ± 3

Significantly different ($p \leq 0.05$) from the chamber control group by Williams' or Dunnett's test.

Source: NTP (1998)

On day 2, minimal increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts occurred in males exposed to ≥ 32 ppm and in females exposed to 200 ppm. At week 13, male and female rats in the 200 ppm groups demonstrated decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts characterized as normocytic, normochromic anemia. Thrombocytopenia, evidenced by a reduction in circulating platelet numbers, occurred in the male and female rats in the 200 ppm groups on day 2 and in the females at 80 and 200 ppm on day 22. Platelet numbers rebounded at study termination in the highest exposure groups for both male and female rats. Activities of serum ALT, GDH, and SDH were elevated on day 22 in both sexes of the 200 ppm group. However, these increases were transient, and serum activities of the enzyme levels returned to control levels by the end of the exposure period. At week 13, an alkaline phosphatase (ALP) enzymeuria occurred in males exposed to ≥ 32 ppm and in females exposed to 200 ppm. In male rats in the 200 ppm group, proteinuria was seen at week 13. Significant reductions in nonprotein sulfhydryl (NPSH) concentrations were observed in the livers from male rats exposed to 200 ppm for 1 day or 12 weeks, as well as in female rats exposed to 200 ppm for 12 weeks. Nonprotein sulfhydryl concentrations were reduced in the lung of 200 ppm female rats after 1 day but not after 12 weeks of exposure to 200 ppm. Significant increases in kidney weights were seen in both male and female rats at 200 ppm and in females at 80 ppm. In male rats exposed to 200 ppm, sperm motility was significantly less than that of the chamber control group. Of the neurobehavioral parameters, horizontal activity was increased in male rats exposed to ≥ 32 ppm compared with chamber control animals. Total activity was increased in male rats in the 32 and 200 ppm groups. There were no exposure-related effects on motor activity, forelimb/hind-limb grip strength, or startle response.

Increased incidences of minimal to mild olfactory epithelial degeneration and respiratory metaplasia occurred in male and female rats exposed to 80 or 200 ppm (Table 4-19). The incidence of olfactory epithelial degeneration in females exposed to 32 ppm was significantly greater than in the chamber control group. In female rats exposed to 200 ppm, the incidence of hepatocellular necrosis was significantly greater than in the chamber control group. Variably sized aggregates of yellow or brown material consistent with hemosiderin appeared in small vessels or lymphatics in or near portal triads or in Kupffer cells of male and female rats exposed to 200 ppm and were significantly increased compared with chamber controls.

Table 4-19. Incidences of selected nonneoplastic lesions in rats in the 13-week inhalation study of chloroprene

	CONTROL	5 ppm	12 ppm	32 ppm	80 ppm	200 ppm
Male						
Nose[a]	10/10	0/10	10/10	10/10	10/10	10/10
Degeneration, olfactory epithelium	0/10	---	0/10	3/10	10/10[c]	10/10[c]
Metaplasia, respiratory, olfactory epithelium	0/10	---	0/10	0/10	4/10[b]	4/10[b]
Liver[a]	10/10	2/10	1/10	1/10	10/10	10/10
Necrosis, centrilobular	0/10	0/10	0/10	01/10	0/10	3/10
Inflammation, chronic	0/10	1/10	0/10	0/10	1/10	2/10
Hemosiderin pigmentation	0/10	0/10	0/10	0/10	0/10	5/10[b]
Female						
Nose[a]	10/10	0/10	10/10	10/10	10/10	10/10
Degeneration, olfactory epithelium	0/10	---	0/10	4/10[b]	9/10[c]	10/10[c]
Metaplasia, respiratory, olfactory epithelium	0/10	---	0/10	0/10	8/10[c]	9/10[c]
Liver[a]	10/10	2/10	5/10	3/10	10/10	10/10
Necrosis, centrilobular	0/10	0/10	0/10	0/10	0/10	5/10[b]
Inflammation, chronic	2/10	0/10	1/10	0/10	1/10	8/10[b]
Hemosiderin pigmentation	3/10	0/10	1/10	0/10	0/10	9/10[c]

[a] Number of animals with tissue examined microscopically.
[b] Significantly different ($p \leq 0.05$) the chamber control group by Fisher's exact test.
[c] Significantly different ($p \leq 0.01$) from the chamber control group by Fisher's exact test.

Source: NTP (1998)

In the mouse portion of the NTP 13-week inhalation study, exposure groups were 0, 5, 12, 32, and 80 ppm. Survival and body weights are given in Table 4-20. There was no increased mortality in any exposure group. Final mean body weights in 80 ppm males were significantly decreased compared with controls.

Table 4-20. Survival and body weights of mice in the 13-week inhalation study of chloroprene

SEX	EXPOSURE (ppm)	SURVIVAL	MEAN BODY WEIGHT (g)		
			Initial	Final	Change (+)
Male	0	10/10	25.5 ± 0.4	35.9 ± 0.9	10.5 ± 0.7
	5	10/10	25.2 ± 0.3	35.1 ± 0.9	10.0 ± 0.7
	12	10/10	25.2 ± 0.2	34.9 ± 0.6	9.7 ± 0.6
	32	10/10	25.4 ± 0.2	36.0 ± 0.9	10.6 ± 0.9
	80	10/10	24.7 ± 0.3	32.7 ± 0.6[a]	7.9 ± 0.5[a]
Female	0	10/10	20.4 ± 0.2	30.3 ± 1.0	9.9 ± 0.9
	5	10/10	20.9 ± 0.3	32.2 ± 0.9	11.3 ± 0.9
	12	10/10	20.4 ± 0.3	30.1 ± 0.6	9.7 ± 0.6
	32	10/10	20.8 ± 0.2	32.6 ± 0.8	11.8 ± 0.7
	80	10/10	20.5 ± 0.2	30.2 ± 1.3	9.7 ± 1.2

[a]Significantly different ($p \leq 0.05$) from the chamber control group by Williams' or Dunnett's test

Source: NTP (1998)

Hematology variables were similar to, although more mild than, in the 13-week rat study. A minimal anemia, including decreased hematocrit values and erythrocyte counts, occurred in female mice exposed to 32 and 80 ppm. Platelet counts were minimally increased in female mice exposed to 32 and 80 ppm, suggesting an increase in platelet production. No significant organ weight effects were observed. Sperm morphology and vaginal cytology parameters were similar to those of the chamber controls. Significantly increased incidences of squamous epithelial hyperplasia of the forestomach were observed in male and female mice exposed to 80 ppm (Table 4-21).

Table 4-21. Incidences of forestomach lesions in mice in the 13-week inhalation study of chloroprene

	CONTROL	5 ppm	12 ppm	32 ppm	80 ppm
Male					
Number examined microscopically	10/10	3/10	0/10	10/10	10/10
Squamous epithelial hyperplasia	0/10	0/10	---	0/10	4/10 [a]
Female					
Number examined microscopically	10/10	0/10	0/10	10/10	10/10
Squamous epithelial hyperplasia	0/10	---	---	0/10	9/10 [b]

[a] Significantly different ($p \leq 0.05$) from the chamber control group by Fisher's exact test.
[b] $p \leq 0.01$.

Source: NTP (1998)

In the 2-year NTP (1998) inhalation study of chloroprene in male and female rats, groups were exposed to 0, 12.8, 32, and 80 ppm chloroprene. Estimates of 2-year survival probabilities are shown in Table 4-22. Survival of males exposed to 32 or 80 ppm was significantly less than that of the chamber control group.

Table 4-22. 2-Year survival probability estimates for F344/N rats chronically exposed (2 years) to chloroprene by inhalation

SEX	STATUS	CONTROL	12.8 ppm	32 ppm	80 ppm
Male	Animals initially in study	50	50	50	50
	Moribund	34	40	41	41
	Natural deaths	3	1	4	5
	Animals surviving to study termination	13	9	5	4
	Percent probability of survival at end of study	26	18	10	8
	Mean survival (days)	646	638	609	609
	Survival analysis[a]	p = 0.013	p = 0.615	p = 0.025	p = 0.025
Female	Animals initially in study	50	50	50	50
	Moribund	19	21	23	27
	Natural deaths	1	1	1	2
	Pregnant	1	0	0	0
	Animals surviving to study termination	29	28	26	21
	Percent probability of survival at end of study	59	56	52	42
	Mean survival (days)	686	685	672	673
	Survival analysis	p = 0.085	p = 1.000	p = 0.473	p = 0.151

[a] The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns

Source: NTP (1998)

All animals were observed twice daily, and body weights were recorded initially, weekly through week 12, approximately every 4 weeks from week 15 through week 91, and every 2 weeks until the end of the study. Clinical findings were recorded initially at weeks 4, 8, 12, and 15, every 4 weeks through week 91, and every 2 weeks until the end of the study. Complete necropsy and microscopic examinations were performed on all rats. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, heart, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular stomach), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. Sperm morphology and vaginal cytology evaluations, clinical pathology evaluations, glutathione evaluations, coagulation studies, and neurobehavioral evaluations were not performed.

The incidences of nonneoplastic and neoplastic lesions observed in rats following 2-year inhalation exposures to chloroprene are given in Tables 4-23 and 4-24 (NTP, 1998). The incidences of squamous cell papilloma and combined squamous cell papilloma and squamous cell carcinoma of the oral cavity (oral mucosa, tongue, pharynx, and gingiva) in male rats exposed to 32 ppm and male and female rats exposed to 80 ppm were significantly greater than those in the chamber controls and exceeded historical control ranges. Squamous hyperplasia was observed in three male rats exposed to

80 ppm chloroprene, and was characterized by focal thickening and folding of the squamous epithelium.

Table 4-23. Incidence and severity of non-neoplastic lesions in F344/N rats chronically exposed (2 years) to chloroprene by inhalation

TISSUE SITE/LESION TYPE	LESION INCIDENCE (SEVERITY)							
	Males (ppm)				Females (ppm)			
	0	12.8	32	80	0	12.8	32	80
Oral cavity Sqamous Cell Hyperplasia	0/50	0/50	0/50	3/50 (2.7)[a]	--	--	--	--
Thyroid gland Follicular Cell Hyperplasia	0/50	2/50 (2.0)	4/49[b] (1.8)	1/50 (1.0)	0/49	0/50	0/50	2/50 (2.5)
Lung Alveolar Hyperplasia	5/50 (1.4)	16/50[c] (1.4)	14/49[b] (1.9)	25/50[c] (1.4)	6/49 (1.8)	22/50[c] 1.4)	22/50[c] (1.5)	34/50[c] (1.3)
Kidney (renal tubules) Hyperplasia	14/50 (2.0)	20/50 (2.6)	28/50[c] (2.1)	34/50[c] (2.9)	6/49 (1.3)	6/50 (1.8)	11/50 (2.1)	21/50[c] (2.0)
Olfactory Atrophy[d]	3/50 (1.7)	12/50[b] (1.8)	46/49[c] (2.2)	48/49[c] (3.6)	0/49	1/50 (1.0)	40/50[c] (1.3)	50/50[c] (2.9)
Basal Cell Hyperplasia	0/50	0/50	38/49[c] (1.6)	46/49[c] (2.2)	0/49	0/50	17/50[c] (1.1)	49/50[c] (2.3)
Metaplasia	6/50 1.7	5/50 (1.0)	45/49[c] (1.8)	48/49[c] (3.1)	0/49	1/50 (1.0)	35/50[c] (1.0)	50/50[c] (2.7)
Necrosis[e]	0/50	11/50[b] (2.0)	26/49[c] (2.0)	19/49[c] (2.2)	0/49	0/50	8/50[c] (2.0)	12/50[c] (1.3)
Chronic Inflammation	0/50	5/50[c] (1.0)	9/49[c] (1.6)	49/49[c] (2.7)	0/49	0/50	2/50 (1.0)	33/50[c] (2.0)

[a] Severity of lesions graded as: 1= minimal, 2 = mild, 3 = moderate, 4 = marked, average severity reported in parenthesis
[b] $p \leq 0.05$, ps correspond to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal
[c] $p \leq 0.01$
[d] Severity of atrophic lesions: Males: control –1 minimal, 2 mild; 12.8 ppm –6 minimal, 3 mild, 3 moderate; 32 ppm – 10 minimal, 19 mild, 15 moderate, 2 marked; 80 ppm – 1 mild, 18 moderate, 29 marked. Females: control –all 0; 12.8 ppm – 1 minimal; 32 ppm – 31 minimal, 7 mild, 2 moderate; 80 ppm – 10 mild, 33 moderate, 7 marked.
[e] Severity of necrotic lesions: Males: control –all 0, 12.8 ppm – 5 minimal, 1 mild, 5 moderate; 32 ppm – 8 minimal, 10 mild, 8 moderate; 80 ppm – 6 minimal, 4 mild, 8 moderate, 1 marked. Females: control –all 0; 12.8 ppm – all 0; 32 ppm – 3 minimal, 2 mild, 3 moderate; 80 ppm – 8 minimal, 4 mild.

Source: NTP (1998)

The incidences of thyroid gland follicular cell adenoma or carcinoma (combined) in male rats exposed to 32 or 80 ppm were significantly greater than those in the chamber control group and exceeded historical control ranges. The incidences of follicular cell adenoma and follicular cell adenoma or carcinoma combined in female rats exposed to 80 ppm were increased but not significantly greater than those in the chamber controls, although they did exceed the historical control range.

Follicular cell carcinomas destroyed the thyroid gland and occasionally invaded the capsule or adjacent structures. The incidence of follicular cell hyperplasia was significantly increased in male rats exposed to 32 ppm. Hyperplasia was characterized by one or a few enlarged follicles with several much smaller follicles inside and to one side.

Table 4-24. Incidence of neoplasms in F344/N rats chronically exposed (2 years) to chloroprene by inhalation

TISSUE SITE/TUMOR TYPE	TUMOR INCIDENCE							
	Males (ppm)				Females (ppm)			
	0	12.8	32	80	0	12.8	32	80
Oral cavity Papillomas or carcinomas	0/50	2/50	5/50[a]	12/50[b]	1/49	3/50	5/50	11/50[b]
Thyroid gland Adenomas or carcinomas	0/50	2/50	4/49[a]	5/50[a]	1/49	1/50	1/50	5/50
Lung Adenomas or carcinomas[c]	2/50	2/50	4/49	6/50	1/49	0/50	0/50	3/50
Kidney (renal tubules) Adenomas or carcinomas (extended and standard evaluations combined)	1/50	8/50[a]	6/50[b]	8/50[b]	0/49	0/50	0/50	4/50
Mammary gland Fibroadenomas	---	---	---	---	24/49	32/50	36/50[a]	36/50[b]

[a] $p \leq 0.05$, ps correspond to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal
[b] $p \leq 0.01$
[c] Adenomas only in females

Source: NTP (1998)

The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in males exposed to 80 ppm were slightly greater than those in the chamber control group. Although the increase in neoplasms was not statistically significantly increased relative to control, the incidences exceeded the historical control range. The incidence of alveolar/bronchiolar adenoma only was increased, though not significantly, in female rats exposed to 80 ppm chloroprene. Alveolar/bronchiolar carcinomas were solid or papillary, obliterated normal pulmonary structure, and sometimes invaded the pleura and other adjacent areas. The incidences of alveolar epithelial hyperplasia (AEH) were significantly greater in all exposed groups of males and females compared with the chamber control groups.

Renal tubule adenoma and hyperplasia were observed in male and female rats. Because renal tubule neoplasms are rare in chamber control F344/N rats, additional kidney sections from male and female control and exposed groups were examined to provide a clearer indication of the potential effects of chloroprene on the kidney. The combined single- and step-section incidences of renal tubule hyperplasia in males exposed to 32 and 80 ppm and in females exposed to 80 ppm and the incidences of adenoma and adenoma or carcinoma combined in all exposed males were significantly greater than those in the chamber controls.

The incidences of multiple fibroadenoma of the mammary gland in all exposed groups of female rats were greater than in the chamber control group. The incidences of fibroadenoma in females exposed to 32 and 80 ppm were significantly greater than in the chamber control group. However, the incidences of fibroadenomas in all exposed females and the chamber control exceeded the historical control range.

A slight increase in the incidence of transitional epithelium carcinoma of the urinary bladder was observed in females exposed at 80 ppm. In addition, one male exposed at 32 ppm had a transitional epithelium carcinoma and one male exposed at 80 ppm had a transitional cell papilloma. No urinary bladder neoplasms have been observed historically in chamber control male or female F344/N rats.

The incidences of atrophy, basal cell hyperplasia, metaplasia, and necrosis of the olfactory epithelium in males and females exposed to 32 and 80 ppm and of atrophy and necrosis in males exposed to 12.8 ppm were significantly greater than those in the chamber control groups. The incidences of chronic inflammation were significantly increased in males exposed to 12.8 or 32 ppm and in females exposed to 80 ppm. The incidences of fibrosis and adenomatous hyperplasia in males and females exposed to 80 ppm were significantly greater than those in the chamber controls. Lesions of the nasal cavity were generally minimal to moderate in average severity. Necrosis of the olfactory epithelium was characterized by areas of karyorrhexis and sloughing of olfactory epithelium with cell debris in the lumen of the dorsal meatus. Atrophy of the olfactory epithelium was characterized by decreased numbers of layers of olfactory epithelium and included loss of Bowman's glands and olfactory axons in more severe cases. Metaplasia was characterized by replacement of olfactory epithelium with ciliated, columnar, respiratory-like epithelium. Basal cell hyperplasia was characterized by proliferation or increased thickness of the basal cell layer in the turbinate and septum.

In the NTP 2-year mouse study, exposure concentrations were 0, 12.8, 32, and 80 ppm. All animals were observed twice daily and body weights were recorded initially, weekly through week 12, approximately every 4 weeks from week 15 through week 91, and every 2 weeks until the end of the study. Clinical findings were recorded initially, at weeks 4, 5, 8, 12, every 4 weeks through week 91, and every 2 weeks until the end of the study. A complete necropsy and microscopic examination were performed on all mice as described for the rat portion of the 2-year study. Estimates of 2-year survival probabilities are shown in Table 4-25.

Table 4-25. 2-Year survival probabilities for B6C3F1 mice chronically exposed (2 years) to chloroprene by inhalation

		CONTROL	12.8 ppm	32 ppm	80 ppm
Male	Animals initially in study	50	50	50	50
	Moribund	15	16	26	34
	Natural deaths	3	7	10	3
	Animals surviving to study termination	27	27	14	13
	Percent probability of survival at end of study	54	54	28	26
	Mean survival (days)	689	683	646	646
	Survival analysis[a]	$p < 0.001$	$p = 1.000$	$p = 0.007$	$p = 0.003$
Female	Animals initially in study	50	50	50	50
	Accidental death	0	1	0	1
	Moribund	13	27	38	41
	Natural deaths	2	6	11	5
	Animals surviving to study termination	35	16	1	3
	Percent probability of survival at end of study	70	33	2	6
	Mean survival (days)	686	641	558	562
	Survival analysis	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

[a] the result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns

Source: NTP (1998)

Survival of males exposed to 32 or 80 ppm and of all exposed female groups was significantly less than that of the chamber controls. The mean body weights of females exposed to 80 ppm were significantly less than those of the chamber control group after week 75.

The incidences of non-neoplastic and neoplastic lesions observed in mice with 2-year inhalation exposure to chloroprene are given in Tables 4-26 and 4-27. The incidences of alveolar/bronchiolar neoplasms in the lungs of all groups of exposed males and females were significantly greater than in the chamber control group and generally exceeded the historical control ranges. The incidences of multiple alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma were increased in all exposed males and females. The morphology of lung neoplasms was similar in control and exposed groups. The incidences of bronchiolar hyperplasia in all exposed groups of males and females were significantly greater than in the chamber control groups. Bronchiolar hyperplasia was characterized by diffuse thickening of the cuboidal cells lining the terminal bronchioles and in some cases caused papillary projections into the lumen. The incidences of histiocytic cell infiltration in males exposed to 80 ppm and in all exposed females were significantly increased relative to chamber controls. This change consisted of histiocytes within alveolar lumens, usually adjacent to alveolar/bronchiolar neoplasms.

Table 4-26. Incidence and severity of non-neoplastic lesions in B6C3F1 mice chronically exposed (2 years) to chloroprene by inhalation

TISSUE SITE/LESION TYPE	LESION INCIDENCE (SEVERITY)							
	Males (ppm)				Females (ppm)			
	0	12.8	32	80	0	12.8	32	80
Lung								
Bronchiolar Hyperplasia	0/50	10/50[c] (2.0)	18/50[c] (1.7)	23/50[c] (2.2)	0/50	15/49[c] (2.0)	12/50[c] (2.2)	30/50[c] (2.2)
Histiocytic Cell Infiltration	7/50 (1.6)	8/50 (3.3)	11/50 (2.5)	22/50[c] (2.9)	1/50 (3.0)	14/49[c] (2.0)	18/50[c] (2.3)	23/23[c] (2.4)
Kidney (renal tubule)								
Hyperplasia	0/50	4/49 (1.3)	5/50[b] (1.2)	5/50[b] (1.4)	--	--	--	--
Mammary Gland								
Hyperplasia	--	--	--	--	0/49	1/49 (1.0)	1/50 (1.0)	3/50 (2.0)
Forestomach								
Epithelial Hyperplasia	4/50 (3.0)	6/48 (1.8)	7/49 (2.3)	29/50[c] (2.2)	4/50 (2.0)	3/49 (3.7)	8/49 (1.6)	27/50[c] (2.7)
Olfactory								
Suppurative Inflammation	2/50 (2.0)	1/48 (1.0)	4/50 (1.0)	6/50 (1.5)	0/50	1/49 (1.0)	3/49[b] (1.7)	4/50[c] (1.5)
Atrophy	7/50 (1.1)	8/48 (1.4)	7/50 (1.1)	49/50[c] (2.5)	6/50 (1.2)	5/49 (1.2)	4/49 (1.3)	47/50[c] (2.0)
Metaplasia	6/50 (1.0)	5/50 (1.4)	5/50 (1.0)	49/50[c] (2.5)	2/50 (1.0)	3/49 91.0)	1/49 92.0)	44/50[c] (2.0)
Spleen								
Hematopoietic Proliferation	26/50	22/49	35/50[d]	31/50[d]	13/50	25/49[d]	42/49[d]	39/50[d]

[a] Severity of lesions graded as: 1= minimal, 2 = mild, 3 = moderate, 4 = marked, average severity reported in parenthesis, average severity not reported for splenic hematopoietic proliferation
[b] $p \leq 0.05$, ps correspond to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal
[c] $p \leq 0.01$
[d] Significantly increased relative to controls, level of significance not reported

Source: NTP (1998)

The incidences of olfactory epithelial atrophy, adenomatous hyperplasia, and metaplasia in males and females exposed to 80 ppm were significantly greater than those in the chamber controls. The incidence of suppurative inflammation in females exposed to 32 and 80 ppm was significantly greater than controls. Atrophy and metaplasia of the olfactory epithelium was similar to lesions observed in rats exposed to chloroprene. Adenomas of the respiratory epithelium were present in one female exposed to 32 ppm and one male exposed to 80 ppm.

In male mice, a pattern of nonneoplastic liver lesions along with silver-staining helical organisms within the liver was observed, consistent with *Helicobacter hepaticus* infection. Polymerase chain reaction-restriction fragment length polymorphism based assay confirmed an

organism compatible with *H. hepaticus*. Historically, NTP studies with *H. hepaticus* associated hepatitis showed increased incidences of hemangiosarcoma in male mice. Therefore, hemangiosarcomas of the liver were excluded from the analyses of circulatory neoplasms in the males in the chloroprene 2-year study. However, even with this exclusion, the combined occurrence of hemangioma or hemangiosarcoma at other sites was significantly increased in all males exposed to chloroprene and in females exposed to 32 ppm. The incidences of neoplasms at other sites were not considered to have been significantly impacted by the infection with *H. hepaticus* or its associated hepatitis. The incidences of hepatocellular carcinoma in all exposed female mice and hepatocellular adenoma or carcinoma combined in females exposed to 32 and 80 ppm were significantly greater than in the chamber control

Table 4-27. Incidence of neoplasms in B6C3F1 mice chronically exposed (2 years) to chloroprene by inhalation

TISSUE SITE/TUMOR TYPE	TUMOR INCIDENCE							
	Males (ppm)				Females (ppm)			
	0	12.8	32	80	0	12.8	32	80
Lung Adenomas or carcinomas	13/50	28/50[c]	36/50[c]	43/50[c]	4/50	28/49[c]	34/50[c]	42/50[c]
All Organs Hemangiomas or hemangiosarcomas	3/50	14/50[b]	23/50[c]	21/50[c]	4/50	6/50	18/50[b]	8/50
Harderian gland Adenomas or carcinomas	2/50	5/50	10/50[a]	12/50[b]	2/50	5/50	3/50	9/50[a]
Kidney (renal tubules) Adenomas or carcinomas (extended and standard evaluations combined)	0/50	2/49	3/50[a]	9/50[b]	---	---	---	---
Mammary gland Carcinomas	---	---	---	---	3/50	4/50	7/50	12/50[a]
Forestomach Papillomas or carcinomas	1/50	0/50	2/50	4/50	1/50	0/50	0/50	4/50
Liver Adenomas or carcinomas	---	---	---	---	20/50	26/49	20/50[a]	30/50[c]
Skin Sarcoma	---	---	---	---	0/50	11/50[b]	11/50[c]	18/50[c]
Mesentery Sarcomas	---	---	---	---	0/50	4/50	8/50[b]	3/50
Zymbal's gland Carcinomas	---	---	---	---	0/50	0/50	0/50	3/50

[a] $p < 0.05$, ps correspond to the pairwise comparisons between the chamber controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal
[b] $p < 0.01$
[c] $p < 0.001$

Source: NTP (1998)

The incidences of Harderian gland adenoma and Harderian gland adenoma or carcinoma combined in males exposed to 32 or 80 ppm and females exposed to 80 ppm were significantly greater than in the chamber controls. The incidences of Harderian gland adenoma or carcinoma combined in these groups exceeded the historical control range.

Although not significantly increased, the incidence of renal tubule adenoma in males exposed to 80 ppm was greater than in the chamber control group. The incidence of this rare neoplasm exceeded the historical control range. The incidences of renal tubule hyperplasia in males exposed to 32 or 80 ppm were significantly greater than in the chamber controls. The combined single- and step-section incidence of renal tubule adenoma in males exposed to 80 ppm and the combined incidences of renal tubule hyperplasia in all groups of exposed male mice were greater than in the chamber controls.

The incidences of mammary gland carcinoma in females exposed to 80 ppm were significantly greater than in the chamber control group. The incidences of mammary gland carcinoma in females exposed to 32 and 80 ppm exceeded the historical control range. Mammary gland hyperplasia was present in a few females exposed to chloroprene, but was not significantly increased relative to chamber controls.

The incidence of forestomach squamous cell papilloma in females exposed to 80 ppm was greater than in the chamber controls but statistically not significant. The incidence observed exceeded the historical control range. In male and female mice exposed to 80 ppm, the incidences of hyperplasia of the forestomach epithelium were significantly greater than in chamber controls, and the lesions were similar to those seen in the 13-week study. Hyperplasia was a focal to multifocal change characterized by an increase in the number of cell layers in the epithelium.

The incidences of sarcoma of the skin were significantly greater in all exposed female mice compared with chamber controls. The incidences of sarcomas of the mesentery were increased in all exposed female mice, with only the mice in the 32 ppm exposure group exhibiting a significant increase.

Carcinomas of Zymbal's gland were observed in three females exposed to 80 ppm, and two carcinomas had metastasized to the lung. Zymbal's gland carcinomas have not been reported in the NTP historical database for control female mice.

Single papillary adenomas were detected in the trachea of one male exposed to 12.8 ppm and in one male exposed to 32 ppm. These adenomas have not been documented in the NTP historical database.

The incidences of splenic hematopoietic proliferation in males exposed to 32 and 80 ppm and in all exposed groups of females were significantly greater than in the chamber controls.

Because of a large number of early deaths of mice exposed to chloroprene for 2-years, survival-adjusted neoplasm rates were estimated by NTP by using the poly-3 survival-adjusted quantal response method of Portier and Bailer (1989). This adjustment accounts for the impact of early mortality on the expression of late-developing neoplasms and provides a clearer indication of exposure-response relationships for neoplasms induced by chloroprene (Table 4-28). The neoplasm incidence values provided represent the ratio of the number of animals in an exposure group bearing the specific neoplasm relative to the adjusted number of animals at risk.

Table 4-28. Survival-adjusted neoplasm rates for mice in the 2-year inhalation study of chloroprene

	MALES (%)				FEMALES (%)			
	0	**12.8**	**32**	**80**	**0**	**12.8**	**32**	**80**
Lung								
Adenoma or carcinoma	14.1[b]	28.3	56.9[b]	66.4[b]	4.6[b]	35.6[b]	53.8[b]	76.0[b]
Alveolar/bronchiolar adenoma or carcinoma	29.8[b]	63.7[b]	79.2[b]	92.9[b]	9.1[b]	68.3[b]	85.8[b]	96.1[b]
All Organs								
Hemangioma or hemangiosarcoma	2.4[b]	28.2[b]	45.2[b]	43.6[b]	9.0[a]	16.0	53.1[b]	27.7[a]
Harderian gland								
Adenoma or carcinoma	4.7[b]	12.0	26.3[b]	32.0[b]	4.5[b]	13.5	11.7	31.2[b]
Kidney (renal tubules)								
Adenoma								
(single section)	0[a]	2.4	2.8	8.2	---	---	---	---
(single + step section)	0[b]	4.8	8.3	23.9[a]	---	---	---	---
Mammary gland								
Adenoacanthoma or carcinoma	---	---	---	---	6.7[b]	12.9	33.7[b]	42.5[b]
Forestomach								
Squamous cell papilloma or carcinoma	2.4[b]	0	5.6	13.3	2.3[b]	0	0	14.6
Liver								
Carcinoma	---	---	---	---	9.0[b]	28.4[a]	47.5[b]	58.2[b]
Adenoma or carcinoma	---	---	---	---	44.8	62.9	63.3	79.7[b]
Skin								
Sarcoma	---	---	---	---	0[a]	27.5[b]	39.0[b]	52.6[b]
Mesentery								
Sarcoma	---	---	---	---	0	10.7[a]	28.9[b]	11.0

In the chamber control column, [a] indicates a significant trend ($p < 0.05$) across all exposure groups by the Poly-3 quantal response test; [b] indicates a significant tend at $p < 0.01$
In the exposed group columns, [a] indicates a significant difference ($p < 0.05$) from the chamber control group by pairwise comparison; [b] indicates a significant difference at $p < 0.01$.

Source: NTP (1998)

In another chronic inhalation study, Trochimowicz et al. (1998) exposed three groups of 100 Wistar rats and Syrian hamsters of each sex to chloroprene at 0, 10, or 50 ppm for 6 hours/day, 5 days/week for up to 18 months (hamsters) or 24 months (rats). Chemical purity of the bulk chloroprene was reported to be 99.6%, with less than 50 ppm of dimers as determined by gas chromatography. Bottles of test material were received weekly and were stored under nitrogen at –20°C. Phenothiazine (0.01%) was added to prevent oxidation. A fresh sample of chloroprene from cold storage was used for each day's exposure. To generate the test atmospheres, sufficient quantities of the bulk material were vaporized with dried and filtered nitrogen at 0°C; vaporization at this temperature was performed to inhibit the formation of degradation products. The saturated chloroprene/nitrogen mixture was then directed into the inhalation chamber inlet, where it was mixed with the main air flow to generate the desired exposure concentration. All animals were observed daily and clinical signs and mortality were recorded. Rats and hamsters were weighed immediately before the first exposure, weekly for the first 8 weeks, and at 4-week intervals thereafter. During the last 6 months of each study, all animals were palpated monthly for the presence of tumors. Time of tumor appearance, size, location, and progression were recorded. At study termination, both hamsters

and rats were sacrificed by exsanguination of the abdominal aorta. A postmortem examination was conducted during which all major organs/tissues were examined for gross abnormalities. Gross pathological examinations were conducted on all animals, including those that died intercurrently or were killed in extremis, unless advanced autolysis or cannibalism prevented this. The following organs were weighed: adrenals, brain (hamster), heart, kidneys, liver, lungs with trachea and larynx, ovaries, pituitary, spleen, testes, and thyroid (rat). The following organs/tissues were preserved and examined microscopically: all gross lesions, adipose tissue, aorta (rat), epididymides, external auditory canal with Zymbal's glands, eyes, exorbital lachrymal glands, femur (with knee joint), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, and colon), lungs, lymph nodes (auxiliary, cervical, and mesenteric), mammary glands, nasal cavity (four transverse sections), pancreas, parotid salivary glands, preputial glands, prostate, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, sternum (bone marrow), sublingual and submaxillary salivary glands, thymus, thyroid with parathyroid (hamster), urinary bladder, and uterus. Microscopic examinations were performed on all organs from all control and high-exposure animals, and on the liver, spleen, pituitary gland, thyroid glands, adrenals, and all grossly visible tumors and tumor-like lesions from the low-exposure animals.

Mortality rate for rats was relatively low in all groups up to week 72, ranging from 1–3%. During week 72, however, 87 males and 73 females of the 10 ppm exposure group died overnight from suffocation resulting from accidental failure of the exposure chamber ventilation system. For hamsters, the mortality rate was negatively correlated with the concentration of chloroprene exposure. After 18 months of exposure, survival rates in the 0, 10, and 50 ppm groups were 88, 92, and 93% in males and 63, 75, and 72% in females, respectively.

A slight but consistent growth retardation was found in male rats (~10%) and female rats (~5%) in the 50 ppm exposure group. Both male and female hamsters showed a slight growth depression in the 50 ppm group throughout the study. Appearance and behavior of the rats were not affected by exposure to chloroprene, except that alopecia occurred more frequently in the 50 ppm group than in the 10 ppm group or in the controls. The alopecia varied from small, focal, mostly bilateral bald areas to severe, diffuse, generalized hair loss. Alopecia was first observed after an exposure period of about 10 weeks, but by 25 weeks the incidence and degree of alopecia gradually decreased and in many animals complete re-growth of hair was observed. No abnormalities were observed in hamsters; alopecia was occasionally seen in each group during the first 64 weeks of study, regardless of exposure.

Body weights are given in Table 4-29. In both male and female rats, mean relative lung weights were significantly lower in both exposure groups than in controls. In females exposed to 50 ppm, the mean relative spleen and thyroid weights were significantly lower. The kidney and pituitary weights in males exposed to 10 ppm were significantly increased compared with controls, although this was not observed in the 50 ppm exposure group. In hamsters, both male and female animals

exposed to 50 ppm had significantly higher brain weights compared with controls. Relative lung weight was significantly higher in males exposed to 50 ppm than in controls.

Table 4-29. Selected mean relative organ weights of rats exposed for 24 months and hamsters exposed for 18 months to chloroprene vapor

GROUP (ppm)	NUMBER[1]	BW (g)	ADRENALS	BRAIN	KIDNEYS	LIVER	LUNGS	SPLEEN	THYROID
Rats									
Males									
0	77	494	---	---	0.61	3.09	0.45	0.154	0.0056
10	9	500	---	---	0.68[a]	3.31	0.37[b]	0.172	0.0056
50	76	496	---	---	0.64	3.15	0.38[b]	0.146	0.0056
Females									
0	81	308	---	---	0.64	3.00	0.53	0.180	0.0080
10	19	309	---	---	0.65	3.23[a]	0.45[a]	0.176	0.0073
50	75	307	---	---	NR[2]	3.13[a]	0.45[a]	0.164[b]	0.0070[b]
Hamsters									
Males									
0	86	101	0.0311	1.10	1.25	5.11	0.85	0.197	---
10	92	101	0.0279[a]	1.11	1.17[b]	4.75[a]	0.84	0.190	---
50	92	93	0.0294	1.19[c]	1.22	4.91	0.90[b]	0.174[b]	---
Females									
0	60	99	0.0340	1.13	1.48	6.73	1.01	0.253	---
10	74	98	0.0356	1.16	1.50	6.54	0.97	0.269	---
50	72	90	0.0383	1.24[c]	1.50	6.37	1.01	0.286	---

[1] Number at sacrifice
[2] Not recorded
[a] Significant, $0.1 \leq p < 0.005$
[b] Significant, $0.001 \leq p < 0.01$
[c] Significant, $p < 0.001$

Source : Trochimowicz et al. (1998)

Gross pathology revealed that lungs from rats exposed at 10 and 50 ppm had markedly lower incidences of nodular pleural surfaces, consolidation, and gross changes consistent with, and characterized as chronic respiratory disease, than did controls. Morphologic indicators of chronic respiratory disease were seen in 28 of 196 controls, 0 of 37 in the 10 ppm group, and 4 of 200 in the 50 ppm group. The incidence of tumors or tumor-like lesions of the mammary glands was slightly higher in the exposed animals terminated at the end of the study (10/24 and 34/100 in 10 and 50 ppm, respectively) compared with controls (23/99); however, these differences were not statistically significant unless animals that were moribund or dead before the terminal sacrifice were included in the analysis. No other remarkable differences in gross pathology were seen in rats. Macroscopic examination of hamsters revealed a slight, concentration-related decrease in the incidence of pale adrenal glands in males.

The only nonneoplastic lesions in rats were observed in liver and lungs (only the livers of animals that died accidentally due to a failure in the ventilation system were available for microscopic

examination). The number of female and male rats with one or more small foci of cellular alteration in the liver was significantly increased in the 50 ppm group than in controls. Mild changes, such as lymphoid aggregates around bronchi, bronchiole, and blood vessels, were observed in males and females exposed to 50 ppm. Acute inflammatory processes in the lungs were found in the 50 ppm exposure group and control animals to a similar extent.

The only nonneoplastic change seen in hamsters was a generalized amyloidosis (in the liver, kidneys, spleen, and adrenals) that was lower in incidence in the 50 ppm exposed group compared with controls.

Tumor incidences for rats and hamsters are shown in Tables 4-30 and 4-31, respectively. With the exception of mammary gland tumors and squamous cell carcinomas, no individual organ or tissue in rats exposed to chloroprene showed a statistically significant excess of tumors compared with controls. The number of females bearing mammary tumors in the 50 ppm group was significantly increased ($p < 0.05$). The observed increase in mammary tumors in the high dose animals was due to the inclusion in the analysis of animals that were moribund or dead before the terminal sacrifice. No difference was observed between control and test group animals that were sacrificed at the end of the study. The number of mammary tumors per rat was not different between the 50 ppm group and the control group. The increased incidence of mammary tumors was almost entirely due to the relatively high number of animals of the test groups bearing benign fibroadenomas. Squamous cell carcinomas involving the nasal cavity, sinus maxillaries, subcutis, and skin were found in 3 of 100 males of the 50 ppm group and in 1 of 99 females of the control group. Neither macroscopic nor microscopic examination could clarify the exact origin of these tumors. If they originated as skin tumors, the total number of squamous-cell carcinomas of the skin would have been 5/100 in the 50 ppm group, which would be a statistically significant ($p < .05$) increase over controls (1/97).

In the hamster, the incidences of cystadenomatous polyps of the gallbladder and pheochromocytoma were slightly, but significantly, elevated in the males exposed to 10 ppm. All other tumors observed were about equally distributed among test and control groups or occurred in only one or two hamsters.

