EPA/635/R-09/010D
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

CHLOROPRENE

(CAS No. 126-99-8)

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

July 2010

NOTICE

This document is an **Interagency Science Discussion/Final Agency Review draft**. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is a final draft for review purposes only and has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
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
ED10	effective dose assocated with 10% excess risk
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
OSHA	Occupational Safety and Health Administration
p	probability value
PBPK	physiologically based pharmacokinetic (model)

PCB	polychlorinated biphenyl
PEL	permissible exposure limit
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
TLV	threshold limit value
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).

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

J. Allen Davis M.S.P.H.
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

AUTHORS

J. Allen Davis, M.S.P.H.
Jeffrey Gift, Ph.D.
Karen Hogan, M.S.
Ines Pagan, D.V.M., Ph.D.
John Stanek, Ph.D.
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C.

J. Michael Wright, Ph.D.
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH

Aparna M. Koppikar, M.D., Ph.D.
E MAGE Inc.

CONTRACTOR SUPPORT

Karla Thrall, Ph.D.
Anthony Fristachi
Nick Heyer, Ph.D.
Paul Hinderliter, Ph.D.
Jessica Sanford, Ph.D.
Battelle Memorial Institute

REVIEWERS

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and has been peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A.

INTERNAL EPA REVIEWERS

Ila Cote, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Lynn Flowers, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Samantha Jones, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Connie Meacham, M.S.
National Center for Environmental Assessment
Office of Research and Development

John Vandenberg, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Debra Walsh, M.S.
National Center for Environmental Assessment
Office of Research and Development

Kate Guyton, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Jennifer Jinot, Ph.D.
National Center for Environmental Assessemnt
Office of Research and Development

EXTERNAL PEER REVIEWERS

Herman J Gibb, Ph.D., M.P.H.
Tetra Tech Sciences
Arlington, VA 22201

Dale Hattis, Ph.D.
Clark University
Worcester, MA 01610

Ronald L. Melnick, Ph.D.
Ron Melnick Consulting, LLC
Chapel Hill, NC 27514

John B. Morris, Ph.D.
University of Connecticut
Storrs, CT 06269

Avima M. Ruder, Ph.D.
National Institute for Occupational Safety and Health (NIOSH)
Cincinnati, OH 45226

Richard B. Schlesinger, Ph.D.
Pace University
Pleasantville, NY 10570

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, 1986, 001468), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986, 001466), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988, 064560), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991, 008567), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994, 076133), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994, 006488), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995, 005992), *Guidelines for*

Reproductive Toxicity Risk Assessment (U.S. EPA, 1996, 030019), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998, 030021), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000, 052149), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000, 052150), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000, 196144), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002, 088824), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005, 088823), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006, 194566), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006, 194567).

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 monomer (C_4H_5Cl) (hereafter referred to as chloroprene) is a volatile, flammable liquid used primarily in the manufacture of polychloroprene or neoprene rubber (U.S., 1989, 625024). Polychloroprene rubber is used to make diverse products, such as adhesives, automotive or industrial parts (e.g., belts/hoses/gaskets), coatings, and dipped goods. While 90% of chloroprene is used to make polychloroprene solid (trade names include neoprene, Bayprene, etc.), about 10% is converted to polychloroprene liquid dispersions, a colloidal suspension of polychloroprene in water (IARC, 1999, 201838). There was one commercial producer of chloroprene in the United States in 1995; chloroprene was produced by other plants for on-site use and processing, as a by-product of vinyl chloride production, or as an impurity in manufacturing processes (NTP, 2005, 093207). Chloroprene is used almost exclusively to produce polychloroprene, and is sold to only three U.S. companies for polychloroprene manufacture; less than 20 lb/yr is sold for research applications[1]. 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 produced from 1995 to 1996 was approximately 200 to 250 million lb (90,700 to 113,000 metric tons) (NTP, 2005, 093207)[2].

There are no known natural occurrences of chloroprene in the environment. The main sources of releases to the environment are or have been 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 totaling 983,888 lbs (NTP, 2005, 093207). Eight of these plants reported individual atmospheric releases from 2 to 481,871 lbs (NTP, 2005, 093207). Three plants in Kentucky, Texas, and Louisiana, each reporting atmospheric releases of > 100,000 lbs, accounted for most of the reported chloroprene releases in 1995[3]. One of these sites produced chloroprene, while the other two converted chloroprene to polychloroprene (NTP, 2005, 093207) The chemical structure of chloroprene is shown in Figure 2-1.

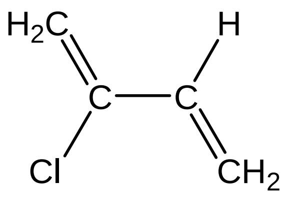


Figure 2-1. The chemical structure of chloroprene.

---

[1] Through the public comment process, DuPont Performance Elastomers provided updated manufacture, transportation, and emission data. In 2008, there was one commercial producer of chloroprene in the United States; this site both manufactured the the monomer and converted it to polymer. Chloroprene is used almost exclusively to produce polychloroprene, with chloroprene monomer sold to only one US company for non-polychloroprene manufacture (1000 lbs in 2008).

[2] According to DuPont's public comments, chloroprene production has decreased since 1996 and in 2008, US production volume was below 40,000 metric tons.

[3] According to DuPont's public comments, in 2008, only one chlorprene plant remained open and reported releases of 210,900 lbs. Domestic production and releases have been decreasing (reported 2002 emissions were 356,700 lbs).

The starting material for the synthesis of chloroprene is currently 1,3-butadiene in the United States (Lynch, 2001, 625182). Chloroprene manufacture using butadiene as the starting material occurs via a two step process consisting of chlorination and subsequent dehydrochlorination reactions. Initial industrial processes (1930s-70s) for chloroprene manufacture involved the dimerization of acetylene and then its hydrochlorination to produce chloroprene monomer. 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 (NLM, 2010, 594343). Chloroprene is volatile and highly reactive; it is not expected to bioaccumulate or persist in the environment (OECD, 1998, 624889). 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, 093207). 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, 625143). 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, 625149).

Of particular relevance to any toxicological studies involving chloroprene is its propensity to spontaneously oxidize and form dimers, peroxides, and other oxygenated species. Stabilizers or inhibitors must be added to prevent peroxide formation and consequent spontaneous polymerization; inhibitors do not reduce dimer formation. Uninhibited chloroprene must be stored under nitrogen at temperatures below 0ºC (e.g., –20ºC) to prevent spontaneous polymerization. If stored at room temperature, chemically uninhibited chloroprene will polymerize to form various byproducts such as cyclic dimers or open-chain polymers (Stewart, 1971, 010705; Trochimowicz et al., 1998, 625008). 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. The polymerization process has been discussed by Lynch (2001, 625182), Kroshwitz and Howe-Grant (1993, 010679), Stewart (1971, 010705), and Nystrom (1948, 003695). Additional information on production and use has been reported by the International Agency for Research on Cancer (IARC, 1999, 201838). Structures have been proposed for some of the chloroprene dimers (Stewart, 1971, 010705); 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; however, rapid volatilization into air may mitigate downward movement into soil. Breakdown via hydrolysis is not likely, as it is only partially soluble in water (OECD, 1998, 624889). 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 (log $K_{ow} = 2.2$). 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, 2001, 625182)[4]. 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 (2010, 594343)
Synonyms	1,3-butadiene, 2-chloro; chlorobutadiene, 2-chlorobutadiene; 2-chlorobutadiene-1,3; beta-chloroprene	NLM (2010, 594343)
Melting point	–130°C	NLM (2010, 594343)
Boiling point	59.4°C	NLM (2010, 594343)
Density	0.956 at 20°C (relative to the density of H_2O at 4°C)	NLM (2010, 594343)
Vapor pressure	215 mm Hg at 25°C	NLM (2010, 594343)
Vapor density	3.0 (air = 1)	NLM (2010, 594343)
Flashpoint (open cup)	–20 °C	NLM (2010, 594343)
Flammability limits	4–20% in air	NLM (2010, 594343)
Water solubility	256-480 mg/L at 20°C	OECD (1998, 624889)
Other solubilities	Miscible with ethyl ether, acetone, benzene; soluble in alcohol, diethyl ether	NLM (2010, 594343)
Log K_{OW}	2.2	OECD (1998, 624889)
Henry's law constant	5.6×10^{-2} atm/m^3-mol at 25°C	NLM (2010, 594343)
Odor threshold	15 ppm (54 mg/m^3)	U.S. EPA (2000, 625036)
Molecular weight	88.54	NLM (2010, 594343)
Conversion factors (in air)	1 mg/m^3 = 0.276 ppm; 1 ppm = 3.62 mg/m^3 at 25°C, 760 torr	NLM (2010, 594343)
Molecular formula	C_4H_5Cl	NLM (2010, 594343)

---

[4] According to DuPont's public comments, as of 2008, occupational exposure potential to chloroprene in the US is limited to one site in Louisiana; other chloroprene manufacturing facilities exist in Germany, France, Armenia/Azerbaijan, India, China, and Japan.