Sanotskii (1976) provided a review of numerous Russian subchronic inhalation studies of chloroprene (chemical purity and exposure regimen not specified) in rats and mice. According to Sanotskii (1976), the studies evaluated the systemic effects of chloroprene exposure in white rats exposed for 4.5 months to 0.051, 0.15, and 1.69 mg/m^3 (0.014, 0.041, and 0.47 ppm) or C57BL/6 mice exposed for 2 months to concentrations as high as 35 mg/m^3 (9.7 ppm). Several "signs of systemic effect" in male rats were reported at 1.69 ± 0.087 mg/m^3, including an increase in a "summation threshold index" (not defined) after 2.5 and 4.5 months, a decrease in the synthesis of hippuric acid from sodium benzoate (Quick's test) at 4.5 months, and an inhibition of gas exchange after 4.5 months. Chloroprene was reported to have had no effect on "the indicators used in the tests" (i.e., summation

threshold index, hippuric acid synthesis, and inhibition of gas exchange) in mice at concentrations as high as 35 ± 0.7 mg/m^3 (9.7 ppm).

Table 4-30. Incidence, site and type of tumor in selected organs and tissues of rats exposed to chloroprene for 24 months

	MALES			FEMALES		
Site and type of tumor[a]	**0 ppm**	**10 ppm**	**50 ppm**	**0 ppm**	**10 ppm**	**50 ppm**
Initial number of rats	100	100	100	100	100	100
Number examined	97	13	100	99	24	100
Number tumor-bearing[b]	51	6	57	66	12	74
Total number primary tumors[b]	73/51	6/6	77/57	100/66	13/12	96/71
Hematopoietic system						
Lymphoid leukemia	1	0	2	0	0	1
Monocytic leukemia	0	0	1	0	0	0
Kidneys						
Lipoma	0	0	1	1	0	1
Adenocarcinoma	0	0	1	0	0	0
Liver						
Unidentified	0	0	0	1	0	0
Lungs						
Anaplastic carcinoma	0	0	0	1	0	0
Mammary glands						
Adenoma	---	---	---	3	1	7
Fibroadenoma	---	---	---	24	6	36
Adenocarcinoma	---	---	---	5	0	3
Papillary carcinoma	---	---	---	1	0	0
Unidentified tumor	---	---	---	1	2	0
Skin						
Squamous cell carcinoma	0	0	2	0	0	0
Skin, nasal cavity, maxillary sinus,						
Squamous cell carcinoma	0	0	3	1	0	0
Spleen						
Hemangiosarcoma	0	0	1	0	0	0
Subcutis, nasal cavity, or maxillary sinus						
Reticulum cell sarcoma	0	0	0	0	0	1
Testes						
Leydig cell tumor	2	2	4	---	---	---
Testes/epididymides						
Mesothelioma	1	0	0	---	---	---
Thyroid gland						
Parafollicular cell adenoma						
Small	6	0	8	11	0	14
Medium/large	3	1	3	3	1	4
Parafollicular cell carcinoma						
Small	1	0	0	0	0	0
Large	1	0	0	0	0	0
Follicular adenoma						
Small	2	0	2	0	0	3
Large	2	0	1	0	0	0
Papillary carcinoma	0	0	0	0	0	2

	MALES			FEMALES		
Site and type of tumor[a]	**0 ppm**	**10 ppm**	**50 ppm**	**0 ppm**	**10 ppm**	**50 ppm**
Urinary bladder Transitional cell carcinoma (metastasizing)	0	0	1	0	0	0
Zymbal's gland Adenoma	0	0	0	0	0	1

[a] Multiple tumors at one site were counted as one tumor
[b] Some animals had more than one tumor

Source: Trochimowicz et al. (1998)

Table 4-31. Incidence, site and type of tumor in selected organs and tissues of hamsters exposed to chloroprene for 18 months

	MALES			FEMALES		
	0 ppm	**10 ppm**	**50 ppm**	**0 ppm**	**10 ppm**	**50 ppm**
Initial number of hamsters	100	100	100	100	100	100
Number examined	100	97	97	94	93	97
Number tumor bearing[a]	14	17	20	10	11	15
Total number primary tumors[a]	15/14	18/17	23/20	11/11	11/11	18/15
Kidney Cortical adenocarcinoma	2	0	0	0	0	0
Liver						
Neoplastic (hepatocellular) nodule	0	1	0	0	0	0
Unidentified tumor-like lesion	0	1	0	0	0	1
Lung tumors	0	0	0	0	0	0
Gallbladder Cystadenomatous polyp	1	6[a]	1	1	2	3
Pancreas						
Islet-cell adenoma	1	0	2	0	0	0
Islet-cell adenocarcinoma	0	0	0	1	0	1
Stomach						
Papilloma	0	0	2	0	0	0
Unidentified papilloma-like lesion	1	1	1	1	2	0
Testes Leydig-cell tumor	1	0	0	---	---	---
Colon Adenomatous polyp	0	0	0	2	0	0
Pituitary Adenoma	0	0	1	2	0	0
Thyroid gland						
Parafollicular cell adenoma	2	0	0	0	2	1
Cystadenoma	1	0	0	0	0	0
Papillary adenoma	0	1	1	1	0	2
Follicular adenoma	2	1	0	1	2	2
Parathyroid Adenoma	0	0	0	0	1	0
Adrenals						
Cortical adenoma	4	1	10	0	0	3
Cortical carcinoma	0	1	0	1	0	1
Pheochromocytoma	0	4[b]	2	0	0	0
Malignant pheochromocytoma	0	0	2	0	0	0
Ovaries Granulosa-theca-cell tumor	---	---	---	0	2	1

	MALES			FEMALES		
	0 ppm	10 ppm	50 ppm	0 ppm	10 ppm	50 ppm
Parotid salivary glands Adenoma	0	0	0	0	0	1
Skin Unidentified tumor-like lesion	0	1	0	0	0	0
Zymbal's gland Sebaceous adenoma	0	0	1	0	0	0
Depot fat Lipoma	0	0	0	0	0	1
Nose						
Adenoma of Bowman's glands	0	0	0	1	0	0
Adenocarcinoma of Bowman's glands	0	0	0	0	0	1
Bone (ribs) Osteosarcoma	0	0	0	1	0	0
Abdominal cavity Reticulum cell sarcoma	1	0	0	0	0	0

[a] Some animals had more than one tumor
[b] Significant, $p < 0.05$ by chi-squared test

Source: Trochimowicz et al. (1998)

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Ponomarkov and Tomatis (1980) administered chloroprene dissolved in olive oil by stomach tube to 17 female BD IV rats at a single dose (100 mg/kg body weight) on gestational day (GD) 17. Progeny from treated females (81 males and 64 females) were treated weekly with 50 mg/kg body weight by stomach tube from the time of weaning for life (120 weeks). A control group of 14 female rats was treated with 0.3 mL olive oil. Litter sizes and preweaning mortality, survival rates, and body weights did not differ between chloroprene-treated animals and controls (see Section 4.2.1 for further study details).

NTP (1998) evaluated sperm morphology and vaginal cytology in rats exposed to 0, 5, 32, or 200 ppm and mice exposed to 0, 12, 32, 80 ppm chloroprene for 13 weeks. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1985). Table 4-32 is a summary of measured epididymal spermatozoal and estrous cycle parameters from these 13-week studies. The sperm motility of male rats exposed to 200 ppm was significantly less than that of controls. This was the only reproductive tissue or estrous cycle parameter affected, compared with controls, in rats or mice at any exposure level.

Table 4-32. Summary of epididymal spermatozoal and estrous cycle parameters for rats and mice in the 13-week study of chloroprene

	RATS				MICE			
	0 ppm	5 ppm	32 ppm	200 ppm	0 ppm	12 ppm	32 ppm	80 ppm
n	10	10	9	9	7	8	10	10
Epididymal spermatozoa - males[a]								
Motility (%)	86.73 ± 1.04	83.62 ± 1.93	82.16 ± 1.84	80.04 ± 1.99[d]	79.09 ± 1.20	81.07 ± 1.13	80.08 ± 1.19	80.04 ± 1.47
Abnormal sperm (%)	0.70 ± 0.05	0.78 ± 0.11	0.73 ± 0.11	1.02 ± 0.14	1.49 ± 0.42	1.30 ± 0.22	0.98 ± 0.10	1.36 ± 0.22
Sperm concentration (10^6/g cauda epididymidis)	698 ± 40	722 ± 62	689 ± 46	683 ± 25	1,632 ± 138	1,447 ± 122	1,575 ± 104	1,672 ± 134
Estrous cycle - females[a]								
Length (days)	5.00 ± 0.15	4.67 ± 0.17[b]	5.00 ± 0.27[c]	5.33 ± 0.17[b]	4.00 ± 0.00	4.30 ± 0.21	4.22 ± 0.15[b]	4.13 ± .13[c]
Diestrus stage (% of cycle)	42.9	35.7	44.3	45.7	31.4	31.4	30.0	35.7
Proestrus stage (% of cycle)	15.7	18.6	11.4	17.1	20.0	20.0	22.9	25.7
Estrus stage (% of cycle)	18.6	22.9	20.0	15.7	24.3	24.3	25.7	20.0
Metestrus stage (% of cycle)	22.9	22.9	24.3	20.0	24.3	24.3	21.4	18.6
Uncertain diagnosis stage (% of cycle)	0.0	0.0	0.0	1.4	----	----	----	----

[a] Epididymal spermatozoal parameters, and estrous cycle lengths are presented as mean ± standard error. Differences from the control group are not significant by Dunn's test (epididymal spermatozoal abnormality and concentration, estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
[c] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[d] Significantly different ($p \leq 0.01$) from the control group by Shirley's test.

Source: NTP, (1998).

Sanotskii (1976) reviewed several Russian studies that exposed white rats (strain unknown) to various concentrations of chloroprene in order to determine the effect on reproductive and developmental parameters. In male rats exposed for 4.5 months to 1.7 mg/m^3 (0.5 ppm) of chloroprene, reductions in the number of normal spermatogonia, increases in the percentage of dead spermatozoa, and decreases in spermatozoal motility were reported. These effects were not observed by NTP (1998) in F344 rats at much higher concentrations (Table 4-32). Sanotskii (1976) also reported an increase in the number of seminiferous tubules with desquamating epithelium in male C57BL/6 mice exposed to 0.32 mg/m^3 (0.09 ppm) for 2 months and increased dominant lethal mutations in germ cells of male and female C57BL/6 mice exposed to 3.5 mg/m^3 (1 ppm) for 2 months.

Sanotskii (1976) also reported on an embryotoxicity study in which pregnant white rats were exposed during their "whole period of pregnancy." Exposure to 4 mg/m^3 (1.1 ppm) chloroprene was reported to have resulted in an increase of embryonic mortality, a decrease in fetal weight, and a disturbance in vascular permeability as evidenced by hemorrhaging into body cavities. Exposure to 0.13 mg/m^3 (~ 0.04 ppm) chloroprene was reported to have resulted in increased postnatal mortality.

Exposure to 4 mg/m^3 (1.1 ppm) chloroprene at various times during pregnancy was reported to have resulted in cerebral hernia and hydrocephalus.

Culik et al. (1978) evaluated the embryotoxic, teratogenic, and reproductive toxicity of chloroprene in rats. Culik et al. (1978) exposed pregnant CD rats to chloroprene by inhalation at 0, 1, 10, or 25 ppm (0.28, 2.8, or 6.9 mg/m^3) for 4 hours daily, either on GDs 1–12 (embryotoxicity study) or GDs 3–20 (teratology study). Pregnant rats in these embryotoxicity and teratology studies were sacrificed and their litters examined on GDs 17 and 21, respectively. Male rats in a separate reproduction study were exposed to 0 or 25 ppm (0 or 6.9 mg/m^3) 4 hours daily for 22 days and bred with untreated females for 8 consecutive weeks. The embryotoxicity study included 200 female rats (50 per exposure group), the teratology study included 100 primigravida rats (25 per exposure group), and the male reproduction study involved 10 male rats (5 per exposure group) and 3 virgin females per male. The test material was reported to be > 99.9% pure and was stored under nitrogen at –20°C in small glass bottles holding one day's supply for generating atmospheres. No chemical decomposition was observed during the experiment.

In both the embryotoxicity and teratogenicity studies, litter size, average numbers of implantation sites per litter, and preimplantation losses among exposed females did not differ significantly from those of the controls (Table 4-33). In the teratology study, there was an increase in the percentage of litters with resorptions that was statistically significant ($p \leq 0.05$, Fisher's exact test) only in the 10 ppm exposure group (62% compared to 29% in the control group). The percentage of litters with resorptions was also elevated in the 25 ppm group (59%), although this increase in effect failed to achieve statistical significance. There was no effect on percentage of litters with resorptions in any exposure group in the larger embryotoxicity study; all groups had approximately 50% of their litters exhibiting resorption. The number of resorptions per litters with resorptions was not affected in either study. The more frequently investigated endpoint of number of resorptions per litter (total) was not reported by the study, but was calculated from the reported data and included in Table 4-33 for reference. There was a slight, but statistically significant ($p < 0.05$), increase in the average body weight of fetuses from dams exposed to chloroprene at 25 ppm in the teratology study. Fetuses from dams in the teratology study exposed to 10 and 25 ppm chloroprene were significantly ($p < 0.05$) longer than the control fetuses. The incidence of minor anomalies (minute subcutaneous hematomas and petechial hemorrhages) in fetuses from exposed dams was similar to that found in control fetuses (Table 4-34). No major compound-induced or concentration-related skeletal or soft tissue anomalies were found. The number of unossified sternebrae and unossified thoracic vertebral centers were similar in all groups regardless of treatment. The combined results of weekly matings for the 8-week reproduction test indicated that there were no significant effects on reproduction due to chloroprene exposure: the mating index, average number of pups per litter, viability index, and lactation index were similar for exposed and control animals.

Table 4-33. Results of teratology and embryotoxicity studies in rats exposed to chloroprene by inhalation

PARAMETER	CONCENTRATION OF CHLOROPRENE (ppm)			
	0	1	10	25
Teratology Study				
Number of litters	21	24	21	19
Pregnancy rate, %	84 (21/25)	96 (24/25)	84 (21/25)	76 (19/25)
Corpora lutea/dam	13 ± 3	12 ± 2	12 ± 2	13 ± 2
Implantation sites/dam	10 ± 2	9 ± 3	9 ± 2	11 ± 1
Median preimplantation loss, %	14.7	29.5	20.0	10.0
Live fetuses/litter	9 ± 2	8 ± 3	8 ± 3	10 ± 1
Litters with resorption, %	29 (6/21)	29 (7/24)	62 (13/21)[a]	59 (11/19)
Litters totally resorbed	0	0	0	0
Median postimplantation loss in litters with resorption, %	11.8	16.7	22.0	16.7
Resorptions/litters with Resorptions	1.3 (8/6)	2.0 (14/7)	1.9 (25/13)	1.6 (17/11)
Resorptions/litters total	0.38 (8/21)	0.58 (14/24)	1.19 (25/21)	0.89 (17/19)
Fetal body weight, g	3.76 ± 0.28	3.94 ± 0.46	3.96 ± 0.26	4.04 ± 0.27[b]
Fetal crown-rump length, mm	32.9 ± 1.4	33.7 ± 1.6	33.8 ± 0.7[b]	34.1 ± 1.2[b]
Embryotoxicity Study				
Number of litters	45	43	43	48
Pregnancy rate, %	90 (45/50)	86 (43/50)	88 (43/49)	94 (48/51)
Corpora lutea/dam	15 ± 3	14 ± 3	14 ± 2	13 ± 3
Implantation sites/dam	11 ± 3	11 ± 4	10 ± 4	10 ± 3
Median preimplantation loss, %	20.0	16.2	17.7	16.0
Live fetuses/litter	10 ± 3	9 ± 4	10 ± 3	10 ± 3
Litters with resorption, %	51 (23/45)	51 (22/43)	53 (23/43)	50 (24/48)
Litters totally resorbed	0	1	0	0
Median postimplantation loss in litters with resorption, %	9.1	12.9	8.3	9.1
Resorptions/litters with resorptions	1.7 (39/23)	2.1 (47/22)	1.6 (37/23)	1.4 (34/24)
Resorptions/litters total	0.87 (39/45)	1.09 (47/43)	0.86 (37/43)	0.71 (34/48)

[a] Significantly different ($p \leq 0.05$) from the control group by Fisher's exact test.
[b] Significantly different ($p \leq 0.05$) from the control group by an analysis of variance and least significant difference (LSD) test

Source: Culik et al. (1978)

Culik et al. (1978) concluded that the statistically significant increase in litters with resorptions observed in the teratology study at 10 ppm was not biologically significant because the increase at 25 ppm was not statistically significant and the effect was not observed in the embryotoxicity study, which had larger numbers of animals per exposure group and was specifically designed to observe such an effect. Further, the control group for the teratology study is the only group in either study (embryotoxicity or teratology) that is far outside of the historical control range for number of resorptions per litter (0.83 ± 0.34) for this strain of rat (Charles River Laboratories, 1996); the corresponding control group in the embryotoxicity study had a response rate equivalent to historical controls. Therefore, if the control group response in the teratology study is abnormally low, this may indicate that the statistically significant increase seen in the 10 ppm group may be a spurious

observation. Chloroprene exerts an effect on fetal weight and size, as evidenced by increases in both at higher exposure levels. However, in the absence of other definitive markers of developmental toxicity, the importance or adversity of this finding remains unclear. Given the lack of a defined dose-response for litters with resorptions in either the embryotoxicity or teratology study, and that the control group in the teratology study may be a statistical outlier compared to historic control data, there is no compelling evidence that chloroprene displays developmental effects in CD rats at exposure levels up to 25 ppm. Therefore, 25 ppm is identified as the NOAEL for this study.

Table 4-34. Incidence of anomalies in litters of rats exposed to chloroprene by inhalation

	CONCENTRATION OF CHLOROPRENE (ppm)			
	0	**1**	**10**	**25**
	Number of litters (fetuses) examined			
Gross anomalies	21 (192)	24 (191)	21 (172)	19 (184)
Soft tissue anomalies	21 (66)	24 (69)	21 (60)	19 (62)
Skeletal anomalies	21 (126)	24 (122)	21 (112)	19 (122)
	Number of litters (fetuses) affected			
Gross anomalies				
Runts[a]	1 (1)	0	1 (1)	1 (1)
Small subcutaneous hematomas	5 (5)	9 (9)	4 (4)	6 (10)
Petechial hemorrhages	5 (5)	2 (6)	3 (3)	2 (2)
Soft tissue anomalies				
Hydronephrosis	8 (9)	4 (6)	1 (1)	5 (7)
Subcutaneous edema	0	1 (1)	0	0
Skeletal anomalies				
Delayed ossification of one or more sternebrae	17 (58)	15 (39)	13 (33)	14 (45)
14th rudimentary ribs(s) or spur(s)	20 (91)	22 (76)	20 (67)	19 (77)
Wavy ribs	4 (4)	4 (5)	2 (3)	3 (4)
Bipartite thoracic centra	2 (2)	2 (3)	2 (2)	4 (8)

[a] Body weight less than control mean weight minus 3 standard deviations

Source: Culik et al. (1978)

Mast et al. (1994) exposed groups of 15-16 pregnant New Zealand white rabbits by inhalation to 10, 40, or 175 ppm chloroprene (36.2 144.8, or 633.5 mg/m^3) for 6 hours/day on gestational days 6-28. Maternal body weights were measured on days 0, 6, 15, 22, and 29 and animals were observed twice daily (7 days/week) during the exposure period for signs of illness or mortality. On GD29, dams were sacrificed and examined for gross tissue abnormalities. Maternal kidneys and liver were removed and weighed. The uterus was removed and weighed, and the number, position, and status (live, resorbed, or dead) of implants were recorded. Live fetuses were weighed and examined from gross, visceral, and skeletal defects. Bulk chemical analysis was performed using infrared spectroscopy to confirm test material identity. Purity and dimer determinations were conducted by gas chromatography. Exposure atmospheres were generated by immersing an evaporation flask containing bulk material in a 150° F water bath and passing a metered flow of nitrogen through the flask to a condenser. The condenser's temperature was maintained at -2° C in order to control the chloroprene vapor

concentration, and to remove low volatility impurities from the vapor. From the condenser, the chloroprene vapor was mixed with an appropriate amount of compressed air in order to achieve the desired exposure concentration. The normal exposure concentrations in the study were between 98-100% target concentrations, and there was no evidence of degradation products greater than 0.1% target concentration.

There were no signs of maternal toxicity due to exposure to chloroprene. A few dams in each group exhibited nasal discharge, vaginal bleeding, and loose stools at various times during the exposure period. The overall pregnancy rate was 89%, with a range of 80-94% for each exposure group. The incidence of clinical signs of toxicity was low during the exposure, and dams appeared to be in excellent health at termination. No exposure-related effects on maternal weight change were noted. Exposure to chloroprene had no effect on the number of implantations, live pups, or resorptions. Fetal body, liver, and kidney weights were not affected by exposure. The incidence of fetal malformations was not affected by exposure to chloroprene. The results of this study indicate that exposure to chloroprene on GD6-28 in rabbits results in no observable developmental toxicity, therefore the high exposure group, 175 ppm, was identified as the NOAEL for this study.

In an unpublished report, Appelman and Dreef van der Meulen (1979) exposed two successive generations (F_0 or F_1) of Wistar rats to 0, 10, 33, or 100 ppm (0, 36.2, 119.5, or 362 mg/m^3) chloroprene. In the F_0-generation, groups of 25 males and females were exposed to chloroprene for 6 hours/day, 5 days/week for 13 weeks. After the termination of the exposure, the treated animals were caged and mated with untreated stock animals for 20 days (1 male per 1 female). After the mating period, the animals were separated: males were sacrificed and their testes were collected and examined whereas females were caged individually and allowed to birth and rear their litters. After their litters were weaned, the females were sacrificed and their uteri were collected and examined for implantation sites. The number of pups in each litter was recorded at birth, as well as the total number of survivors and total litter weight at days 1, 3, 14, and 28. Litters containing more than 8 siblings were randomly culled to that number at day 4. From the F_1-litters, 20 males and females were selected randomly from each exposure group one week after weaning and exposed to the same concentrations of chloroprene from 10 weeks (6 hours/day, 5 days/week). In both the F_0 and F_1 rats, the general condition, behavior, and signs of possible intoxication were checked daily and all signs of illness or reaction to exposure were recorded. Individual body weights were recorded weekly during exposure. In the F_1 rats, blood samples were collected from 15 rats/sex/exposure group at an age of 4 weeks and analyzed for hemoglobin concentration. At the end of the exposure period, 10 F_1 rats/sex/exposure group were sacrificed and their liver, lungs, and gonads were weighed and examined. Test atmospheres were generated by evaporating bulk material with a metered flow of filtered and dried nitrogen at 0° C. The chloroprene-saturated chloroprene was then mixed with air to achieve the desired test concentration. Nominal concentrations were within 98-100% target concentrations.

The general condition and behavior of F_0 rats did not differ between exposure groups. At 100 ppm, slight (less than 10% decrease relative to control), but significant, growth retardation was observed in males in weeks 3, 6, 7, 8, and 10 and in females from week 2 to termination of exposure ($p < 0.05$). There were statistically significant decreases in body weights in both sexes at various time points in the low and mid-exposure groups compared to controls, but no consistent exposure-related pattern was observed. No data on food consumption were provided, but the authors note that decreases in body weight were most likely attributable to occasional shortages in food availability. The percentage of females (exposed and non-exposed) that successfully mated was not affected by chloroprene exposure. Sex ratios, mortality during lactation, and resorption quotients were not significantly altered in any exposure group. The body weight of offspring descended from treated females and untreated males was statistically reduced in the high exposure group. Body weights of offspring descended from treated males and untreated females were not affected.

The general condition and behavior of F_1 rats did not differ between exposure groups. Statistically significant decreases in body weight (greater than 10% reduction compared to control) were observed in females descended from treated females during week 1 of exposure ($p < 0.01$), in males descended from treated males during weeks 4, 6, 7, and 10 ($p < 0.01$), and in females descended from treated males during weeks 5 and 6 ($p < 0.01$). Again, no food consumption data were provided, precluding a determination of whether these decreases in body weight were related to exposure. Hemoglobin levels were similar in all groups. The relative weights of testes from F_1 males were statistically increased in all exposure groups in males descended from treated females ($p < 0.05$ at 10 and 33 ppm, $p < 0.01$ at 100 ppm) and at 33 and 100 ppm in males descended from treated males ($p < 0.05$). F_1 females descended from treated males and exposed to 100 ppm chloroprene had significantly increased liver ($p < 0.01$), ovary ($p < 0.001$), and lung ($p < 0.05$) weights. Gross and microscopic histopathological examinations revealed no treatment-related abnormalities in these organ systems. Given the lack of histopathological findings in any examined organ system, the significant increases in lung, liver, and gonad weights in F_1 males and females are not considered to be adverse.

The NOAEL for this study was identified as 33 ppm based on decreases in body weight during lactation in pups descended from treated females and untreated males.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute and Subchronic Studies

Clary et al. (1978) conducted a study to investigate chloroprene's acute and subchronic toxicity and to determine the dose range for a 2 year chronic inhalation study (chronic study by Trochimowicz et al., 1998) in rats and hamsters. Groups of six male albino rats (from Charles River laboratories) were exposed to chloroprene by the dermal (200 mg/kg), oral (50 mg/kg), or inhalation (2 mg/L [~550 ppm]) routes for 4 hours and sacrificed for histological examinations 14 days after exposure. This exposure protocol was referred to as a "modified Class B poison test" (extension of sacrifice from 2–

14 days after exposure). A lethal concentration test was also conducted by exposing male rats to 0, 530, 1,690, 2,280, 3,535, or 3,610 ppm (0, 146, 467, 630, 976, or 997 mg/m^3). The approximate lethal concentration by inhalation (4 hours) in rats was determined to be 2,280 ppm (Table 4-35). In the 4-week range-finding inhalation study, Wistar rats were exposed to chloroprene at 0, 50, 200, or 800 ppm (actual mean concentrations were 0, 39, 161, or 625 ppm [0, 11, 44, or 173 mg/m^3], respectively). A similar study was conducted (after completion of the 4-week rat study) with Syrian golden hamsters exposed to 0, 40, 160, or 625 ppm (actual mean concentrations were 0, 39, 162, or 630 ppm [0, 11, 45, or 174 mg/m^3], respectively). The purity of chloroprene used in this study was 99.9% with 0.01% phenothiazine added as a polymerization inhibitor. Test atmospheres were generated by low temperature (0°C) vaporization in nitrogen.

Table 4-35. Chloroprene-induced mortality in male rats

CONCENTRATION (ppm)	MORTALITY (DEAD/TOTAL)
530	0/6
1,690	0/6
2,280	1/6
3,535	2/6
3,610	2/6

Source: Clary et al. (1978)

Clary et al. (1978) reported no deaths from dermal, oral, or inhalation administration in the standard Class B poison test (sacrifice 2 days after the 4-hour exposure period). There were mild to moderate skin irritation and erythema after the dermal exposure. Irregular respiration, mild lacrimation, and slight initial weight loss were reported after the inhalation exposure. For the modified Class B poison test (sacrifice 14 days after the 4-hour exposure period), 2/6 and 3/6 animals died on the sixth and seventh days, respectively.

In the 4-week range-finding study, exposure to 625 ppm chloroprene was associated with eye irritation, restlessness, lethargy, nasal discharge, and orange-colored urine in rats and hamsters. Hair loss was observed in female rats exposed to the two highest exposure groups (161 and 625 ppm). Increased mortality in rats was observed at the two highest concentrations starting in week 1 (5/10 males and 3/10 females died at 625 ppm; 3/10 males died at 161 ppm at the end of the exposure period, 4 weeks). Mortality was 100% for male and female hamsters in the highest dose group (630 ppm) by the end of week 1, and 1/10 males and 3/10 females at the mid-exposure (162 ppm) by the end of week 4. 1 male hamster died in the low exposure (39 ppm) group by week 4. Decreases in body weight were observed at all concentrations in rats and at 162 ppm in hamsters. There were changes in the relative weights of all organs except for the heart. The relative organ weights for kidneys were increased at the 162 ppm exposure level for both male and female hamsters, the 625 ppm level for male rats, and the 161 and 625 ppm level for female rats. Liver weights were increased in the high exposure group in both species except for female hamsters. Male rats exhibited decreased liver

weights at 39 and 161 ppm. Relative lung weights were increased at 625 ppm for male and female rats. Clary et al. (1978) noted that these increases in the relative weight of the kidneys, liver, and lungs may have indicated a direct effect of chloroprene exposure, whereas weight changes in other organs (spleen, brain, thyroid, and adrenal glands) may have been secondary to decreases in body weight.

In rats, gross pathological examination of the animals that died during exposure revealed dark, swollen livers and grayish lungs with hemorrhagic areas. Dark swollen livers were also observed in several animals exposed to the highest concentration when they were sacrificed at the end of the study. Microscopic examination revealed slight to severe centrilobular liver degeneration in all male rats and in 8/10 of the females at the high concentration. This change was also observed in 2/3 male rats exposed to 161 ppm that died during the study. The kidneys of male and female rats exposed to 625 ppm had enlarged tubular epithelial cells. In addition, one male and one female rat exposed to 625 ppm showed foci of necrotic tubules in the intramedullary area of the kidneys.

In hamsters, the lungs of most of the animals that died within the first 24 hours of exposure (all animals died after a single exposure to 630 ppm and 1/10 males and 1/10 females at 162 ppm) showed gray-reddish edematous areas. Fecal and urinary incontinence were observed in 1/10 male and 3/10 females at 630 ppm. The heart of 1/2 females that died on the second day of exposure was pale with severe myocarditis, and the thoracic cavity contained a considerable amount of fluid. The other female had a small spleen and a pale liver with a pronounced lobular pattern. Significant body weight decreases were observed only in the 162 ppm group. Histopathology examinations revealed necrosis and midzonal degeneration of hepatocytes in most of the survivors of the 162 ppm group. Several males and females (number not specified) exposed to either 39 or 162 ppm showed irritation of the mucous membranes of the nasal cavity. This irritation was described as a slight flattening and thinning of the layer of the olfactory epithelium in the dorsomedial part of the cavity.

4.4.2. Immunotoxicity

There are some laboratory animal data suggesting potential immunomodulatory effects in of chloroprene; however the data are from standard toxicological studies and no targeted immunotoxicological studies of chloroprene were identified. The studies discussed below were described in detail previously in the assessment and only the relevant immune data are presented here. NTP (1998) observed that thymus weights in adult male and female B6C3F1 mice exposed to 80 ppm chloroprene for 16 days were significantly decreased compared to controls ($p < 0.01$) and thymic necrosis, characterized by karyorrhexis of thymic lymphocytes, was observed in both sexes at 200 ppm. No changes in thymus weight or histopathology were reported in mice after chloroprene exposure for a longer period (i.e., 13-week exposure) as part of the same NTP (1998) study. Alterations in differential white blood cell counts (i.e., increased leukocyte, neutrophil, and monocyte numbers) were observed at 500 ppm in male rats after 16 days exposure and segment neutrophils were decreased in male rats at 200 ppm after 13 weeks of exposure. In the 2-year chronic portion of the NTP study, splenic hematopoietic cell proliferation was significantly increased over controls in male

mice at 32 and 80 ppm, and in all exposed females (level of significance not reported). Hyperplasia of the mediastinal lymph node was observed in females exposed to 32 or 80 ppm (significance not stated).

Trochimowicz et al. (1998) observed that mean relative spleen and thymus weights were significantly ($p < 0.01$) lower in female Wistar rats exposed to 50 ppm chloroprene for 2 years, but did not report any accompanying histopathological changes in either organ. Clary et al. (1978) also observed small spleens in hamsters (qualitative description) and decreased spleen weights (possibly secondary to decreased body weights) in rats exposed to 625-630 ppm chloroprene for 4 weeks. Sanotskii (1976) reported that chromosomal aberrations were observed in the bone marrow of mice exposed to chloroprene and in leukocyte cultures of exposed chloroprene production workers.

These findings provide some evidence of immunomodulatory effects of chloroprene in laboratory animals. The immune-related data for chloroprene include altered lymphoid organ weights and histopathology, and chromosomal aberrations in bone marrow. However, it has been shown that changes in lymphoid organ weights and genotoxicity observed in lymphoid organs are both poor predictors of compound-related changes in immune function (Luster et al., 1992). The changes in thymic histopathology reported after 16 days of exposure were not observed with longer exposure, suggesting no chronic effects. The remaining data on increased hematopoietic cell proliferation and lymph node hyperplasia are nonspecific effects that are difficult to interpret as potential immuntoxicity of chloroprene. They may be related to general hematopoietic effects of chloroprene rather than an effect on the immune system or immune function. In general, measures such as these (i.e., morphological disturbances) are not clear measures of a chemical's potential to cause changes in immune function (Putman et al., 2003). Direct measures of immune function, such as antibody production to a T-cell dependent antigen, are usually preferred to delineate a chemical's immunotoxic potential (Luster et al., 1992; Putman et al., 2003).

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

4.5.1. Mode-of-Action Studies

Many of the available studies addressing the mode of action (MOA) of chloroprene have focused on investigating the metabolic profile for chloroprene including identifying epoxide metabolites, their reactivity with DNA, and adduct formation in vitro (Munter et al., 2002; Hurst and Ali, 2007). Other studies have used molecular analysis to study alterations in ras proto- oncogenes from lung and Harderian gland tumors identified in the NTP (1998) chronic bioassay that may indicate events in chloroprene-induced neoplasia (Ton et al., 2007; Sills et al., 1999).

The metabolism of chloroprene into reactive epoxides has been primarily evaluated in vitro with liver and lung tissue fractions from rat, mouse, hamster, and humans. Only a limited number of studies have investigated the in vivo metabolism of chloroprene. In studies using mouse and human

liver microsomes, Bartsch et al. (1979) showed that 2-chloro-2-ethynyloxirane and/or (1-chloroethenyl)oxirane could be intermediates in the biotransformation of chloroprene. Metabolism of chloroprene into (1-chloroethenyl)oxirane was confirmed by Himmelstein et al. (2001b); oxidation of chloroprene to (1-chloroethenyl)oxirane was evident in rodent and human liver microsomes and most likely involved CYP2E1, as evidenced by the near complete in vitro inhibition with 4-methylpryazole. A comparison across species suggested that a greater amount of (1-chloroethenyl)oxirane was present in B6C3F1 mice and F344 rat liver microsomes, followed by the Wistar rat, then humans and hamsters. A maximum concentration of (1-chloroethenyl)oxirane of 0.01-0.02 μM was detected in mouse liver microsomes between 5-10 minutes after initiation of exposure with 0.05 μM chloroprene. Preliminary data also showed that hydrolysis of (1-chloroethenyl)oxirane was slowest in the liver microsomes of B6C3F1 mice. Further comparing metabolism between species, Cottrell et al. (2001) observed that qualitative profiles of metabolites from liver microsomes obtained from B6C3F1 mice, Sprague-Dawley or F344 rats, and humans were similar, with (1-chloroethenyl)oxirane being the major metabolite in all species and genders. Himmelstein et al. (2004a) developed a two-compartment closed vial model to describe both chloroprene and (1-chloroethenyl)oxirane metabolism in liver and lung fractions from rat (two strains, F344 and Wistar), mouse, hamster, and humans. Estimates for V_{max} and K_m for oxidation of chloroprene (into (1-chloroethenyl)oxirane) in liver microsomes ranged from 0.068–0.29 μmol/hour/mg protein and 0.53–1.33 μM, respectively. Oxidation (V_{max}/K_m) of chloroprene in the liver was slightly faster in the mouse and hamster than in rats or humans. In lung microsomes, V_{max}/K_m was much greater for mice compared with the other species. Conversely, hydrolysis (V_{max}/K_m) of (1-chloroethenyl)oxirane in liver and lung microsomes was faster for the human and hamster, than for rat or mouse. The observation that mice generally metabolized chloroprene into its epoxide metabolite at equal or faster rates than other species and hydrolyzed the epoxide more slowly may, in part, explain why mice were observed to be the most sensitive species in regards to chloroprene's observed carcinogenicity.

The in vivo rodent studies support the postulated metabolic pathway for chloroprene. For example, male Wistar rats administered 100 or 200 mg/kg chloroprene by gavage demonstrated a rapid depletion of hepatic GSH and a dose-dependent increase in excreted urinary thioethers (presumably GSH-conjugates), which is consistent with in vitro studies using isolated liver hepatocytes (Summer and Greim, 1980). Pretreatment of rats or hepatocytes with phenobarbital or a polychlorinated biphenyl (PCB) mixture (Clophen A50) to induce the mixed-function oxidase enzymes enhanced the GSH depletion effect.

Munter et al. (2002) investigated the reactivity of the chloroprene metabolite (1-chloroethenyl)oxirane towards DNA nucleosides and calf thymus DNA. The adducts were isolated by reverse-phase chromatography and characterized by their mass spectrometric features. The reaction of (1-chloroethenyl)oxirane with the nucleoside 2'-deoxyguanosine yielded one major adduct derived by nucleophilic attack of N-7 guanine on C-3′ of the epoxide. In addition, another chloroprene

metabolite 2-chlorobut-2-en-1-al (See Figure 1, metabolite labeled as number 15) described as an unsaturated aldehyde, yielded 2 major adducts. The reaction of (1-chloroethenyl)oxirane with double stranded calf thymus DNA yielded N7-(3-chloro-2-hydroxy-3-buten-1-yl)-guanine (dGI) as the major adduct, the same adduct seen when the chloroprene metabolite was incubated with 2'-deoxyguanosine individually. N3-(3-chloro-2-hydroxy-b-buten-1-yl)-2'-deoxyuridine (dCI) was also detected. The reaction of (1-chloroethenyl)oxirane with deoxycytidine in DNA may be significant because such adducts are difficult to repair and may therefore be implicated in mutagenesis (Koskinen et al., 2000).

The in vitro reactivity of (1-chloroethenyl)oxirane with hemoglobin (adduct formation) and enantiomer detoxification (i.e., disappearance of R- vs. S-enantiomer from the test system) in vitro have been investigated by Hurst and Ali (2007). Mouse (C57BL/6) erythrocytes (RBCs) were incubated with the R- and S-enantiomers of (1-chloroethenyl)oxirane in vitro. The authors reported a greater persistence of the R- over the S-enantiomer upon incubation with RBCs in the in vitro system tested. The authors also reported a greater amount of globin adducts formed with the R- than with the S-enantiomer.

As part of the 2-year bioassay of chloroprene, NTP (1998) evaluated possible oncogene-activating mechanisms for lung and Harderian gland neoplasms in the B6C3F1 mouse at 0, 12.8, 32, and 80 ppm. The results were published by Sills et al. (1999). After isolation and amplification of DNA from the neoplasms, H-ras and K-ras mutations were identified. A higher frequency (80%) of K-ras mutations was detected in chloroprene-induced lung neoplasms than in spontaneous neoplasms of control mice (30%). The predominant mutation was an A→T transversion (CAA→CTA) at K-ras codon 61: 80% (8/10) of low dose, 71% (10/14) of mid dose, and 18% (4/22) of high dose lung tumors were observed to have this mutation). This specific mutation was not observed in spontaneously occurring lung neoplasms. A similar pattern of ras mutations was observed also with isoprene-induced lung neoplasms but not in those induced by butadiene. Rare point mutations, not seen in spontaneous lung neoplasms, were detected at codon 12. No consistent morphological pattern (papillary, solid, or mixed) or type (benign or malignant) of neoplasm was co-observed with specific K-ras mutations. Although definitve evidence is currently unavailable, there are a number of factors that may explain the observation of the lower frequency of codon 61 CTA transversions in lung tumors of high dose animals. In the lung, the lower frequencies in CTA transversions at high doses may be due to non-ras mutation mechanisms of genotoxicity or carcinogenicity. Alternatively, differences in DNA-adduct formation or induction of repair or removal mechanisms may explain the pattern observed.