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 (Cottrell et al., 2001, 157445; Himmelstein et al., 2001, 019013; Himmelstein et al., 2001, 019012; Himmelstein et al., 2004, 625152; Munter et al., 2003, 625214; Munter et al., 2007, 576501; Munter et al., 2007, 625213; Summer and Greim, 1980, 064961). Hurst and Ali (2007, 625159) 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., 2004, 625152; Himmelstein et al., 2004, 625154). 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, 625192; NLM, 2010, 594343). 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, 042076).

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, 042076). Himmelstein et al. (2004, 625154) determined tissue-to-air 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, 063084). Briefly, gas-tight vials (10 ml) were prepared in triplicate as either reference vials or containing samples of blood, lung, liver, fat, muscle, or kidney. The vials were sealed and 100 ppm chloroprene was added after preheating to 37°C for 5 minutes. 100 µl samples were taken at 1.5, 3, and 4.5 hours from the start of incubation. For measurement of the human blood-to-air partition coefficient, blood samples were drawn from three healthy male subjects and analyzed in triplicate (Himmelstein et al., 2004, 625154). Results are 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 relatively low blood:air partition coefficients across species suggests that chloroprene would not likely be efficiently scrubbed in the upper airways. 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. (2004, 625154)

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 (Cottrell et al., 2001, 157445; Himmelstein et al., 2001, 019013; Himmelstein et al., 2001, 019012; Himmelstein et al., 2004, 625152; Munter et al., 2003, 625214; Munter et al., 2007, 576501; Munter et al., 2007, 625213; Summer and Greim, 1980, 064961). 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 that of vinyl chloride and vinylidene chloride (Haley, 1978, 010685). 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, 010689) showed that chloroprene was enzymatically converted into a reactive metabolite and postulated that this metabolite was probably an epoxide. This was based on the finding that 4-(4-nitrobenzyl)pyridine trapped a volatile metabolite produced during reaction of mouse liver microsomes with chloroprene. The authors proposed that the epoxidation of the carbon double bonds in chloroprene yields one of two isomeric oxiranes (or both): 2-chloro-2-ethynyloxirane and/or (1-chloroethenyl)oxirane. A report by Himmelstein et al. (2001, 019012) 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, 010689). Microsomal suspensions were isolated through differential centrifugation of livers pooled from male B6C3F1 mice, Fisher and Wistar rats, and Syrian hamsters.

Human liver microsomal suspensions were prepared from a mixed pool of 15 different individuals. Chloroprene (800 ppm) was incubated with the microsomal suspensions (1 mg) in sealed vials for all species. Incubations were stopped after 30 minutes by the addition of cold diethyl ether containing 1-butanol as an internal standard and analyzed using gas chromatography mass spectroscopy. Himmelstein et al. (2001, 019012) reported that incubation of chloroprene with liver microsomes of all species resulted in an apparent spectrographic peak that was consistent with (1-chloroethenyl)oxirane (based on comparison to synthesized (1-chloroethenyl)oxirane standard). Comparisons of the amount of (1-chloroethenyl)oxirane to the amount of the 1-butanol standard indicated that a greater amount of (1-chloroethenyl)oxirane was present in B6C3F1 mice and F344 rat liver microsomes, followed by the Wistar rat, humans, and hamsters (Table 3-2). Additional time course experiments showed that the decline of chloroprene (from 3 to 0.1 µM between 5 – 10 minutes after start of incubation with [0.05 µM]100 ppm chloroprene) from the headspace of mouse liver microsomes coincided with an increase of (1-chloroethenyl)oxirane (0.01 – 0.02 µM). Metabolism of chloroprene into (1-chloroethenyl)oxirane most likely involved CYP 2E1, as evidenced by nearly complete *in vitro* inhibition with 4-methylpyrazole hydrochloride.

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

METABOLITE PEAK[a]	LIVER MICROSOMAL SUSPENSION				
	B6C3F1 mouse[b]	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.
[b] One vial was used for each species

Source: Himmelstein et al. (2001, 019012)

Further metabolism of (1-chloroethenyl)oxirane was observed in time-course evaluations with liver microsomes (Himmelstein et al., 2001, 019012). *In vitro* uptake of (1chloroethenyl)oxirane from vial headspace of liver microsomes was observed, with preliminary results indicating that the ranking of (1-chloroethenyl)oxirane hydrolysis in liver microsomes was as follows: hamsters ~ humans > Wistar rats > B6C3F1 mice and F344 rats. The uptake of (1-chloroethenyl)oxirane was attributable to either epoxide hydrolase-mediated hydrolysis or further oxidative metabolism. Time course experiments demonstrated the uptake of (1-chloroethenyl)oxirane from hepatic cytosol from mice, rats, or hamsters. Uptake was absent in boiled cytosol, or glutathione depleted cytosol, indicating that conjugation of (1-chloroethenyl)oxirane to glutathione was enzyme-dependent. The relative activity of glutathione conjugation was as follows: hamsters > rats > mice (human cytosol was not evaluated).

Studies by Cottrell et al. (2001, 157445) are in agreement with reports from Himmelstein et al. (2001, 019013; 2001, 019012) 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.

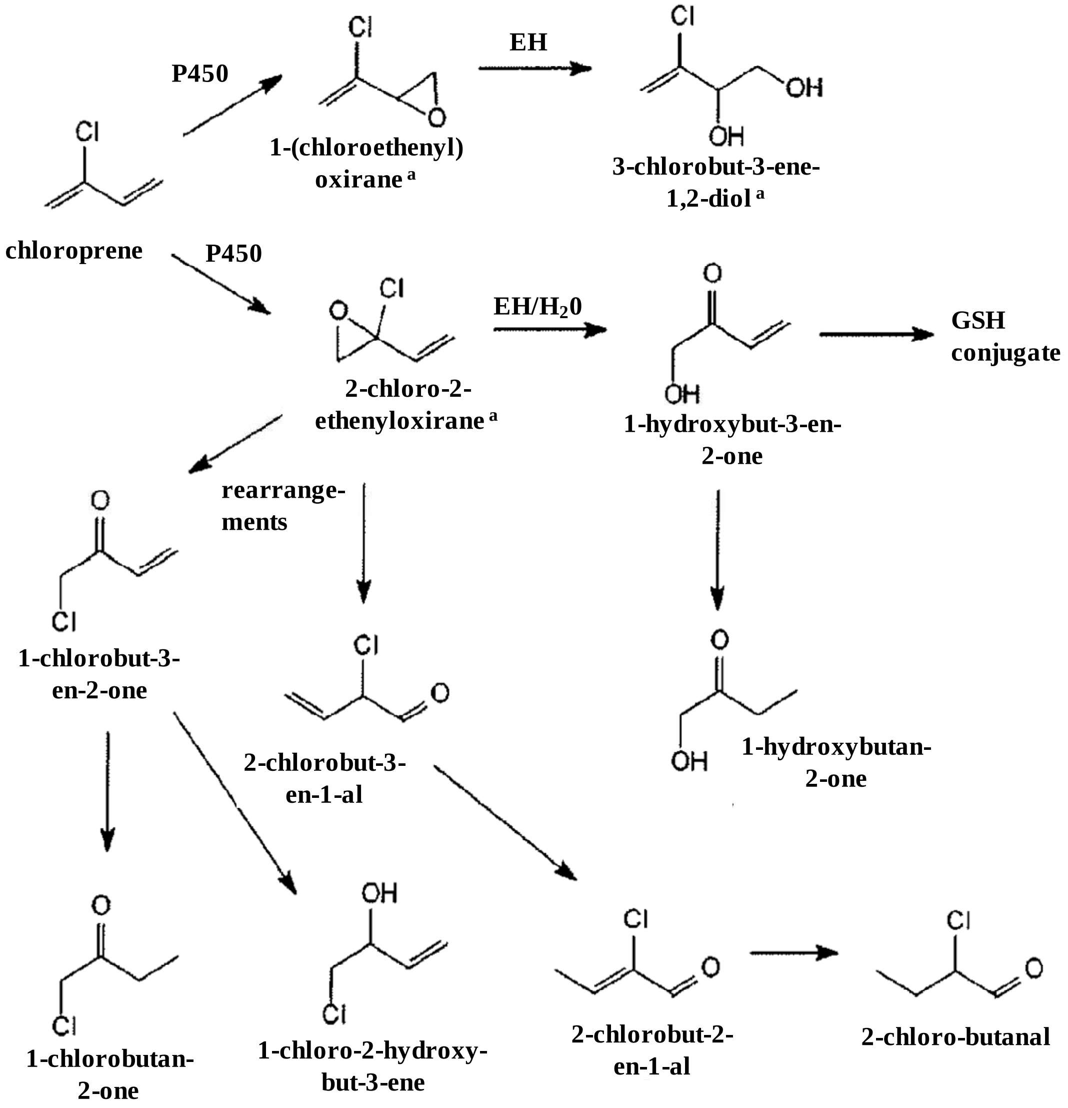


Figure 3-1. Proposed metabolism of chloroprene.

[a] R- and S- enantiomers

Source: Adapted from Cottrell et al. (2001, 157445).

Comparing metabolism between species, Cottrell et al. (2001, 157445) observed that qualitative profiles of metabolites from liver microsomes obtained from B6C3F1 mice, Sprague-Dawley or F344 rats, and humans were similar. Microsomal suspensions were prepared by differential centrifugation from livers pooled from male and female B6C3F1 mice and Sprague-Dawley rats. Human liver microsomal suspensions were prepared from a mixed pool of 15 different individuals. 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 P-450 mediated formation of R- and S-enantiomers of (1-chloroethenyl)oxirane in the presence of a epoxide hydrolase inhibitor (cyclohexene oxide) (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. Both female and male B6C3F1 mice and humans showed slight enantioselectivity in metabolism to the S-enantiomer. In incubations without an inhibitor of epoxide hydrolase present, (1-chloroethenyl)oxirane was not detected as a metabolite. Instead, 3-chlorobut-3-ene-1,2-diol was observed, indicating that epoxide hydrolase is effective in the detoxification of the epoxide metabolite of chloroprene. In incubations supplemented with an epoxide hydrolase inhibitor and glutathione, there was no change in the observed levels of (1-chloroethenyl)oxirane, suggesting that conjugation with glutathione may not be an active detoxification pathway for the active epoxide metabolite of chloroprene. Glutahione conjugation was apparent with 1–hydroxybut-3-en-2-one, the downstream product of the minor epoxide metabolite of chloroprene, 2-chloro-2-ethenyloxirane.

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,b]	**% R**	**% S**	**Chloroprene (mM)**	**Species/strain**[a,b]	**% 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, 157445)

A further study by this group (Munter et al., 2003, 625214) verified significant differences between species in the amounts of R- and S-enantiomers of (1-chloroethenyl)oxirane formed in liver microsomes from rats, mice, or humans without epoxide hydrolase inhibitor present. Microsomal samples were prepared in the same manner as for Cottrell et al. (2001, 157445). After incubation with 10 µM chloroprene, the relative ratio of the R-enatiomer of (1-chloroethenyl)oxirane formed in mice, rat, or human microsomes was 20:4:1. This ratio was also observed in incubations with 100 µM and 10 mM chloroprene. For the S-enantiomer, the presence of (1-chloroethenyl)oxirane was detected in only mouse microsomes after incubations with 10 µM chloroprene. After incubations with 100 µM chloroprene, S-(1-chloroethenyl)oxirane was detected in rat microsomes, but at levels approximately 10-fold less than observed in mouse microsomes. The formation of S-(1-chloroethenyl)oxirane was not observed in human microsomes at any incubation concentration. Therefore, in the presence of epoxide hydrolase, microsomal oxidation of chloroprene to (1-chloroethenyl)oxirane was most effective in the mouse, and epoxide hydrolase preferentially hydrolyzed the S-enantiomer of (1-

chloroethenyl)oxirane, leading to an accumulation of the R-enantiomer. Levels of detected 3-chlorobut-3-ene-1,2-diol were highest in mouse microsomes compared to rats or humans (which had similar levels). Additional experiments identified 3 conjugates when racemic (1-chloroethenyl)oxirane was incubated with glutathione at 37°C in an aqueous phosphate buffer solution, but further indicated that (1-chloroethenyl)oxirane either did not react with glutathione or did so very slowly in microsomal incubations with chloroprene. Addition of liver cytosol (containing glutathione transferase) only marginally affected the formation of glutathione conjugates. Downstream metabolites formed from the minor epoxide metabolite, 2-chloro-2-ethenyloxirane, were shown to rapidly react with glutathione even in the absence of glutathione transferase. At all concentrations of chloroprene, the total amount of glutathione-conjugated metabolites formed in liver microsomes was highest for the mouse, followed by the rat, and then humans.

Hurst and Ali (2007, 625159) evaluated the kinetics of R- and S-enantiomers of (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, which indicates that rapid removal is dependent on cellular glutathione. The study by Hurst and Ali (2007, 625159) suggested that the R-enantiomer of (1-chloroethenyl)oxirane is potentially more toxic because of slower detoxification.

Summer and Greim (1980, 064961) reported that *in vitro* incubation of hepatocytes isolated from male Wistar rats with chloroprene decreased cellular glutathione levels to approximately 50% that of controls after 15 minutes of exposure to 3mM chloroprene. This effect was dose-dependent and was observed with exposures to 0.5 and 1.0 mM as well. The limited *in vivo* rodent studies support the postulated metabolic pathway for chloroprene. In male Wistar rats (four per experiment) exposed orally to either 100 or 200 mg/kg chloroprene via gavage (Summer and Greim, 1980, 064961), hepatic glutathione levels fell to 55 and 39% that of controls three hours after exposure, respectively. These results indicate that glutathione conjugation plays an active role in the detoxification of chloroprene. 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. (2004, 625152) investigated the *in vitro* metabolism of chloroprene in mouse, rat, hamster, and human liver and lung microsomes. Rodent microsomes and cytosol were prepared from pooled liver and lungs using differential centrifugation. Human microsomes and cytosol were prepared from pooled individuals as follows: pooled liver microsomes from 15 individuals for experiments involving hydrolysis of (1-chloroethenyl)oxirane, pooled liver microsomes from 10 individuals for simultaneous measurement of chloroprene and (1-chloroethenyl)oxirane, pooled lung microsomes from 5 individuals, pooled liver cytosol from 15 individuals, and lung cytosol from 1 individual. Experiments investigating the microsomal metabolism of chloroprene or (1-chloroethenyl)oxirane were conducted in closed vials and headspace samples were analyzed using gas chromatography. A two-compartment closed vial model was developed to describe both chloroprene and (1-chloroethenyl)oxirane metabolism in the rodent and human liver and lung microsomes. Liquid-to-air partition coefficients measured in Himmlestein et al. (2001, 019012) (0.69 ± 0.05 for chloroprene and 57.9 ± 1.6 for (1-chloroethenyl)oxirane) were used to calculate liquid phase concentrations for modeling purposes.

Chloroprene oxidation in liver microsomes for all species was described as a saturable Michaelis-Menten mechanism. In liver microsomes, the rate (as expressed by V_{max}/K_m, ml/h/mg protein) of chloroprene oxidation was faster in the mouse and hamster than in rats or humans (Table 3-4). Chloroprene oxidation in mouse lung microsomes was also saturable, and oxidation appeared saturated at all doses in hamsters, rats, and humans; the rate was optimized as V_{max}/K_m rather than individual measurements of V_{max} or K_m for these species (see Table 3-4). Chloroprene oxidation in lung microsomes was much greater (approximately 50-fold) for mice compared with the other species. Microsomal hydrolysis of (1-chloroethenyl)oxirane also operated via saturable Michaelis-Menten mechanics, especially in human and hamster liver and lung microsomes (see Table 3-5). Hydrolysis (V_{max}/K_m) of (1-chloroethenyl)oxirane in liver and lung microsomes was fastest for humans, followed by hamsters, rats, and mice..

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

TISSUE	SPECIES	ACTIVITY OF MICROSOMAL OXIDATION		
		V_{max}[a]	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[b]
	Wistar rat	--	--	1.3[b]
	Hamster	--	--	1.3[b]
	Human	--	--	1.3[b]

[a] Values derived from modeling of vial headspace concentration time-course data (using liquid-to-air partition coefficient = 0.69) (Himmelstein et al., 2001, 019012).V_{max} , µmol/h/mg protein, K_m, µmol/l, V_{max}/K_m, ml/h/mg protein

[b] 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. (2004, 625152)

.