A high incidence (100%) of both K-ras and H-ras mutations was detected in chloroprene-induced Harderian gland neoplasms, compared with 56% in spontaneous Harderian gland tumors in control mice, 100% in neoplasms from isoprene-exposed mice, or 69% in neoplasms from butadiene-exposed mice. The predominant mutation was also a CAA→CTA transversion at K-ras codon 61 (93%), which only occurred in 7% (2/27) spontaneously occurring Harderian gland neoplasms. The concentration-response was similar across exposure groups. It was suggested that the large number of

ras mutations at A:T base pairs after exposure to chloroprene, isoprene, or butadiene indicated an interaction with DNA to form adenine adducts that may be important for tumor induction. Sills et al. (2001) reported higher frequencies of K- and H-ras mutations (57%) in chloroprene-induced forestomach tumors in B6C3F1 mice compared to spontaneous tumors (36%). The A→T transversion (CAA→CTA) in H-ras codon 61 was identified in 29% of the chemically induced forestomach neoplasms, but was not observed in spontaneous control tumors. Mutations at K-ras codon 61 were not observed in chloroprene-induced forestomach tumors.

Ton et al. (2007) evaluated mutations in the K-ras oncogenes and loss of heterozygosity in the region of K-ras on distal chromosome 6 in lung tumor samples collected from mice exposed to chloroprene in the NTP 2-year inhalation study. DNA analysis included isolation from formalin fixed tissue sections, and amplification, cycle sequencing of ras gene and analysis for loss of heterozygosity (LOH). Chloroprene-induced mouse lung tumors had a high frequency of LOH on chromosome 6 in the region of K-ras. The correlation between K-ras mutation and loss of the wildtype allele was high in the tumors examined: of the 19 lung tumors with LOH from B6C3F1 mice exposed to chloroprene, 16 (84%) of them also had K-ras mutations.

4.5.2. Genotoxicity Studies

This section presents the findings of several genotoxicity studies that are summarized in Table 4-36.

Table 4-36. Genotoxicity assays of chloroprene

TEST SYSTEM	CELLS/STRAIN	TESTED CONCENTRATIONS	RESULTS[a]	REFERENCE
Bacterial assays				
Salmonella typhimurium	TA100	0.5 to 8% (volume/ volume) in air	+	Bartsch et al. (1979)
	TA100, TA1535		+	Willems (1980)
	TA98		–	Willems (1980)
	TA100, TA1535	10,000-40,000 ppm	+	Willems (1978)
	TA98, TA1537, TA1538	10,000-40,000 ppm	–	Willems (1978)
	TA100, TA1535, TA1537, TA98	up to 3,333 µg/plate	–	NTP (1998)
	TA100	0-5 µmol/plate	–	Westphal et al. (1994)
	TA100	0-5 µmol/plate[b]	+	Westphal et al. (1994)
	TA100, TA1535, TA97A, TA98	0 to 69 mM[c]	+	Himmelstein et al. (2001a)
Mammalian cell assays				
Micronucleus	Chinese hamster V79	10% (v/v)	–	Drevon and Kuroki (1979)
Micronucleus	Chinese hamster V79	0.175 mM[a]	–	Himmelstein et al. (2001a)

TEST SYSTEM	CELLS/STRAIN	TESTED CONCENTRATIONS	RESULTS[a]	REFERENCE
		In vivo bioassays		
Sex-linked recessive lethal mutation	Drosophila (Canton-S)		–	Foureman et al. (1994)
Sex-linked recessive lethal mutation	Drosophila (Berlin-K)		+	Vogel (1979)
Sister chromatid exchange: bone marrow	B6C3F1 mice	12.8, 32, 80 ppm	–	NTP (1998); Shelby (1990); Tice (1988a, 1988b)
Chromosomal aberration: bone marrow	B6C3F1 mice	12.8, 32, 80 ppm	–	NTP (1998)
Chromosomal aberration: bone marrow	C57BL/6 mice	up to 1 ppm	+	Sanotskii (1976)
Micronucleus: peripheral blood	B6C3F1 mice	12.8, 32, 80 ppm	–	NTP (1998)
Micronucleus: bone marrow	B6C3F1 mice		–	Shelby and Witt (1995)

[a] For bacterial assays, tests were performed in the absence or presence of the exogenous S9 metabolism system. In all cases of positive mutagenicity (except Westphal et al., 1994), addition of S9 mixture enhanced the observed mutagenicity
[b]Aged chloroprene distillates tested (in the absence of the exogenous S9 metabolism system).
[c] Epoxide metabolite (1-chloroethenyl)oxirane tested .

4.5.2.1. *Bacterial Mutagenicity Assays*

Both positive and nonpositive mutagenic responses have been observed in bacterial mutagenic assays.

Bartsch et al. (1979) exposed *Salmonella typhimurium* strain TA100 to 0.5–8% (volume/volume [v/v]) of chloroprene within sealed desiccators for 4 hours at 37°C in the absence or presence of the exogenous S9 metabolism system. Batch solutions were freshly prepared before use and kept at –20°C. Chloroprene purity was 99% and contained a negligible amount of dimers. A positive mutagenic response that was concentration-dependent was observed without S9 fraction; this response increased threefold when S9 fractions from either phenobarbital-pretreated or untreated mice were used.

Willems (1978, 1980) found that chloroprene (purity not stated, but sample was "freshly supplied") was mutagenic with *S. typhimurium* strains TA100 and TA1535 in the presence or absence of S9 (mutagenicity was more pronounced in the presence of the S9 fraction), indicating base pair substitution mutations. Chloroprene, however, was not mutagenic in S. *typhimurium* strains TA98, TA1537, and TA1538 indicating a lack of frameshift mutations. Petri plates were incubated at 37°C in desiccators for either 48 or 24 hours, removed, and then incubated for another 24 hours. Positive controls were used. Four dimers (chemical characterization not stated) were also tested under the same conditions. Three of the four were mutagenic against both salmonella base pair substitution strains (TA100 and TA1535).

Westphal et al. (1994) investigated the mutagenicity of chloroprene with respect to the compound stability and reactivity with solvents used in the test system. The Ames test was performed

using the *S. typhimurium* (strain TA100) with or without S9, in gas-tight chambers to prevent chloroprene volatilization. Chloroprene was freshly distilled from a 50% xylene solution. The distillates were stored at –20°C and checked for purity immediately before testing. The authors noted that 2–5% xylenes remained in the chloroprene distillates. Another set of distillates were prepared in the same manner and stored either under air or under argon and kept at room temperature (referred to as aging) for 1, 2, or 3 days. Chromatographic analysis of the aged chloroprene revealed the presence of decomposition products reported to be cyclic dimers. The influence of solvents was also tested in this study by using either ethanol or dimethyl sulfoxide (DMSO) as vehicles. Propylene oxide (a volatile direct mutagen) and benzo(a)pyrene were used as positive controls.

Freshly distilled chloroprene dissolved in either DMSO or ethanol as vehicles, with or without S9, was not mutagenic in TA100. Aged chloroprene had a mutagenic effect on TA100 that increased linearly with increasing age of the chloroprene distillates. Westphal et al. (1994) confirmed these findings by obtaining positive results with 10 additional distillates containing different proportions (quantitative details not specified) of the decomposition products, without S9. The mutagenicity of the distillates correlated with the proportion of the decomposition products (which increased over time in the aged samples). The mutagenicity of aged chloroprene towards TA100 was the same whether chloroprene was stored under air or under an inert gas. The authors speculated that the mutagenic products in aged chloroprene were less volatile than those in the fresh distillates, thus remaining in the test medium long enough to cause toxicity.

Addition of GSH, both with and without S9, reduced the mutagenicity of aged chloroprene but was less effective as the amount of decomposition products increased. Westphal et al. (1994) stated that chloroprene diluted in DMSO was markedly more toxic and more mutagenic than chloroprene dissolved in ethanol, although no data were provided to support this statement.

Chloroprene did not show any evidence of mutagenicity in any of four strains of *S. typhimurium* (TA98, TA100, TA1535, or TA1537) tested at concentrations up to 3,333 µg/plate, in the presence or absence of aroclor-induced rat or hamster liver S9 fraction (NTP, 1998).

Chloroprene monoepoxide, (1-chloroethenyl)oxirane (> 98% purity), was found to be mutagenic in salmonella strains TA100 and TA1535. Some activity was also observed in strains TA97A and TA98 without Aroclor-induced S9 activation (Himmelstein et al., 2001a); inclusion of S9 had no effect in the mutagenic response in all tester strains. Test concentrations were 0–69 mM in DMSO. Toxicity was noted at > 14 mM in plates without S9 and at > 34 mM in plates with S9.

4.5.2.2. *Mammalian Cell Assays*

Chloroprene (99% pure) was evaluated for mutagenic potential in V79 Chinese hamster cells in the presence of a liver supernatant (S15 fraction) from phenobarbitone-pretreated rats and mice (Drevon and Kuroki, 1979). Cells were incubated at 37°C for 5 hours or longer in 2.5 mL of reaction

mixture with or without S15 fraction from mice pretreated with phenobarbitone, plus cofactors, either in liquid suspension or in 0.3 % agar. The petri dishes were placed in a desiccator and exposed to 0, 0.2, 1, 2, and 10% (v/v) chloroprene vapors for 5 hours. Toxicity was evaluated as a measure of plating efficiency. Mutations were evaluated in terms of resistance to a purine analogue (8-azaguanine) and ouabain (inhibitor of adenosine triphosphatase in cell membranes). Chloroprene toxicity was observed at concentrations above 1%; this effect was enhanced with addition of the S15 fraction. The authors noted that this suggested the formation of a toxic metabolite. No mutations were observed in the absence or presence of S15.

Himmelstein et al. (2001a) evaluated the clastogenic potential of the (1-chloroethenyl)oxirane (> 98% purity) using the cytochalasin-B blocked micronucleus test in Chinese hamster V79 cells without metabolic activation. The V79 cells plated on tissue culture slides were placed inside sterile bottles filled with culture medium followed by injection of 0–0.943 mM (1-chloroethenyl)oxirane dissolved in DMSO into the bottles and incubation for 3 hours. Cells were then transferred to fresh medium containing cytochalasin-B and incubated for an additional 16 hours. A minimum of 500 binucleated cells were scored for micronuclei. Cytotoxicity, reported as a reduction in the number of binucleated cells, and altered cell morphology were observed starting at 0.175 mM. No clastogenic response was noted at concentrations up to 0.175 mM.

4.5.2.3. *In Vivo Bioassays*

Vogel (1979) evaluated chloroprene (99% pure with negligible dimer content) dissolved in DMSO (final DMSO concentration = 1%) in an experiment for induction of recessive lethal mutations on the X chromosome of male *Drosophila melanogaster* (Berlin-K). Storage conditions and the elapsed time between receipt and use were not reported. After mating, the F_3 generation was evaluated for recessive lethality. The increase in the percentage of observed recessive-lethal mutations was marginal in several experiments and was not concentration dependent. However, when the data from pooled samples from several experiments (53 lethals in 15,941 X chromosomes) were compared with seven control experiments, the difference was statistically significant at $p < 0.01$. The authors noted that the possible variation among samples could be related to the instability of chloroprene.

In a study by Foureman et al. (1994), chloroprene (purity not reported) dissolved in ethanol was nonpositive ($p > 0.01$) for sex-linked recessive lethal mutations in postmeiotic and meiotic germ cells of adult male *D. melanogaster* (Canton-S) when exposed by either the injection or feeding route. The investigators suggested that the discrepancy between their nonpositive findings and those of Vogel (1979) may be due to (1) differences in purity of the chloroprene sample, (2) differences between the Berlin-K and Canton-S strains, (3) differences in sample sizes, and (4) possible genetic drift within the female populations used by the two groups of investigators. Another possibility for the conflicting results could be that chloroprene in ethanol is less genotoxic than if dissolved in DMSO (Westphal et al., 1994; Gahlmann, 1993).

Cytogenetic tests using chloroprene were nonpositive. In studies performed by Brookhaven National Laboratories for the NTP (1998), sister chromatid exchanges and chromosomal aberrations (bone marrow cells) and the frequency of micronuclei in peripheral blood erythrocytes were evaluated in male mice exposed by inhalation to chloroprene in the NTP (1998) bioassay. Results were published separately by Shelby (1990), Tice (1988), and Tice et al (1988). Mice were exposed by inhalation to chloroprene at 0, 12.8, 32, 80, or 200 ppm (0, 3.5, 8.8, 22, or 55 mg/m^3) 6 hours/day for 12 days. Mortality was 100% at 200 ppm. There were no exposure-related effects compared with controls in numbers of sister chromatid exchanges, chromosomal aberrations, or micronucleus frequency in polychromatic or normochromatic erythrocytes. Tice (1988) and Tice et al (1988) did report that the mitotic index (frequency of cells in metaphase) in mouse bone marrow cells was elevated in chloroprene-exposed animals, with the increase being significant in the 80 ppm group. Tice (1988), and Tice et al (1988) suggested that the lack of chloroprene-induced genotoxicity in bone marrow may imply that any carcinogenic activity attributable to chloroprene would likely be localized to tissues directly exposed to chloroprene (e.g., lung) or to tissues with a high metabolic activity that form reactive intermediates.

The frequency of micronucleated cells in peripheral blood erythrocytes was not affected when mice were exposed to chloroprene for 13 weeks to 0, 12.8, 32, or 80 ppm (0, 3.5, 8.8, or 22 mg/m^3) (NTP, 1998; MacGregor et al., 1990).

Sanotskii (1976) reported on a study identifying an increase in chromosomal aberrations in bone marrow cells of mice exposed for 2 months to chloroprene concentrations of 3.5 mg/m^3 (1 ppm) and below. The protocol details and information about the purity and storage of chloroprene were not provided.

Shelby and Witt (1995) found nonpositive results in vivo in the mouse bone marrow micronucleus test and in chromosomal aberration tests when male B6C3F1 mice were injected intraperitoneally with chloroprene in corn oil, three times, at 24-hour intervals. Dose levels, protocol details, and information about the purity and storage of chloroprene were not provided.

Chloroprene was also tested in a dominant lethal assay with male Swiss mice (Immels and Willems, 1978). Groups of 12 males were exposed to 0, 10, or 100 ppm (0, 2.8, or 28 mg/m^3) chloroprene 6 hours/day, 5 days/week for 2 weeks. Immediately after exposure, each male was mated with two virgin females for seven days. Females were replaced each week for 8 weeks. There was no sign of dominant lethal mutations or effects on mating performance or fertility.

4.5.3. Structural Alerts

Chloroprene is the 2-chloro analog of 1,3-butadiene, a multiorgan, cross-species carcinogen, and is structurally similar to isoprene (2-methyl-1,3-butadiene). Inhalation studies have demonstrated that, similar to butadiene and isoprene, chloroprene is a multisite carcinogen in rats and mice. Butadiene and isoprene are both metabolized to epoxides and diepoxides that are known mutagens and

are believed to be responsible for their carcinogenicity. Chloroprene is also metabolized to an epoxide intermediate that may mediate its carcinogenic effects; however, there is no evidence of diepoxide formation in the metabolism of chloroprene. The similarities in the sites of tumor induction in rodents (see Table 4-37) between butadiene, isoprene, and chloroprene provide further evidence for a similar MOA for these epoxide-forming compounds. A comparative report of the carcinogenicity of these compounds highlights the qualitative and quantitative concordance of their tumorigenic effects (Melnick and Sills, 2001). The female mouse lung was the most sensitive site of carcinogenicity for both chloroprene and butadiene.

Table 4-37. Sites of increased incidences of neoplasms in the 2 year inhalation studies of 1,3-butadiene, isoprene, and chloroprene in rats and mice

Site	Mice			Rats		
	Butadiene	Isoprene	Chloroprene	Butadiene	Isoprene	Chloroprene
Lymphatic/ hematopoietic	M, F[a]	M	M, F			
Circulatory	M, F	M				
Lung	M. F	M	M, F			M
Liver	M, F	M	F			
Forestomach	M, F	M	M, F			
Harderian gland	M, F	M, F	M, F			
Mammary gland	F		F	F	M, F	F
Brain				M		
Thyroid				F		M, F
Pancreas				M		
Testis				M	M	
Zymbal's gland			F	F		
Kidney	M		M		M	M, F
Oral Cavity						M, F

[a] M = males ; F = females

Source: NTP (1998); Melnick et al. (1994); Placke et al. (1996); U.S. EPA (2002b)

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

There is a limited body of information on the toxicological consequences to humans who are exposed to chloroprene. In a summary by Nystrom (1948), chloroprene was reported to cause respiratory, eye, and skin irritation, chest pains, temporary hair loss, dizziness, insomnia headache, and fatigue in occupationally exposed workers. Chest pains accompanied by tachycardia and dyspnea were also reported. In a Russian review (Sanotskii, 1976) of the effects of chloroprene, medical examinations of chloroprene production workers revealed changes in the nervous system (lengthening of sensorimotor response to visual cues and increased olfactory thresholds), cardiovascular system

(muffled heart sounds, reduced arterial pressure, and tachycardia), and hematology (reduction in RBC counts, decreased hemoglobin levels, erythrocytopenia, leucopenia, and thrombocytopenia). The ambient concentration of chloroprene in work areas ranged from 1–7 mg/m^3 (3.6–25 ppm).

4.6.1. Animal Studies

4.6.1.1. *Oral Exposure*

Chloroprene's toxic potential by the oral route has been assessed in only one study (Ponomarkov and Tomatis, 1980). This was a reproductive study involving exposure of BDIV rats to a single dose (100 mg/kg) of chloroprene on the 17th day of pregnancy and of their progeny to weekly doses (50 mg/kg) for 120 weeks. Animals treated with chloroprene that died within the first 30 weeks of treatment showed severe congestion of the lungs and kidneys.

4.6.1.2. Inhalation Exposure

The database for inhalation toxicity studies in animals on chloroprene includes two range-finding studies for 16 days and 13 weeks (NTP, 1998 [also reported by Melnick et al., 1999]), two chronic inhalation bioassays (NTP, 1998 [also reported by Melnick et al., 1999]; Trochimowicz et al., 1998) and four reproductive developmental studies (Mast et al., 1994: Culik et al., 1978; Appelman and Dreef van der Meulen, 1979; Sanotskii, 1976). These studies associate chloroprene inhalation exposure with respiratory, kidney, liver, spleen, forestomach, reproductive, and developmental effects.

Inhalation exposure for 16 days (first range-finding study for the NTP [1998] chronic bioassay) to chloroprene was associated with a range of effects. In rats, there were increased mortality at the high exposure concentration (500 ppm) and decreased body weight starting at 200 ppm. Minimal to mild olfactory epithelial degeneration was observed in all exposed groups (males and females). Mild to moderate centrilobular hepatocellular necrosis was observed in male and female rats exposed to 200 or 500 ppm. Hematological and clinical chemistry parameters indicated increased serum enzyme (ALT, GDH, and SDH) activities, as well as anemia and thrombocytopenia (decreased platelet count) in the 200 and 500 ppm groups on day 4 only. In females, significant increases in kidney weights (right kidney only) were seen at 80 and 500 ppm, and significantly increased liver weights were seen at 200 and 500 ppm.

In mice, all male and female animals in the high-exposure (200 ppm) group died, exhibiting signs of narcosis, hepatocellular and thymic necrosis, and hypertrophy of the myocardium. Significantly decreased body weight gains were seen in males at 32 and 80 ppm. There were no other clinical findings related to chloroprene exposure in the mouse.

In the second (13-week) range-finding study, inhalation to chloroprene was associated with a range of effects across several organ systems. In rats, on day 2, minimal increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts occurred in males exposed to 32 ppm or higher and

in females exposed to 200 ppm. At week 13, male and female rats in the 200 ppm group demonstrated decreased hematocrit values, decreased hemoglobin concentrations, and decreased erythrocyte counts characterized as normocytic, normochromic anemia. Thrombocytopenia, evidenced by a reduction in circulating platelet numbers, occurred in the male and female rats in the 200 ppm group on day 2 and in the females at 80 and 200 ppm on day 22. Transient increases in platelet numbers were observed at 80 and 200 ppm in treated males and females. Transient increases in activities of serum enzymes (ALT, GDH, and SDH) were also observed on day 22 in both sexes at 200 ppm. Alkaline phosphatase enzymeuria was observed in males at ≥ 32 ppm and in females at 200 ppm. In male rats proteinuria was observed at 200 ppm. Reductions in liver NPSH levels were observed in both sexes of rats at 200 ppm. Nonprotein sulfhydryl concentrations were also reduced in the lungs of female rats at 200 ppm. Increases in kidney weights were seen in both male and female rats at 200 ppm and in females at 80 ppm. In male rats, sperm motility was decreased at 200 ppm. Of the neurobehavioral parameters tested, horizontal activity was increased in male rats exposed to ≥ 32 ppm. Total activity was increased in male rats at 32 and 200 ppm. There were no exposure-related effects on motor activity, forelimb/hind-limb grip strength, or startle response.

Increased incidences of minimal to mild olfactory epithelial degeneration and respiratory metaplasia occurred in male and female rats at 80 or 200 ppm. Olfactory epithelial degeneration was observed in females at 32 ppm. The incidence of hepatocellular necrosis was increased in female rats at 200 ppm. Variably sized aggregates of yellow or brown material, consistent with hemosiderin accumulation, appeared in small vessels or lymphatics in or near portal triads or in Kupffer cells of male and female rats exposed to 200 ppm.

In mice, the hematological changes were similar to those observed in rats; however, they were less severe. Minimal anemia, including decreased hematocrit values, erythrocyte counts, and platelet counts were observed in females at 32 and 80 ppm. Sperm morphology and vaginal cytology parameters were similar to those of the chamber controls. Significantly increased incidences of squamous epithelial hyperplasia of the forestomach were observed in male and female mice at 80 ppm.

Exposure to chloroprene for 2 years (NTP, 1998) was associated with effects to the respiratory tract (lung and nose) in rats and mice. The forestomach was also a target for chloroprene-induced effects in mice.

In rats, the incidences of atrophy, basal cell hyperplasia, metaplasia, and necrosis of the olfactory epithelium in males and females were increased at 32 and 80 ppm; atrophy and necrosis were also increased in males at 12.8 ppm. The incidence of chronic inflammation was increased in males exposed to 12.8 ppm and greater and in females exposed to 80 ppm. The incidences of fibrosis and adenomatous hyperplasia of the olfactory epithelium were increased in males and females at 80 ppm. The incidence of alveolar/bronchiolar hyperplasia was statistically significantly increased in males and females in every exposure group.

In mice, there was increased mortality in males at 32 or 80 ppm and in females at all concentrations tested. A decrease in mean body weights was observed in females at 80 ppm. Increases in the incidences of olfactory epithelial atrophy, adenomatous hyperplasia, and metaplasia were observed in males and females at 80 ppm. An increase in the incidence of forestomach epithelial hyperplasia was observed at 80 ppm. Bronchiolar hyperplasia was increased in males and females at doses 12.8 ppm and greater, whereas pulmonary histiocytic cellular infiltration was increased in every dose group in females only. Hematopoietic cell proliferation was increased in males at 12.8 ppm and greater. Renal tubule hyperplasia was observed in males at 32 and 80 ppm.

In the study by Trochimowicz et al. (1998), the only remarkable nonneoplastic lesions in rats were observed in liver and lungs. The number of rats with one or more small foci of cellular alteration in the liver was higher in the 50 ppm group than in controls. In males, there was an increased incidence of hepatocellular lesions described as one or several small clear cell foci in the 50 ppm group. Mild changes, such as lymphoid aggregates around bronchi, bronchioles, and blood vessels, were observed in males and females exposed to 50 ppm. Acute inflammatory processes in the lungs were found in the 50 ppm group and control animals to a similar extent. In hamsters, the only exposure-related effect was a generalized amyloidosis (liver, kidney, spleen, and adrenal glands) that was lower in incidence in the 50 ppm exposed group compared with controls.

Culik et al. (1978) exposed pregnant rats by inhalation to chloroprene at 0, 1, 10, or 25 ppm 4 hours/day on days 1–12 (embryotoxicity study) or days 3–20 (teratology study). In the teratology study, an increase in the percentage of litters with resorptions was observed at 10 and 25 ppm, with only the change in the 10 ppm group achieving statistically significance ($p \leq 0.05$) relative to controls. An increase in the percentage of litters with resorptions was not observed by Culik et al. (1978) in the larger embryotoxicity portion of the study, which was specifically designed to detect such effects. The equally high numbers of litters with resorptions (~50%) in all experimental groups, including controls, in the embryotoxicity study correspond well to the level of response observed at 10 ppm and 25 ppm in the teratology study (62% and 59%, respectively). The observation that the control rates of litters with resorptions differ so much between the teratology and embryotoxicity portions of the Culik et al. (1978) warrants further consideration. When the potential increase in resorptions is expressed in numbers of resorbed fetuses per litter, the control group for the teratology study is the only exposure group which falls far outside of the historical control range for this strain of rat (Charles Rivers Laboratories, 1976). This suggests that the control group response in the teratology study may be a statistical outlier and that the finding of a statistically significant increase of litters with resorptions at 10 ppm is spurious. Chloroprene exposure did result in statistically significant increases in average body weight of fetuses in the 25 ppm group ($p < 0.05$) and in the length of fetuses from dams in the 10 and 25 ppm groups ($p < 0.05$). No major compound-induced or dose-related skeletal or soft tissue anomalies were found.

Mast et al. (1994) exposed groups of 15-16 pregnant New Zealand white rabbits by inhalation to 10, 40, or 175 ppm chloroprene (36.2 144.8, or 633.5 mg/m^3) for 6 hours/day on gestational days 6-28. There were no signs of maternal toxicity due to exposure to chloroprene. A few dams in each group exhibited nasal discharge, vaginal bleeding, and loose stools at various times during the exposure period. The overall pregnancy rate was 89%, with a range of 80-94% for each exposure group. The incidence of clinical signs of toxicity was low during the exposure, and dams appeared to be in excellent health at termination. No exposure-related effects on maternal weight change were noted. Exposure to chloroprene had no effect on the number of implantations, live pups, or resorptions. Fetal body, liver, and kidney weights were not affected by exposure. The incidence of fetal malformations was not affected by exposure to chloroprene.

Appelman and Dreef van der Meulen (1979, unpublished report) exposed two successive generations (F_0 or F_1) of Wistar rats to 0, 10, 33, or 100 ppm (0, 36.2, 119.5, or 362 mg/m^3) chloroprene via inhalation. The F_0 generation was exposed for 13 weeks and then allowed to mate with untreated animals. After weaning, the F_1 was exposed for an additional 10 weeks. There were statistically significant decreases in body weight reported in the F_0 animals, however, concurrent food consumption data was not reported and the authors report that the observed decreases were most likely related to inadequacies in food availability. There were no reported effects on resorptions, sex ratio, or mortality during lactation, although F_1 pups from treated females had reduced body weights at birth and weaning compared to controls. The exposed F_1 rats also had decreased body weights relative to controls, but as with the F_0 animals, no concurrent food consumption data were available to assess whether those decreases were treatment-related. Exposed F_1 males had significantly smaller testes and females had larger ovaries, livers, and lungs compared to controls, but no histopathological changes were observed in those organs.

The general lack of effects in the Mast et al. (1994), Culik et al. (1978) and Appelman and Dreef van der Meulen (1979) studies are not consistent the many positive effects seen in previous Russian studies reviewed in Sanotskii (1976). Similar to other conflicting results in the chloroprene toxicity database, the differences in the results in the reproductive and developmental studies may be attributed to the purity of the test substance, differences in the species and strains used, and experimental design and parameters evaluated in the individual studies.

4.7. EVALUATION OF CARCINOGENICITY

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is evidence that chloroprene is "likely to be carcinogenic to humans" based on (1) statistically significant and dose-related information from an NTP (1998) chronic inhalation bioassay demonstrating the early appearance of tumors, development of malignant tumors, and the occurrence of multiple tumors within and across animal species; (2) evidence of an association between liver cancer risk and occupational exposure to chloroprene; (3) some evidence of an association between lung cancer risk and

occupational exposure; (4) the proposed mutagenic mode of action; and (5) structural similarities between chloroprene and known human carcinogens, butadiene and vinyl chloride (see Table 4-38).

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of chloroprene via the inhalation route demonstrates that tumors occur in tissues remote from the site of absorption. Information on the carcinogenic effects of chloroprene via the oral and dermal routes in humans or animals is limited or absent. Data regarding the absorption via any route of exposure are unavailable. However, based on the observance of systemic tumors following inhalation exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, chloroprene is considered "likely to be carcinogenic to humans" by all routes of exposure.

4.7.1. Summary of Overall Weight of Evidence

According to NTP (1998), there is clear evidence of carcinogenicity in the F344/N rat and B6C3F1 mouse due to lifetime inhalation exposure to chloroprene. In rats, increased incidences of neoplastic lesions primarily occurred in the oral cavity and lung (males only), kidney, and mammary gland (females). In mice, increased incidences in neoplasms occurred in the lungs, circulatory system (all organs), Harderian gland, forestomach, liver, skin and mesentery (females only), and kidney (males only).

In the current document, a total of nine studies covering 11 cohorts of human subjects exposed to chloroprene were reviewed to assess the occurrence of cancer. The most consistent findings across the database were excess cancers of the liver (Bulbulyan et al., 1999, 1998; Li et al., 1989; Leet and Selevan, 1982) and lung (Marsh et al., 2007b; Colonna and Laydevant, 2001; Bulbulyan et al., 1999, 1998; Leet and Selevan, 1982; Pell, 1978). The epidemiologic evidence for increased lung cancer mortality due to chloroprene exposures is limited. The few studies that reported increased risk were not statistically significant. In addition to lack of a consistent association and the small increased risks that were detected, other study limitations, such as lack of smoking data, limit the ability to determine possible causal associations between lung cancer and humans exposed occupationally to chloroprene based on the available data.

Table 4-38. Summary of animal and human tumor data and weight of evidence descriptor for chloroprene

Statistically Significant Tumor Types	• In male F344/n rats, increased incidence of kidney (renal tubule) adenoma or carcinoma in all dose groups, and oral papilloma or carcinoma and thyroid adenoma or carcinoma at the two highest dose groups • In female F344/n rats, increased incidence of mammary fibroadenoma at the two highest dose groups and oral papilloma or carcinoma at the highest dose • In male B6C3F1 mice, increased incidence of lung adenoma or carcinoma and hemangioma/hemangiosarcoma in all organs in all dose groups, and harderian gland adenoma or carcinoma and kidney (renal tubule) adenoma or carcinoma at the two highest dose groups • In female male B6C3F1 mice, increased incidence of lung adenoma or carcinoma and skin sarcoma in all dose groups, liver adenoma or carcinoma at the two highest dose groups, and harderian gland adenoma or carcinoma and mammary gland fibroadenomas at the highest dose. Hemangiomas/hemangiosarcomas in all organs and mesentery sarcomas were observed in the middle dose. • In humans, significant increases in liver cancer mortality were observed in 4 occupational epidemiology studies (out of 9 total studies). Relative risk estimates for liver cancer (while not statistically significant) increased with increasing exposure, indicating a dose-response trend.
Rare Tumors	• Statistically significant increase in rare kidney (renal tubule) adenoma in male rats and mice. Non-statistical increase in females at the high dose. • Statistically significant increases in primary (assumed) liver cancer in four cohort studies and lung cancer mortality in two studies in workers occupationally exposed to chloroprene
Multiple Studies	• Animals – NTP (1998) • Humans – Leet and Sullivan (1982), Li et al. (1989), Bulbulyan et al. (1989), and Bulbulyan et al. (1999)
Conclusions	• Tumors in both sexes of rats and mice • Decreased time to tumor in both sexes of rats and mice • Tumors in occupationally exposed workers • Methodological limitations of the occupational epidemiology studies (e.g., no available data for some potential confounders which precluded adjustment, limited statistical power due to small sample sizes, and lack of precise quantitative exposure ascertainment) make it difficult to draw firm conclusions regarding the human cancer data • Rare tumors (kidney renal tubule adenomas in animals, primary liver cancer in humans) • Metabolites include DNA-reactive epoxides and a mutagenic mode of action is proposed.
Weight of Evidence characterization	• Likely to be carcinogenic to humans

There was a statistically significant excess of liver cancers in four of the cohorts reviewed (Bulbulyan et al., 1999, 1998; Li et al., 1989; Leet and Selevan, 1982), with a two- to more than five-fold increased risk in the SMR seen among these studies. Although no statistically significant increase in risk of liver cancer was detected in the most recent and comprehensive cohort study involving workers at four plants (Marsh et al., 2007b), the observed RR increased with increasing cumulative exposure in the plant with the highest exposure levels, indicating a dose-response trend. Similar to the Colonna and Laydevant (2001) study, there was only one case of liver cancer in the other three plants included in this study (Marsh et al., 2007a, 2007b). Limitations in the existing epidemiological database included the lack of information on individual workers' habits (i.e., alcohol consumption) needed to control for potential confounding, incomplete enumeration of incidence and mortality cases, and potential for biases that may lead to an underestimation of the risk (e.g., the healthy worker effect). These limitations are further discussed in Section 4.7.2.1.

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

4.7.2.1. *Human*

A number of occupational cohort studies have examined cancer mortality and incidence among workers exposed to chloroprene monomer and/or polychloroprene latex in the United States, Russia (Moscow), Armenia, France, China, and Ireland (Marsh et al., 2007a, 2007b; Colonna and Laydevant, 2001; Bulbulyan et al., 1999, 1998; Romazini et al., 1992; Li et al., 1989; Leet and Selevan, 1982; and Pell, 1978). Concern that exposure to chloroprene may result in liver cancer derives principally from its structural similarity to vinyl chloride, a chemical known to cause liver angiosarcoma in humans. Exposed workers have included those involved in chloroprene monomer production using both the acetylene process in which exposure to vinyl chloride was possible and the more recent butadiene process which does not involve vinyl chloride exposure. Other workers were involved with handling/sampling of partially finished products such as polychloroprene latex which contains various amounts of dissolved monomer. Some studies span eras in which little or no worker safety protection measures were likely used in contrast with years in which process improvements and concern for worker safety were gradually instituted. Therefore, it is difficult to compare results across studies given a wide range of exposure variability within and between these cohorts.

Despite these differences in occupational exposure to chloroprene and other chemicals, four of the cohorts with observed liver/biliary passage cancer cases showed statistically significant associations (i.e., two- to five-fold increased risk) with chloroprene exposure. Four mortality studies reported SMRs of 571, 482, 240, and 339 when compared to external populations (Bulbulyan et al., 1999, 1998; Li et al, 1989; and Leet and Selevan, 1982). Although sample size and statistical power were limited (thus limiting the precision of risk estimates), Bulbulyan et al. (1999, 1998) observed

significantly elevated relative risk estimates for liver cancer incidence and mortality among intermediate and highly exposed workers. The study involving four plants by Marsh et al. (2007b), which had the largest sample size and most extensive exposure assessment, also observed increased relative risk estimates for liver cancer in relation to cumulative exposure in the plant with the highest exposure levels (trend p-value = 0.09, RRs 1.0, 1.90, 5.10, and 3.33 across quartiles of exposure, based on 17 total cases). Athough not statistically significant, these findings were comparable to results (RR range: 2.9-7.1) detected in two other studies for high and intermediate cumulative exposures (Bulbulyan et al., 1999, 1998). Though several studies noted higher SMRs for lung cancer among workers exposed to chloroprene, the evidence was not considered as strong as liver cancer. This was mostly due to the inability to adequately control for confounding by smoking status, a strong risk factor for lung cancer. There was also no evidence of exposure-response relationship across various chloroprene exposure categories.

One of the strengths of several of the more recent epidemiologic studies was improved exposure assessment data. These studies utilized industrial hygiene information to determine which areas or jobs were most likely to have received higher chloroprene exposures. This allowed for examination of various exposure contrasts and helped reduce the potential for exposure misclassification. As such, valid internal analyses were conducted which were less impacted by bias due to the healthy worker effect. Despite these improvements, several study limitations added to the uncertainty in addressing the weight of evidence of the epidemiologic data.

A key limitation of most of the chloroprene studies (and other occupational studies) is the potential for bias due to the healthy worker effect. Although this may be less of a concern for cancer mortality outcomes, SMR analyses are based on external comparisons to the general population and will often result in reduced SMR values for the occupational cohort. Two studies with more advanced chloroprene exposure assessment conducted internal analyses to reduce this source of bias (Bulbulyan et al., 1999; Marsh et al., 2007b). Among these studies, only Bulbulyan et al. (1999) observed a statistically significant association between chloroprene exposure and liver cancer mortality. As with most epidemiological research, the potential for bias due to residual confounding is another limitation that exists in these studies. With respect to liver cancer, the lack of data on alcohol consumption is a key limitation which precludes its examination, although there is no direct evidence that alcohol is related to the exposure of interest (i.e., chloroprene). Given the nature of the work environment for most of the study participants in these occupational studies, there is also a high likelihood of co-exposures which may be confounders. Despite this potential, there is little evidence of substantial exposure to liver carcinogens in these populations. One study with data on a co-exposure (vinyl chloride) reported evidence of negative confounding (Marsh et al., 2007b). This would result in an underestimate of the reported association between chloroprene and liver cancer if adjusted for vinyl chloride which suggests that this co-exposure was unlikely to explain the association observed between chloroprene and liver cancer in that population.

An additional limitation in several studies was incomplete enumeration of both incident cases and deaths. In some studies, there were many workers who were exposed during time periods when chloroprene levels were relatively high who could not be identified or located for inclusion in the studies. This raises the possibility that the actual number of liver cancer cases might have been higher than indicated from the data on the subset of individuals that were included in the studies. Another concern in these occupational studies is the reliance on death certificates for outcome ascertainment in the mortality analyses. Although misclassification of cause of death can be minimized by the review of medical records or by histological confirmation, this was not done in any of the studies. The lack of histological review of the liver cancer cases is an important limitation of the available studies using internal controls.

These epidemiologic study results, when examined in the context of different plant operating and worker exposure conditions over different time periods and a low number of incident liver cancers, offer evidence of an association for exposure to chloroprene with an increase of liver cancer in humans. Despite various limitations (e.g., healthy worker bias, potential co-exposure, and incomplete enumeration of cases), internal and external comparisons showed consistent evidence of an association between chloroprene exposures and liver cancer. The associations detected in some studies add support to the cancer weight of evidence determination.

4.7.2.1.1. Evidence for Causality

The evidence for causality for cancer from the human studies is summarized in the paragraphs that follow and is based on recommendations from the EPA (2005a) guidelines for carcinogen risk assessment. It should be noted that there exists a number of methodological limitations of the epidemiologic studies that may preclude drawing firm conclusions regarding the following criteria. These limitations include lack of control of personal confounders and risk factors associated with the outcomes in question, imprecise exposure ascertainment resulting in crude exposure categories, incorrect enumeration of cases leading to misclassification errors, limited sample sizes, and the healthy worker effect.

Temporality – exposure must precede the effect for causal inference. Furthermore, and particularly with cancers, exposure must precede the effect with a sufficient latency to be considered causal. In all the occupational studies reviewed the chloroprene exposure has preceded effect (either incidence of or mortality due to liver cancer) with sufficient latency to be considered causally associated. Several of the studies have specifically evaluated latencies of 15 to 20 years (Marsh et al., 2007a, 2007b; Colonna and Laydevant, 2001; Bulbulyan et al., 1998; and Pell, 1978).