Table 3-5. Kinetic parameters used to describe the microsomal epoxide hydrolase activity of (1-chloroethenyl)oxirane

TISSUE	SPECIES	ACTIVITY OF MICROSOMAL EPOXIDE HYDROLASE		
		V_{max}[a]	K_m	V_{max}/K_m
Liver	Mouse	0.14	20.9	6.7
	F344 rat	0.60	41.5	14.5
	Wistar rat	0.64	53.0	12.1
	Hamster	2.49	73.8	33.7
	Human	3.66	99.7	36.7
Lung	Mouse	0.11	51.5	2.1
	F344 rat	0.12	90.9	1.3
	Wistar rat	0.16	91.6	1.7
	Hamster	1.34	187.6	7.1
	Human	0.58	72.2	8.0

[a] Values derived from modeling of vial headspace concentratioin time-course data (using liquid-to-air partition coefficient = 57.9) (Himmelstein et al., 2001, 019012). V_{max}, µmol/h/mg protein, K_m, µmol/l, V_{max}/K_m, ml/h/mg protein

Source: Himmelstein et al. (2004, 625152)

Further hydrolysis experiments, conducted in the presence or absence of $NADP^+$, demonstrated oxidation of (1-chloroethenyl)oxirane in mouse liver microsomes, but not in human, rat, or hamster liver microsomes. When experiments were carried out in the presence of $NADP^+$, pretreatment of mouse microsomal preperations with 4-methylpyrazole (4-MP) or 1-aminobenzotriazole (ABT), both inhibitors of P450 monooxygenase, did not affect hydrolysis but completely inhibited oxidation. Results were similar when experiments were carried out in the absence of $NADP^+$. Although oxidation of (1-chloroethenyl)oxirane could potentially produce diepoxides, only 3-chloro-3-butene-1,2,-diol was detected, in agreement with Cottrell et al. (2001, 157445). The potential for (1-chloroethenyl)oxirane oxidation was not evaluated in lung microsomes.

The cytochrome P450 dependent oxidation of chloroprene in both liver and lung microsomes coincided with an increase in (1chloroethenyl)oxirane in the vial headspace. Peak concentrations of (1chloroethenyl)oxirane ranged from 0.01 to 0.1 nmol/ml for liver microsomes, and the greatest concentration (0.1 nmol/ml) was observed in the mouse due to the faster rate of chloroprene oxidation compared to the rat, hamster, or human. The chloroprene-dependent formation of (1-chloroethenyl)oxirane was apparent in mouse lung microsomes with headspace concentrations approximate to mouse liver microsomes. (1-chloroethenyl)oxirane was detected in rat and hamster lung microsomes despite lower levels of chloroprene oxidation compared to mice. Only one detectable value of (1-chlororethenyl)oxirane was recorded in human lung microsomes due to the high activity of epoxide hydrolase. A satisfactory model fit to (1-chloroethenyl)oxirane formation was obtained when the oxidative metabolism of chloroprene was split into (1-chloroethenyl)oxirane and other uncharacterized metabolites, and then the measured epoxide hydrolase kinetics were applied.

Formation of (1-chloroethenyl)oxirane was best modeled as making up only 2-5% of total oxidation of chloroprene in the liver across all species (see Table 3-6). Similar adjustment in lung microsomes indicated that formation of (1-chlororethenyl)oxirane accounted for 3-22% of total chloroprene metabolism in rodents, although the adjustment was less robust than for the liver due to limited time course data. The value of 78% total metabolism for human lung microsomes was most likely an overestimate due to the rapid removal of (1-chloroethenyl)oxirane by epoxide hydrolase. In the lung, the rate of (1-chloroethenyl)oxirane formation appeared to be 10-fold greater in mice compared to rats, and 2-fold greater compared to humans.

Table 3-6. Kinetic parameters used to describe the time course of (1-chloroethenyl)oxirane formation from microsomal oxidation of chloroprene

TISSUE	SPECIES	(1-CHLOROETHENYL)OXIRANE FORMATION			
		V_{max}[a]	K_m	V_{max}/K_m	Ratio of (1-chloroethenyl)oxirane/ total (%)[b]
Liver	Mouse	0.149	36.6	4.1	2
	F344 rat	0.184	23.7	7.8	5
	Wistar rat	0.148	25.3	5.8	5
	Hamster	0.048	9.0	5.4	2
	Human	0.108	20.7	5.2	5
Lung	Mouse	0.050	25.0	2.0	3
	F344 rat	0.0075	40.4	0.19	15
	Wistar rat	0.0082	30.1	0.27	22
	Hamster	0.013	81.2	0.16	13
	Human	0.024	24.6	0.98	78

[a] Optimized oxidative rate constants used to describe the amount of (1-chloroethenyl)oxirane derived from total chloroprene oxidation. V_{max}, µmol/h/mg protein, K_m, µmol/l, V_{max}/K_m, ml/h/mg protein

[b] V_{max}/K_m for (1-chlororethenyl)oxirane formation divided by the V_{max}/K_m for total chloroprene oxidation (from Table 3-4) multipled by 100

Source: Himmelstein et al. (2004, 625152)

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 0.0016–0.0130 hour/mg cytosolic protein in liver and 0.00056–0.0022 hour/mg in lung. In the liver the rates were as follows: hamster > Fischer rat ≈ Wistar rat > mouse > human. In the lung cytosol the rates were as follows: mouse > Fischer rat > human > Wistar rat > hamster. The half-life of the spontaneous first-order reaction between (1-chloroethenyl)oxirane and glutathione was approximately 10 hours.

Table 3-7. Kinetic parameters used to describe the cytosolic glutathione S-transferase activity towards (1-chloroethenyl)oxirane

TISSUE	SPECIES	ACTIVITY OF CYTOSOLIC GLUTATHIONE *S*-TRANSFERASE[a]		
		ks	$C^{BS(0)}$	ks × $C^{BS(0)}$
Liver	Mouse	0.0015	2.7	0.0040
	F344 rat	0.0074	0.92	0.0068
	Wistar rat	0.011	0.56	0.0063
	Hamster	0.024	0.54	0.0130
	Human	0.0017	0.94	0.0016
Lung	Mouse	0.0011	2.01	0.0022
	F344 rat	0.0023	0.70	0.0016
	Wistar rat	0.0051	0.18	0.00092
	Hamster	0.015	0.038	0.00056
	Human	0.0028	0.44	0.0012

Note: ks (1/µmol/h/mg cytosolic protein), rate constant $C^{BS(0)}$ (µmol/l) as initial concentration of protein binding sites and ks × $C^{BS(0)}$ (h/mg protein) describing enzymatic (1-chloroethenyl)oxirane-glutathione conjugate formation as a pseudo-second order reaction

[a] First order reaction of (1-chloroethenyl)oxirane with glutathione was measured as kf = 0.07 h^{-1} independent of protein

Source: Himmelstein et al. (2004, 625152)

Himmelstein et al. (2004, 625154) conducted closed-chamber gas uptake exposures to evaluate chloroprene metabolism rates in rats (Wistar and F344), mice (B6C3F1), and hamsters (Syrian Golden). The first exposure scenario investigated chemical distribution with or without metabolic inhibition with 4-methyl pyrazole. Exposure concentrations ranged from 160–240 parts per million (ppm) chloroprene. Animals (Wistar and F344 rats and B6C3F1 mice, n = 3) were placed in the exposure chamber 30 minutes prior to exposure. The chamber atmosphere was circulated through the system at 2 L/min and chloroprene concentrations were analyzed by gas chromatography flame ionization detection for up to six hours. The second exposure scenario measured the uptake of chloroprene over a range of starting concentrations. Only one rat was used per exposure chamber at one time and hamsters were substituted for Wistar rats. A known volume of concentrated chloroprene was added to the chamber at the start of the exposures, with starting concentrations ranging from 2 to 400 ppm for mice and rats and 10 to 270 ppm for hamsters. 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., 2004, 625152). The *in vitro* scaling of total chloroprene metabolism (Table 3-7) was sufficient to explain the *in vivo* gas uptake data. Inhibition of uptake was obtained with pre-treatment with 4-methyl pyrazole, indicating the the loss of chamber chloroprene was due to metabolic oxidation via P-450 monooxygenases. Setting V_{max} to zero for liver and lung metabolism allowed the PBPK model to obtain sufficient fit to the observed inhibition data.

Table 3-8. Metabolic parameters of chloroprene

BIOCHEMICAL PARAMETERS[a]		SPECIES			
		Mouse	F344 rat	Wistar rat	Hamster
Liver	V_{max} (mg/h/kg BW)	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/h/kg BW)	431.0	244.0	208.0	363.0
Lung	V_{max} (mg/h/kg BW)	1.02	---	---	---
	K_m (mg/L)	0.13	---	---	---
	V_{max}/K_m (L/h/kg BW)	7.67	0.14	0.14	0.14

[a] Scaled from Himmelstein et al. (2004, 625152) using microsomal protein content.