Strength of Association – refers to the magnitude of measures of association such as the ratio of incidence or mortality (e.g., SMRs, SIRs, RRs or odds ratios) irrespective of statistical significance. Studies reporting large, precise risks are less likely to be doing so due to chance, bias, or confounding. Reports of modest risk, however, do not preclude a causal association and may reflect lower levels of

exposure or an agent of lower potency. When compared to external populations, there was a stasticially significant two- to five-fold increased risk of liver cancer in four cohort studies in China (Li et al, 1989), United States (Leet and Selevan, 1982), Russia (Bulbulyan et al., 1998) and Armenia (Bulbulyan et al., 1999) despite evidence of healthy worker effect bias. Despite relatively small numbers, there were also suggestive data from the Louisville cohort (Marsh et al., 2007b) which found RRs ranging from 1.9-5.1 (not statistically significant) for cumulative exposures to chloroprene and liver cancer mortality. These data were consistent in magnitude to two other studies (Bulbulyan et al. 1999, 1998) examining intermediate and high cumulative exposures to chloroprene and liver cancer incidence (RRs = 2.9-4.9, statistically signficant) and mortality (RRs = 4.4-7.1, not statistically significant), respectively.

Consistency – the observation of the same site-specific effect across several independent study populations strengthens an inference of causality. Four different studies have shown an association between chloroprene exposure and liver cancer incidence and mortality (Bulbulyan et al. 1998, 1999; Li et al., 1989; Leet and Selevan, 1982), while a fifth study showed evidence suggesting an association when examined in relation to detailed exposure data (Marsh et al, 2007b). Larger effect estimates for liver cancer risk have been observed in diverse populations working in chloroprene monomer and polymer production, neoprene manufacturing, and manufacturing utilizing polychloroprene products in the U.S., China, Armenia, and Russia. The studies with internal comparisons showed consistently elevated liver cancer relative risk estimates for intermediate (RR range: 2.9-7.1) and high cumulative risk exposures (Range: 3.3-4.9) as noted above.

Specificity – as originally intended, this refers to increased inference of causation if a single site effect as opposed to multiple effects is observed and associated with exposure. Based on current understanding, this is now considered one of the weaker guidelines for causality (for example, many agents cause respiratory disease and respiratory disease has multiple causes). However, when specificity of effect is found, it strengthens causal inference. Chloroprene exposure has been found to be associated specifically with increased risk of liver cancer in four cohorts (Bulbulyan et al. 1998, 1999; Li et al., 1989; Leet and Selevan, 1982).

Biological Gradient – refers to the presence of a dose-response and/or duration-response between a health outcome and exposure of interest. The aforementioned internal analyses for chloroprene (Bulbulyan et al., 1999; Marsh et al., 2007b) show a biological gradient by comparing highly exposed workers to low or unexposed workers. This has also been observed in comparisons between long-term employees and short-term employees

Biological Plausibility – refers to the observed effect having some biological link to the exposure. Chloroprene has been found to be metabolized by humans and other species to epoxides, which are known genotoxic metabolites, and has been shown to be a potent (early appearance, multiplicity, malignancy of observed tumors) carcinogen in mice and rats. In addition, the structurally

related carcinogen, butadiene, is also metabolized to epoxides and produces a tumor profile resembling that observed with chloroprene.

In summary, the temporality of exposure prior to occurrence of liver cancer, strength of association, consistency, biological gradient, and biological plausibility provide some evidence for chloroprene's carcinogenicity in humans.

4.7.2.2. *Laboratory Animal*

According to the NTP (1998), there is clear evidence of carcinogenicity in the F344/N rat and B6C3F1 mouse due to lifetime inhalation exposure to chloroprene. The mouse is regarded as the most sensitive species because tumor incidence and multisite distribution were greater than with the rat. There was decreased survival in chloroprene-exposed rats and mice, and survival in mice was significantly associated with the burden of neoplastic lesions. Mortality in rats was likely due to overt toxicity across many organ systems. In rats, increased incidences of neoplastic lesions primarily occurred in the oral cavity and lung (males only), kidney, and mammary gland (females). In mice, increased incidences in neoplasms occurred in the lungs, circulatory system (all organ, Harderian gland, forestomach, liver, skin and mesentery (females only), and kidney (males only). In contrast to the neoplastic findings in the F334/N rat, only small numbers of neoplastic lesions were observed in Wistar rats or Syrian golden hamsters (Trochimowicz et al., 1998). There is no unequivocal explanation for why the results for the rat differ between these two studies. The stability of the bulk material in the NTP (1998) study was monitored by gas chromatography, and the material was analyzed for peroxide content. In addition, stabilizer concentrations were in an acceptable range and no dimer peaks were found in the distribution lines leading to the exposure chamber. Concentrations of volatile degradation products (e.g., 1-chlorobutadiene) never exceeded 0.6% of the atmospheric concentration of chloroprene when sampled from either the distribution line or exposure chamber. In the study in the Wistar rat by Trochimowicz et al. (1998), there was no evidence of degradation of the freshly distilled chloroprene, and dimer concentrations were stated to be less than the limit of detection. Thus, it is unlikely that the bulk materials or generated atmospheres differed to an extent that would have caused the differences in results. The discrepancy between the carcinogenicity of chloroprene observed in the two studies may be due to species and/or strain differences. Himmelstein et al. (2001b) observed that liver microsomes from B6C3F1 mice and the F344 rats, the two species used in the NTP (1998) study, produced more (1-chloroethenyl)oxirane than those from hamsters or Wistar rats, the two species used in the Trochimowicz et al. (1998) study. These differences in production of (1-chloroethenyl)oxirane were as great as 15-fold greater (F344 rats vs. hamsters).

The inhalation study by Dong et al. (1989) found that a 7-month exposure of the Kunming strain of albino mice, a strain reported to have a low spontaneous rate of lung tumor formation, resulted in a chloroprene-associated increase in lung tumors. Although quality assurance procedures

regarding histopathology were not reported, these study results are considered to support the findings in the B6C3F1 mice in the NTP (1998) chronic bioassay.

In the only long-term oral cancer study (an F1 generation of inbred BD IV rats given weekly doses of 50 mg/kg chloroprene by gavage), no significant neoplastic effects were reported (Ponomarkov and Tomatis, 1980). The number of tumor-bearing animals was similar to controls.

4.7.3. Mode-of-Action Information

4.7.3.1. *Hypothesized Mode of Action*

The proposed hypothesis is that chloroprene acts via a mutagenic mode of action involving reactive epoxide metabolites formed at target sites. DNA-epoxide adduct formation is an effect observed for a number of carcinogens structurally related to chloroprene, including those with a known mutagenic mode of action (i.e., vinyl chloride; EPA, 2005b, 2000f) and those for which a preponderance of evidence strongly suggests a mutagenic mode of action (i.e., isoprene and 1,3-butadiene) (Begemann et al., 2004; EPA, 2002b; Sills et al., 1999). This hypothesized mode of action is presumed to apply to all tumor types. Mutagenicity is a well-established cause of carcinogenicity.

4.7.3.2. *Experimental Support for the Hypothesized Mode of Action*

Compelling evidence for the hypothesized mutagenic mode of action for chloroprene includes: 1) chloroprene, like butadiene and isoprene, is metabolized to epoxide intermediates and both compounds are carcinogens; 2) chloroprene forms DNA adducts via its epoxide metabolite, and is a point mutagen in vitro (in some but not all bacterial assays) and in vivo (in carcinogenicity bioassays, with mutations occurring in proto-oncogenes); 3) observation of the genetic alterations (base-pair transversions) in proto-oncogenes in chloroprene-induced lung, Harderian gland, and forestomach neoplasms in mice and positive results in *Salmonella typhimurium* strains that test for base-pair substitution mutations ; and 4) similarities in tumor sites and sensitive species between chloroprene and butadiene in chronic rodent bioassays (NTP 1998 and 1999, respectively). These lines of evidence are elaborated on below.

Evidence for the formation of reactive epoxide metabolites following exposure to chloroprene has been observed in both genders of multiple species. Currently, *in vivo* data are unavailable for blood or tissue-specific epoxide metabolism rates or concentrations. However, in studies using mouse and human liver microsomes, Bartsch et al. (1979) showed that 2-chloro-2-ethynyloxirane and/or (1-chloroethenyl)oxirane could be intermediates in the biotransformation of chloroprene. Himmelstein et al. (2001b) confirmed the identity of the volatile metabolite reported by Bartsch et al. (1979) as the epoxide (1-chloroethenyl)oxirane. Himmelstein et al. (2001b) reported that the oxidation of chloroprene to (1-chloroethenyl)oxirane was evident in rodent and human liver microsomes and most likely involved CYP 2E1. The oxidation of chloroprene to (1-chloroethynyl)oxirane is more prevalent

in B6C3F1 mice and F344 rat liver microsomes than in Wistar rats, humans, or hamsters. Comparing metabolism between species, Cottrell et al. (2001) confirmed the results of Himmelstein et al. (2001b), and further showed that the quantitative profiles of metabolites from liver microsomes obtained from mice, rats, and humans were similar. In all species and either gender, (1-chloroethynyl)oxirane was the major metabolite detected. One distinct difference between species was the stereospecificity of epoxide metabolites formed. In 2 strains of rats (Sprague-Dawley and F344), the R-enantiomer was preferentially formed, whereas this enantioselectivity was not observed in mice or humans. Hurst and Ali (2007) reported that the S-(1-chloroethynyl)oxirane enantiomer was more quickly detoxified in mouse erythrocytes than the R-enantiomer, suggesting that the R-enantiomer may be more toxic due to its slower elimination. 1,3-butadiene exhibits similar biotransformation to reactive epoxide metabolites. Oxidation of 1,3-butadiene to 1,2-epoxy-3-butene has been observed in hepatic, lung, and kidney microsomes, as well as lung tissue and bone marrow, in rats, mice, and humans (EPA, 2002b). Further oxidation of 1,2-epoxy-3-butene to 1,2,3,4-diepoxybutane has been observed rat, mouse, and human liver microsomes, as well as in blood and tissues of mice and rats exposed by inhalation to 1,3-butadiene (EPA, 2002b). Vinyl chloride and isoprene are also readily converted into their reactive epoxide metabolites; vinyl chloride is converted to chloroethylene epoxide in rats and isoprene to (2,2')-2-methylbioxirane in rats and mice (Watson et al., 2001; EPA, 2002f).

Chloroprene's metabolites have been shown to form DNA adducts when reacted with nucleosides and double stranded DNA in vitro. Reaction of (1-chloroethenyl)oxirane with the nucleoside 2'-deoxyguanosine yielded one major adduct derived by nucleophilic attack of N-7 guanine on C-3′ of the epoxide, whereas another metabolite, 2-chlorobut-2-en-1-al, yielded 2 major adducts (Munter et al., 2002). The reaction of (1-chloroethenyl)oxirane with double stranded calf thymus DNA yield the same adduct observed when the chloroprene metabolite was incubated with 2'-deoxyguanosine individually. (1-chloroethenyl)oxirane also reacted with deoxycytidine in double stranded DNA to yield an adduct which may be significant as such adducts are difficult to repair and may therefore be implicated in mutagenesis (Koskinen et al., 2000).

Evidence for the mutagenic potential of chloroprene has been shown in molecular analysis of the genetic alteration of cancer genes including the ras proto-oncogenes (Sills et al., 1999, 2001; Ton et al., 2007), which are alterations commonly observed in human cancers. Tissues from lung, forestomach, and Harderian gland tumors from mice exposed to chloroprene in the NTP chronic bioassay (1998) were shown to have a higher frequency of mutations in K- and H-ras proto-oncogenes than in spontaneous occurring tumors (Sills et al., 1999, 2001). Further, there was a high correlation between K-ras mutations and loss of heterozygosity in the same chromosome in chloroprene-induced lung neoplasms in mice (Ton et al., 2007). Similar increases in the frequencies of K-ras mutations in rodents were observed in isoprene-induced lung neoplasms and vinyl chloride-induced heptatocellular carcinomas (NTP, 1998; U.S. EPA, 2002f). Activated K-ras oncogenes were observed in lung tumors, hepatocellular carcinomas, and lymphomas in B6C3F1 mice exposed to 1,3-butadiene (EPA, 2002b).

Activated K-ras oncogenes have not been found in spontaneously occurring liver tumors or lymphomas, and are found in only 1/10 spontaneous forming lymphomas in B6C3F1 mice (EPA, 2002b).

Although the genetic toxicity database for chloroprene includes numerous studies covering a range of standard test batteries, their results have been conflicting. In general, bacterial base pair substitution mutation (*Salmonella typhimurium* strains TA100 and TA 1535) assays have been positive (Willems 1980; Bartsch et al., 1979) while the bacterial frame shift (*S. typhimurium* strains TA 97 and TA 98) assays have been nonpositive (NTP, 1998; Willems 1980; Willems 1978). The observation of positive results in bacterial base pair substitution assays is in concordance with the finding that mutations in H- and K-ras oncogenes in select neoplasms of exposed mice manifest in base pair tranversions (Sills et al., 1999, 2001). In contrast, other studies (NTP, 1998) have reported nonpositive results for all bacterial strains. Westphal et al. (1994) suggested that decomposition products of chloroprene may be responsible for the mutagenicity seen in positive tests. Westphal et al. (1994) exposed bacteria directly to liquid chloroprene in solution and observed no increase in mutagenicity, whereas positive tests (Willem 1978, 1980; Bartsch et al., 1979) were conducted by exposure of bacteria to chloroprene in the air. Atmospheric exposures of chloroprene may result in more degradation products being formed, thereby increasing the mutagenicity of the parent compound. A positive result with all bacterial strains was observed when exposed to chloroprene's epoxide metabolite (1-chloroethenyl)oxirane in solution (Himmelstein et al., 2001).

Conflicting results (positive in Vogel, 1979; nonpositive in Foureman et al., 1994) have also been reported for the in vivo Drosophila melanogaster sex-linked lethal mutation assay. Differences observed may be due to differences in purity, strain susceptibilities, and sample size. Chloroprene has been primarily nonpositive in the in vitro micronucleus assay (Himmelstein et al., 2001; Drevon and Kuroki, 1979), in vivo chromosomal damage (NTP 1998) assay, and bone marrow micronucleus assays (NTP 1998; Shelby and Witt, 1995). The lack of genotoxic damage induced in bone marrow or blood by chloroprene suggests that the carcinogenic activity of this chemical may be site specific. The *in vivo* uptake of chloroprene involves a balance of reactive epoxide formation and glutathione- or epoxide hydrolase-dependent detoxification pathways. These pathways may be enhanced or more active in some tissues, thus limiting DNA damage in those tissues. Bone marrow was not a target for cancer in the chronic carcinogenicity bioassays (NTP, 1998), and the endpoints for chromosomal damage in this tissue were nonpositive. Evidence for target organ-dependent mutagenicity is further supported by the findings of K- and H-ras oncogene mutations in lung, forestomach, and Harderian gland neoplasms in B6C3F1 mice (Sills et al., 1999, 2001). However, a positive result with all bacterial strains was observed with chloroprene's epoxide intermediate (1-chloroethenyl)oxirane (Himmelstein et al., 2001).

A comparative analysis by Melnick and Sills (2001) has shown that chloroprene, isoprene, and butadiene share several tumor sites in rats (mammary gland, thyroid, and kidney) and mice

(hemangiomas and hemangiosarcomas [all organs], lung, liver, forestomach, Harderian gland, and mammary gland). Similar to butadiene, the female mouse lung was the most sensitive site of chloroprene carcinogenicity (see Section 4.5.3 and Tables 4-24 and 4-27). There are also remarkable similarities in the potency and shape of the dose response between both compounds. Detailed quantitative analysis (Melnick and Sills, 2001) has rated butadiene as being of slightly greater or equal in potency at some of the common sites of tumor induction (mammary gland and Harderian gland), and more importantly, of equal potency in the induction of the most sensitive tumor, lung neoplasms in female mice.

In summary, the evidence supports the hypothesized mutagenic mode of action for chloroprene . A mutagenic mode of carcinogenic action of chloroprene is supported by chloroprene's epoxide metabolite formation, DNA-adduct formation, observation of in vivo and in vitro mutagenicity, and the well known structure-activity relationship of similar epoxide-forming carcinogens. Chloroprene has been found to be metabolized to epoxides by humans and rodents. The hypothesized mutagenic mode of action is supported by evidence of base pair substitution mutations seen in H- and K-ras proto-oncogenes in chloroprene-induced lung, forestomach, and Harderian gland neoplasms observed in the NTP (1998) study.

4.7.3.3 *Conclusions about the Hypothesized Mode of Action*

As noted above, the hypothesis is that chloroprene carcinogenicity has a mutagenic mode of action. This hypothesized mode of action is presumed to apply to all of the tumor types. The *key events* in the hypothesized mutagenic mode of action are metabolism to reactive epoxide intermediates followed by binding to DNA, which leading to mutation. Epoxide-forming agents are generally capable of forming DNA adducts which in turn have the potential to cause genetic damage, including mutations; mutagenicity, in turn, is a well-established cause of carcinogenicity. This chain of key events is consistent with current understanding of the biology of cancer. Further, the mutagenic mode of action hypothesis is strongly supported by analogy with another epoxide-forming compound, 1,3-butadiene. In addition, alternative or additional modes of action for chloroprene carcinogenicity have not been hypothesized or have supporting evidence.

Strength, Consistency, Specificity of Association – Data from NTP (1998) and Sills et al. (1999, 2001) show codon-specific (codons 12, 13, and 61) mutations in the H- and K-ras proto-oncogenes in chloroprene-induced lung, forestomach, and Harderian gland neoplasms. The high incidence of ras proto-oncogene activation (37/46 lung, 27/27 Harderian gland, 4/7 forestomach) in tumors in treated animals, in contrast with the lower incidence of oncogene activation in spontaneously occurring tumors (25/82 lung, 15/27 Harderian gland, 4/11 forestomach), provides support for the role of mutation in the ras oncogene as a precursor to tumor formation in animals treated with chloroprene. Similar findings of ras oncogene activation for isoprene (11/11 lung, 30/30 Harderian gland, 7/10 forestomach) and 1,3-butadiene (6/9 lung, 20/29 Harderian gland, 20/24 forestomach) were observed

in tumors from animals treated with these structurally-related compounds (Sills et al., 2001, 1999). These findings provide additional support for the importance of ras proto-oncogene activation via mutation in the carcinogenesis of chloroprene and related compounds.

Dose-Response Concordance – High frequencies of K-ras codon 61 CTA mutations were observed in lung tumors from animals exposed to the low- and mid-dose of chloroprene, but not the high dose. Similarly high frequencies of K-ras mutations were observed at all doses in Harderian gland tumors. There are a number of factors that might explain such observations. The higher frequency of mutations at lower doses in lung neoplasms may indicate the saturation of one or more metabolic pathways at higher doses or may suggest that non-ras mechanisms of genotoxicity are operating at those doses. Dose-dependent differences in the mutation profile in the lung and Harderian gland may be explained by differences in DNA-adduct formation or repair in low doses vs high doses.

Temporal Relationships – In mice exposed to chloroprene, tumors were observed in a significant fraction of the exposed animals after 2 years of exposure. DNA-adduct formation and subsequent ras mutations were most likely early mutagenic events in the development of lung, Harderian gland, and forestomach neoplasms. The observation that ras mutations occured in benign neoplasms in these organ systems (lung and Harderian gland adenomas and forestomach papillomas) is supportive evidence of this. Additionally, in mice exposed to isoprene for 6 months and then allowed a 6 month recovery period, forestomach neoplasm with ras mutations did not regress (Melnick et al., 1994). This suggests that ras mutations may have transformed forestomach epithelial cells at an early time point and that the transformed cells progressed to neoplasia even after chemical exposure had been terminated.

Biological Plausibility and Coherence – The biological plausibility of a mutagenic mode of action for chloroprene is supported by evidence of mutations leading to ras proto-oncogene activation in tumors from mice treated with chloroprene (Sills et al., 2001, 1999; NTP, 1998). These studies provide the critical link between the in vitro evidence of mutagenicity (positive results in *S. typhiimurium* strains 100 and 1535 that test for point mutations) and tumor formation in a specific species. Similar findings with the structurally related chemicals 1,3-butadiene and isoprene and the lower incidence of spontaneously occurring tumors displaying ras mutations in untreated animals (Sills et al., 2001, 1999) enhance the database supporting this particular mode of action for chloroprene.

Additional evidence for the association between mutagenesis and tumor formation is the observation that chloroprene exposure caused tumors in a wide variety of mouse tissues, including lung, kidney, Harderian gland, mammary gland, forestomach, liver, skin, mesentery, and Zymbal's gland (NTP, 1998). Tumors were also observed in a number of rat tissues, including oral cavity, thyroid, lung, kidney, and mammary gland. Induction of tumors at multiple sites and in different species is characteristic of carcinogens acting via mutagenesis (US EPA, 2005a).

Early-Life Susceptibility – According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* (U.S. EPA, 2005b) those exposed to

carcinogens with a mutagenic mode of action are assumed to have increased early-life susceptibility. Data on chloroprene are not sufficient to develop separate risk estimates for childhood exposure. There are no data comparing the carcinogenicity of chloroprene after exposure during early life with the carcinogenicity after exposure during adulthood. Exposure to chloroprene commenced at about 6 weeks of age in mice and rats, and continued through adulthood in the 2-year chronic assay (NTP, 1998).

Therefore, because the weight of evidence supports a mutagenic mode of action for chloroprene carcinogenicity (see Section 4.7.3.2), and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the age-dependent adjustment factors (ADAFs) should be applied, in accordance with the Supplemental Guidance.

In conclusion, *the weight of evidence supports a mutagenic mode of action for chloroprene carcinogenicity and application of ADAFs to address assumed early-life susceptibility*.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No direct evidence has been found that indicates children are more susceptible to the toxic effects of chloroprene exposure than adults: exposures of children have not been reported and the metabolic fate of chloroprene in humans has not been sufficiently characterized. However, there are a number of issues that, when considered together, suggest that childhood may represent a lifestage with increased susceptibility to chloroprene effects.

There are indications of reduced metabolic capacity and elimination in children relative to adults that may be a source of susceptibility. Glutathione levels are rapidly depleted in response to in vitro (rat hepatocytes) and in vivo (Wistar rats) chloroprene exposures, suggesting a GSH-dependent detoxification pathway (Summer and Greim, 1980). Additionally, chloroprene's major metabolite, (1-chloroethenyl)oxirane, is rapidly detoxified via epoxide hydrolase-mediated hydrolysis in mouse liver microsomes (Himmelstein et al., 2001b). The levels of both epoxide hydrolase and glutathione transferase (GST) have been shown to be lower in infants than adults (Ginsberg et al., 2004). Epoxide hydrolase is active at birth, but only at 50% of adult function for as long as 2 years. Evidence, although limited, suggests that two forms of GST (GSTmu and α_{B2}) may be deficient (40-60% of adult levels) in early life. This decrement in GST activity is especially relevant as GSTmu is critical to epoxide conjugation to glutathione. Therefore, as both epoxide hydrolase and certain forms of GST exhibit decreased activity in early life, newborns and young infants may experience higher and more persistent blood concentrations of chloroprene and/or its metabolite than adults at similar dose levels. Compensating mechanisms (i.e., other GST isozymes such as GSTpi) may be active in early life. Reduced renal clearance in children may be another important source of potential susceptibility.

Excretion of chloroprene in exposed rats occurs through the elimination of urinary thioesters (presumably glutathione conjugates) (Summer and Greim, 1980). Data indicating reduced renal clearance for infants up to 2 months of age may suggest a potential to affect chloroprene excretion, thus prolonging its toxic effects.

Further, a mutagenic mode of action is proposed for chloroprene's observed carcinogenicity (See Section 4.7.3). In the absence of chemical-specific data to evaluate the differences between adults and children, chemicals with such a mode of action are assumed to have increased early-life susceptibility and age-dependent adjustment factors (ADAFs) should be applied, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

4.8.2. Possible Gender Differences

In lifetime studies conducted in the rat, mouse, and hamster, chloroprene was not shown to exhibit any remarkable gender-related differences in effects with the exception of a more pronounced neoplastic response in B6C3F1 female mice compared to males.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RFD)

The available data are inadequate to derive an oral RfD for chloroprene. There are no human data involving oral exposure. The only lifetime oral study exposed rats to chloroprene at one dose (50 mg/kg/day) and only qualitatively reported non-cancer effects (Ponomarkov and Tomatis, 1980).

In summary, this study identifies the liver (multiple liver necroses and degenerative lesions of parenchymal cells), lung (severe congestion), and kidney (severe congestion) as potential target organs for the oral toxicity of chloroprene; although, the available information is insufficient to characterize toxicity outcomes or dose-response relationships. An RfD was not derived due to the significant uncertainty associated with the oral database for chloroprene.

5.2. INHALATION REFERENCE CONCENTRATION (RFC)

RfCs are derived for exposures via the inhalation route. In general, the RfC is an estimate of a daily exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a statistical lower confidence limit on the benchmark dose (BMDL), a no-observed-adverse-effect level (NOAEL), a lowest-observed-adverse-effect level (LOAEL), or another suitable point of departure (POD), with uncertainty/variability factors applied to reflect limitations of the data used. The inhalation RfC 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) effects and systems peripheral to the respiratory system (extra-respiratory or systemic effects). It is generally expressed in mg/m^3.

5.2.1. Choice of Principal Study and Critical Effect(s)

While literature exists on the carcinogenic potential of chloroprene exposure in humans, no human studies are available that would allow for the quantification of sub-chronic or chronic non-cancer effects. Two inhalation studies investigating portal-of-entry (nasal and pulmonary) and systemic effects were identified in the literature and considered for the principal study for derivation of an RfC: a 2-year chronic study in B6C3F1 mice and F344 rats (NTP, 1998), and a 2-year chronic study in Wistar rats and Syrian gold hamsters (Trochimowicz et al., 1998).

The chronic NTP inhalation bioassay (1998) exposed groups of 50 mice and rats of each sex to 0, 12.8, 32 or 80 ppm chloroprene for 6 hours/day, 5 days/week for 2 years. This study observed a range of chloroprene-induced nonneoplastic effects across several organ systems including the respiratory tract (from the nose to the alveolar region) in both mice and rats, the kidneys of rats and male mice, the forestomach of male and female mice and the spleen of male and female mice (NTP, 1998). In addition, many histopathological lesions were significantly increased compared to controls at the lowest level tested (12.8 ppm), including alveolar epithelial hyperplasia in male and female rats,

bronchiolar hyperplasia in male and female mice, lung histiocytic cell infiltration in female mice, hematopoietic cell proliferation in the spleen in female mice, and atrophy, necrosis, and chronic inflammation of the nasal olfactory epithelium in male rats.

Trochimowicz et al. (1998) exposed three groups of 100 Wistar rats and Syrian hamsters of each sex to chloroprene at 0, 10, or 50 ppm for 6 hours/day, 5 days/week for up to 18 months (hamsters) or 24 months (rats). Unlike the NTP (1998) study, this study did not observe a wide range of nonneoplastic effects in multiple organ systems. Gross pathology revealed that the lungs from rats exposed at 10 and 50 ppm had markedly lower incidences of pathological changes consistent with, and characterized as, chronic respiratory disease than did controls. Male hamsters exhibited a concentration-related decrease in the incidence of pale adrenal glands. The only remarkable nonneoplastic lesions statistically increased in male and female rats were observed in the liver and lungs at 50 ppm: an increase in foci of cellular alteration in the liver and mild changes, such as lymphoid aggregates around the bronchi, bronchiole, and blood vessels, in the lungs. Accidental failure of the exposure chamber ventilation system suffocated 87 males and 73 females in the low exposure (10 ppm) group during week 72 of exposure, and limited the histopathological examinations performed in this study. Only the livers of rats that died accidentally were processed for microscopic examination. No morphological disturbances were noted in the liver of low exposure group animals. The only nonneoplastic change seen in hamsters was a generalized amyloidosis (in the liver, kidneys, spleen, and adrenals) that was lower in incidence in the 50 ppm exposed group compared with controls.

The chronic NTP (1998) study was chosen as the principal study for the derivation of the RfC. Based on the non-cancer database for chloroprene, this study demonstrated exposure concentration-related effects more extensively than any other study. It was a well conducted study that utilized 50 animals per sex, per exposure group, a range of exposure concentrations based on the results of preliminary, shorter-duration studies (16 day and 13 weeks), and thoroughly examined chloroprene's observed toxicity in two species. Trochimowicz et al. (1998) was not chosen as the principal study primarily due to the lack of observed effects at similar exposure levels as the NTP (1998) study (Trochimowicz et al., 1998; see Section 4.7.2.2 for discussion of potential causes of differences in observed toxicity between the NTP and Trochimowicz studies). Concerns regarding the abnormally high mortality in the low dose animals in the Trochimowicz et al. (1998) also influenced the choice to disregard it as the principal study.

From the NTP (1998) study, all portal-of-entry and systemic nonneoplastic lesions that were statistically increased at the lowest exposure concentration (12.8 ppm), compared to chamber controls, were considered candidates for the critical effect. The candidate endpoints included bronchiolar hyperplasia, pulmonary histiocytic cell infiltration, and splenic hematopoietic cell proliferation in mice, and alveolar epithelial hyperplasia, nasal chronic inflammation, olfactory necrosis, and olfactory epithelium atrophy in rats (Table 5-1).

The incidence data for atrophic and necrotic olfactory epithelial lesions in male rats were mostly coincident, suggesting an interdependence between these two lesions. In the 12 ppm group, 10 of the 13 males affected with one of these lesions showed the other as well, and all male rats with necrosis at higher exposures also showed atrophy. Because both are degenerative lesions and not enough information was available to establish if one lesion was a precursor for the other (e.g., atrophy progressing to necrosis), these lesions were combined and considered as one critical effect, degenerative nasal lesions. In addition, with the exception of one high concentration male rat, all rats with nasal chronic inflammation showed nasal atrophy.

Table 5-1. Incidences of nonneoplastic lesions statistically significantly increased at lowest exposure concentration resulting from chronic exposure (ppm) to chloroprene

SPECIES	TISSUE	ENDPOINT	MALE				FEMALE			
			0	12.8	32	80	0	12.8	32	80
Mice	Lung	Bronchiolar hyperplasia	0/50	10/50[b]	18/50[b]	23/50[b]	0/50	15/49[b]	12/50[b]	30/50[b]
		Histiocytic cell infiltration	--	--	--	--	1/50	14/49[b]	18/50[b]	23/50[b]
	Spleen	Hematopoietic cell proliferation	--	--	--	--	13/50	25/49[c]	42/49[c]	39/50[c]
Rats	Nose	Inflammation, chronic	0/50	5/50[a]	9/49[b]	49/49[b]	--	--	--	--
		Atrophy	3/50	12/50[a]	46/49[b]	48/49[b]	--	--	--	--
		Necrosis	0/50	11/50[b]	26/49[b]	19/49[b]	--	--	--	--
		Atrophy or Necrosis[d]	3/50	13/50	47/49	48/49	--	--	--	--
	Lung	Alveolar epithelial hyperplasia	5/50	16/50[b]	14/49[a]	25/50[b]	6/49	22/50[b]	22/50[b]	34/50[b]

[a] $p < 0.05$
[b] $p < 0.01$
[c] Reported as significantly greater than controls, but level of significance not reported
[d] Combination of two separately reported endpoints

-- Endpoint not observed

Source: NTP (1998)

5.2.2. Methods of Analysis

This assessment uses benchmark dose (BMD) methodology to estimate a POD for the derivation of an RfC for chloroprene. The use of the BMD approach improves the assessment by including consideration of the shape of the dose-response curve, providing independence from experimental doses, and providing estimation of the experimental variability associated with the calculated dose-response relationship. Use of BMD methods involves fitting mathematical models to dose-response data and provides a BMD and its 95% lower confidence limit (BMDL) associated with a

predetermined benchmark response (BMR). The BMDL is then used as the POD for deriving the RfC. The suitability of these methods to determine a POD is dependent on the nature of the toxicity database for a specific chemical. Alternatively, the NOAEL/LOAEL approach was used when the data could not be appropriately modeled.

A BMR of 10% extra risk was chosen for these endpoints under the assumption that it represents a minimal biologically significant change. In any case, a 10% increase in incidence relative to controls is recommended for the BMR when using dichotomous models, to facilitate a consistent basis of comparison across assessments and endpoints (U.S. EPA, 2000b). All available dichotomous models in the EPA BMD software (BMDS) (version 2.0) were fit to the incidence data for lung, spleen, and nasal effects in rats and mice (Table 5-1). Details of the BMD modeling analysis are included in Appendix B1.

The models selected for a particular endpoint were chosen based on global and local goodness-of-fit criteria (Akaike Information Criterion [AIC] and chi-square [χ^2] residual values, respectively) and visual inspection. The BMDs and BMDLs associated with an extra risk of 10% for the best-fitting models for each endpoint are shown in Table 5-2. NOAELs and LOAELs were used as potential PODs for the bronchiolar hyperplasia and splenic hematopoietic cell proliferation endpoints in female mice that could not be modeled adequately.

5.2.3. Exposure Duration and Dosimetric Adjustments

Because an RfC is a measure that assumes continuous human exposure over a lifetime, data derived from animal studies need to be adjusted to account for the noncontinuous exposure protocols used in animal studies. In the NTP (1998) study, rats were exposed to chloroprene for 6 hours/day, 5 days/week for 2 years. Therefore, the duration-adjusted PODs for lung, nasal, and spleen lesions in rats and mice are calculated as follows:

$$POD_{ADJ}\,(ppm) = POD\,(ppm) \times \text{hours exposed per day}/24\ \text{hours} \times \text{days exposed per week}/7\text{days}$$

RfCs are typically expressed in units of mg/m^3; the above ppm value needs to be converted using the chemical specific conversion factor of 1 ppm = 3.62 mg/m^3 (see Table 2-1) for chloroprene. Therefore, the final POD_{ADJ} values are calculated as follows:

$$POD_{ADJ}\,(mg/m^3) = POD_{ADJ}\,(ppm) \times 3.62\ mg/m^3/1ppm$$

Finally, this POD_{ADJ} value must be converted to a human equivalent concentration (HEC). Chloroprene is a relatively water-insoluble, non-reactive gas that primarily induces nasal (i.e., olfactory atrophy) and thoracic (i.e., bronchiolar/alveolar hyperplasia) non-cancer effects. Water-insoluble, non-reactive chemicals typically do not partition greatly into the aqueous mucus coating of the upper respiratory system. Rather, they tend to distribute to the lower portions of the respiratory

tract where larger surface areas and the thin alveolar-capillary barrier facilitate uptake (Medinsky and Bond, 2001). However, the pattern of respiratory effects seen following chloroprene exposure is consistent with what is known about its metabolism and the expression of cytochrome P450 enzymes in the olfactory and respiratory mucosa in rats. The proposed mode of action of chloroprene involves the conversion of the parent compound into its reactive epoxide metabolite by P450 isoform CYP2E1. The olfactory and respiratory mucosa of rats have been shown to specifically express CYP2E1 (Thornton-Manning and Dahl, 1997). Further, the olfactory mucosa of rats contains levels of P450s that are more similar to hepatic levels than any other non-hepatic tissue examined and respiratory mucosa P450 concentrations are approximately 25% that of the olfactory mucosa. Conversion of chloroprene into its reactive epoxide metabolite in the olfactory and respiratory epithelia may facilitate its uptake in those regions.

Therefore, in accordance with the U.S. EPA (1994b) RfC methods, chloroprene is characterized as a Category 1 gas for portal-of-entry effects, and as such the HECs for lung and nasal lesions in rats and mice were calculated by the application of a dosimetric adjustment factor (DAF). DAFs are ratios of animal and human physiologic parameters, and are dependent on the nature of the contaminant (particle or gas) and the target site (e.g., respiratory tract or remote to the portal-of-entry) (U.S. EPA, 1994b). For gases with extrathoracic portal-of-entry effects (i.e. nasal), the DAF is the regional gas dose ratio ($RGDR_{ET}$) and is expressed (in this particular case, for male rats) as follows:

$$RGDR_{ET} = (MV_r/S_{ET\_r})/ (MV_h/S_{ET\_h})$$

where:
MV_r = F344/N male rat minute volume (0.294 L/min)[2]
MV_h = human minute volume, (13.8 L/min)
S_{ET_r} = surface area of the extrathoracic region in rats (15 cm^2)
S_{ET_h} = surface area of the extrathoracic region in humans (200 cm^2),

$$RGDR_{ET} = (.294/15)/(13.8/200)$$

$$RGDR_{ET} = 0.28$$

Therefore, the HEC for portal-of-entry effects in male rats is calculated as follows:

$$HEC\ (mg/m^3) = POD_{ADJ}\ (mg/m^3) \times DAF$$
$$= POD_{ADJ}\ (mg/m^3) \times 0.28$$

The calculated HEC values for all considered endpoints are presented in the last column of Table 5-2.

[2] Calculated according to U.S. EPA (1994b): $\ln(MV_r) = b_0 + b_1 \times \ln(BW)$. Default minute volume is in L/min; b_0 and b_1 = species-specific (rat) intercept and coefficient used; body weight in kg. Time-weighted average body weight was 0.456, kg for male rats

Table 5-2. Human equivalent concentration estimates for best fitting models of the BMD from chronic exposure to chloroprene

Endpoint	Species/ Sex	NOAEL (ppm)	LOAEL (ppm)	Model[a]	BMD_{10}[b] (ppm)	$BMDL_{10}$[b] (ppm)	POD_{adj}[c] (mg/m^3)	DAF[d,e]	POD_{HEC} (mg/m^3)
Lung									
Alveolar epithelial hyperplasia	Rat/male	n/a	12.8	Log-logistic	11.4	7.1	4.6	3.4	15.6
	Rat/female	n/a	12.8	Log-logistic	4.9	3.3	2.1	2.3	4.9
Bronchiolar hyperplasia	Mouse/ male	n/a	12.8	Log-logistic	7.5	5.6	3.6	4.1	14.8
	Mouse/ female	n/a	12.8	[f]	--	--	8.3	4.1	33.9
Histiocytic cell infiltration	Mouse/ female	n/a	12.8	[f]	--	--	8.3	4.1	33.9
Nasal									
Chronic Inflammation	Rat/male	n/a	12.8	Log-logistic[g]	14.6	9.3	6.0	0.28	1.7
Atrophy	Rat/male	n/a	12.8	Logistic[g]	7.7	6.0	3.9	0.28	1.1
Necrosis	Rat/male	n/a	12.8	Log-probit[g]	7.9	6.4	4.1	0.28	1.2
Atrophy or Necrosis	Rat/male	n/a	n/a	Logistic[g]	7.4	5.7	3.7	0.28	1.0
Systemic Effects									
Hematopoietic proliferation	Mouse/ female	n/a	12.8	Probit[g]	4.0	3.3	2.1	1.0	2.1

[a]Best fitting model as determined by goodness-of-fit statistics. Bold numbers indicate which value (NOAEL, LOAEL, or BMDL) is used in determination of POD_{HEC}.

[b]At BMR = 10% extra risk.

[c]Duration adjusted POD [mg/m^3] (POD_{adj}) = POD [ppm] × (3.62 mg/m^3/ppm) × (5 days/7 days) × (6 hours/24 hours), in accordance with EPA policy (2002a)

[d] DAF = dosimetric adjustment factor.

[e] For portal-of-entry effects (lung and nasal effects) the DAF = the regional gas dose ratio (RGDR) and is expressed as: $(MV/SA)_{animals}/(MV/SA)_{human}$, where MV = minute volume and SA = respiratory surface area. F344/N male rat MV = 0.294 L/min, F344/N female rat MV = 0.203 L/min, B6C3F1 male mouse MV = 0.052L/min, B6C3F1 female mouse MV = 0.053 L/min, human minute volume = 13.8 L/min. Surface area of the pulmonary region in F344/N rats =0.34 m^2, surface area of the pulmonary region in B6C3F1 mice = 0.05 m^2, surface area of the pulmonary region in humans = 54 m^2, surface area of the extrathoracic region in rats = 15 cm^2, surface area of the extrathoracic region in humans = 200 cm^2. Minute volumes were calculated according to U.S. EPA 1994b using the following time-weighted average body weights: 0.456, 0.290, 0.0437, and 0.0443 kg for male rats, female rats, male mice, and female mice, respectively. For systemic effects (splenic effects) the DAF is expressed as the ratio of animal to human blood:air partition coefficients. As given in Table 3-1, the blood:air partition coefficients for rats are in the range of 7.3-8.0, while that for humans is 4.5. A default value of 1 is substituted when the laboratory animal value exceeds the human.

[f] No model fits appropriately according fit statistics or visual inspection. Therefore, the NOAEL/LOAEL approach is recommended to determine a POD

[g] High dose group was dropped in order to obtain adequate model fit.

Source: NTP (1998)

The BMD modeling indicated that degenerative nasal lesions were the most sensitive endpoint, when comparing the BMD_{10}s and $BMDL_{10}$s. Selection of the most relevant BMR for this endpoint to use for developing the RfC involved evaluating the relative biological significance of the degenerative nasal lesions. The nasal lesion data for chloroprene over the range of exposure concentrations tested (NTP, 1998) indicate a progression in both incidence and severity from no necrosis and minimal atrophy in the controls to manifestations of cellular injury and diminished tissue function at the highest exposures (see Table 4-23 for severity grade details). Considering the mild to moderate degenerative nasal lesions observed in 26% of male rats exposed to 12.8 ppm (average severity 1.8 for atrophy and 2.0 for necrosis), a BMR of 10% extra risk was selected for the POD based on the assumption that a 10% increase in incidence of this effect (with presumably less than moderate severity) is minimally biologically significant. Therefore, 1.0 mg/m^3 was selected to serve as the human equivalent POD for the derivation of the RfC.

5.2.4. RfC Derivation—Including Application of Uncertainty Factors

A POD_{HEC} value of 1.0 mg/m^3 for increased incidence of degenerative nasal lesions in male F344/N rats (NTP, 1998) was used as the POD to derive the chronic RfC for chloroprene because it was the lowest POD_{HEC} calculated after duration and dosimetric adjustments. A total UF of 100 was applied to this POD as described below:

- A 3-fold UF_A was used to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). This uncertainty factor is comprised of two separate and equal areas of uncertainty to account for differences in the toxicokinetics and toxicodynamics of animals and humans. In this assessment, toxicokinetic uncertainty was accounted for by the calculation of a human equivalent concentration by the application of a dosimetric adjustment factor as outlined in the RfC methodology (U.S. EPA, 1994b). As the toxicokinetic differences are thus accounted for, only the toxicodynamic uncertainties remain, and a UF of 3 is retained to account for this residual uncertainty.

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

- An UF_S was not needed to account for subchronic-to-chronic extrapolation because a chronic inhalation study is being used to derive the chronic RfC.

- An UF for LOAEL-to-NOAEL extrapolation was not applied 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 10% change in degenerative nasal lesions was selected under an assumption that is represents a minimal biologically significant change.

- A 3-fold UF was used to account for deficiencies in the database. The major strength of the database is the observation of exposure-response effects in multiple organ systems in a well-designed chronic inhalation study that utilized 50 animals per sex per dose group, a range of doses based on the results of preliminary, shorter-duration studies (16 day and 13 weeks), and thoroughly examined chloroprene's observed toxicity in two species (rat and mouse). The database further contains another chronic inhalation bioassay investigating outcomes in another species (hamster), and well-designed embryotoxicity, teratological, and reproductive toxicity studies. The database also contains subchronic studies and chronic studies observing potential neurotoxic and immunotoxic effects. A limitation in the database is the lack of a two-generation reproductive toxicity study.

Application of this 100-fold composite uncertainty factor yields the calculation of the chronic RfC for chloroprene as follows:

$$RfC = POD_{HEC} \div UF = 1.0 \text{ mg/m}^3 \div 100 = 1.0 \times 10^{-2} \text{ mg/m}^3$$

5.2.5. Previous RfC Assessment

The IRIS Program has not previously evaluated the noncancer inhalation toxicity of chloroprene.

5.2.6. RfC Comparison Information

Figure 5-1 presents PODs, applied UFs, and derived sample RfCs for all of the endpoints from the chronic inhalation NTP (1998) study that were considered for the critical effect for determination of an RfC. Of the considered studies, the NTP (1998) study was considered the most suitable to derive an RfC. The endpoints considered for the critical effects from the NTP (1998) study included any histopathological lesion that was significantly increased in the lowest dose group relative to controls. The PODs are either based on the best fit models from BMD models or the LOAEL of 12.8 ppm and were adjusted for duration and dosimetry before applications of uncertainty factors.

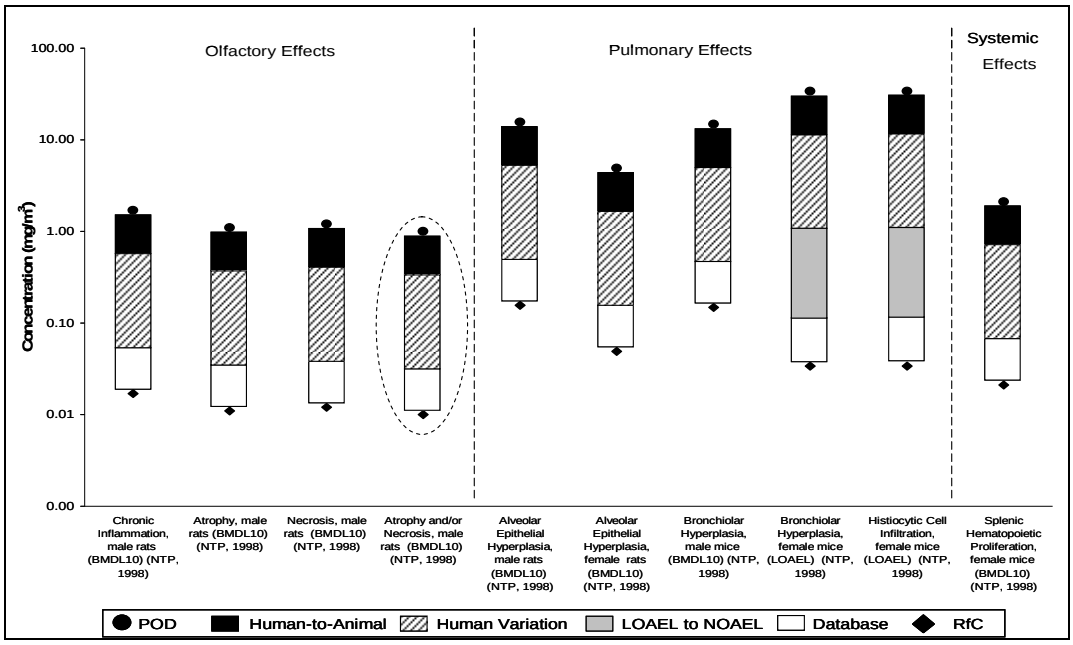

Figure 5-1. Points of departure (in mg/m^3) for selected endpoints with corresponding applied uncertainty factors and derived sample RfCs (chosen RfC value is circled).

5.3. UNCERTAINTIES IN THE INHALATION REFERENCE CONCENTRATION

As presented earlier in the previous section, the UF approach, following EPA practices and RfC guidance (U.S. EPA, 1994b), was applied to the POD_{HEC} in order to derive 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, POD determination methodologies, and to account for database deficiencies. The following is a more extensive discussion of the uncertainties associated with the RfC for chloroprene beyond which is described quantitatively in Section 5.2.4. A summary is provided in Table 5-3.

Choice of endpoint. Sample RfCs considered from the NTP (1998) chronic inhalation study ranged from 1.0×10^{-2} to 3.4×10^{-1} mg/m^3. Sample RfC values primarily depended on whether olfactory or pulmonary effects were considered. The chosen critical effect, increased incidence of degenerative nasal lesions in male rats, is considered to be the most sensitive endpoint because it returned the lowest POD_{HEC} value compared to all other considered endpoints. Its portal-of-entry nature is consistent with the other portal-of-entry effects (e.g., nasal chronic inflammation, alveolar epithelial hyperplasia, etc.) observed in the NTP (1998) study. The location of the effect is also consistent with what is currently proposed for chloroprene's metabolism: conversion of chloroprene into its reactive metabolite (1-chloroethenyl)oxirane by CYP2E1. The olfactory mucosa of rats has been shown to specifically express CYP2E1 (Thornton-Manning and Dahl, 1996) and levels of total P450 enzymes most similar to hepatic levels than any other non-hepatic tissue examined. Choice of

other olfactory effects (chronic inflammation, necrosis or atrophy considered separately) as the critical effect would not appreciably alter the derived RfC (1.7×10^{-2} for chronic inflammation, 1.2×10^{-2} for olfactory necrosis, or 1.1×10^{-2} for olfactory atrophy) relative to degenerative nasal lesions (i.e., atrophy and necrosis considered together). Choice of degenerative nasal lesions as the critical effect is supported as the RfC derived is marginally lower than RfCs for other olfactory effects, and thus this value is presumed to be protective for any individual lesion type. Choice of pulmonary effects (e.g., alveolar epithelial hyperplasia) as the critical effect would result in RfC values up to 30-fold higher than the RfC for degenerative nasal lesions. Choice of the only systemic effect considered from the NTP (1998) study, splenic hematopoietic proliferation, would result in an RfC approximately 2-fold higher than the RfC for degenerative nasal lesions.

Choice of model for BMDL derivation. The logistic model fit the data for degenerative nasal lesions in male rats adequately (global goodness of fit p-value = 0.231). Data points are well-predicted near the BMD (χ^2 residual = 0.905). Use of sample models would either increase the RfC by approximately 50% or decrease the RfC by approximately 3-fold. However, the logistic model was chosen over these models based on current BMD technical guidance (U.S. EPA, 2000b).

Choice of BMR. There is uncertainty in the selection of the benchmark response (BMR) level. For increased incidence of degenerative nasal lesions in male rats, definitive data do not exist to further inform the selection of what the appropriate BMR should be; therefore a BMR of 10% extra risk was chosen based on the assumption that a 10% increase in incidence of this effect (with presumably less than moderate severity) is minimally biologically significant.

Statistical uncertainty at POD. For the logistic model applied to degenerative nasal lesions in male rats, there is a reasonably small degree of statistical uncertainty at the 10% extra risk level (the point of departure for derivation of the RfC), with the BMDL being about 25% below the BMD.

Choice of bioassay. The NTP (1998) chronic inhalation study was used for development of the RfC because it was a well designed study that was conducted in 2 relevant species, used 50 animals per sex per exposure group, and thoroughly examined a wide-range of appropriate toxicological endpoints. The other bioassays were discounted for use as the principal study due either a general lack of effects at similar exposure levels (i.e., Trochimowicz et al., 1998) or interpretational difficulties in respect to the observed effects (i.e., Culik et al., 1978).

Choice of species. The RfC was based on increased incidence of degenerative nasal lesions in male rats exposed to chloroprene via inhalation for 2 years. Use of other effects that occurred in another species, B6C3F1 mice, would result in RfCs approximately 2-30 times greater than the current RfC.

Human population variability. The extent of inter-individual variation of chloroprene metabolism in humans has not been well characterized. However, a number of issues, including lower enzyme levels and renal clearance in children, potential distribution of chloroprene to breast milk, and chloroprene's proposed mutagenic mode of action suggest that childhood may represent a potentially

susceptible lifestage to chloroprene's toxicity. The 10-fold default uncertainty value is applied to the POD_{HEC} primarily due to the limited data on human variability or potential susceptible subpopulations.

Table 5-3. Summary of Uncertainties in the Chloroprene noncancer risk assessment

CONSIDERATION	POTENTIAL IMPACT[a]	DECISION	JUSTIFICATION
Choice of endpoint	Use of other endpoints could ↑ RfC by up to 30-fold	RfC is based on the endpoint with the lowest POD_{HEC} , increased incidence of degenerative nasal lesions in male rats,	Chosen endpoint is considered to be the most sensitive (based on POD_{HEC} values). Its portal-of-entry nature is consistent with other portal-of-entry effects observed in the NTP (1998) study.
Choice of model for BMDL derivation	Other models would ↑ or ↓ RfC	Logistic model used	U.S. EPA (2000b) BMD technical guidance used to choose model based on global and local measures of model fit
Choice of BMR	Other BMRs would ↑ or ↓ RfC	BMR of 10% extra risk chosen	BMR of 10% extra risk was chosen based on the assumption that a 10% increase in incidence of this effect (with presumably less than moderate severity) is minimally biologically significant
Statistical uncertainty at POD	RfC would be ~ 25% higher if BMD (vs. BMDL) were used	BMDL used as POD per U.S. EPA guidance (2000b)	Size of bioassay results in sampling variability; lower bound is 95% confidence interval on administered exposure
Choice of bioassay	Other bioassays could ↑ or ↓ RfC	NTP (1998) used as critical study	Other bioassays were available but were discounted as principal study due to lack of effects or interpretational difficulties. The chosen bioassay was well-conducted and reported and resulted in the lowest BMDL for derivation of RfC
Choice of species	RfC would ↑ if based on another species	Rats chosen	RfC is based on the most sensitive endpoint (incidence of degenerative nasal lesions) in the most sensitive species (rat), based on POD_{HEC}
Human population variability	RfC could ↑ or ↓ if a non-default value of UF was used	10-fold uncertainty factor applied to derived the RfC	10-fold UF, the default value, is applied principally because of limited data on human variability or potential susceptible subpopulations

[a] ↑ = increase, ↓ = decrease

5.4. CANCER ASSESSMENT

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

The NTP (1998) study was used for development of an inhalation unit risk. This was a well-designed study, conducted in both sexes of two species with 50 animals per sex per dose group and 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 respiratory system and more distant locations. The Trochimowicz et al. (1998) was not considered for quantification purposes, primarily due to the general lack of observed neoplastic effects at similar exposure levels as the NTP (1998) study (see Section 4.2.2. for study details).

5.4.2. Dose-Response Data

In the NTP (1998) study, groups of 50 male and female F344 rats and B6C3F1 mice were exposed via inhalation to 0, 12.8, 32, or 80 ppm chloroprene for 6 hours/day, 5 days/week for 2 years. These data are summarized in Tables 5-4 (mice) and 5-5 (rats).

Mice were the more sensitive species, with statistically significant increases in tumor incidence observed at multiple sites: all organs (hemangiomas, hemangiosarcomas), lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas [males]), skin and mesentery (sarcomas), liver, and mammary gland (females). 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, $p \leq 0.001$). Etiologically similar tumor types, benign and malignant tumors of the same cell type, were combined for these tabulations because of the possibility that the benign tumors could progress to the malignant form (U.S. EPA, 2005a). Survival for all chloroprene-exposed female mice and for male mice in the two higher exposed groups was statistically significantly lower than for the corresponding control mice.

Table 5-4. Tumor incidence in female and male B6C3F1 mice exposed to chloroprene via inhalation

TISSUE	CUMULATIVE INCIDENCE; TIME OF FIRST OCCURRENCE	ADMINISTERED CHLOROPRENE CONCENTRATION (ppm)			
		Control	12.8	32	80
Females					
All organs: hemangioma or hemangiosarcoma	Unadjusted KM (%) First incidence (days)	4/50 11% 541	6/49 26% 482	18/50 100% 216	8/50 50.8% 523
Lung: alveolar/bronchiolar adenoma or carcinoma	Unadjusted KM[a] (%) First incidence (days)	4/50 11% 706	28/49 84% 447	34/50 100% 346	42/50 100% 324
Liver: hepatocellular adenoma or carcinoma	Unadjusted KM (%) First incidence (days)	20/50 52% 493	26/49 82% 440	20/50 100% 503	30/50 100% 384
Skin or mesentery: sarcoma	Unadjusted KM (%) First incidence (days)	0/50 0% -	15/49 63% 285	18/50 100% 463	19/50 72% 443
Mammary gland: adenocarcinoma, carcinoma or adenoacanthoma	Unadjusted KM (%) First incidence (days)	3/50 7.4% 527	6/49 23% 440	11/50 42% 394	14/50 61% 336
Forestomach: squamous cell papilloma or carcinoma	Unadjusted KM (%) First incidence (days)	1/50 2.9% 734	0/49 0% -	0/50 0% -	4/50 19% 576
Harderian gland: adenoma or carcinoma	Unadjusted KM (%) First incidence (days)	2/50 5% 527	5/49 24% 621	3/50 12% 524	9/50 77% 427
Zymbal's gland: carcinoma	Unadjusted KM (%) First incidence (days)	0/50 0% -	0/50 0% -	0/50 0% -	3/50 11% 565
Males					
All organs: hemangioma or hemangiosarcoma	Unadjusted KM (%) First incidence (days)	3/50 11% 733	14/50 42% 659	23/50 71% 495	21/50 74% 454
Lung: alveolar/bronchiolar adenoma or carcinoma	Unadjusted KM (%) First incidence (days)	13/50 39% 635	28/50 79% 530	36/50 89% 382	43/50 100% 523
Forestomach: squamous cell papilloma or carcinoma	Unadjusted KM (%) First incidence (days)	1/50 4% 733	0/50 0% -	2/50 14% 733	4/50 15% 587
Harderian gland: adenoma or carcinoma	Unadjusted KM (%) First incidence (days)	2/50 5.8% 596	5/49 18% 701	10/50 42% 596	12/50 58% 589
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	Unadjusted KM (%) First incidence (days)	0/50 0% -	2/49 7.1% 722	3/50 20% 715	9/50 41% 567

[a] Kaplan-Meier estimated neoplasm incidence rate at the end of the study, involving adjustment for intercurrent mortality and under the assumption that the observed tumors were fatal.

Source: NTP (1998).

Table 5-5. Tumor incidence in female and male F344 rats exposed to chloroprene via inhalation

TISSUE	CUMULATIVE INCIDENCE; TIME OF FIRST OCCURRENCE	ADMINISTERED CHLOROPRENE CONCENTRATION (ppm)			
		Control	12.8	32	80
Females					
Oral cavity: papillomas or carcinomas	Unadjusted KM[a] (%) First incidence (days)	1/49 3.0% 687	3/50 9.2% 681	5/50 17% 588	11/50 41% 660
Thyroid gland: follicular cell adenomas or carcinomas	Unadjusted KM (%)a First incidence (days)	1/49 3.4% 733	1/50 3.2% 721	1/50 3.8% 733	5/50 17% 617
Mammary gland: fibroadenomas	Unadjusted KM (%) First incidence (days)	24/49 65% 366	32/50 86% 302	36/50 85% 470	36/50 90% 433
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	Unadjusted KM (%) First incidence (days)	0/49 0% -	0/50 0% -	0/50 0% -	4/50 10% 609
Males					
Oral cavity: papillomas or carcinomas	Unadjusted KM (%) First incidence (days)	0/50 0% -	2/50 14% 701	5/50 28% 609	12/50 75% 539
Thyroid gland: follicular cell adenomas or carcinomas	Unadjusted KM (%) First incidence (days)	0/50 0% -	2/50 14% 597	4/49 30% 569	5/50 36% 307
Lung: alveolar/bronchiolar adenoma or carcinoma	Unadjusted KM (%) First incidence (days)	2/50 6.9% 616	2/50 18% 702	4/49 20% 505	6/50 59% 540
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	Unadjusted KM (%) First incidence (days)	1/50 7.7% 733	8/50 43% 600	6/50 53% 679	8/50 71% 625

[a] Kaplan-Meier estimated neoplasm incidence rate at the end of the study, involving adjustment for intercurrent mortality and under the assumption that the observed tumors were fatal.

Source: NTP (1998).

5.4.3. Dose Adjustments and Extrapolation Methods

The current EPA *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a) emphasize that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA 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 MOA because of DNA reactivity or if another MOA that is anticipated to be linear is applicable A mutagenic mode of carcinogenic action of chloroprene is supported by chloroprene's epoxide metabolite formation, DNA-adduct formation, observation of in vivo and in vitro mutagenicity, and the well known structure-activity relationship of similar epoxide-forming carcinogens. The determination of a mutagenic mode of action is also supported by evidence of base pair substitution mutations seen in H- and K-ras proto-

oncogenes in chloroprene-induced lung, forestomach, and Harderian gland neoplasms observed in the NTP (1998) study.

. For these reasons, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with chloroprene exposure. Because the weight of evidence supports a mutagenic mode of action for chloroprene carcinogenicity, and in the absence of chemical-specific data on early-life susceptibility, increased early-life susceptibility should be assumed and, if there is early-life exposure, the age-dependent adjustment factors (ADAFs) should be applied, as appropriate, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

Due to the occurrence of multiple tumor types, earlier occurrence with increasing exposure, and increased mortality with increasing exposure level, methods that 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 following form:

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

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 (ICF, Fairfax, VA), which is based on Weibull models drawn from Krewski et al. (1983). Parameters were estimated using the method of maximum likelihood estimate (MLE).

Other characteristics of the observed tumor types were considered prior to modeling, including allowance for different, although possibly unidentified, MOAs 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 Table 5-4 were modeled separately. A further consideration allowed by the software program is the distinction between tumor types as being either fatal or incidental in order to adjust for competing risks. Incidental tumors are those tumors thought not to have caused the death of an animal, while fatal tumors are thought to have resulted in animal death.

Specific multistage 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, 2002b). If twice the difference in log-likelihoods was less than a χ^2 with degrees of freedom equal to the

difference in the number of stages included in the models being compared, the models were considered comparable, and the most parsimonious model (i.e., the lowest-stage model) was selected contingent on visual fits of the data as follows. For incidental tumors, 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). For fatal tumors, plots of model fits were compared with Kaplan-Meier estimates of cumulative incidence. 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.

Tumor types were categorized by tumor context as either fatal or incidental tumors. Incidental tumors are those tumors thought not to have caused the death of an animal, and fatal tumors are thought to have resulted in animal death. Hemangiosarcomas were treated as fatal tumors, unless observed at an interim or terminal sacrifice, in which case they were considered incidental. Furthermore, these fatal tumors were deemed rapidly fatal, and t_0 was set equal to 0 (the data were considered insufficient to reliably estimate t_0 in any event, without any interim sacrifices for example). Tumors at all other sites were treated as incidental. This is consistent with the tumor context determinations in the IRIS assessment of 1,3-butadiene (US EPA, 2002b)

PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, generally corresponding to 10% extra risk, defined as the extra risk over the background tumor rate, $[P(d) - P(0)]/[1 - P(0)]$. PODs were converted to continuous human-equivalent exposure levels by multiplying by (6 hours)/(24 hours) × (5 days)/(7 days), or 0.178. Additionally, in accordance with the U.S. EPA (1994b) RfC methodology, the HECs for the various tumors were calculated by the application of DAFs (see section 5.2.3 and Table 5-2 footnote f for explanations of derivation). With the exception of the lung tumors, all tumors were treated as systemic effects. For these sites a DAF of 1.0 was applied as the value for the rat blood:air partition coefficient exceeded the human value . For alveolar/bronchiolar tumors, the HEC was calculated treating the neoplasms alternatively as portal-of-entry effects or systemic effects. As there is evidence that chloroprene and/or its metabolite are distributed systemically (i.e., the observation of tumors in multiple organ systems), there is the potential that chloroprene is redistributed to the lungs. In this manner, chloroprene may induce lung tumors as a systemically delivered carcinogen in addition to inducing tumors via inhalation. However, the contribution of either route of delivery (i.e., inhalation vs. bloodstream) to the induction of lung tumors is currently unknown. Therefore, chloroprene-induced lung tumors were treated as either point-of-entry lesions using a DAF of 4.1, or as systemic lesions using a DAF of 1.0 due to a lack of data clarifying whether one or both modes were more likely to be operating.

The lifetime continuous inhalation unit risk for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD. This 95% upper confidence limit represents a plausible upper bound on the true risk. Unit risks for each tumor site were calculated by dividing the

BMR level (usually 10%) by its corresponding lower bound on the benchmark concentration ($BMDL_{10}$).

5.4.4. Oral Slope Factor and Inhalation Unit Risk

In the absence of any data on the carcinogenicity of chloroprene via the oral route, or a suitable PBPK model allowing route-to-route extrapolation, no oral slope factor was derived. An inhalation unit risk was derived based on the carcinogenic effects of chloroprene via the inhalation route. The results of applying the multistage Weibull models to the male and female mouse tumor incidence data are provided in Tables 5-6 and 5-7, respectively. Human equivalent unit risks estimated from the mouse tumor sites with statistically significant increases ranged from 7.2×10^{-6} to 1.7×10^{-4} per $\mu g/m^3$, approximately a 25-fold range. The highest unit risk (1.7×10^{-4} per $\mu g/m^3$) corresponded to lung tumors (treated as systemic lesions) in female mice; the highest unit risk in male mice was associated with lung tumors (treated as systemic lesions) at 8.5×10^{-5} per $\mu g/m^3$. Lung tumors were the most sensitive response in mice for 1,3-butadiene as well. Alternatively, if the lung tumors resulted strictly from portal-of-entry processes, the estimated risks associated with this site would be four-fold lower, and the highest unit risk estimates come from hemangiomas and hemangiosarcomas, at 8.3×10^{-5} per $\mu g/m^3$ for female mice and 6.6×10^{-5} per $\mu g/m^3$ for male mice.

Given the multiplicity of tumor sites, however, basing the unit risk on one tumor site may underestimate the carcinogenic potential of chloroprene. An approach suggested in the cancer guidelines would be to estimate cancer risk from tumor-bearing animals. EPA traditionally used this approach until the document *Science and Judgment in Risk Assessment* (National Research Council [NRC], 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 substantially, as is suggested by the variation in estimates of *z* (see Tables 5-6 and 5-7) which shows an association of increasing incidence with time, from about 1.0–10 for female mice and from about 2–10 for male mice. Fitting a model like the multisage-Weibull with mechanism-related parameters to composite data would not characterize the evident range of variation. A simpler empirical model could be used for the composite data, such as the multistage model, but available biological information (time of tumor observation) would then be ignored.

Table 5-6. Dose-response modeling summary for male mouse tumor sites associated with inhalation exposure to chloroprene

TUMOR TYPE*	MLE COEFFICIENTS[a]	POINT OF DEPARTURE[b] (μg/m^3)				UNIT RISK[d] (μg/m^3)$^{-1}$	OVERALL UNIT RISK[e] (μg/m^3)$^{-1}$
		Modeled from bioassay		Continuous, human equivalent[c]			
		$BMDL_{10}$	BMD_{10}	$BMDL_{10}$	BMD_{10}		
Lung: alveolar/ bronchiolar adenoma or carcinoma[f]	$q_0 = 4.01 \times 10^{-8}$ $q_1 = 4.46 \times 10^{-9}$ $z = 3.5$	6.64×10^3	9.08×10^3	*1.18×10^3* 4.84×10^3	*1.62×10^3* 6.62×10^3	*8.5×10^{-5}* 2.01×10^{-5}	*1.7×10^{-4}* 1.1×10^{-4}
All organs: hemangiosarcomas, hemangiomas	$q_0 = 8.38 \times 10^{-22}$ $q_1 = 1.16 \times 10^{-22}$ $z = 10$	8.50×10^3	1.10×10^4	1.51×10^3	1.95×10^3	6.6×10^{-5}	
Forestomach: squamous cell papilloma or carcinoma	$q_0 = 3.03 \times 10^{-6}$ $q_1 = 2.34 \times 10^{-7}$ $z = 1.8$	1.68×10^5	4.01×10^5	2.99×10^3	7.14×10^4	3.3×10^{-5}	
Harderian gland: adenoma or carcinoma	$q_0 = 3.26 \times 10^{-13}$ $q_1 = 3.60 \times 10^{-14}$ $z = 5.6$	3.86×10^4	6.15×10^4	6.87×10^3	1.10×10^4	1.5×10^{-5}	
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	$q_1 = 2.03 \times 10^{-15}$ $z = 6.1$	6.07×10^4	9.85×10^4	1.08×10^4	1.75×10^4	9.3×10^{-6}	

[a] Model: multistage–Weibull: $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t - t_0)^z]$, coefficients estimated in terms of ppm as administered in bioassay; lower stage q_i not listed were estimated to be zero.
[b] BMD_{10} = Concentration at 10% extra risk; $BMDL_{10}$ = 95% lower bound on concentration at 10% extra risk.
[c] Continuous equivalent estimated by multiplying exposures by (6 hr)/(24 hr) × (5 days)/(7 days).
[d] Unit risk estimated by dividing the BMR (0.1) by the $BMDL_{10}$.
[e] Overall unit risk estimate, across all sites listed; see text for method.
[f] Values in italics indicate BMD/BMDL when lung tumors are treated as systemic lesions
* Tumor incidence data from NTP (1998)

Table 5-7. Dose-response modeling summary for female mouse tumors associated with inhalation exposure to chloroprene

TUMOR TYPE*	MLE COEFFICIENTS[a]	POINT OF DEPARTURE[b] (μg/m^3)				UNIT RISK[d] /(μg/m^3)	OVERALL UNIT RISK[e] /(μg/m^3)
		Modeled from bioassay		Continuous, Human equivalent[c]			
		$BMDL_{10}$	BMD_{10}	$BMDL_{10}$	BMD_{10}		
Lung: alveolar/ bronchiolar adenoma or carcinoma[f]	$q_0 = 5.95 \times 10^{-10}$ $q_2 = 5.76 \times 10^{-10}$ $z = 4.1$	3.38×10^3	4.22×10^3	*6.02×10^2* 2.47×10^3	*7.51×10^2* 3.08×10^3	*1.7×10^{-4}* 4.1×10^{-5}	*3.3×10^{-4}* 2.1×10^{-4}
Skin: sarcoma	$q_1 = 7.40 \times 10^{-7}$ $z = 2.1$	2.54×10^4	3.91×10^4	4.53×10^3	6.95×10^3	2.2×10^{-5}	
All organs: hemangiosarcomas, hemangiomas	$q_0 = 1.35 \times 10^{-15}$ $q_2 = 1.97 \times 10^{-17}$ $z = 6.8$	1.97×10^4	3.56×10^4	3.51×10^3	6.34×10^3	8.3×10^{-5}	
Mammary gland: adenocarcinoma, carcinoma or adenoacanthoma	$q_0 = 6.82 \times 10^{-4}$ $q_1 = 4.97 \times 10^{-5}$ $z = 1.0$	4.88×10^4	7.52×10^4	8.69×10^3	1.34×10^4	1.2×10^{-5}	
Liver: hepatocellular adenoma or carcinoma	$q_0 = 1.15 \times 10^{-10}$ $q_1 = 5.27 \times 10^{-12}$ $z = 4.8$	9.74×10^3	1.30×10^4	1.73×10^3	2.31×10^3	5.78×10^{-5}	
Forestomach: squamous cell papilloma or carcinoma	$q_0 = 6.98 \times 10^{-23}$ $q_1 = 1.33 \times 10^{-23}$ $z = 10$	6.07×10^4	1.97×10^5	1.08×10^4	3.51×10^4	9.3×10^{-6}	
Harderian gland: adenoma or carcinoma	$q_0 = 4.68 \times 10^{-14}$ $q_1 = 6.62 \times 10^{-15}$ $z = 5.9$	4.81×10^4	6.05×10^4	8.56×10^3	1.08×10^4	1.2×10^{-5}	
Zymbal's gland: carcinoma	$q_3 = 2.22 \times 10^{-27}$ $z = 10$	7.86×10^4	2.52×10^5	1.40×10^4	4.49×10^4	7.2×10^{-6}	

[a] Model: multistage-Weibull: $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t - t_0)^z]$, coefficients estimated in terms of ppm as administered in bioassay; lower stage q_i not listed were estimated to be zero.
[b] BMD_{10} = Concentration at 10% extra risk; $BMDL_{10}$ = 95% lower bound on concentration at 10% extra risk.
[c] Continuous equivalent estimated by multiplying exposures by (6 hours)/(24 hours) × (5 days)/(7 days).
[d] Unit risk estimated by dividing the BMR (0.1) by the $BMDL_{10}$.
[e] Overall unit risk estimate, across all sites listed; see text for method.
[f] Values in italics indicate BMD/BMDL when lung tumors are treated as systemic lesions
* Tumor incidence data from NTP (1998)

Following the recommendations of the NRC (1994) and the current *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) to consider total risk, a statistically-based upper bound on total risk was estimated in order to gain some understanding of the total risk from multiple tumor sites in female and male B6C3F1 mice. Note that this upper bound estimate of overall risk describes the risk of developing any combination of the tumor types considered, not just the risk of developing all simultaneously. Statistical methods which can accommodate the underlying distribution of slope factors are optimal, such as through maximum likelihood estimation or through bootstrapping or Bayesian analysis. However, these methods have not yet been extended to models such as the multistage-Weibull model. Consequently, this analysis used the same method as in several previous

assessments (e.g., 1,3-butadiene (US EPA, 2002b), 1,2-dibromoethane (US EPA, 2005)) which involves assuming that variability in slope factors can be characterized by a normal distribution. Each overall risk estimate involved the following steps (detailed in Appendix C):

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

- The models previously fitted to estimate the BMDs and BMDLs were used to extrapolate to a lower level of risk (R) where the BMDs and BMDLs were in a linear range. For these data a 10^{-2} risk was generally the lowest risk necessary. Although this step appears to differ from the explicit recommendation of the cancer guidelines (U.S. EPA, 2005a) to estimate cancer risk from a POD "near the lower end of the observed range, without significant extrapolation to lower doses," this method is recommended in the cancer guidelines as a method for combining multiple extrapolations. A sensitivity analysis considering risks nearer the lower end of the observed ranges for each tumor type was also considered and is described below with the results. The unit risk for each site was then estimated by $R/BMDL_R$, as for the estimates for each tumor site above.

- The central tendency estimates of unit potency (that is, risk per unit of exposure) at each BMD_R, estimated by R/BMD_R, were summed across the sites listed in Table 5-6 for male mice and similarly across the sites for female mice listed in Table 5-7.

- An estimate of the 95% upper bound on the overall unit risk 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 following formula:

$$95\%\ UCL = MLE + 1.645 \times SD \quad (1)$$

rearranged to:

$$SD = (UCL - MLE)/1.645 \quad (2)$$

where 1.645 is the t-statistic corresponding to a one-sided 95% confidence interval and > 120 degrees of freedom, and the standard deviation (SD) is the square root of the variance of the MLE. The variances (variance = SD^2) for each site-specific estimate were summed across tumor sites to obtain

the variance of the sum of the MLEs. The 95% UCL on the sum of the individual MLEs was calculated from expression (1) using the variance of the MLE to obtain the relevant SD (SD = variance$^{1/2}$).

The resulting combined unit risk for all tumor types for female mice was 3.3×10^{-4} per $\mu g/m^3$ (with lung tumors treated as a systemic effect). Overall, the consideration of the other tumor sites increased the unit risk by two-fold from the highest unit risk for any individual tumor type, 1.7×10^{-4} per $\mu g/m^3$ for lung tumors treated as a systemic lesion. The increase was due largely to the hemangiosarcomas and liver tumors, with little contribution from the other tumor sites. A sensitivity analysis (not included in this document) showed that the overall risk was essentially the same (to 2 significant digits) whether or not the individual risks were estimated in the region of 10^{-2} risk or near the PODs.

For male mice the combined unit risk for all tumor types was 1.7×10^{-4} per $\mu g/m^3$ (with lung tumors treated as a systemic lesion), a 2-fold increase compared to the highest unit risk for any individual tumor type, 8.5×10^{-5} per $\mu g/m^3$ for lung tumors treated as a systemic lesion. The increase was due almost entirely to the risk associated with the hemangiosarcomas. As with the overall risk for female mice, there was a trivial difference whether or not the individual risks were estimated in the region of 10^{-2} risk or near the PODs.

For estimates in both species, if the lung tumors are primarily site of contact lesions, the estimated overall risk decreases to 2.1×10^{-4} per $\mu g/m^3$ (females) and 1.1×10^{-4} per $\mu g/m^3$ (males). Based on the relatively high fat:air partition coefficients (see Section 3.2.) in rodents and humans, chloroprene is likely to be absorbed rapidly (U.S. EPA, 1994), consistent with the possibility that the lung tumors are both portal-of-entry and systemic lesions.

Based on the analyses discussed above, the recommended upper bound estimate on human extra cancer risk from continuous lifetime exposure to chloroprene is 3×10^{-4} per $\mu g/m^3$, rounding the overall risk for female mice above to one significant digit. This unit risk should not be used with continuous lifetime exposures greater than 600 $\mu g/m^3$ (0.6 mg/m^3), the human equivalent POD for the female lung 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 chloroprene. The recommended unit risk estimate reflects the time-to-tumor dimension of the responses as well as the exposure-response relationships for the multiple tumor sites in both sexes of mice.

5.4.5 Application of Age-Dependent Adjustment Factors

Because a mutagenic mode of action for chloroprene carcinogenicity is sufficiently supported by *in vivo and in vitro* data and relevant to humans (see Section 4.7.3.1), and in the absence of chemical-specific data to evaluate the differences in susceptibility, increased early-life susceptibility is assumed and the age-dependent adjustment factors (ADAFs) should be applied, as appropriate, along with specific exposure data in accordance with EPA's *Supplemental Guidance for Assessing*

Susceptibility From Early-Life Exposure to Carcinogens (U.S. EPA, 2005b). The inhalation unit risk of 3×10^{-4} per $\mu g/m^3$, calculated from data for adult exposures, does not reflect presumed early-life susceptibility for this chemical. Example evaluations of cancer risks based on age at exposure are given in Section 6 of the *Supplemental Guidance*.

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

To illustrate the use of the ADAFs established in the *Supplemental Guidance* (U.S. EPA, 2005b), sample calculations are presented for a lifetime risk estimate for continuous exposure from birth with a life expectancy of 70 years. The ADAFs are first applied to obtain risk estimates for continuous exposure over the three age groups:

- Risk for birth through < 2 yr = 3×10^{-4} per $\mu g/m^3 \times 10 \times 2yr/70yr = 8.6 \times 10^{-5}$ per $\mu g/m^3$
- Risk for ages 2 through < 16 = 3×10^{-4} per $\mu g/m^3 \times 3 \times 14yr/70yr = 1.8 \times 10^{-4}$ per $\mu g/m^3$
- Risk for ages 16 until 70 = 3×10^{-4} per $\mu g/m^3 \times 1 \times 54yr/70yr = 2.3 \times 10^{-4}$ per $\mu g/m^3$

To calculate the lifetime risk estimate for continuous exposure from birth for a population with default life expectancy of 70 years, the risk associated with each of the three relevant time periods is summed:

- Risk = $8.6 \times 10^{-5} + 1.8 \times 10^{-4} + 2.3 \times 10^{-4} = 5.0 \times 10^{-4}$ per $\mu g/m^3$

Using the above full lifetime unit risk estimate of 5×10^{-4} per $\mu g/m^3$ for continuous exposure from birth to 70 years, the lifetime chronic exposure level of chloroprene corresponding to an extra risk of 1×10^{-6} can be estimated as follows:

$$1 \times 10^{-6} \div 5 \times 10^{-4} \text{ per } \mu g/m^3 = 0.002 \ \mu g/m^3$$

5.4.6. Previous Cancer Assessment

The carcinogenicity of chloroprene has not been evaluated previously for the IRIS program.