Source: Himmelstein et al. (2004, 625154)

3.4. ELIMINATION

Limited information is available regarding the elimination of chloroprene in rodents. Summer and Greim (1980, 064961) exposed male Wistar rats (four per experiment) to 100 or 200 mg/kg chloroprene by gavage and observed a dose-dependent, nonlinear increase in excreted urinary thioethers (presumably glutathione conjugates and mercaptic acids). This increase in urinary thioesters was reversible and levels of urinary thioesters returned to control levels within 24 hours, indicating that elimination was rapid. At higher concentrations of chloroprene, a decline in the excretion rate of urinary thioesters was observed

Consideration of physiological and biological factors suggests there may exist differences in chloroprene clearance across species. For example, while the fat:air partition coefficient is similar for all species investigated (see Table 3-1), humans have a much greater amount of fat as a percentage of body weight compared to rodents. This may mean that a greater total amount of chloroprene partitions into the fat of humans thereby increasing the time necessary to eliminate chloroprene from the body for humans. Also, it has been shown that metabolic oxidation and hydrolysis rates vary substantially across species. These differences in enzyme activity may lead to differences in chloroprene body burdens and elimantion profiles.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Himmelstein et al. (2004, 625154) published a physiologically based toxicokinetic (PBPK) model of chloroprene to describe gas uptake data and calculate internal dose metrics for use in dose-response analyses. Construction of the mathematical model was based on physicochemical, physiological, and metabolic parameters for chloroprene from mouse, rat, hamster, and humans (Table 3-9). The model consisted of distinct compartments for liver and lung, as well as lumped compartments for fat and slowly and rapidly perfused tissues. Individual tissues were modeled as homogenous, well-mixed compartments connected by systemic circulation. Metabolism of chloroprene was localized to the lung and liver compartments and described by Michaelis-Menten type saturable kinetics. Standard physiological values were used to parameterize the model. Tissue-to-blood partition coefficients were calculated from tissue-to-air values and the *in vivo* metabolic parameters (see Table 3-7) were scaled from *in vitro* metabolic parameters for total chloroprene metabolism in the liver and lung (Himmelstein et al., 2004, 625152) using microsomal protein content. Microsomal protein contents for the liver differ among species and were obtained from the literature. The microsomal protein content for the lungs was set as equal for all species. Gas uptake was modeled by subtracting the amount taken up by the animal from the chloroprene concentration in the chamber. Physiological and metabolic parameters were not adjusted except for alveolar ventilation and cardiac output as need to obtain adequate model fit to the gas uptake data.

Table 3-9. Physiological parameters used for chloroprene PBPK modeling

PHYSIOLOGICAL PARAMETERS	**SPECIES**				
	Mouse	**F344 rat**	**Wistar rat**	**Hamster**	**Human**
Values for dose reponse modeling [a,b]					
Body weight (kg)	0.03	0.25	0.25	0.11	70
Ventilation (L/h/kg)	30	21	21	30	16.2
Cardiac output (L/h/kg)	30	18	18	30	16.2
Values for simulation of chamber gas uptake[c]					
Body weight (kg)	0.024–0.034	0.16–0.28	0.20–0.34	0.10–0.18	NA
Ventilation (L/h/kg)	15	10.5	10.5	12	NA
Cardiac output (L/h/kg)	15	9	9	12	NA
Tissue volumes (% body weight)[a,d]					
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)[a,d]					
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

[a] Parameters for mouse, rats, and humans drawn from the literature. Hamster ventilation, cardiac output, tissue volume, and blood flow values were based on the mouse and rat.

[b] Values used for the dose-response modeling based on average body weight data from chronic inhalation studies and ssumption that literature values for ventilation and cardiac output are representative of repear inhalation exposure conditions

[c] Values used specifically for simulation of closed chamber gas uptake data

[d] Tissue volumes and blood flows are calculated by the model with resulting units of liters (L) and L/h, respectively

Source: Himmelstein et al. (2004, 625154)

Although the model was used to estimate the chloroprene concentration in each of the defined compartments (including blood), 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 metabolic inhibition was obtained by setting V_{max} to zero for both liver and lung metabolism. Model simulations demonstrated good agreement with chamber uptake data for a wider range of starting chloroprene concentrations for mice, rats, and hamsters. Scaling of *in vitro* metabolic parameters was sufficient to explain the *in vivo* gas uptake data. The alveolar ventilation and cardiac output values used to simulate the chamber gas uptake data were lower than the standard values used in the dose-response modeling. Justification for application of lower alveolar ventilation and cardiac output values for the gas uptake simulations included decreased ventilation due to sensory irritation and anesthetic effects. The decision to use standard values as reported in the literature for the dose-response modeling was that these values more likely represent bioassay conditions involving chronic, whole-body exposures. Use of a model-calculated internal dose metric (total chloroprene metabolism/g lung tissue/day) was used in a dose-response analysis of bronchiolar adenoma/carcinoma in male rodents (NTP, 1998, 042076; Trochimowicz et al., 1998, 625008), and was found to fit the incidence data much better than the external dose metric. Lastly, the model was used to calculate exposure concentrations for humans that would result in internal doses equivalent to the internal dose calculated from the dose-response analysis in rodents.

DeWoskin (2007, 202141) reviewed the chloroprene PBPK model and suggested the following potential applications of the model for developing an IRIS assessment:

1. Correlate parent compound concentration or total amount metabolized with cancer and non cancer endpoints in order to determine the relevant mode(s) of action.
2. Investigate observed species differences in the external dose-response relationship
3. Estimate the human dose-response based on the most relevant internal dose metric for the proposed mode of action
4. Use PBPK model parameter distributions to represent variability in intra-population rates of chemical absorption, distribution, metabolism, and elimination in order to estimate human variability.

Himmelstein et al. (2004, 625154) addressed the first three of these suggestions in the application the the PBPK model. DeWoskin (2007, 202141) also notes that in order for a PBPK model to be applied in the IRIS process, it must be reviewed in detail in regard to the scientific assumptions used in its construction and application. Currently, the Himmelstein et al. (2004, 625154) model has a number of limitations. The model currently predicts blood chloroprene and delivery of chloroprene to metabolizing tissues based on metabolic constants and partition coefficients based on *in vitro* data. Loss of chamber chloroprene is attributed to uptake and metabolism by test animals and was used to test the metabolic parameters and validate the model. However, Himmelstein et al. (2004, 625154) did not provide results of sensitivity analyses indicating whether chamber loss was sensitive to metabolism, and therefore it is uncertain whether chamber loss is useful for testing the metabolic parameters used in the model.   Also, the chamber data were fit by varying alveolar ventilation and cardiac output. This method does not result in adequate testing of the model and does not validate the scaled *in vitro* metabolic parameters. Additionally, there are currently no blood or tissue time-course concentration data available for model validation. Therefore, as the model is currently constructed, the PBPK model for chloroprene is inadequate for application for calculation of internal dose metrics or interspecies dosimetry extrapolations.

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 published from 1978 to 2008.

4.1.1. Chloroprene Exposure and Cancer Effects

4.1.1.1. Overview

The NTP (1998, 042076; 2005, 093207) 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, 064957) and Li et al. (1989, 625181). Rice and Boffetta (2001, 624894) briefly examined evidence from five epidemiologic studies (Pell, 1978, 064957; Li et al., 1989, 625181; Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Colonna and Laydevant, 2001, 625112). 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, 624894). To date, there have been nine occupational epidemiological studies conducted covering eight cohorts. It is important to note that where different studies investigated the same cohort (as with Leet and Selevan (1982, 094970); and Marsh et al. (2007, 625188), which investigated the Louisville Works cohort), differences in cohort recruitment, follow-up time, and exposure ascertainment were deemed sufficient to present those study findings independently. This epidemiological database is reviewed in the following section.

4.1.1.2. Individual Occupational Studies

Pell (1978, 064957) 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 either internal (DuPont) or 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, 094970). 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 the 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, 064957) study and the NIOSH reanalysis (Leet and Selevan, 1982, 094970) include absence of quantitative exposure information and a lack of data on smoking history and other potential risk factors which precluded further consideration. 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, 094970), 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])	LOW-EXPOSURE CASES, SMR (95% CI)	HIGH-EXPOSURE CASES, SMR (95% CI)
All Causes	193, 79 (68–91)	102, 82 (67–100)	91, 75 (61–92)
All Cancers	51, 107 (80–141)	26, 107 (70–157)	25, 107 (69–158)
Digestive	19, 145 (87–227)	11, 164 (82–294)	8, 125 (54–246)
Biliary/liver	4, 571 (156–1463)	1, 250 (6–1393)	3, 750 (155–2192)
Trachea, bronchus, lung	17, 106 (62–170)	7, 86 (35–178)	10, 128 (61–236)
Lymphatic, hematopoietic	7, 140 (56–288)	3, 120 (25–351)	4, 160 (44–410)

[a] CI = confidence interval.

Source: Leet and Selevan (1982, 094970)

Pell (1978, 064957) evaluated a second cohort in New Jersey 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 of 55 deaths was observed in this cohort. Study exclusions included thirteen 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 higher than expected (SMR = 140) but the SMR was not statistically significant. There were three deaths due to digestive cancer compared to 2.7 expected and four deaths due to lung cancer compared to 3.0 expected. With five observed cancers of the urinary system (3 bladder and 2 kidney), the SMR was significantly elevated compared to the DuPont population (SMR = 300; $p < 0.01$) and compared to the U.S. general population (SMR = 250; $p < 0.01$). 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, 625181) 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 accrued at least one year of chloroprene-related work prior to June 30, 1980, was 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 estimated 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. Males had statistically significant ($p < 0.005$) greater exposure to chloroprene than 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 subgroups (see Table 4-2) starting from July 1, 1969 or when the individual first started working with chloroprene through June 30, 1983 for live individuals or until their dates of death.[5] SMRs were calculated using sex- and age- specific local area rates in 1973-1975. The results presented in Table 4-2 are for male workers only as all sixteen reported cancer deaths occurred among male workers. The all-cancer SMR for the male workers was 271 ($p < 0.01$). Among the 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, $p < 0.05$).

---

[5] 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

EXPOSURE AREA	NUMBER OF DEATHS/SMR		
	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[**]	2/1,667[**]	1/5,000[**]
Neoprene workshop	5/176	2/165	1/556
Polymer operator[a]	5/394[**]	2/357	1/1250
Final treatment	0/---		
Maintenance mechanic[a]	0/---		
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, 625181)

One limitation of the Li et al. (1989, 625181) study was the availability of only three years (1973-1975) of local area data to calculate SMRs. If these years were not representative of the entire study period, then the SMRs could be biased. For example, if the general population experienced higher mortality during the time periods not examined (i.e., 1969-1972 & 1976-1983) then the SMRs reported in the study would be overestimated due to a lower expected number of deaths. If mortality was lower during the other time periods not examined, then the reported SMRs would be overestimated. Lack of quantitative exposure information precluded conducting internal analyses by latency or duration of exposure. Additionally, there were 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. The authors also noted that the chemical plant investigated in the study used the acetylene process for chloroprene manufacture, and therefore there was no possibility of co-exposure to 1,4-dichloro-2-butene, which is only produced as a by-product using the butadiene process of chloroprene manufacture.

Li et al. (1989, 625181) 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 earlier 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, 625105) 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 location and by different years. They therefore devised a relative scoring system to assign exposures: workers in the high-exposure departments were 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 to 1993 which included 70,328 (62,492 in females and 7,836 in males) person-years of observation. Thirty-seven percent of cohort members (female/male distribution not provided) contributing 26,063 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 the 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 available only for 1992-1993. Therefore, the rate 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 significantly elevated for all cancers, liver cancer and leukemia (Table 4-3). 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* (107–137)	56;158* (119–205)	209;115* (100–131)
Liver cancer	10; 240* (110–430)	2; 240 (30–860)	8; 230* (100–460)
Lung cancer	31; 140 (90–200)	17; 170* (100–270)	14;110 (60–190)
Leukemia	13; 190* (100–330)	2; 190 (20–700)	11;190* (100–350)

* Statistical significance $p < 0.05$.

Source: Bulbulyan et al. (1998, 625105)

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	HIGH-EXPOSURE CASES	HIGH-EXPOSURE RR (95% CI)[a]
All causes	194	1.23* (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)

* Statistical significance $p < 0.05$.

[a] Reference group is defined as workers with no chloroprene exposure.

Source: Bulbulyan et al. (1998, 625105)

Although there were only a few deaths in each group, analysis by categories of duration of employment among workers with the highest exposure to chloroprene (1–9 years, 10–19 years, 20+ years) relative to no exposure showed a significant trend ($p = 0.02$) 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 (10.1-30 unit-years, RR = 7.1, 95% CI: 0.8-61) and three deaths in the highest exposure category (30.1+ unit years, RR = 4.4, 95% CI: 0.4-44). Kidney cancer was increased in all cumulative exposure 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, 625105)

The most prominent finding in the Bulbulyan et al. (1998, 625105) 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 the Li et al. study (1989, 625181), the minimal data on observed deaths for some cancers among the general population may have also resulted in biased SMR values if mortality during these years was not representative of mortality during the entire study period.

Bulbulyan et al. (1999, 157419) 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 to 1988, and vital status was accessed through the Yerevan Address Bureau. Death certificates were coded by using the 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 the summer average ranged from 0.80–3.60 mg/m^3 (0.22–0.99 ppm) and concentrations ranged from 0.55–2.10 mg/m^3 (0.15–0.58 ppm) for the winter. 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 were available for 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 in 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	68 (49–94)
Lung cancer	6	53 (24–119)
Liver cancer	6	327* (147–727)

* Statistical significance $p < 0.05$.

Source: Bulbulyan et al. (1999, 157419)

Internal trend analyses of plant workers 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 =345, 95% CI: 129-920). 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 = 612, 95% CI: 230-1630). Similar findings were noted in analyses using cumulative exposure,(unit-years) with a statistically significant SIR of 486 (95% CI: 202-1170) among the five cases in the highest cumulative exposure category of 40+ units. All six cases of liver cancer in this study occurred among highly exposed operators. These internal analyses suggest 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 health outcomes among the workers, the authors acknowledged that misclassification might have also occurred due to 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 in either the mortality or incidence analyses are liver carcinogens.

Romazini et al. (1992, 624896) investigated cancer mortality in a retrospective French 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 era 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(odds ratio = 5.34; 95% CI: 1.28-22.3). 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, 625112) 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 exposures of < 2 ppm, 2-5 ppm, and > 5 ppm respectively. Exposure duration was divided into three groups of ≤ 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 in the group employed 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)	COHORT EXPOSED BEFORE 1977 CASES, SIR (95% CI)
All Cancers	34, 126 (88-177)	32, 146 (100-206)
Head and Neck	9, 189 (87-359)	8, 209 (90-411)
Larynx	3, 243 (50-713)	3, 297 (61-868)
Lung	9, 184 (84-349)	8, 199 (86-391)
Liver	1, 136 (4-763)	1, 164 (5-913)
Colon/Rectal	2, 66 (8-239)	2, 79 (10-287)

Source: Colonna and Laydevant (2001, 625112)

Although none of the SIRs were statistically significant, a trend was observed when the data were analyzed by duration of exposure. Five lung cancers were reported in workers with > 20 years of exposure (SIR = 257), 3 in those with 11-20 years exposure (SIR = 149) and 1 in those with ≤ 10 years exposure (SIR = 106). 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 463 (95% CI: 127-1191), 125 (95% CI: 15-451), and 123 (95% CI: 26-361) 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 cancer excess was unlikely to be due to chloroprene exposure. Although smoking and alcohol consumption were 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. (2007, 625187) evaluated mortality patterns of four chloroprene production facilities by using external regional rates and internal comparisons (Marsh et al., 2007, 625188). This study attempted to address the problems identified with earlier studies by conducting a detailed exposure assessment for both chloroprene and a potential confounding co-exposure, vinyl chloride monomer (Esmen et al., 2007, 625114; Esmen et al., 2007, 625118; Esmen et al., 2007, 625121; Hall et al., 2007, 625243). As described in detail by Esmen et al. (2007, 625121), 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., 2007, 625118).

Two of the facilities evaluated were in the U.S. - DuPont/Dow plants at Louisville (L), Kentucky and Pontchartrain (P), Louisiana. The third facility was the Maydown (M) plant in Northern Ireland, and the fourth facility was the Enichem Elastomer plant in Grenoble (G), France. These plant cohorts included all employees with possible chloroprene exposure from plant start-up through 2000: 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 (L), 0.13 (P), 0.084 (M), and 1.01 (G) ppm-years. The median average intensity of chloroprene exposure (in ppm) at these plants were: 5.23 (L), 0.0283 (P), 0.160 (M), and 0.149 (G). Vinyl chloride exposures occurred at only two plants, Louisville and Maydown. Their median cumulative vinyl chloride exposures were 1.54 and 0.094 ppm-years, respectively. The median average intensity of vinyl chloride exposures were 1.54 and 0.030 ppm, respectively.

The study period for the cohorts encompassed 52 (L), 41(M), 39 (P), and 34 (G) years resulting in 197,919 (L), 127,036 (M), 30,660 (P), and 17,057 (G) person-years (Marsh et al., 2007, 625187). 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 cohorts 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 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 respiratory cancer deaths (including bronchus, trachea, and lung) were 266 (L), 48 (M), 12 (P), and 10 (G), while liver cancer deaths were 17 (L), 1 (M), 0 (P), and 1 (G) for each plant. Compared to the local population rates, fewer deaths than expected from liver cancer were observed in the Louisville (SMR = 90, 95% CI: 53-144) 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 reported in the occupational cohorts compared to general population estimates. An additional paper by this group (Leonard et al., 2007, 625179) further explored the healthy worker effect in an analysis of the Louisville and Pontchartrain workers. Compared to the local county population estimates, SMRs were decreased for all cancers, respiratory cancers, and liver cancers.