5.4.7. Uncertainties in Cancer Risk Values

A number of uncertainties underlie the cancer unit risk for chloroprene. These are discussed in the following paragraphs. Specifically addressed is the impact on the assessment of issues such as the use of models and extrapolation approaches, the use of other bioassay data, and the choices made and the data gaps identified. In addition, the use of assumptions, particularly those underlying the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) is explained and the decision

concerning the preferred approach is given and justified. Principal uncertainties are discussed below and summarized in Table 5-8.

Table 5-8. Summary of uncertainties in chloroprene cancer unit risk estimate

Consideration	Potential Impact[a]	Decision	Justification
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk could ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity. Mutagenic MOA indicates potentially increased early-life susceptibility.
Low-dose extrapolation procedure	Unknown; not clear what departure from Cancer Guidelines would be plausible	Multistage-Weibull model to determine POD, linear low-dose extrapolation from POD	Multistage-Weibull model addresses competing risks from other tumors and intercurrent mortality. Mutagenic MOA supports linear low-dose extrapolation.
Dose metric	Alternatives could ↑ or ↓ low-dose risk per unit concentration by an unknown extent	Used administered concentration	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are neither clearly identified nor quantifiable. Use of administered concentration provides an unbiased estimate if proportional to the actural carcinogen(s).
Bioassay	Others unavailable	NTP study	Standard design, well conducted, extensively peer reviewed; carcinogenic response consistently observed across all 4 species/sex combinations.
Species /gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Multiple sites in female mice	Unit risk is based on the most sensitive endpoint (risk of any tumor type) in the most sensitive species and gender (female mouse), based on POD_{HEC} It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. Site concordance for liver tumors for humans and female mice was observed, but human data not sufficient to rule out other types seen in mice or rats.
Cross-species extrapolation	Alternatives for lung tumors differ by 4-fold: human risk for any site could ↓ or ↑. Low-dose risk would ↓ approximately 40% if lung tumors were treated as portal-of-entry effects	RfC methodology: Equal risk per unit of air concentration for all sites; for lung also considered relative surface areas of affected region. Treat lung tumors as systemic effects.	There are no data to support other alternatives. There is evidence that chloroprene is distributed systematically (observation of tumors at multiple sites), and correspondingly the possibility that chloroprene is redistributed to the lungs. The contribution of one route of delivery (i.e., inhalation vs. bloodstream) to the induction of lung tumors is currently unknown, therefore the derivation approach that returns the highest unit risk was used

Consideration	Potential Impact[a]	Decision	Justification
Statistical uncertainty at POD	↓ risk per unit concentration 1.2-fold if BMD_{10} used rather than $BMDL_{10}$	BMCL (default approach for calculating plausible upper bound)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on concentration.

[a] ↑ = increase, ↓ = decrease

Human population variability. The extent of inter-individual variability in chloroprene metabolism has not been characterized. A separate issue is that the human variability in response to chloroprene is also poorly understood. The effect of metabolic variation, including potential implications for differential toxicity, has not been well studied. Although a mutagenic MOA indicates increased early-life susceptibility, there are no data exploring whether there is differential sensitivity to chloroprene carcinogenicity across human life stages. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source 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 multistage Weibull time-to-tumor model was the preferred model because it can account for differences in mortality and other competing risks between the exposure groups in the mouse bioassay; however, it is unknown how well this model predicts low-dose extrapolated risks for chloroprene.

Dose metric. Chloroprene is metabolized to intermediates with carcinogenic potential, most likely an epoxide. However, data sufficient to estimate quantities were not available. Under the assumption that the carcinogenic form(s) of chloroprene are produced in proportion to low exposures of chloroprene, the derived unit risk is an unbiased estimate.

Choice of bioassay/species/gender. The NTP inhalation bioassay followed an accepted protocol, was well conducted, and extensively peer reviewed. The carcinogenic response occurs in both species and sexes of rodents as well as in humans. The calculated combined unit risk is based on the most sensitive endpoint (risk of any tumor type) in the most sensitive species and gender (female mouse). There is no information on chloroprene to indicate that the observed rodent tumors are not relevant to humans. Further, no data exist to guide quantitative adjustment for differences in sensitivity among rodents and humans. While site concordance generally is not assumed across species, e.g., due to potential differences in pharmacokinetics, DNA repair, other protective systems across species and tissues (U.S. EPA, 2005a), it is notable that human-mouse site concordance was observed for liver tumors. In addition, rat and mouse tumor types overlapped but included different tumor types observed for each species/sex combination. Human data were insufficient to rule out the occurrence of these additional tumor types in humans.

Cross-species scaling. Another source of uncertainty comes from the interspecies extrapolation of risk from mouse to human. The two rodent species for which bioassay data were available— mouse

and rat—vary in their carcinogenic responses to chloroprene, in terms of both site specificity and magnitude of response (see Chapter 4). Ideally, a PBPK model for the internal dose(s) of the reactive metabolite(s) would decrease some of the quantitative uncertainty in interspecies extrapolation; however, current PBPK models are inadequate for this purpose (Chapter 3). Existing pharmacokinetic models cannot yet adequately explain the species differences in carcinogenic response, and it is possible that there are pharmacodynamic as well as pharmacokinetic differences between the mouse and rat with respect to their sensitivities to chloroprene.

While concordance of specific sites between rodents and humans (e.g., liver tumors) tends to support the relevance of rodent species to humans, lack of specific site concordance (other tumors) does not diminish concern for human carcinogenic potential. The mouse was the more sensitive species to the carcinogenic effects of chloroprene exposure. Although the derivation took into account some known differences between mice and humans in tissue dosimetry (US EPA, 1994), differences in anatomy of the upper respiratory tract and resulting differences in absorption or in local respiratory system effects are sources of uncertainty.

Statistical uncertainty at the Point of Departure (POD). Parameter uncertainty within the chosen model reflects the limited sample size of the cancer bioassay. For the multistage-Weibull model applied to this data set, there is a reasonably small degree of uncertainty at the 10% extra risk level (the POD for linear low-dose extrapolation). Central estimates of risk differed from their upper bounds by about 1.2-fold for lung tumors and for the overall risk estimates.

HEC derivation. A source of uncertainty in the derivation of the HEC comes from whether or nor chloroprene induces lung tumors due to portal-of-entry or systemic effects. Systemic distribution of chloroprene is evidenced by the induction of tumors in multiple organs and suggests that chloroprene may be redistributed back to the lungs and may potentially act as a systemically delivered carcinogen rather than, or in addition to, a portal-of-entry toxicant. However, the contribution of either route of delivery (i.e., inhalation vs. bloodstream) to the induction of lung tumors is currently unknown. Treating lung tumors as systemic effects returns the highest combined unit risk (approximately 60% greater than if lung tumors are treated as portal-of-entry effects).

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Chloroprene (C_4H_5Cl, 2-chloro-1,3-butadiene, CASRN 126-99-8) is a volatile and flammable liquid monomer that can be produced by dimerization of acetylene and addition of hydrogen chloride or by chlorination of 1,3-butadiene. Chloroprene is polymerized to form elastomers for use in the manufacture of belts, hoses, gloves, wire coatings, tubing, solvents, and adhesives. Chloroprene is also a structural analogue of isoprene (2-methyl-1,3-butadiene) and resembles vinyl chloride as far as having a chlorine bound to a double-bonded carbon (alkene) backbone.

Toxicokinetic information on the absorption, distribution, and in vivo metabolism and excretion of chloroprene and/or its metabolites is nonexistent for humans and limited for animals. Several in vitro studies have focused on chloroprene metabolism in lung and liver tissue fractions from rat, mouse, hamster, and humans (Hurst and Ali, 2007; Munter et al., 2007a, b, 2003; Himmelstein et al., 2004a, 2001a, 2001b; Cottrell et al., 2001; Summer and Greim, 1980). These studies suggest that chloroprene is metabolized via the CYP450 enzyme system to monoepoxides [(1-chloroethenyl)oxirane and 2-chloro-2-ethynyloxirane], further metabolized to aldehydes and ketone intermediates and subsequent mercapturic acid derivatives, and cleared via hydrolysis and/or glutathione conjugation reactions. Similar to 1,3-butadiene, the epoxide metabolite is considered to be the toxic moiety. The metabolic profile for chloroprene is qualitatively similar across species. However, in vitro kinetic studies using tissues from rodents and humans suggest quantitative species and tissue-specific differences that, if operative in vivo, could contribute to the species, strain, and gender differences observed in chloroprene-induced effects.

Limited information exists on the noncancer effects of chloroprene due to oral ingestion. In rats, oral exposures from weaning until death (at 120 weeks) resulted in indices of liver toxicity (liver necroses and degenerative lesions of th eparenchymal cells). No information is available on chloroprene's oral toxicity in humans.

Limited information exists on the noncancer effects of chloroprene via the inhalation route in humans. Chloroprene was reported to cause respiratory, ocular, and dermal irritation, chest pains, temporary hair loss, dizziness, insomnia, headache, and fatigue. Chest pains accompanied by tachycardia and dyspnea were also reported. In a Russian review of the effects of chloroprene, Sanotskii (1976) reported that medical examinations of chloroprene production workers revealed changes in the nervous system (lengthening of sensorimotor response to visual cues and increased olfactory thresholds), cardiovascular system (muffled heart sounds, reduced arterial pressure, and tachycardia), and hematology (reduction in red blood cell (RBC) counts, decreased hemoglobin levels, erythrocytopenia, leucopenia, and thrombocytopenia). The ambient concentration of chloroprene associated with these effects ranged from 1–7 mg/m^3.

Chloroprene's toxic and carcinogenic potential by the inhalation route has been assessed in several laboratory animal studies, including a rat and mouse subchronic (16 days and 13 weeks) and chronic inhalation bioassays conducted by NTP (1998), a subchronic range-finding and a chronic study in rats and hamsters conducted by Trochimowicz et al. (1998), an embryotoxicity and a teratology study by Culik et al. (1978), and a series of Russian reproductive and developmental toxicity studies reviewed by Sanotskii (1976). These studies associate chloroprene inhalation exposure with respiratory, kidney, liver, forestomach, reproductive, and developmental effects. The pulmonary (alveolar hyperplasia) and nasal (olfactory epithelium) lesions were the most sensitive endpoints in chronically exposed test animals, having been observed at all the doses tested (12.8–80 ppm) in the NTP (1998) study of rats and mice. In the chronic study by Trochimowicz et al. (1998), lesions in lungs (inflammation, lymphoid aggregates around the bronchi, bronchiole, and blood vessels) and livers (small foci of cellular alteration) of rats were observed at 50 ppm. Embryotoxicity and fetal resorptions were reported in the inhalation developmental toxicity study (Culik et al., 1978). However, interpretational difficulties obscure whether this effect is an actual outcome or rather a statistical artifact of an abnormally low background rate in control animals.

Chloroprene's carcinogenic potential in humans has been assessed in a number of occupational epidemiologic studies among workers exposed to chloroprene monomer and/or polychloroprene latex conducted in 11 cohorts from the United States, Russia, Armenia, France, China, and Ireland. Five cohorts with sufficient numbers of liver/biliary passage cancer cases showed some evidence of association with occupational chloroprene exposure. Four mortality studies reported elevated SMRs when compared to external populations (Marsh et al., 2007a; Bulbulyan et al., 1999; Li et al., 1989; Leet and Selevan, 1982). These measures of association were strong, especially in the presence of the healthy worker effect bias. Several studies were able to use more advanced exposure assessments and internal reference populations, which should reduce this bias. These studies showed relatively consistent elevated relative risk estimates among intermediate and highly exposed workers, despite limited sample size and statistical power (Marsh et al., 2007a; Bulbulyan et al., 1999, 1998). Several studies also reported higher SMRs for lung cancer among workers exposed to chloroprene. These associations are not considered as strong as those with liver cancer due to the inability to control for confounding by smoking status, a strong indicator of lung cancer.

Chloroprene has been shown to induce multisite, malignant tumors in rats and mice in the 2-year NTP (1998) bioassay. Dose-related increasing trends in tumors were noted in rats at the following sites: oral cavity, thyroid gland, lung, kidney, mammary gland. Dose related increasing trends in tumors were noted in mice at the following sites: lung, all organs (hemangiomas and hemangiosarcomas), Harderian gland, forestomach, kidney, skin, liver, mammary gland, mesentery, Zymbal's gland. All of these tumor sites showed statistically significantly positive trends with increasing exposure level (Cochran-Armitage test for trend $p < 0.05$, most with $p \leq 0.001$). In

addition, many early deaths and moribund sacrifices were associated with chloroprene-induced neoplasms.

The genetic toxicity database includes numerous studies covering a range of standard genotoxicity test batteries; however, the results have been conflicting, making it difficult to ascertain the mutagenic potential of chloroprene. In general, bacterial base pair substitution (*S. typhimurium* strains TA100 and TA 1535) mutation assays have been positive (Willems, 1980; Bartsch et al., 1979), while the bacterial frame shift (*S. typhimurium* strains TA97 and TA98) mutation assays have been nonpositive (NTP, 1998; Willems, 1980). In contrast, other studies (NTP, 1998) have reported nonpositive results for all bacterial strains. A positive result with all bacterial strains was observed with chloroprene's epoxide intermediate epoxide (1-chloroethenyl)oxirane (Himmelstein et al., 2001a). Chloroprene has been primarily nonpositive in in vitro micronucleus assays (Himmelstein et al., 2001a; Drevon and Kuroki, 1979), in vivo chromosomal damage assays (NTP, 1998), and bone marrow micronucleus assays (NTP, 1998; Shelby and Witt, 1995). Conflicting results (positive in Vogel [1979]; nonpositive in Foureman et al. [1994]) have been reported for the in vivo drosophila sex-linked lethal mutation assay. Further in vivo evidence for chloroprene's mutagenicity is the observation that tissues from lung, forestomach, and Harderian gland tumors from mice exposed to chloroprene in the NTP chronic bioassay (1998) were shown to have a higher frequency of mutations in K- and H-ras proto-oncogenes than in spontaneous occurring tumors (Stills et al., 1999, 2001). There was also a high correlation between K-ras mutations and loss of heterozygosity in the same chromosome in chloroprene-induced lung neoplasms in mice (Ton et al., 2007). Possible explanations for the conflicting mutagenic responses of chloroprene in standard genotoxicity assays include methods of exposure that do not control for the high volatility of chloroprene (i.e., chloroprene is not present in the test system), the presence of more stable (perhaps more toxic) chloroprene dimers, the use of microsomal inducers that did not elicit a broad range of metabolic enzymes (specifically, in bacterial assays), and the reactivity (perhaps deactivation) of chloroprene with treatment vehicle (e.g., DMSO vs. ethanol).

The likely MOA for chloroprene is via mutagenicity involving epoxide metabolites formed at the target sites. The MOA determination is supported by chloroprene's epoxide metabolite formation, DNA-adduct formation, observation of in vivo and in vitro mutagenicity, and the well known structure-activity relationship of similar epoxide-forming carcinogens. Chloroprene has been found to be metabolized to epoxides by humans and rodents. The hypothesized mutagenic mode of action is supported by evidence of base pair substitution mutations seen in H- and K-ras proto-oncogenes in chloroprene-induced lung, forestomach, and Harderian gland neoplasms observed in the NTP (1998) study.

In addition, chloroprene is the 2-chloro analog of 1,3-butadiene. Inhalation studies have demonstrated that, similar to 1,3-butadiene and isoprene, chloroprene is a multisite carcinogen in rats and mice. Butadiene and isoprene are metabolized to epoxides and diepoxides which are believed to be responsible for their carcinogenicity. Chloroprene is also metabolized to epoxide intermediates

that, similarly to butadiene, may mediate its carcinogenic effects. The similarities in the sites of tumor induction in rodents (mammary gland and thyroid gland in rats, lung, Harderian gland, forestomach, kidney, and liver in mice) between butadiene and chloroprene provide further evidence for a similar MOA for these epoxide-forming compounds. In addition, the mouse lung was the most sensitive site of carcinogenicity for both chloroprene and butadiene. Similar to butadiene, DNA reactivity and adduct formation have been described for chloroprene. Areas of uncertainty exist in the data supporting a mutagenic MOA for chloroprene carcinogenicity, more specifically in the genotoxicity database. There is conflicting evidence in the bacterial genotoxocity assays and generally nonpositive findings in mammalian in vivo tests, but these results are weighed against the base pair substitution mutations seen in H- and K-ras proto-oncogenes in chloroprene-induced lung, forestomach, and Harderian gland neoplasms observed in the NTP (1998) study.

6.2. DOSE RESPONSE

The chronic inhalation study conducted by NTP (1998) was considered as the principal study for both the non-neoplastic and neoplastic effects of chloroprene exposure.

The respiratory system is the primary targets of chloroprene-induced non-neoplastic effects via inhalation. A range of portal-of-entry non-neoplastic effects from the NTP study (1998), including alveolar epithelial hyperplasia, bronchiolar hyperplasia, pulmonary histiocytic cell infiltration, olfactory epithelial atrophy, chronic inflammation, and necrosis were considered as candidates for the selection of the critical effect for derivation of the RfC. BMD modeling was used to determine potential PODs for deriving the chronic RfC by estimating the effective dose at a specified level of response (benchmark concentration [BMD_{10}]) and it's $BMDL_{10}$ for each selected chloroprene-induced respiratory and systemic effect (see Table 5-2). The HEC for each of the selected endpoints was then estimated for the best fitting models of the BMD at a BMR of 10% extra risk. Degenerative nasal lesions in male rats (characterized by atrophy or necrosis of the olfactory epithelium) resulted in the lowest $POD_{(HEC)}$ value of approximately 1.0 mg/m^3. This POD was then divided by a 100-fold UF (3 for uncertainty associated with animal to human differences, 10 for consideration of human variability, and 3 for database deficiencies) to obtain a chronic RfC of 1×10^{-2} mg/m^3.

Statistically significant increases in tumor incidence were observed at multiple sites in the mouse (the most sensitive species) in the NTP study: all organs (hemangiomas and hemangiosarcomas), lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas), skin and mesentery, liver, and mammary glands. These tumors generally appeared earlier with increasing exposure level and showed statistically significantly increasing trends with increasing exposure level (by life table test or logistic regression, $p \leq 0.001$). Dose-response modeling was used to determine potential PODs for deriving the inhalation unit risk by estimating the effective dose at a specified level of response (benchmark concentration [BMD_{10}]) and its $BMDL_{10}$ for each selected chloroprene-induced tumor (see Tables 5-6

and 5-7). Lung tumors, treated as a systemic lesion (see Section 5.4.3 and 5.4.7 for details), in female mice resulted in the highest inhalation unit risk (1.7×10^{-4} per $\mu g/m^3$) when modeled as an individual lesion. When etiologically different tumors were considered together (given the multiplicity of the tumor sites, basing unit risk on only one tumor site may underestimate the carcinogenic potential of chloroprene), the resulting combined inhalational unit risk for female mice was 3.3×10^{-4} per $\mu g/m^3$ (when lung tumors were considered systemic lesions). Based on these modeling results, the upper bound estimate on human extra lifetime cancer risk from continuous lifetime (adult) exposure to chloroprene is 3×10^{-4} per $\mu g/m^3$. Application of the ADAFs to account for early-life susceptibility to chloroprene's proposed mutagenic mode of action yields an adjusted human lifetime cancer risk of 5 x 10^{-4} per $\mu g/m^3$.

Confidence in the principal study (NTP, 1998) is judged to be high as it was a well-designed study using two test species (rats and mice) with 50 animals per dose group. This study appropriately characterizes a range of chloroprene-induced non-neoplastic and neoplastic lesions. In addition, the key histopathological lesions observed are appropriately described, and suitable statistical analysis is applied to all animal data.

Confidence in the critical non-cancer effect identified in the principal study is medium to high. The critical non-cancer effect, increased incidence of degenerative nasal lesions in male rats, is consistent with what is known about chloroprene's metabolism and the expression of cytochrome p450 enzymes in the olfactory and respiratory mucosa of rats, as well as the effects of structurally analogous chemicals (i.e. 1,3-butadiene).

Confidence in the overall database specific to chloroprene is medium to high. The major strength of the database is the observation of dose-response effects in multiple organ systems in a well-designed chronic inhalation study that utilized 50 animals per sex per dose group, a range of doses based on the results of preliminary, shorter-duration studies (16 day and 13 weeks), and thoroughly examined chloroprene's observed toxicity in two species (rat and mouse). The database further contains another chronic inhalation bioassay investigating outcomes in another species (hamster), and well-designed embryotoxicity, teratological, and reproductive toxicity studies. The database also contains subchronic studies and chronic studies observing potential neurotoxic and immunotoxic effects. A major limitation in the database is the lack of a two-generation reproductive toxicity study. Therefore, confidence in the RfC is judged to be medium to high.

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

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

Benchmark Dose (BMD) modeling was performed to identify the point of departure for the derivation of the chronic RfC for chloroprene. The modeling was conducted in accordance with the draft EPA guidelines (U.S. EPA, 2000b) using Benchmark Dose Software Version 2.0 (BMDS). The BMDS model outputs for the derivation of the chronic RfC are attached.

The following critical effects were modeled using BMDS: alveolar epithelial hyperplasia, bronchiolar hyperplasia, lung histiocytic cell infiltration, nasal epithelial chronic inflammation, nasal epithelium atrophy, nasal epithelial necrosis, and splenic hematopoietic cell proliferation. The endpoint being modeled specified which set of models, continuous (linear, polynomial, power, and Hill) or dichotomous (gamma, logistic, multi-stage, probit, quantal-linear, quantal-quadratic, Weibull, and dichotomous Hill), 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).

The critical endpoint selected for the derivation of the chronic RfC was increased incidence of degenerative nasal lesions in male rats. The logistic model provided the best fit for this data set. The following tables (B-1 through B-9) are summaries of the modeling results for all considered endpoints. The best fitting model for each endpoint is indicated in **bold** and the model plot and output are included immediately after the table.

Table B-1. Modeling Results for Alvelolar Epithelial Hyperplasia in Male F344/N Rats

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	14.8657	24.4838	11.4228	28.604	23.3986	14.866	14.866	3.54712
BMDL	10.0883	19.1571	7.06934	19.5927	18.2584	10.0883	10.0883	9.05E-08
Chi^2	4.05	5.12	3.48	6.63	5.02	4.05	4.05	1.989357
Goodness-of-fit p-value	0.1317	0.0775	0.1753	0.0363	0.0813	0.1317	0.1317	NA
AIC	231.042	232.34	230.479	233.859	232.209	231.042	231.042	233.164
Fitted Log-Likelihood	-113.521	-114.17	-113.24	-114.93	-114.104	-113.521	-113.521	-112.582
Fitted p-value	0.1421	0.0743	0.1883	0.03475	0.07933	0.1421	0.1421	NA

Scaled Residuals:								
0	-0.928	-0.928	-0.649	-1.676	-1.427	-0.928	-0.928	-0.1573
12.8	1.698	1.698	1.566	1.939	1.711	1.698	1.698	0.6599
32	-0.509	-0.509	-0.763	-0.087	-0.126	-0.509	-0.509	-1.122
80	-0.224	-0.224	-0.16	-0.23	-0.198	-0.224	-0.224	0.5197

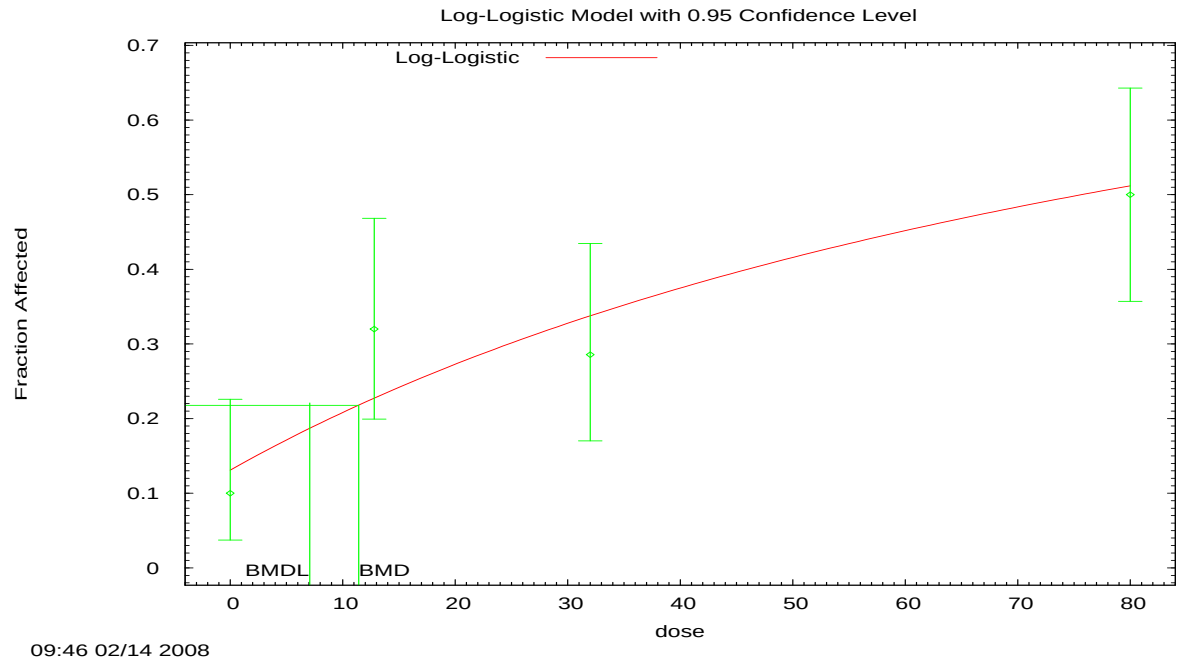


Figure B-1. Log-logistic model fit for alveolar epitheliala hyperplasia in male F344/N rats

```
1   ==========================================================================
2   Logistic Model. (Version: 2.10; Date: 09/23/2007)
3   Input Data File: U:\Chloroprene\Male_F344_rat\alv_hyper_loglog.(d)
4   Gnuplot Plotting File:  U:\Chloroprene\Male_F344_rat\alv_hyper_loglog.plt
5   Thu Feb 14 09:46:06 2008
6    ==========================================================================
7    BMDS Model Run
8   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9   The form of the probability function is:
10  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
11  Dependent variable = alveolar_hyper
12  Independent variable = DOSE
13  Slope parameter is restricted as slope >= 1
14  Total number of observations = 4
15  Total number of records with missing values = 0
16  Maximum number of iterations = 250
17  Relative Function Convergence has been set to: 1e-008
18  Parameter Convergence has been set to: 1e-008
19  User has chosen the log transformed model
20
21  Default Initial Parameter Values
22  background =         0.1
```

intercept = -4.4782
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	background	intercept
background	1	-0.66
intercept	-0.66	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	S td. Err.	Lower Conf. Limit	Upper Conf. Limit
background	0.130984	*	*	*
intercept	-4.63283	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-111.57	4			
Fitted model	-113.24	2	3.33902	2	0.1883
Reduced model	-121.815	1	20.4898	3	0.0001343

AIC: 230.479

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1310	6.549	5	50	-0.649
12.8000	0.2272	11.360	16	50	1.566
32.0000	0.3373	16.526	14	49	-0.763
80.0000	0.5113	25.564	25	50	-0.160

Chi^2 = 3.48 d.f. = 2 P-value = 0.1753

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 11.4228
BMDL = 7.06934

Table B-2. Modeling Results for Alveolar Epithelial Hyperplasia in Female F344/N Rats

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	8.0322	14.8564	4.90719	15.342	14.4844	8.03223	8.03223	1.42027
BMDL	5.89582	11.9857	3.27097	10.7468	11.8082	5.89582	5.89582	0.001489
Chi^2	5.59	8.24	3.45	9.68	8.14	5.59	5.59	1.853207
Goodness-of-fit p-value	0.0612	0.0163	0.1779	0.0079	0.0171	0.0612	0.0612	0.1734
AIC	245.78	248.949	243.677	249.954	248.806	245.78	245.78	244.161
Fitted Log-Likelihood	-120.89	-122.475	-119.839	-122.977	-122.403	-120.89	-120.89	-119.081
Fitted p-value	0.06481	0.01328	0.1854	0.00804	0.01427	0.06481	0.06481	0.1733
Scaled Residuals:								
0	-1.098	-2.002	-0.453	-1.77	-1.96	-1.098	-1.098	-0.05399
12.8	1.998	1.99	1.536	2.446	2.006	1.998	1.998	0.6151
32	-0.328	0.271	-0.91	-0.201	0.275	-0.328	-0.328	-1.089
80	-0.531	-0.442	-0.246	-0.723	-0.439	-0.531	-0.531	0.5347

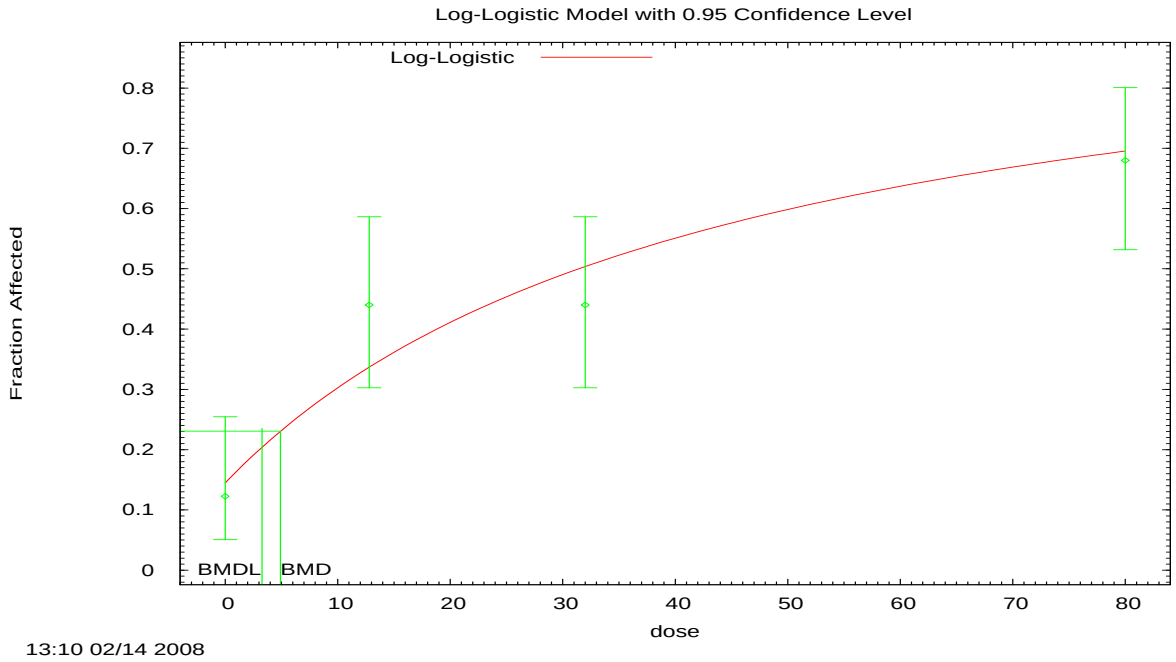


Figure B-2. Log-logistic model fit for alveolar epithelial hyperplasia in female F344/N rats

```
 ========================================================================
Logistic Model. (Version: 2.10; Date: 09/23/2007)
Input Data File: U:\Chloroprene\Female_F344_rat\alv_hyper_loglog.(d)
Gnuplot Plotting File:  U:\Chloroprene\Female_F344_rat\alv_hyper_loglog.plt
                                                    Thu Feb 14 13:10:35 2008
 ========================================================================

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

   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
   Dependent variable = alveolar_hyper
   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 Parameter Values
   background =     0.122449
   intercept  =    -3.74532
   slope      =           1


Asymptotic Correlation Matrix of Parameter Estimates

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

	background	intercept
background	1	-0.62
ntercept	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.145252	*	*	*
intercept	-3.78793	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-118.153	4			
Fitted model	119.839	2	3.37005	2	0.1854
Reduced model	-135.512	1	34.7167	3	<.0001

AIC: 243.677

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1453	7.117	6	49	-0.453
12.8000	0.3373	16.866	22	50	1.536
32.0000	0.5044	25.218	22	50	-0.910
80.0000	0.6960	34.799	34	50	-0.246

Chi^2 = 3.45 d.f. = 2 P-value = 0.1779

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 4.90719
BMDL = 3.27097

Table B-3. Modeling Results for Bronchiolar Hyperplasia in Male B6C3F1 Mice

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	9.962	25.582	7.54241	18.0076	23.8731	9.962	9.962	6.46897
BMDL	7.95025	20.9208	5.60381	12.7086	19.6205	7.95025	7.95025	0.13456
Chi^2	6.24	12.55	2.32	13.97	12.13	6.24	6.24	0
Goodness-of-fit p-value	0.1003	0.0019	0.5085	0.0009	0.0023	0.1003	0.1003	0.9997
AIC	192.219	206.147	188.645	203.779	205.312	192.219	192.219	190.376
Fitted Log-Likelihood	-95.1094	-101.073	-93.3224	-99.8893	-100.656	-95.1094	-95.1094	-92.1882
Fitted p-value	0.1195	0.000138	0.5186	0.000452	0.00021	0.1195	0.1195	0.9997
Scaled Residuals:								
0	0	-2.549	0	-1.669	-2.442	0	0	0
12.8	1.561	0.897	0.8	2.278	0.992	1.561	1.561	-0.0003
32	1.139	2.136	0.6	1.259	2.094	1.139	1.139	-0.00015
80	-1.585	-0.826	-1.149	-2.098	-0.891	-1.585	-1.585	0.000113

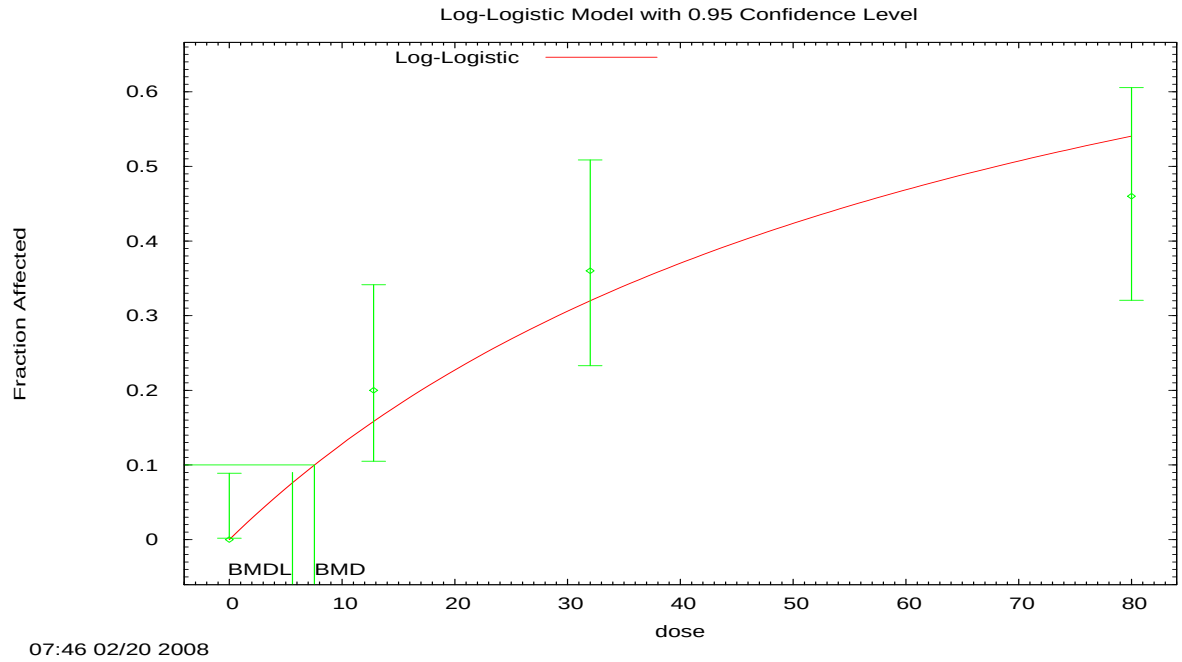


Figure B-3. Log-logistic model fit for bronchiolar hyperplasia in male B6C3F1 Mice