However, when comparisons were based on Dupont national and Dupont Region 1 comparison populations (in order to control for the healthy worker effect), the authors found statistically significant elevated risks for: all cancers, SMR = 111 (Dupont national population only); and respiratory cancer mortality, SMRs = 137 (Dupont national population) and 120 (Dupont Region 1 population). Elevated SMRs were observed for liver, cancer, SMRs = 127 (Dupont national population) and 121 (Dupont Region 1 population), although these liver cancer risks were smaller than reported in other studies and were non-significant,

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

CAUSE OF DEATH	LOUISVILLE (L) CASES, SMR (95% CI)[a]	MAYDOWN (M) CASES, SMR (95% CI)[b]	PONTCHARTRAIN (P) CASES, SMR (95% CI)[a]	GRENOBLE (G) CASES, SMR (95% CI)[b]	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)

[a] Local county comparisons

[b] National comparisons

Source: Marsh et al. (2007, 625187)

When chloroprene exposed and unexposed workers were analyzed separately in this cohort, 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 (Marsh et al., 2007, 625187). The very small number of unexposed workers (n=28) across all four plants limits the conclusions that can be drawn based on the crude exposure classification approach (see Table 4-8). In their companion paper (Marsh et al., 2007, 625188), the authors conducted internal RR analyses of more detailed worker exposure levels at each of these four plants. Exposure-response trends across quartiles of exposure 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 (due to an inability to locate complete smoking histories for employees who died from respiratory cancers). 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. This was confirmed by the detection of higher SMRs for all cancer, respiratory cancer and liver cancer mortality in the Louisville and Pontchartrain cohorts based on Dupont national and Dupont Region 1 comparison populations (Leonard et al., 2007, 625179).

The internal analysis for liver cancer could only be conducted in the Louisville cohort, which included 17 of the 19 observed deaths and also had the highest chloroprene levels (Marsh et al., 2007, 625188). Despite the limited number of deaths, these data show some potential evidence of a dose-response effect across the four exposure levels ($p = 0.09$). Although the individual RRs were not statistically significant, the RRs for the highest three exposure levels were 1.9 (95% CI = 0.21-23.81), 5.1 (95% CI = 0.88-54.64), and 3.3 (95% CI = 0.48-39.26).

As shown in Table 4-9, 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 (Marsh et al., 2007, 625188). 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 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 was so highly correlated with chloroprene exposures.

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

PLANT	LEVEL 1* (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

*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. (2007, 625188)

The authors also conducted internal analyses of cancer mortality and vinyl chloride exposure (the primary co-exposure in this study) at the Louisville plant (Marsh et al., 2007, 625188). They found inverse associations (many of them statistically significant) between risk of both respiratory and liver cancer in relation to vinyl chloride exposures; however, these associations were based on limited numbers of cancer deaths in the vinyl chloride exposure groups. 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. However, the authors reported that there was no correlation between cumulative exposures to vinyl chloride and chloroprene among these workers. Given this, it is highly unlikely that confounding by vinyl chloride could explain the associations observed between chloroprene and these cancers.

The recent Dupont studies (Marsh et al., 2007, 625187; Marsh et al., 2007, 625188; Leonard et al., 2007, 625179) represent some 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, there was some evidence that this may in part be due to the healthy worker effect (Leonard et al., 2007, 625179). The cancer specific findings suggest that the association between chloroprene exposure and liver cancer mortality risk was smaller but comparable with other studies. There was also some suggestion of elevated risk of respiratory cancer mortality at the upper two exposure levels in several of the cohorts (Table 4-9). Although statistical power to detect mortality trends across exposure levels appeared limited, the relative risks in the upper two exposure groups were all in excess of 1.8 relative to the unexposed populations with the exception of the Louisville plant (Marsh et al., 2007, 625188). Despite study limitations, findings from this cohort add to the weight of evidence that chloroprene exposure may be associated with cancer mortality especially when comparisons are based on internal populations or other regional or national Dupont workers.

4.1.1.3. *Summary and Discussion of Relevant Methodological Issues*

Nine studies covering eight cohorts were reviewed to assess the relationship between exposure to chloroprene and cancer incidence and mortality. Four cohorts had fewer than 1000 workers, while the remaining cohorts had fewer than 6000. The most consistent finding was excess liver (Bulbulyan et al., 1999, 157419; Bulbulyan et al., 1998, 625105; Li et al., 1989, 625181; Leet and Selevan, 1982, 094970) and lung/respiratory system (Marsh et al., 2007, 625188; Colonna and Laydevant, 2001, 625112; Bulbulyan et al., 1999, 157419; Bulbulyan et al., 1998, 625105; Leet and Selevan, 1982, 094970; Pell, 1978, 064957) 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) or pre-existing disease status (i.e., hepatitis B infection), 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. Internal comparisons however may also not completely eliminate the healthy worker effect as the healthy worker survivor effect (e.g., shorter-term exposed workers having increased mortality) can also lead to attenuation of effect measures (Arrighi and Hertz-Picciotto, 1994, 625164).

Another limitation of occupational cohort 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. Since there is no direct evidence of substantial misclassification of health outcomes in these studies, 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: Standardized mortality ratios (SMRs) and standardized incidence ratios (SIRs) for the overall cohort populations relative to external comparison populations[a] and relative risks (RRs) for intermediate and high chloroprene exposures

STUDY	TOTAL COHORT SMR/SIR (95% CI)[a]	INTERMEDIATE-EXPOSURE SMR/SIR/RR[b] (95% CI)	HIGH-EXPOSURE SMR/SIR /RR[b] (95% CI)
(Bulbulyan et al., 1998, 625105)	140 (90-200)	1.0 (0.4–2.5) [c,d]	0.8 (0.3–2.4)[c,d]
(Bulbulyan et al., 1999, 157419)	50 (16-155)	-----	-----
(Colonna and Laydevant, 2001, 625112)	184 (84-349)[e]	125 (15–451)[e]	123 (26-361)[e]
(Leet and Selevan, 1982, 094970)	106 (62-170)	86 (35-178)[f]	128 (61-236)
(Marsh et al., 2007, 625187; Marsh et al., 2007, 625188) - Louisville	75 (66-85)	92 (73-115)[d,]	65 (50-84)[d,]
(Marsh et al., 2007, 625187; Marsh et al., 2007, 625188) - Maydown	79 (58-105)	97 (50-169)[d,]	113 (60-192)[d,]
(Marsh et al., 2007, 625187; Marsh et al., 2007, 625188-Pontchartrain) - Pontchartrain	62 (32-109)	96 (12-348)[d,]	85 (23-218)[d,]
(Marsh et al., 2007, 625187; Marsh et al., 2007, 625188) - Grenoble	85 (41-156)	119 (32-304)[d]	128 (26-373)[d,]

[a] SMRs and SIRs calculated relative to external population rates and are reported on a 100-base scale, unless noted all values are SMRs

[b] Relative to low or unexposed Groups

[c] Relative risk of death from lung cancer

[d] Cumulative chloroprene exposures

[e] Standardized incidence ratios

[f] Low-exposure group

Table 4-11. Epidemiologic summary results of liver/biliary passage cancers: Standardized mortality ratios (SMRs) for the overall cohort populations relative to external comparison populations and SMRs and relative risks (RRs) for intermediate and high chloroprene exposures

STUDY	TOTAL COHORT SMR[a] (95% CI)	INTERMEDIATE-EXPOSURE SMR/RR[b] (95% CI)	HIGH-EXPOSURE SMR/RR[b] (95% CI)
(Bulbulyan et al., 1998, 625105)	240 (110-430)	7.1 (0.8–61)[c,d]	4.4 (0.4–44)[c,d]
(Bulbulyan et al., 1999, 157419)	339 (109-1050)	293 (41-2080)[d,e]	486 (202-1170)[d,e]
(Colonna and Laydevant, 2001, 625112)	136 (4–763)[e]	------	------
(Leet and Selevan, 1982, 094970)	571 (156-1463)	250 (6-1393) [a]	750 (155-2192) [a]
(Li et al., 1989, 625181)	482 (N/R, p < 0.01)	-----	------
(Marsh et al., 2007, 625187; Marsh et al., 2007, 625188) - Louisville	90 (52-144)	5.1 (0.9, 54.5)[c,d]	3.3 (0.5, 39.3)[c,d]
(Marsh et al., 2007, 625187; Marsh et al., 2007, 625188) – Maydown	24 (1-134)	------	------
(Marsh et al., 2007, 625187; Marsh et al., 2007, 625188) - Pontchartrain	------	------	------

N/R: Not Reported

[a] SMRs and SIRs calculated relative to external population rates and are reported on a 100-base scale, unless noted all values are SMRs

[b] Relative to low or unexposed groups

[c] Relative risk of death from liver cancer

[d] Cumulative chloroprene exposures

[e] Standardized incidence ratio

Lung Cancer Summary

An increased risk of lung cancer incidence and mortality was observed in a few studies (Colonna and Laydevant, 2001, 625112; Bulbulyan et al., 1998, 625105; Pell, 1978, 064957; Li et al., 1989, 625181; Leonard et al., 2007, 625179), 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. (2007, 625188) 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 not consistent evidence of an exposure-response relationship across various chloroprene exposure categories.