```
1   ==========================================================================
2   Logistic Model. (Version: 2.10; Date: 09/23/2007)
3   Input Data File: U:\Chloroprene\Male_B6C3F1_mouse\bronc_hyper_loglog.(d)
4   Gnuplot Plotting File:  U:\Chloroprene\Male_B6C3F1_mouse\bronc_hyper_loglog.plt
5   Wed Feb 20 07:46:23 2008
6   ==========================================================================
7
8    BMDS Model Run
9   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10  The form of the probability function is:
11  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
12  Dependent variable = bronchiolar_hyper
13  Independent variable = DOSE
14  Slope parameter is restricted as slope >= 1
15  Total number of observations = 4
16  Total number of records with missing values = 0
17  Maximum number of iterations = 250
18  Relative Function Convergence has been set to: 1e-008
19  Parameter Convergence has been set to: 1e-008
20  User has chosen the log transformed model
21
22
23  Default Initial Parameter Values
24   background =           0
25   intercept =     -4.24694
26  slope =           1
27
28
29  Asymptotic Correlation Matrix of Parameter Estimates
30
31  ( *** The model parameter(s)  -background    -slope
32  have been estimated at a boundary point, or have been specified by the user,
33  and do not appear in the correlation matrix )
34
35   intercept
```

intercept 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-4.21777	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-92.1882	4			
Fitted model	-93.3224	1	2.26827	3	0.5186
Reduced model	-113.552	1	42.7283	3	<.0001

AIC: 188.645

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	50	0.000
12.8000	0.1586	7.932	10	50	0.800
32.0000	0.3204	16.019	18	50	0.600
80.0000	0.5410	27.049	23	50	-1.149

Chi^2 = 2.32 d.f. = 3 P-value = 0.5085

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.54241

BMDL = 5.60381

Table B-4. Modeling Results for Bronchioloar Hyperplasia in Female B6C3F1 Mice

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	8.36521	21.7233	6.16449	16.922	20.2623	8.36519	8.36519	2.97514
BMDL	6.74162	17.9832	4.6013	11.5061	16.905	6.74162	6.74162	0.149667
Chi^2	11.72	14.42	7.96	18.88	14.34	11.72	11.72	5.982981
Goodness-of-fit p-value	0.0084	0.0007	0.0469	0.0001	0.0008	0.0084	0.0084	0.0502
AIC	194.774	205.342	192.407	206.563	204.825	194.774	194.774	192.953
Fitted Log-Likelihood	-96.387	-100.671	-95.2036	-101.282	-100.412	-96.387	-96.387	-94.4763
Fitted p-value	0.01856	9.29E-05	0.05422	5.04E-05	0.00012	0.01856	0.01856	0.04552
Scaled Residuals:								
0	0	-2.5	0	-2.04	-2.409	0	0	0
12.8	3.092	2.835	2.128	3.615	2.889	3.092	3.092	1.122
32	-1.377	-0.285	-1.847	-1.163	-0.359	-1.377	-1.377	-1.956
80	-0.513	-0.235	0.137	-0.549	-0.249	-0.513	-0.513	0.9482

Note: No model fits appropriately. Therefore, recommend using the NOAEL/LOAEL approach to determining a POD

Table B-5. Modeling Results for Histiocytic Cell Infiltration in Male B6C3F1 Mice

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	11.1361	24.8436	7.44347	22.6862	23.4156	11.1363	11.1363	
BMDL	8.17864	19.8574	5.28235	15.5226	18.7645	8.17864	8.17864	
Chi^2	8.97	12.27	5.38	14.62	12.04	8.97	8.97	
Goodness-of-fit p-value	0.0113	0.0022	0.0679	0.0007	0.0024	0.0113	0.0113	
AIC	215.559	222.023	211.84	223.144	221.531	215.559	215.559	1615.32
Fitted Log-Likelihood	-105.78	-109.012	-103.92	-109.572	-108.765	-105.78	-105.78	-807.661
Fitted p-value	0.01235	0.000488	0.07927	0.000278	0.000624	0.01235	0.01235	1.31E-304
Scaled Residuals:								
0	-1.224	-2.606	-0.49	-2.356	-2.524	-1.224	-1.224	
12.8	2.188	1.653	1.762	2.379	1.714	2.188	2.188	
32	0.816	1.488	0.222	1.188	1.458	0.817	0.817	
80	-1.421	-0.733	-1.409	-1.414	-0.779	-1.421	-1.421	

Note: No model fits appropriately. Therefore, recommend using the NOAEL/LOAEL approach to determining a POD

Table B-6. Modeling Results for Olfactory Chronic Inflammation in Male F344/N Rats

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	15.2489	23.8087	14.6428	17.7991	15.2489	15.2489	15.2489	--
BMDL	10.1164	18.9473	9.27776	13.7362	10.1164	10.1164	10.1164	--
Chi^2	0.22	2.83	0.12	3.08	0.22	0.22	0.22	--
Goodness-of-fit p-value	0.8964	0.0925	0.9398	0.2144	0.8964	0.8964	0.8964	--
AIC	81.4586	87.0594	81.3682	83.9766	81.4586	81.4586	81.4586	--
Fitted Log-Likelihood	-39.7293	-41.5297	-39.4291	-40.9883	-41.3298	-39.7293	-39.7293	--
Fitted p-value	0.8993	0.05085	0.9409	0.2553	0.06467	0.8993	0.8993	--

Scaled Residuals:								
0	0.000	-1.120	**0.000**	0.000	-1.027	0.000	0.000	--
12.8	0.390	1.221	**0.286**	1.458	1.195	0.390	0.390	--
32	-0.258	-0.291	**-0.207**	-0.976	-0.345	-0.258	-0.258	--

Note: High dose group was dropped in order to obtain adequate model fit

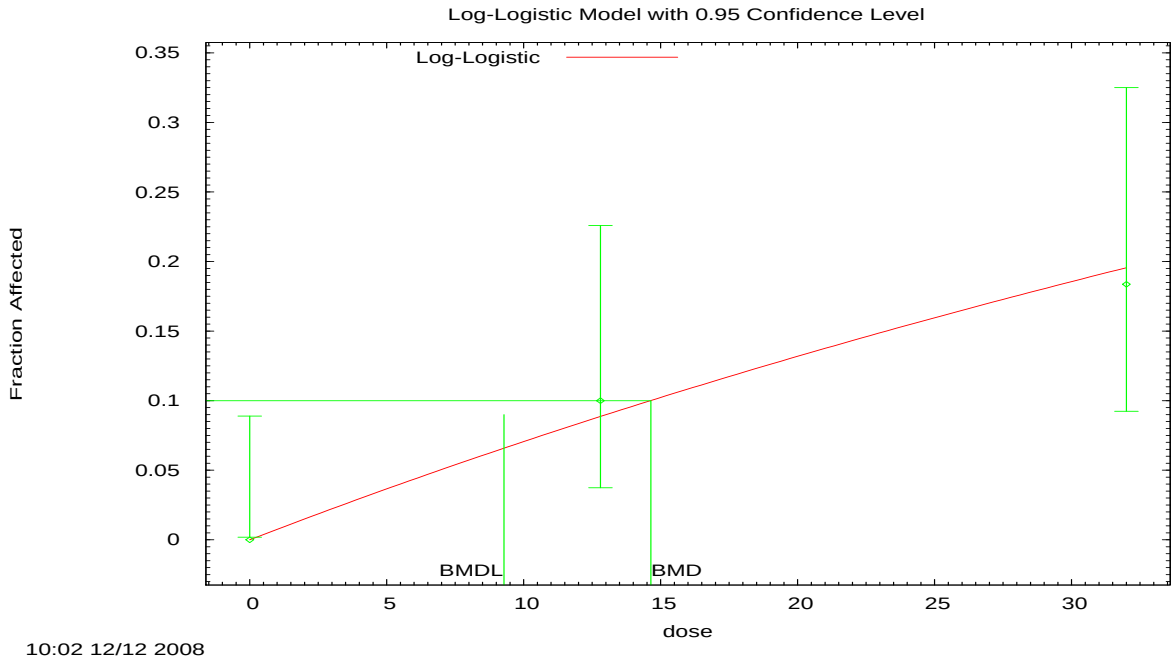


Figure B-4. Log-logistic model fit for olfactory chronic inflammation in male F344/N rats

```
1   ================================================================
2   Logistic Model. (Version: 2.12; Date: 05/16/2008)
3   Input Data File: M:\Chloroprene\Male_F344_rat\ntp_inflammation_hdd_loglog.(d)
4   Gnuplot Plotting File:  M:\Chloroprene\Male_F344_rat\ntp_inflammation_hdd_loglog.plt
5   Fri Dec 12 08:22:05 2008
6    ================================================================
7
8    BMDS Model Run
9   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10  The form of the probability function is:
11  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
12  Dependent variable = Col3
13  Independent variable = Col1
14  Slope parameter is restricted as slope >= 1
15  Total number of observations = 3
16   Total number of records with missing values = 0
17   Maximum number of iterations = 250
18   Relative Function Convergence has been set to: 1e-008
19   Parameter Convergence has been set to: 1e-008
20  User has chosen the log transformed model
21
```

Default Initial Parameter Values
background = 0
intercept = -4.79799
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	*	*	*
intercept	-4.88117	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-39.6231	3			
Fitted model	39.6841	1	0.121914	2	0.9409
Reduced model	-46.4291	1	13.6119	2	0.001107

AIC: 81.3682

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
12.8000	0.0885	4.426	5.000	50	0.286
32.0000	0.1954	9.574	9.000	49	-0.207

Chi^2 = 0.12 d.f. = 2 P-value = 0.9398

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 14.6428
BMDL = 9.27776

Table B-7. Modeling Results for Olfactory Atrophy in Male F344/N Rats

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	10.6003	7.70048	10.81	10.9386	6.91725	9.95012	2.28431	--
BMDL	7.99938	5.97454	8.62799	8.79455	5.40111	7.06875	1.80011	--
Chi^2	0.00	1.24	0.00	0.00	2.02	0.00	18.03	--
Goodness-of-fit p-value	NA	0.2655	NA	NA	0.1555	NA	0.0000	--
AIC	106.376	105.53	106.376	106.376	106.283	106.376	125.166	--
Fitted Log-Likelihood	-50.1882	-50.7651	-50.1882	-50.1882	-51.1417	-50.1882	-60.5831	--
Fitted p-value	NA	0.2828	NA	NA	0.1673	NA	5.12E-006	--
Scaled Residuals:				--				
0	-0.000	0.847	0.000	0.000	0.977	0.000	0.459	--
12.8	-0.000	-0.597	0.000	-0.000	-0.901	0.000	-3.280	--
32	0.000	0.406	-0.000	0.000	0.502	-0.000	2.658	--

Note: High dose group was dropped in order to obtain adequate model fit

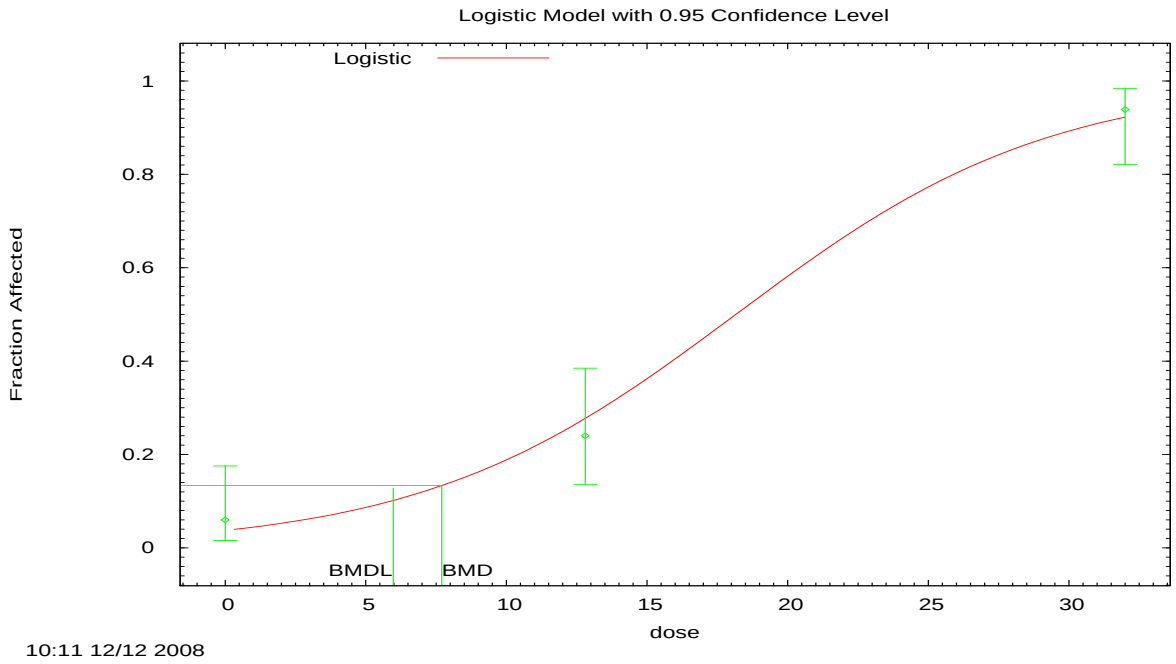


Figure B-5. Logistic model fit for olfactory atrophy in male F344/N rats

==

Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File: M:\Chloroprene\Male_F344_rat\ntp_atrophy_hdd_logistic.(d)
Gnuplot Plotting File: M:\Chloroprene\Male_F344_rat\ntp_atrophy_hdd_logistic.plt
Fri Dec 12 08:51:22 2008

==

BMDS Model Run

~~~~~~

The form of the probability function is:
P[response] = 1/[1+EXP(-intercept-slope*dose)]
Dependent variable = Col3
Independent variable = Col1
Slope parameter is not restricted
Total number of observations = 3
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
background = 0 Specified
intercept = -2.84277
slope = 0.164779

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	-0.85
slope	-0.85	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
intercept	-3.25094	0.484263	-4.20007	-2.3018
slope	0.179356	0.0262753	0.127857	0.230855

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-50.1882	3			
Fitted model	-50.7651	2	1.15379	1	0.2828
Reduced model	-100.819	1	101.262	2	<.0001

AIC: 105.53

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0373	1.865	3.000	50	0.847
12.8000	0.2778	13.892	12.000	50	-0.597
~~~~~~

1 32.0000 0.9233 45.243 46.000 49 0.406
2
3 Chi^2 = 1.24 d.f. = 1 P-value = 0.2655
4
5
6 Benchmark Dose Computation
7 Specified effect = 0.1
8 Risk Type = Extra risk
9 Confidence level = 0.95
10 BMD = 7.70048
11 BMDL = 5.97454

Table B-8. Modeling Results for Olfactory Necrosis in Male F344/N Rats

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	6.46561	12.1684	6.92124	7.98173	11.3581	6.31726	4.75407	--
BMDL	3.70666	9.77545	2.96263	6.41755	9.13936	3.70666	3.65317	--
Chi^2	0.00	4.56	0.00	0.06	3.86	0.00	0.30	--
Goodness-of-fit p-value	1.0000	0.0328	1.0000	0.9686	0.0494	1.0000	0.8622	--
AIC	124.435	130.942	124.435	122.499	129.762	124.435	122.737	--
Fitted Log-Likelihood	-60.2177	-63.4712	-60.2177	-60.2494	-62.881	-60.2177	-60.3685	--
Fitted p-value	1.00	0.01075	1.00	0.9688	0.021	1.00	0.86	--
Scaled Residuals:								
0	0.000	-1.510	0.000	0.000	-1.313	0.000	0.000	--
12.8	-0.000	1.450	0.000	0.188	-0.461	-0.000	-0.443	--
32	0.000	-0.418	-0.000	-0.169	1.387	0.000	0.317	--

Note: High dose group was dropped in order to obtain adequate model fit

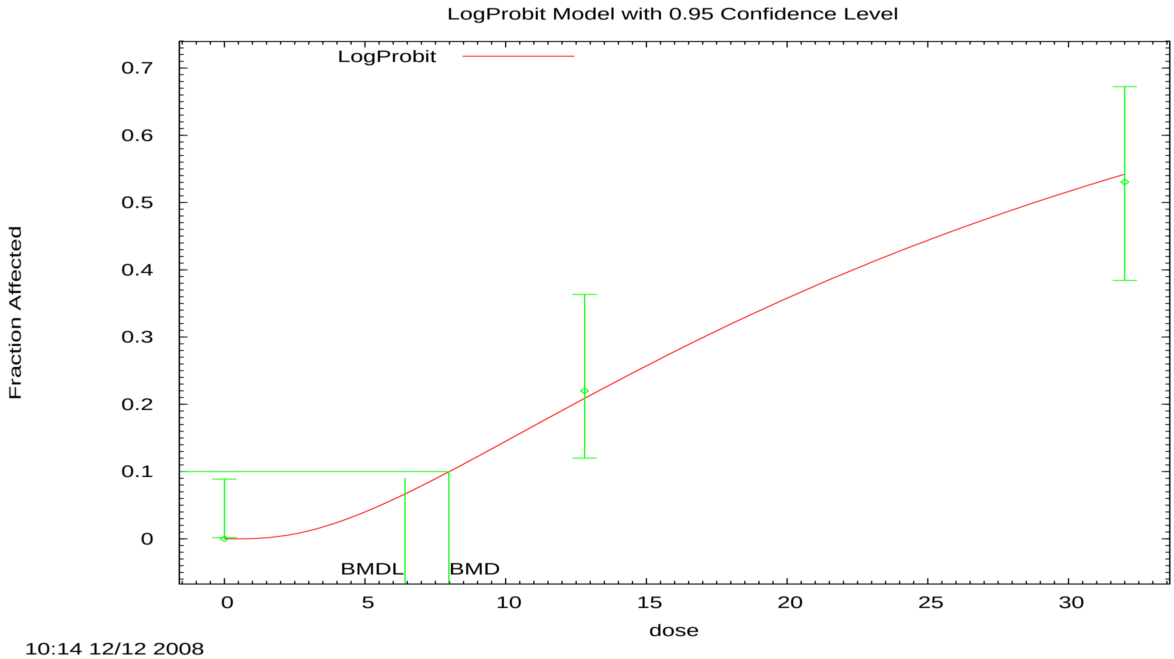


Figure B-6. Logistic model fit for olfactory necrosis in male F344/N rats

===
Probit Model. (Version: 3.1; Date: 05/16/2008)
Input Data File: M:\Chloroprene\Male_F344_rat\ntp_necrosis_hdd_logprobit.(d)
Gnuplot Plotting File: M:\Chloroprene\Male_F344_rat\ntp_necrosis_hdd_logprobit.plt
Fri Dec 12 09:00:57 2008
===

BMDS Model Run ~~~~~~

The form of the probability function is:
P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
where CumNorm(.) is the cumulative normal distribution function
Dependent variable = Col3
Independent variable = Col1
Slope parameter is restricted as slope >= 1
Total number of observations = 3
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 = -3.33803
slope = 1

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

intercept

intercept 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-3.35871	0.133307	-3.61998	-3.09743
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	-60.2177	3			
Fitted model	-60.2494	1	0.063351	2	0.9688
Reduced model	-83.5122	1	46.5889	2	<.0001

AIC: 122.499

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
12.8000	0.2092	10.459	11.000	50	0.188
32.0000	0.5426	26.588	26.000	49	-0.169

Chi^2 = 0.06 d.f. = 2 P-value = 0.9686

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 7.98173
BMDL = 6.41755

Table B-9. Modeling Results for Degenerative Nasal Lesions (olfactory atrophy or necrosis) in Male F344/N Rats

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes			Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	10.3392	7.38383	10.6373	10.7236	6.61322	9.55894	2.13213	--
BMDL	7.89967	5.71667	8.62242	8.71969	5.14757	6.8742	1.68322	--
Chi^2	0	1.44	0	0	2.11	0	18.61	--
Goodness-of-fit p-value	n/a	0.2309	n/a	n/a	0.1460	n/a	0.0	--
AIC	102.417	102.053	102.714	102.714	102.721	102.714	122.619	--
Fitted Log-Likelihood	-48.3572	-49.0267	-48.3572	-48.3572	-49.3605	-48.3572	-59.3096	--
Fitted p-value	n/a	0.2472	n/a	n/a	0.1566	n/a	2.8E-006	--
Scaled Residuals:								
0	-0.0	0.905	0.0	0.0	0.993	-0.0	0.445	--
12.8	0.0	-0.614	0.0	-0.0	-0.903	0.0	-3.304	--
32	-0.0	0.490	-0.0	0.0	0.558	0.0	2.738	--

Note: High dose group was dropped in order to obtain adequate model fit

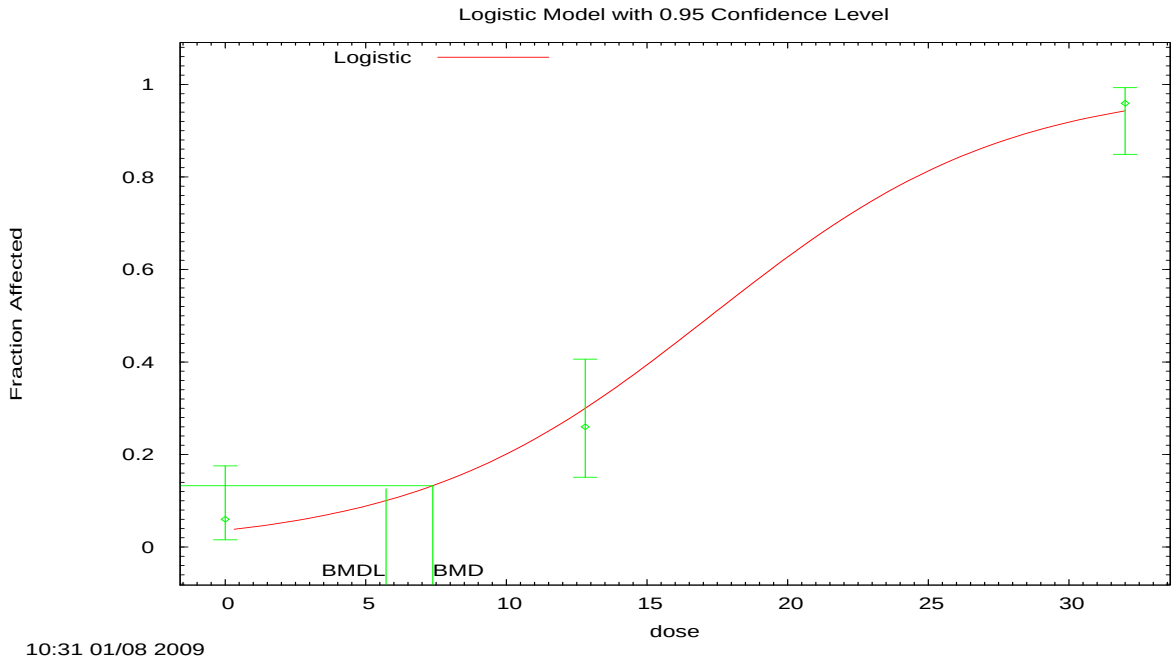


Figure B-7. Logistic model fit for degenerative nasal lesions (olfactory atrophy or necrosis) in male F344/N rats

```
1   ================================================================
2   Logistic Model. (Version: 2.12; Date: 05/16/2008)
3   Input Data File: M:\Chloroprene\Male_F344_rat\olfactory_logistic_hdd.(d)
4    Gnuplot Plotting File:  M:\Chloroprene\Male_F344_rat\olfactory_logistic_hdd.plt
5    Tue Jan 06 12:25:14 2009
6    ================================================================
7
8    BMDS Model Run
9   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10
11  The form of the probability function is:
12  P[response] = 1/[1+EXP(-intercept-slope*dose)]
13  Dependent variable = Col3
14  Independent variable = Col1
15  Slope parameter is not restricted
16  Total number of observations = 3
17  Total number of records with missing values = 0
18  Maximum number of iterations = 250
19  Relative Function Convergence has been set to: 1e-008
20  Parameter Convergence has been set to: 1e-008
21
22
23  Default Initial Parameter Values
24  background =          0   Specified
25  intercept =          -2.85849
26   slope =             0.176122
27
28  Asymptotic Correlation Matrix of Parameter Estimates
29  ( *** The model parameter(s)  -background
30  have been estimated at a boundary point, or have been specified by the user,
31  and do not appear in the correlation matrix )
```

intercept	slope	
intercept	1	-0.85
slope	-0.85	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
intercept	-3.28386	0.49695	-4.25787	-2.30986
slope	0.190277	0.0288302	0.133771	0.246783

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance Test	d.f.	P-value
Full model	-48.3572	3			
Fitted model	-49.0267	2	1.33895	1	0.2472
Reduced model	-101.497	1	106.279	2	<.0001

AIC: 102.053

Goodness of Fit

					Scaled
Dose	Est._Prob.	Expected	Observed	Size	Residual
0.0000	0.0361	1.806	3.000	50	0.905
12.8000	0.2998	14.989	13.000	50	-0.614
32.0000	0.9429	46.204	47.000	49	0.490

Chi^2 = 1.44 d.f. = 1 P-value = 0.2309

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 7.38383
BMDL = 5.71667

Table B-10. Modeling Results for Splenic Hematopoietic Cell Proliferation in Female B6C3F1 Mice

Model:	Gamma	Logistic	Log-Logistic	Log-Probit	Probit	Weibull	Quantal-Linear	Hill
Restricted:	Yes		Yes	Yes		Yes		Yes
BMR:	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BMR Type:	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk	Extra Risk
BMD	5.73584	4.06642	6.5828	6.91076	4.03306	5.17994	2.34557	--
BMDL	1.90919	3.28512	2.43228	3.48982	3.33147	1.90919	1.7616	--
Chi^2	0.00	0.02	0.00	0.00	0.00	0.00	1.35	--
Goodness-of-fit p-value	NA	0.8993	NA	NA	0.9466	NA	0.2455	--
AIC	171.405	169.421	171.405	171.405	169.41	171.405	171.405	--
Fitted Log-Likelihood	-82.7026	-82.7106	-82.7026	-82.7026	-82.7048	-82.7026	-82.7026	--
Fitted p-value	NA	0.8993	NA	NA	0.9466	NA	NA	--
Scaled Residuals:								
0	0.000	0.064	0.000	0.000	0.033	-0.000	-0.000	--
12.8	-0.001	-0.095	0.000	0.000	-0.052	0.000	0.000	--
32	0.000	0.054	-0.000	0.000	0.026	-0.000	-0.000	--

Note High dose group was dropped in order to obtain adequate model fit

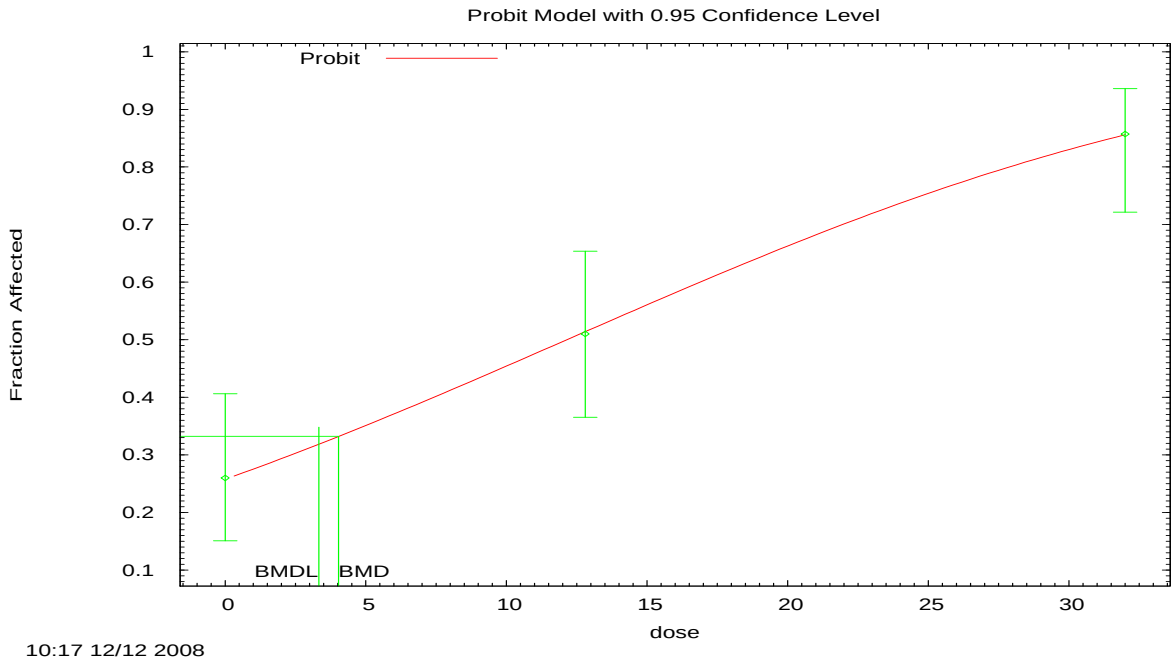


Figure B-8. Probit model fit for splenic hematopoietic cell proliferation in female B6C3F1 mice

---

```
 ================================================================================
Probit Model. (Version: 3.1;  Date: 05/16/2008)
Input Data File: M:\Chloroprene\Female_B6C3F1_mouse\ntp_hemato_prolif_hdd_probit.(d)
Gnuplot Plotting File:  M:\Chloroprene\Female_B6C3F1_mouse\ntp_hemato_prolif_hdd_probit.plt
Fri Dec 12 09:20:03 2008
 ================================================================================

 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 = Col3
Independent variable = Col1
Slope parameter is not restricted
Total number of observations = 3
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 =          -0.643083
 slope =              0.0528681


 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	-0.73
slope	-0.73	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
intercept	-0.649733	0.16595	-0.97499	-0.324476
slope	0.0534876	0.00913534	0.0355826	0.0713925

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-82.7026	3			
Fitted model	-82.7048	2	0.00449095	1	0.9466
Reduced model	-102.099	1	38.7924	2	<.0001

AIC: 169.41

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2579	12.897	13.000	50	0.033
12.8000	0.5139	25.182	25.000	49	-0.052
32.0000	0.8559	41.937	42.000	49	0.026

Chi^2 = 0.00 d.f. = 1 P-value = 0.9466

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 4.03306
BMDL = 3.33147

APPENDIX C. CANCER DOSE-RESPONSE MODELING RESULTS

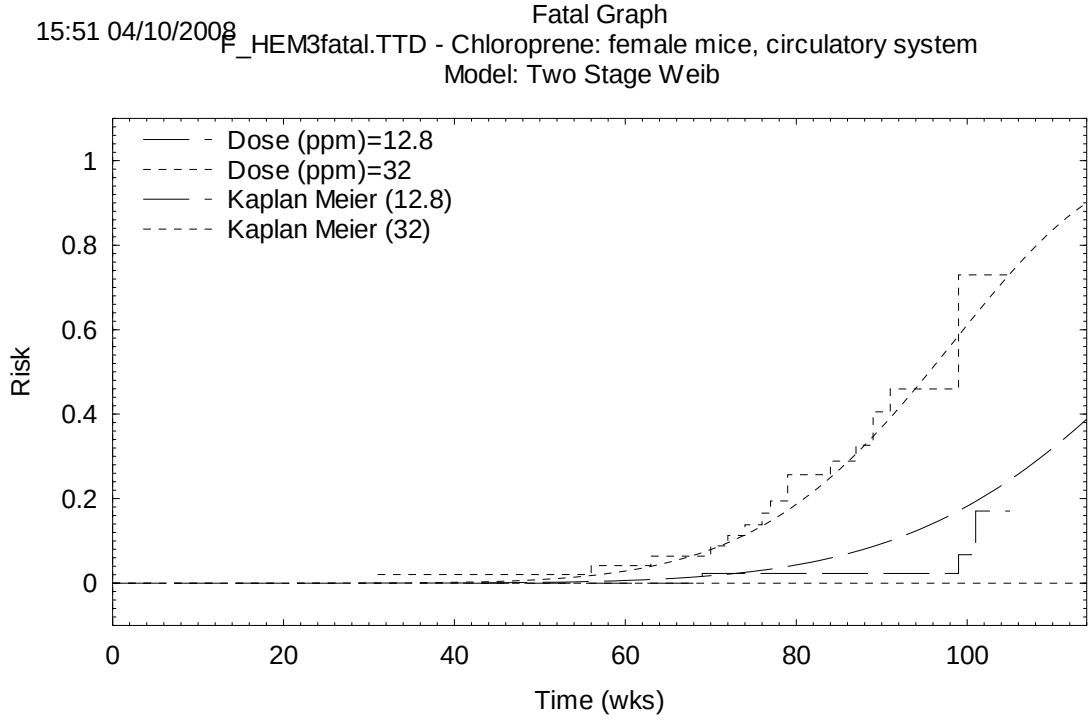


Figure C-1. Female mice, hemangiomas and hemangiosarcomas in all organs; high dose dropped,) hemangiosarcomas occurring before termination considered fatal

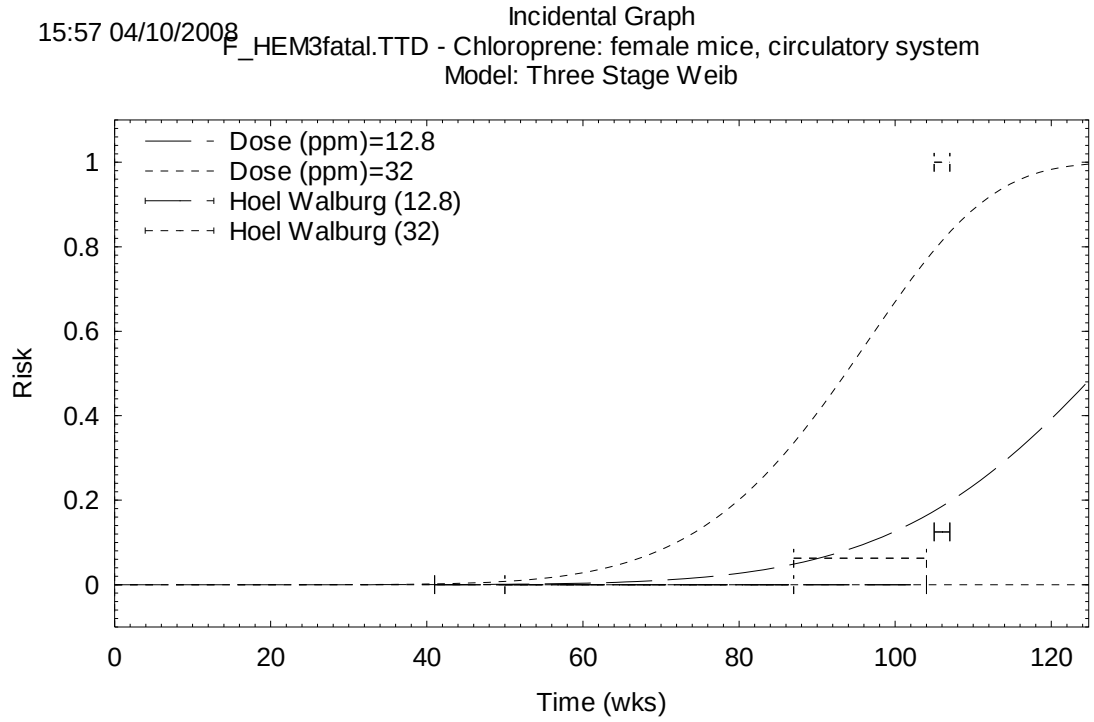


Figure C-2. Female mice, hemangiomas and hemangiosarcomas in all organs; high dose dropped, hemangiosarcomas occurring before termination considered fatal. Details below.

```
Model: Two Stage Weib          Dataset: M:\_ToxRiskData\Chloroprene\F_HEM3fatal.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
Maximum Log-Likelihood =  -1.431146e+002

       Parameter Estimates :
                              Q 0 = 1.352245E-015
                              Q 1 = 0.000000E+000
                              Q 2 = 1.970859E-017
                              Z   = 6.820986E+000
                              T0  = 0.000000E+000   Set by User

Avg. Doses          -------------------- Number --------------------
(ppm)               of animals       with fatal    with incidental
                                      tumors          tumors
 0                    50                1              3
12.80                 50                4              2
32                    50               16              2

                                        Exposure Pattern
         Model: Two Stage Weib     Age Begins: 0     Age Ends: 70
Target Species: Human              Weeks/Year: 52   Days/Week:  7
         Route: Air                                 Hours/Day : 24

Animal to human conversion method: PPM IN AIR

                Human Equivalent Dose Estimates (ug/m^3)

                                95.00 %                          95.00 %
Incid Extra Risk   Time (yr)  Lower Bound          MLE          Upper Bound
     1.0000E-006      70.00   6.3992E-002      8.5868E-002     Not Reqstd
     1.0000E-005      70.00   6.3992E-001      8.5868E-001     Not Reqstd
     0.0001           70.00   6.3995E+000      8.5872E+000     Not Reqstd
     0.0010           70.00   6.4024E+001      8.5911E+001     Not Reqstd
     0.01             70.00   6.4314E+002      8.6300E+002     Not Reqstd
     0.10             70.00   6.7422E+003      9.0471E+003     Not Reqstd
```

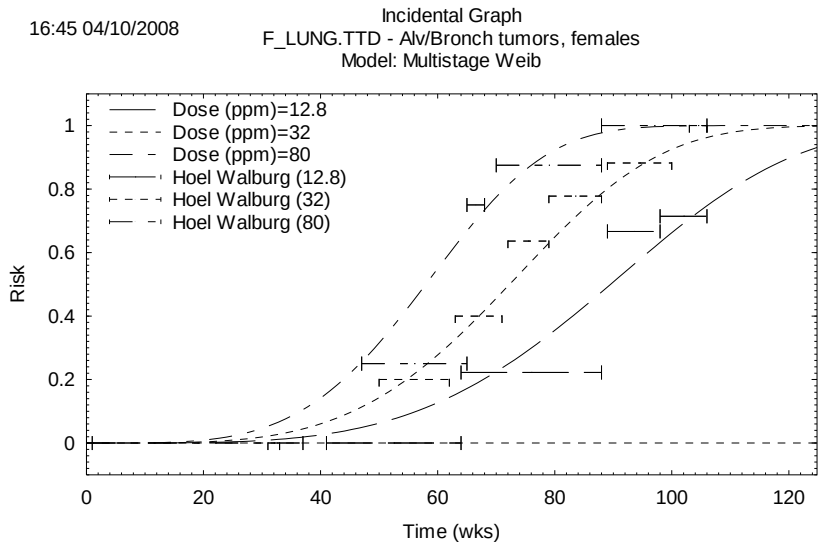


Figure C-3. Female mice, alveolar/bronchiolar tumors. Details below.

```
              Model: Three Stage Weib       Dataset: M:\_ToxRiskData\Chloroprene\F_LUNG.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3 )
                 * (T - T0)^Z]
         Maximum Log-Likelihood =  -8.624362e+001

       Parameter Estimates :
                            Q 0 = 5.948316E-010
                            Q 1 = 5.755951E-010
                            Q 2 = 0.000000E+000
                            Q 3 = 0.000000E+000
                            Z   = 4.067307E+000
                            T0  = 0.000000E+000   Set by User

      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal    with incidental
                                               tumors          tumors
         0                    50                0              4
        12.80                 50                0             28
        32                    50                0             34
        80                    50                0             42


                                          Exposure Pattern
              Model: Three Stage Weib   Age Begins: 0     Age Ends: 70
     Target Species: Human              Weeks/Year: 52   Days/Week:  7
              Route: Air                                 Hours/Day : 24

    Animal to human conversion method: PPM IN AIR


                          Human Equivalent Dose Estimates (ug/m^3)
                                95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound          MLE         Upper Bound
     1.0000E-006      70.00   3.2072E-002      4.0061E-002     Not Reqstd
     1.0000E-005      70.00   3.2072E-001      4.0061E-001     Not Reqstd
     0.0001           70.00   3.2074E+000      4.0063E+000     Not Reqstd
     0.0010           70.00   3.2088E+001      4.0081E+001     Not Reqstd
     0.01             70.00   3.2234E+002      4.0263E+002     Not Reqstd
     0.10             70.00   3.3791E+003      4.2208E+003     Not Reqstd
```

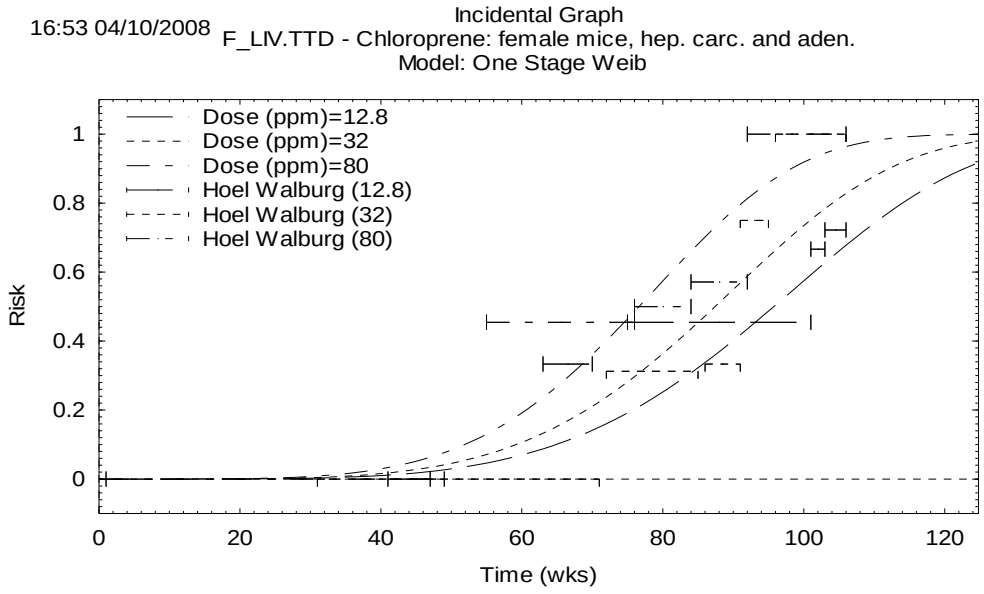


Figure C-4. Female mice, hepatocellular adenomas and carcinomas. Details below.

```
            Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\F_LIV.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -1.233781e+002

       Parameter Estimates :
                           Q 0 = 1.153457E-010
                           Q 1 = 5.270331E-012
                           Z   = 4.834857E+000
                           T0  = 0.000000E+000   Set by User


      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal    with incidental
                                               tumors         tumors
         0                    50                0             20
        12.80                 49                0             26
        32                    50                0             20
        80                    50                0             30



                                        Exposure Pattern
             Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human            Weeks/Year: 52   Days/Week:  7
             Route: Air                               Hours/Day : 24
    Animal to human conversion method: PPM IN AIR

                        Human Equivalent Dose Estimates (ug/m^3)
                                95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound          MLE          Upper Bound
     1.0000E-006      70.00   9.2486E-002      1.2383E-001     Not Reqstd
     1.0000E-005      70.00   9.2486E-001      1.2383E+000     Not Reqstd
     0.0001           70.00   9.2490E+000      1.2384E+001     Not Reqstd
     0.0010           70.00   9.2532E+001      1.2389E+002     Not Reqstd
     0.01             70.00   9.2951E+002      1.2445E+003     Not Reqstd
     0.10             70.00   9.7443E+003      1.3047E+004     Not Reqstd
```

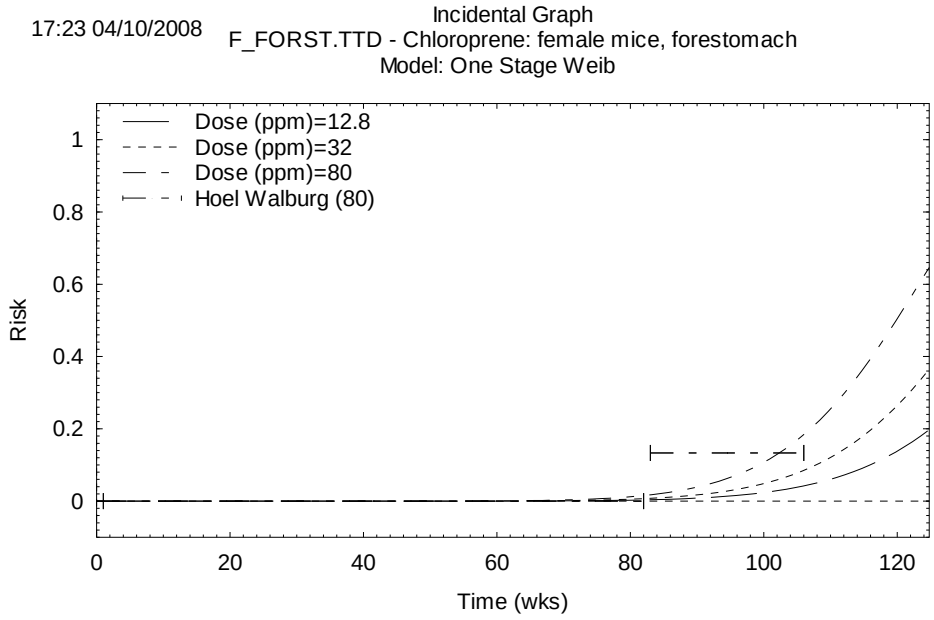


Figure C-5. Female mice, forestomach tumors. Details below.

```
             Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\F_FORST.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -3.359600e+001

       Parameter Estimates :
                            Q 0 = 6.983602E-023
                            Q 1 = 1.330500E-023
                            Z   = 1.000000E+001
                            T0  = 0.000000E+000   Set by User

      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal     with incidental
                                               tumors          tumors
         0                    50                 0              1
        12.80                 50                 0              0
        32                    49                 0              0
        80                    50                 0              4

                                          Exposure Pattern
              Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human             Weeks/Year: 52   Days/Week:  7
              Route: Air                                 Hours/Day : 24

     Animal to human conversion method: PPM IN AIR


                          Human Equivalent Dose Estimates (ug/m^3)
                                95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound          MLE          Upper Bound
     1.0000E-006      70.00   5.7604E-001      1.8723E+000     Not Reqstd
     1.0000E-005      70.00   5.7604E+000      1.8723E+001     Not Reqstd
     0.0001           70.00   5.7607E+001      1.8724E+002     Not Reqstd
     0.0010           70.00   5.7632E+002      1.8733E+003     Not Reqstd
     0.01             70.00   5.7894E+003      1.8818E+004     Not Reqstd
     0.10             70.00   6.0692E+004      1.9727E+005     Not Reqstd
```

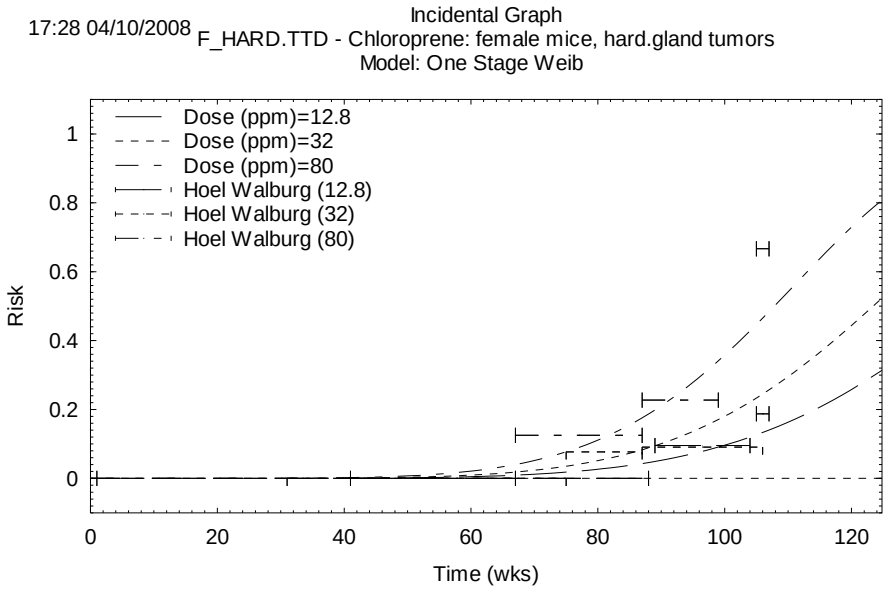


Figure C-6. Female mice, Harderian gland tumors. Details below.

```
            Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\F_HARD.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -6.640761e+001

       Parameter Estimates :
                           Q 0 = 4.680639E-014
                           Q 1 = 6.624162E-015
                           Z   = 5.942561E+000
                           T0  = 0.000000E+000   Set by User

      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal     with incidental
                                               tumors           tumors
         0                    50                 0               2
        12.80                 50                 0               5
        32                    50                 0               3
        80                    50                 0               9

                                                          Exposure Pattern
              Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human             Weeks/Year: 52   Days/Week:  7
              Route: Air                                Hours/Day : 24

     Animal to human conversion method: PPM IN AIR


                          Human Equivalent Dose Estimates (ug/m^3)
                                95.00 %                          95.00 %
Incid Extra Risk  Time (yr)     Lower Bound          MLE         Upper Bound
     1.0000E-006      70.00   4.5697E-001      5.7446E-001     Not Reqstd
     1.0000E-005      70.00   4.5697E+000      5.7446E+000     Not Reqstd
     0.0001           70.00   4.5699E+001      5.7449E+001     Not Reqstd
     0.0010           70.00   4.5720E+002      5.7474E+002     Not Reqstd
     0.01             70.00   4.5927E+003      5.7735E+003     Not Reqstd
     0.10             70.00   4.8146E+004      6.0525E+004     Not Reqstd
```

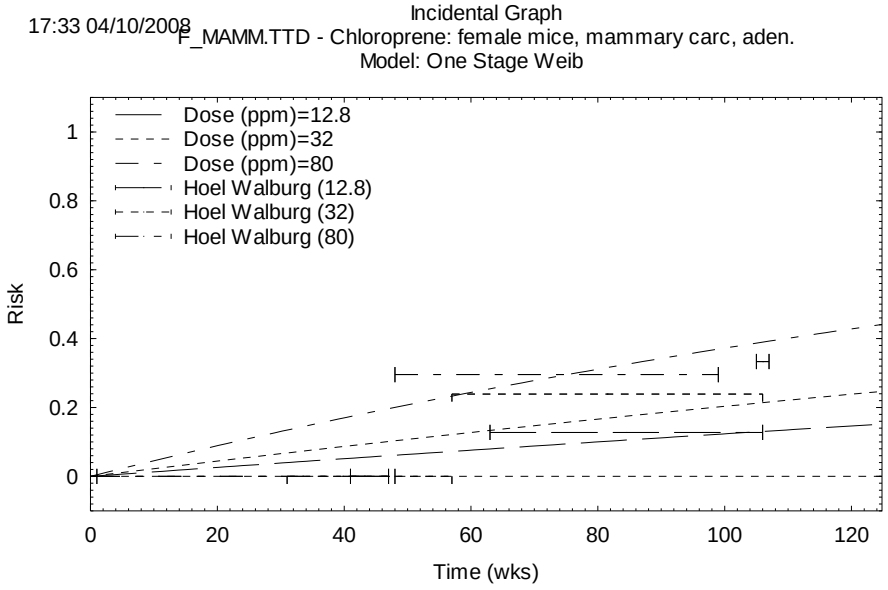


Figure C-7. Female mice, mammary gland tumors. Details below.

```
            Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\F_MAMM.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
        Maximum Log-Likelihood =  -9.075190e+001

       Parameter Estimates :
                              Q 0 = 6.820061E-004
                              Q 1 = 4.969301E-005
                              Z   = 1.000000E+000
                              T0  = 0.000000E+000   Set by User

      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal    with incidental
                                               tumors          tumors
         0                    50                0              3
        12.80                 50                0              6
        32                    50                0             11
        80                    50                0             14

                                          Exposure Pattern
              Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human             Weeks/Year: 52   Days/Week:  7
              Route: Air                                 Hours/Day : 24

     Animal to human conversion method: PPM IN AIR

                          Human Equivalent Dose Estimates (ug/m^3)
                               95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound          MLE         Upper Bound
     1.0000E-006      70.00   4.6346E-001      7.1352E-001     Not Reqstd
     1.0000E-005      70.00   4.6347E+000      7.1352E+000     Not Reqstd
     0.0001           70.00   4.6349E+001      7.1355E+001     Not Reqstd
     0.0010           70.00   4.6369E+002      7.1387E+002     Not Reqstd
     0.01             70.00   4.6580E+003      7.1711E+003     Not Reqstd
     0.10             70.00   4.8831E+004      7.5176E+004     Not Reqstd
```

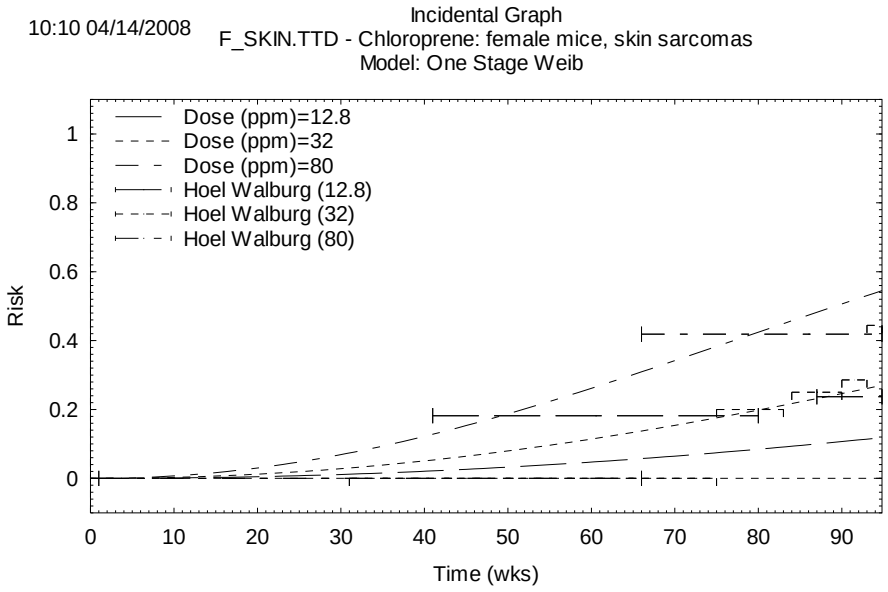


Figure C-8. Female mice, skin sarcomas. Details below.

```
            Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\F_SKIN.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -9.120449e+001

       Parameter Estimates :
                           Q 0 = 0.000000E+000
                           Q 1 = 7.398518E-007
                           Z   = 2.086144E+000
                           T0  = 0.000000E+000   Set by User

      Avg. Doses         -------------------- Number --------------------
        (ppm)              of animals       with fatal     with incidental
                                              tumors           tumors
         0                    50                0               0
        12.80                 50                0              11
        32                    50                0              11
        80                    50                0              18

                                          Exposure Pattern
             Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human            Weeks/Year: 52   Days/Week:  7
             Route: Air                                Hours/Day : 24

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

                           Human Equivalent Dose Estimates (ug/m^3)
                                95.00 %                          95.00 %
Incid Extra Risk  Time (yr)     Lower Bound          MLE         Upper Bound
     1.0000E-006      70.00   2.4134E-001      3.7066E-001     Not Reqstd
     1.0000E-005      70.00   2.4134E+000      3.7066E+000     Not Reqstd
     0.0001           70.00   2.4135E+001      3.7068E+001     Not Reqstd
     0.0010           70.00   2.4146E+002      3.7085E+002     Not Reqstd
     0.01             70.00   2.4255E+003      3.7253E+003     Not Reqstd
     0.10             70.00   2.5428E+004      3.9053E+004     Not Reqstd
```

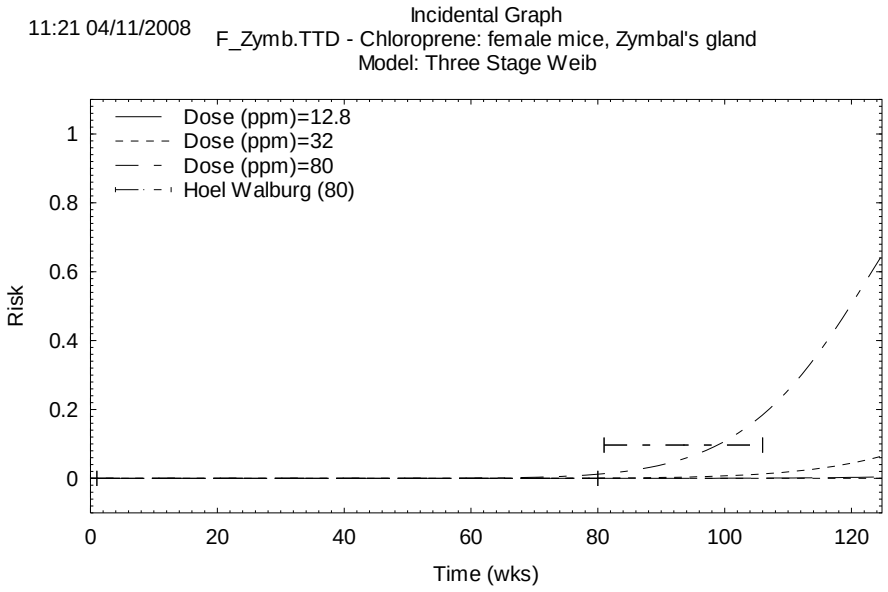


Figure C-9. Female mice, Zymbal's gland tumors. Details below.

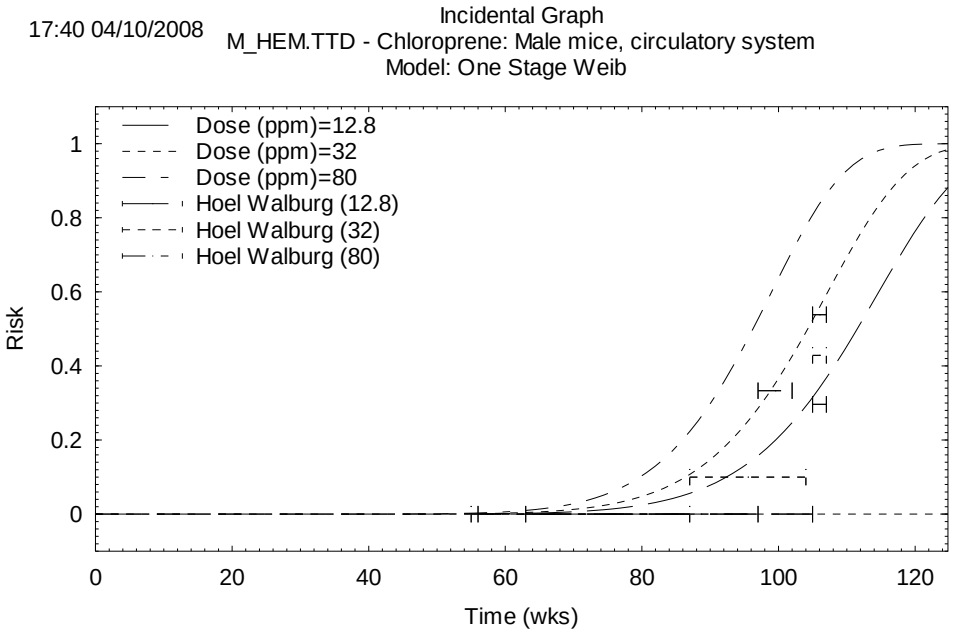
```
            Model: Three Stage Weib       Dataset: M:\_ToxRiskData\Chloroprene\F_Zymb.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3 )
                 * (T - T0)^Z]
         Maximum Log-Likelihood =  -2.897667e+001

       Parameter Estimates :
                            Q 0 = 0.000000E+000
                            Q 1 = 0.000000E+000
                            Q 2 = 0.000000E+000
                            Q 3 = 2.224776E-027
                            Z   = 1.000000E+001
                            T0  = 0.000000E+000   Set by User

      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal    with incidental
                                               tumors          tumors
         0                    50                0              0
        12.80                 50                0              0
        32                    49                0              0
        80                    50                0              3

                                       Exposure Pattern
             Model: Three Stage Weib   Age Begins: 0     Age Ends: 70
      Target Species: Human            Weeks/Year: 52   Days/Week:  7
             Route: Air                                 Hours/Day : 24

     Animal to human conversion method: PPM IN AIR
                          Human Equivalent Dose Estimates (ug/m^3)
                                95.00 %                          95.00 %
Incid Extra Risk  Time (yr)     Lower Bound          MLE         Upper Bound
     1.0000E-006      70.00   4.8038E-001      5.3398E+003     Not Reqstd
     1.0000E-005      70.00   4.8039E+000      1.1504E+004     Not Reqstd
     0.0001           70.00   4.8041E+001      2.4786E+004     Not Reqstd
     0.0010           70.00   4.8062E+002      5.3407E+004     Not Reqstd
     0.01             70.00   1.1261E+004      1.1524E+005     Not Reqstd
     0.10             70.00   7.8634E+004      2.5220E+005     Not Reqstd
```



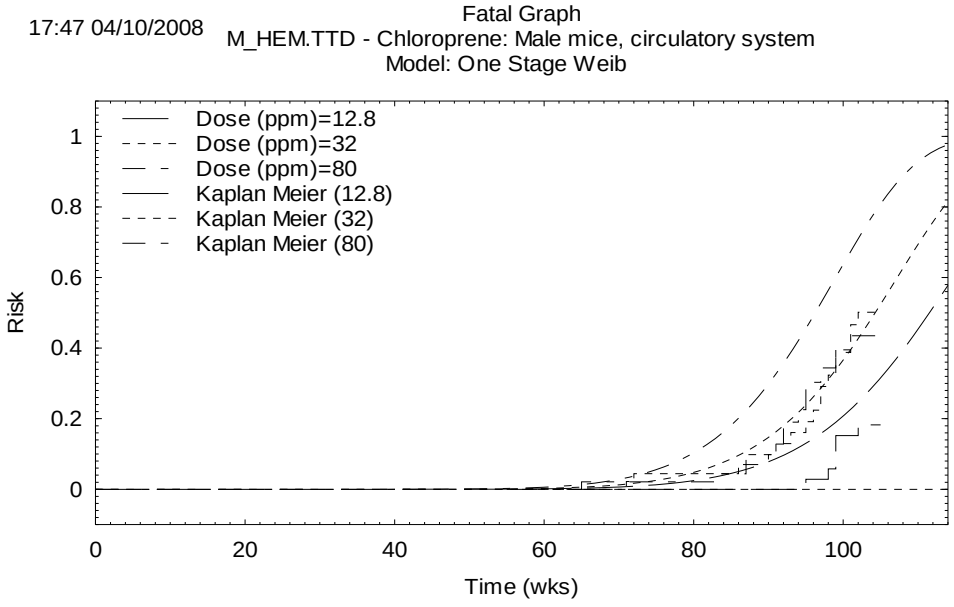


Figure C-10. Male mice, hemangiomas and hemangiosarcomas; hemangiosarcomas occurring before termination considered fatal. Details below.

```
            Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\M_HEM.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -2.022502e+002

       Parameter Estimates :
                            Q 0 = 8.379828E-022
                            Q 1 = 1.163863E-022
                            Z   = 1.000000E+001
                            T0  = 0.000000E+000   Set by User


      Avg. Doses         -------------------- Number --------------------
        (ppm)              of animals       with fatal    with incidental
                                              tumors          tumors
         0                    50                0              3
        12.80                 50                6              8
        32                    50               16              7
        80                    50               13              8


                                      Exposure Pattern
              Model: One Stage Weib   Age Begins: 0     Age Ends: 70
      Target Species: Human           Weeks/Year: 52   Days/Week:  7
              Route: Air                              Hours/Day : 24

     Animal to human conversion method: PPM IN AIR

                           Human Equivalent Dose Estimates (ug/m^3)
                                  95.00 %                          95.00 %
IncidExtra Risk  Time (yr)   Lower Bound          MLE          Upper Bound
     1.0000E-006      70.00   8.0694E-002      1.0423E-001     Not Reqstd
     1.0000E-005      70.00   8.0694E-001      1.0423E+000     Not Reqstd
     0.0001           70.00   8.0698E+000      1.0424E+001     Not Reqstd
     0.0010           70.00   8.0734E+001      1.0428E+002     Not Reqstd
     0.01             70.00   8.1100E+002      1.0475E+003     Not Reqstd
     0.10             70.00   8.5019E+003      1.0982E+004     Not Reqstd
```

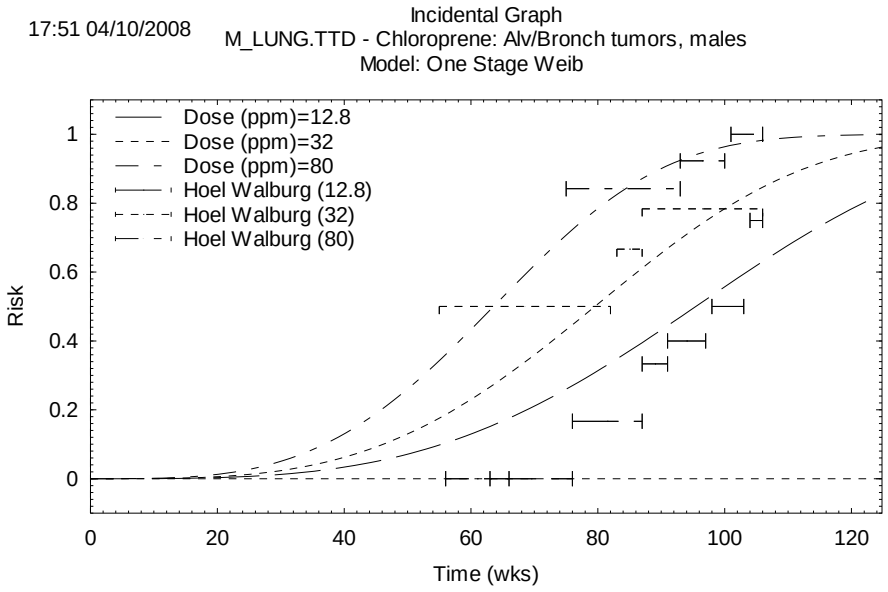


Figure C-11. Male mice, alveolar/bronchiolar tumors. Details below.

```
            Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\M_LUNG.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -1.049275e+002
  Parameter Estimates :
                             Q 0 = 4.009395E-008
                             Q 1 = 4.460491E-009
                             Z   = 3.461551E+000
                             T0  = 0.000000E+000   Set by User

      Avg. Doses         -------------------- Number --------------------
        (ppm)              of animals       with fatal    with incidental
                                              tumors          tumors
         0                    50                0             13
        12.80                 50                0             28
        32                    50                0             36
        80                    50                0             43
Generating Extrapolated Doses Table ---
TITLE: Chloroprene: Alv/Bronch tumors, males

             Dataset: M:\_ToxRiskData\Chloroprene\M_LUNG.TTD
                                          Exposure Pattern
               Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human              Weeks/Year: 52   Days/Week:  7
               Route: Air                                 Hours/Day : 24

     Animal to human conversion method: PPM IN AIR


                          Human Equivalent Dose Estimates (ug/m^3)
                                  95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound          MLE          Upper Bound
     1.0000E-006      70.00   6.2992E-002      8.6156E-002     Not Reqstd
     1.0000E-005      70.00   6.2992E-001      8.6157E-001     Not Reqstd
     0.0001           70.00   6.2995E+000      8.6160E+000     Not Reqstd
     0.0010           70.00   6.3024E+001      8.6199E+001     Not Reqstd
     0.01             70.00   6.3309E+002      8.6590E+002     Not Reqstd
     0.10             70.00   6.6369E+003      9.0774E+003     Not Reqstd
```

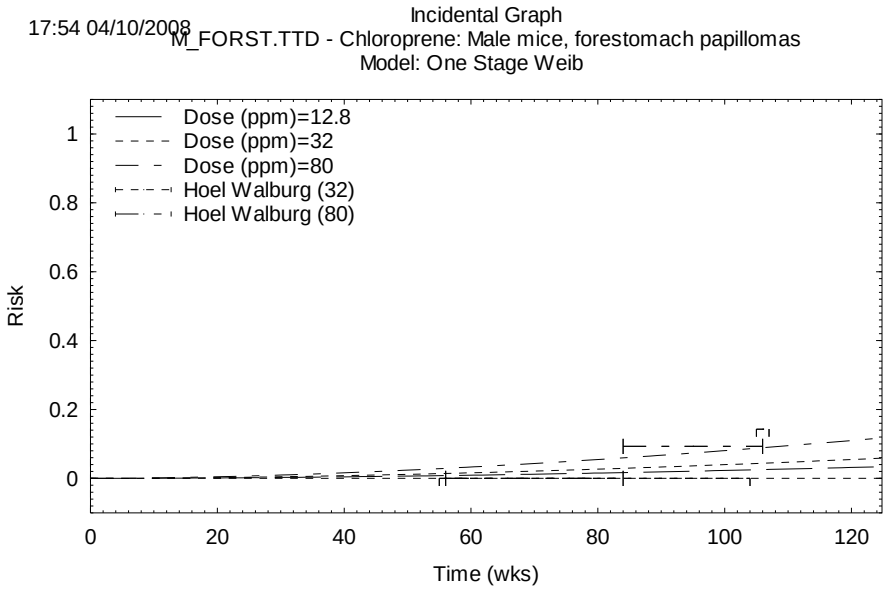


Figure C-12. Male mice, forestomach tumors. Details below.

```
            Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\M_FORST.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -2.831876e+001

       Parameter Estimates :
                              Q 0 = 3.027002E-006
                              Q 1 = 2.341463E-007
                              Z   = 1.793090E+000
                              T0  = 0.000000E+000   Set by User

      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal    with incidental
                                               tumors          tumors
         0                    50                0              1
        12.80                 50                0              0
        32                    50                0              2
        80                    50                0              4

                                          Exposure Pattern
              Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human             Weeks/Year: 52   Days/Week:  7
              Route: Air                                 Hours/Day : 24

     Animal to human conversion method: PPM IN AIR

                          Human Equivalent Dose Estimates (ug/m^3)
                              95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound          MLE         Upper Bound
     1.0000E-006      70.00   1.5961E+000      3.8065E+000     Not Reqstd
     1.0000E-005      70.00   1.5961E+001      3.8065E+001     Not Reqstd
     0.0001           70.00   1.5962E+002      3.8067E+002     Not Reqstd
     0.0010           70.00   1.5969E+003      3.8084E+003     Not Reqstd
     0.01             70.00   1.6041E+004      3.8256E+004     Not Reqstd
     0.10             70.00   1.6816E+005      4.0105E+005     Not Reqstd
```

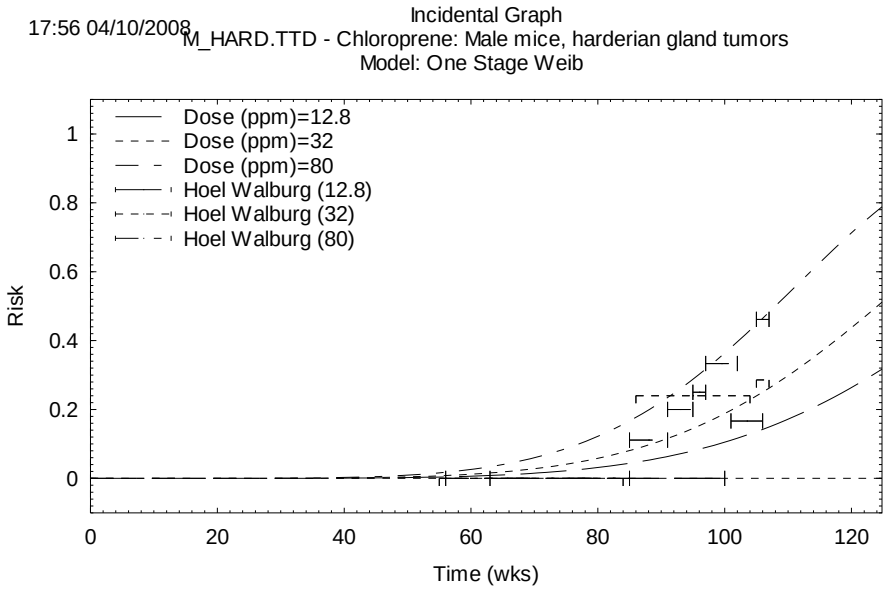


Figure C-13. Male mice, Harderian gland tumors. Details below.

```
Generating Model Fit Table ---
TITLE: Chloroprene: Male mice, Harderian gland tumors
              Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\M_HARD.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -7.366394e+001
 Parameter Estimates :
                              Q 0 = 3.258829E-013
                              Q 1 = 3.597993E-014
                              Z   = 5.574590E+000
                              T0  = 0.000000E+000   Set by User
      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal    with incidental
                                               tumors          tumors
          0                    50                0              2
        12.80                  50                0              5
        32                     50                0             10
        80                     50                0             12
Generating Extrapolated Doses Table ---
TITLE: Chloroprene: Male mice, Harderian gland tumors
             Dataset: M:\_ToxRiskData\Chloroprene\M_HARD.TTD
                                          Exposure Pattern
               Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human              Weeks/Year: 52   Days/Week:  7
               Route: Air                                 Hours/Day : 24
     Animal to human conversion method: PPM IN AIR

          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd   MLE = 5.9938E-003   Upper Bound(q1*) = 9.5529E-003

 Induction Time (T0) Set by User to 0

                         Human Equivalent Dose Estimates (ug/m^3)
                                95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound          MLE          Upper Bound
     1.0000E-006      70.00   3.6653E-001      5.8417E-001     Not Reqstd
     1.0000E-005      70.00   3.6653E+000      5.8417E+000     Not Reqstd
     0.0001           70.00   3.6655E+001      5.8420E+001     Not Reqstd
     0.0010           70.00   3.6671E+002      5.8446E+002     Not Reqstd
     0.01             70.00   3.6837E+003      5.8711E+003     Not Reqstd
     0.10             70.00   3.8618E+004      6.1548E+004     Not Reqstd
```

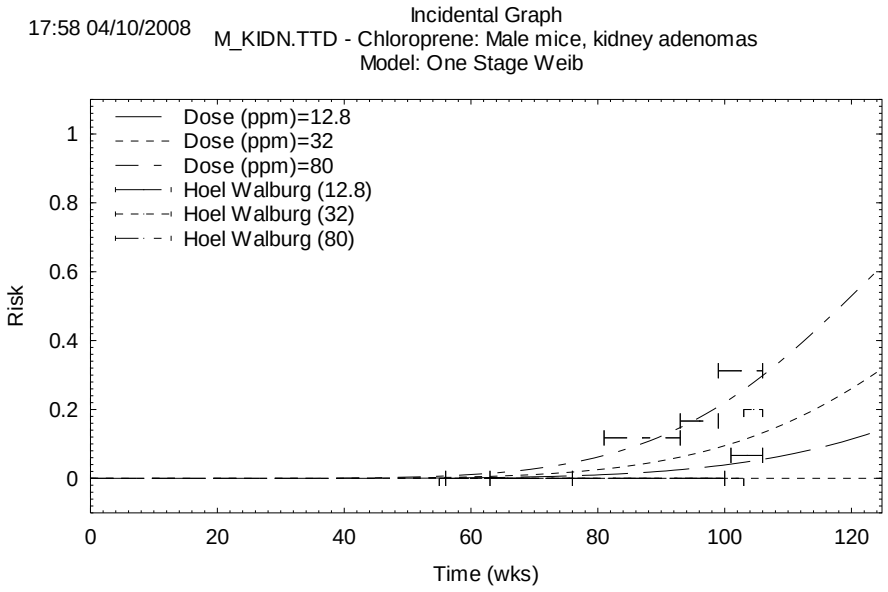


Figure C-14. Male mice, renal tubule tumors. Details below.

```
          Model: One Stage Weib         Dataset: M:\_ToxRiskData\Chloroprene\M_KIDN.TTD
Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
         Maximum Log-Likelihood =  -4.100330e+001
  Parameter Estimates :
                              Q 0 = 0.000000E+000
                              Q 1 = 2.031241E-015
                              Z   = 6.092308E+000
                              T0  = 0.000000E+000   Set by User

      Avg. Doses          -------------------- Number --------------------
        (ppm)               of animals       with fatal    with incidental
                                               tumors          tumors
         0                    50                 0              0
        12.80                 50                 0              2
        32                    50                 0              3
        80                    50                 0              9
                                              Exposure Pattern
              Model: One Stage Weib     Age Begins: 0     Age Ends: 70
      Target Species: Human             Weeks/Year: 52   Days/Week:  7
              Route: Air                                 Hours/Day : 24

     Animal to human conversion method: PPM IN AIR

                          Human Equivalent Dose Estimates (ug/m^3)
                                 95.00 %                          95.00 %
Incid Extra Risk  Time (yr)   Lower Bound         MLE          Upper Bound
     1.0000E-006      70.00   5.7583E-001      9.3451E-001     Not Reqstd
     1.0000E-005      70.00   5.7583E+000      9.3451E+000     Not Reqstd
     0.0001           70.00   5.7586E+001      9.3455E+001     Not Reqstd
     0.0010           70.00   5.7612E+002      9.3498E+002     Not Reqstd
     0.01             70.00   5.7873E+003      9.3921E+003     Not Reqstd
     0.10             70.00   6.0670E+004      9.8460E+004     Not Reqstd
```

Table C-1. Summary of human equivalent overall cancer risk values estimated by R/BMD_R, based on male and female mouse tumor incidence (NTP, 1998)

Tumor site	Assumed Dosimetry	Risk, R	BMD_R, µg/m^3	$BMDL_R$, µg/m^3	Unit Risk at BMD_R[a], per µg/m^3	Unit risk[b], per µg/m^3	SD	SD^2	Proportion of total variance	
Female Mice										
Lung	Systemic	0.01	3.22×10^2	4.03×10^2	3.10×10^{-5}	2.48×10^{-5}	3.76×10^{-6}	1.41×10^{-11}		0.55
	Portal-of-Entry	0.01	1.32×10^3	1.65×10^3	$7.57. \times 10^{-6}$	6.06×10^{-6}	9.17×10^{-7}	8.41×10^{-13}	0.07	
Skin	Systemic	0.01	2.43×10^3	3.73×10^3	4.12×10^{-6}	2.68×10^{-6}	8.74×10^{-7}	7.65×10^{-13}	0.06	0.03
All hemangiomas, hemangiosarcomas	Systemic	0.01	6.43×10^2	8.63×10^2	1.55×10^{-5}	1.16×10^{-5}	2.41×10^{-6}	5.80×10^{-12}	0.46	0.22
Mammary adenomas, carcinomas, adenocanthomas	Systemic	0.01	4.66×10^3	7.17×10^3	2.15×10^{-6}	1.39×10^{-6}	4.57×10^{-7}	2.09×10^{-13}	0.02	0.01
Hepatocellular tumors	Systemic	0.01	9.30×10^2	1.24×10^3	1.08×10^{-5}	8.04×10^{-6}	1.66×10^{-6}	2.74×10^{-12}	0.22	0.11
Forestomach	Systemic	0.01	5.79×10^3	1.88×10^4	1.73×10^{-6}	5.31×10^{-7}	7.27×10^{-7}	5.29×10^{-13}	0.04	0.02
Harderian gland	Systemic	0.01	4.59×10^3	5.77×10^3	2.18×10^{-6}	1.73×10^{-6}	2.71×10^{-7}	7.33×10^{-14}	0.01	0.00
Zymbal's gland	Systemic	0.001	4.81×10^2	5.34×10^4	2.08×10^{-6}	1.87×10^{-8}	1.25×10^{-6}	1.57×10^{-12}	0.13	0.06
Sum, MLE Cancer Risks[c]:					5.08×10^{-5}[c] 3.20×10^{-5}		Sum, SD^2:	2.58×10^{-11} 1.25×10^{-11}		
Upper Bound on Sum of MLE Risk Estimates[e]:						5.92×10^{-5} 3.79×10^{-5}	Overall SD[d]:	5.08×10^{-6} 3.54×10^{-6}		
Continuous Human Equivalent Overall Unit Risk[f]:						3.32×10^{-4} 2.13×10^{-4}				

Tumor site	Assumed Dosimetry	Risk, R	BMD_R, $\mu g/m^3$	$BMDL_R$, $\mu g/m^3$	Unit risk at BMD_R[a], per $\mu g/m^3$	Unit risk[b], per $\mu g/m^3$	SD	SD^2	Proportion of total variance	
Male Mice										
Lung	Systemic	0.01	6.33×10^2	8.66×10^2	1.58×10^{-5}	1.15×10^{-5}	2.58×10^{-6}	6.67×10^{-12}		0.66
	Portal-of-Entry	0.01	2.60×10^3	3.55×10^3	3.85×10^{-6}	2.82×10^{-6}	6.30×10^{-7}	3.96×10^{-13}	0.10	
All hemangiomas, hemangiosarcomas	Systemic	0.01	8.11×10^2	1.05×10^3	1.23×10^{-5}	9.55×10^{-6}	1.69×10^{-6}	2.86×10^{-12}	0.74	0.28
Forestomach	Systemic	0.01	1.60×10^4	3.83×10^4	6.23×10^{-7}	2.61×10^{-7}	2.20×10^{-7}	4.84×10^{-14}	0.01	0.00
Harderian gland	Systemic	0.01	3.68×10^3	5.87×10^3	2.71×10^{-6}	1.70×10^{-6}	6.15×10^{-7}	3.78×10^{-13}	0.10	0.04
Kidney	Systemic	0.01	5.79×10^3	9.39×10^3	1.73×10^{-6}	1.06×10^{-6}	4.03×10^{-7}	1.63×10^{-13}	0.04	0.02
Sum, MLE Cancer Risks[c]:					2.41×10^{-5} *1.54×10^{-5}*		Sum, SD^2:	1.01×10^{-11} *3.85×10^{-12}*		
Upper Bound on Sum of Risk Estimates[e]:						2.94×10^{-5} *1.86×10^{-5}*	Overall SD[d]:	3.18×10^{-6} *1.96×10^{-6}*		
Continuous Human Equivalent Overall Unit Risk[f]:						1.65×10^{-4} *1.05×10^{-4}*				

[a] R/BMD_R

[b] $R/BMDL_R$

[c] Summary statistics in italics were calculated using the "systemic" entries. The other summary statistics were calculated using the "portal-of-entry" estimate for lung tumors and all entries for the other tumor sites.

[d] Overall SD = $(\text{Sum, } SD^2)^{0.5}$

[e] Upper bound on the overall risk estimate = Sum of MLE cancer risks + 1.645 × Overall SD.

[f] Adjusted for continuous exposure by multiplying unit risk in previous line by 6/24 (hours) × 5/7 (days) = 5.6.