Liver Cancer Summary

Statistically significant excesses of liver cancers were detected in four studies examining four cohorts (Bulbulyan et al., 1999, 157419; Bulbulyan et al., 1998, 625105; Li et al., 1989, 625181; Leet and Selevan, 1982, 094970). Although no statistically significant increase in the risk of liver cancer (compared to the general population) was detected when the Louisville cohort was analyzed by Marsh et al. (2007, 625188), the SMRs for liver cancer mortality exceeded 1.2 when based on comparisons to national and regional Dupont worker populations (Leonard et al., 2007, 625179). The relative risk of liver cancer mortality also increased with increasing cumulative exposures indicating a potential dose-response trend. In the French (Grenoble/Isere) cohort, there was only one case of liver cancer or mortality from liver cancer (Marsh et al., 2007, 625187; Marsh et al., 2007, 625188; Colonna and Laydevant, 2001, 625112) detected, while the Pontchartrain cohort study had no reported liver cancer deaths (Marsh et al., 2007, 625188). The small numbers of liver cancer deaths especially in the latter studies precluded further examination of the 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, 157419) 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. (2007, 625188) 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 who 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 an increased liver cancer risk.

No adjustments for known 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, although unlikely, this might be a source of residual confounding. Other risk factors for liver cancer that were not controlled for, including hepatitis infection and aflatoxin ingestion, are not likely to be associated with chloroprene exposure among these occupational cohorts. Although the lack of adjustment for these known risk factors of liver cancer may be a cause of concern when considering the studies individually, the consistent observation of increased liver/biliary cancer in multiple heterogeneous occupational cohorts ameliorates this concern to some degree. 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, 625160). There are also few identified chemicals that have been associated with primary liver cancer, so co-exposures are unlikely to confound the association between chloroprene exposure and liver cancer mortality. The observation of an increased risk of liver cancer mortality is fairly consistent and there is some suggestive evidence of an exposure-response relationship among workers exposed to chloroprene in different cohorts on different continents (i.e. U.S., China, Russia, and Armenia) (see further discussion in Section 4.7.1.1.1 – Biological Gradient).

4.1.2. Chloroprene Exposure and Noncancer Effects

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

Nystrom (1948, 003695) 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 polymerization and distillation, in both the pilot plant and early period of regular production. Over the time period from 1944–1947, the author conducted a series of employee medical examinations. 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. Dermatitis was present in four workers (30%), and all other symptoms evaluated were limited to no more than one worker. 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 lower numbers (3–6 employees) complaining of palpitations, giddiness, irritability, and dermatitis. No workers experienced loss of hair.

Guided by animal studies and reports from other companies, Nystrom (1948, 003695) 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, 003695) 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.

Biochemical and hematological effects of occupational chloroprene exposure of workers in a chloroprene manufacturing plant were reported by Gooch and Hawn (1981, 064944). The study investigated exposed and non-exposed workers at the DuPont Louisville Works plant and included any workers employed as of December 31, 1977. Workers were categorized into three exposure groups: currently exposed (workers assigned to the chloroprene polymerization area of the plant as of

December 31, 1977); not currently exposed (workers with a history of work in the chloroprene polymerization area of the plant); and never exposed (workers with no history of being assigned to the chloroprene polymerization area of the plant). Exposure groups were based on a job description indicating the worker was assigned to the chloroprene polymerization area of the plant. Additionally, seven employees in supervisory roles familiar with chloroprene manufacture independently rated each job as "high", "medium", "low", or "varied" in regard to the actual potential for exposure to chloroprene. At the Louisville plant, all new hires were required to undergo a physical examination upon employment and at specificed intervals thereafter that included clinical chemistry and hematological analyses, chest x-rays, and pulmonary function tests (Jones et al., 1975, 625203).. The results for tests conducted between 1974 and 1977 were included in the analysis. When clinical chemistry parameters were compared between exposure groups no effect was seen in currently exposed workers and those workers never exposed to chloroprene; this lack of effect was also observed when currently exposed workers with "high" potential for chloroprene exposure were compared to workers never exposed to chloroprene. Paired analyses (comparisons of clinical chemistry in workers with test results before and after being assigned to chloroprene manufacture) showed that glucose and cholesterol values were lower and LDH values were higher in workers after being assigned to chloroprene manufacture compared to test results before assignment. However, all values were well within normal ranges, indicating the results were likely due to normal variability and not to any chemically-related effect. No hematological effects were observed.

In a subsequent NIOSH industrial hygiene investigation of the DuPont Louisville Works plant, ambient and personal monitoring was conducted to assess worker exposure to chloroprene (McGlothlin et al., 1984, 625204). Additionally, medical interviews and medical record examinations were conducted to determine if adverse health outcomes due to workplace exposures could be detected. In the air quality monitoring portion of the study, personal breathing zone and area air samples were collected in the manufacturing areas that dealt with both the monomer (chloroprene) and polymer (polychloroprene). The range of chloroprene air concentrations detected by fixed location area samples ranged from below detection limits (32 out of 79 total samples) to 1200 ppm. The two highest concentrations (910 and 1200 ppm) were detected at "drainage trenches" and may not have been representative of normal workday exposures experienced in the manufacture areas. In the remaining fixed location samples, the average chloroprene concentration (over 6-7 hours) was 5.6 ppm, which was below the OSHA PEL of 25 ppm for an 8 hour workday. Only one fixed location area air sample (excluding thoses taken at the drainage trenches) exceeded the OSHA PEL (26 ppm). Of the 194 personal air samples taken from workers in the monomer and polymer portions of the plant, 103 (54%) exceeded the NIOSH 15-minute recommendation of 1 ppm, 5 (3%) exceeded the ACGIH TLV of 10 ppm, and only 1 (0.5%) exceeded the OSHA PEL of 25 ppm. It is important to note that the magnitude of worker exposure detected in this study may not be representative of exposures workers experience currently due to increased safety procedures and improved manufacturing processes. In the medical examination portion of the study, 37 workers were interviewed and demographic and occupational information was collected. Smoking histories, medical problems, past illnesses, and current symptoms were covered in the interviews and any relation to current work exposures was sought. None of the workers indicated in the interviews that they felt that their current health status was related to their workplace exposure to chloroprene. Some workers indicated that they had occasionally experienced lightheadedness and eye, nose, and throat irritation. Workers experiencing respiratory disease had medical histories indicating heavy smoking, heart disease, or other medical issues. An examination of medical records for 8 of the 37 workers found that the only significant problem observed was a large deviation in pulmonary function tests year-to-year that may be due to faulty test equipment. In summary, no major health effects were observed in workers involved in chloroprene manufacture and polymerization even though personal and ambient monitoring indicated that occupational safety limits were occasionally exceeded.

In a Russian review of the effects of chloroprene, Sanotskii (1976, 063885) 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 (0.28 – 1.93 ppm) 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, 063885), women aged 19–23 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

Gooch and Hawn (1981, 064944) investigated the effects on clinical chemistry parameters in workers chronically exposed to chloroprene (study description above). When currently exposed workers were compared to never exposed workers stratified by duration of exposure (< 1 year, 1-5 years, 6-10 years, > 10 years), cholesterol and alkaline phosphatase were higher in workers exposed > 10 years (cholesterol) and 6-10 years (alkaline phosphatase). This pattern was also observed when only workers with a “high” potential for exposure were analyzed. When cholesterol values were adjusted for the age of the workers, no chemically-related effect was observed. The differences seen in alkaline phosphatase were attributed to two workers with abnormally high alkaline phosphatase levels due to bone injury and blood pressue medication. Therefore, no chemically-related effects were seen in clinical chemistry parameters in workers chronically exposed to chloroprene.

Chronic effects in exposed workers at an electrical engineering plant were also reported in the review by Sanotskii (1976, 063885). 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 the rate of spontaneous abortion in the wives of chloroprene workers was more than 3-fold greater when compared to 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, 017624) concluded that recall bias associated with a retrospective questionnaire, such as was used in the study reviewed by Sanotskii (1976, 063885), 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 spontaneous abortions occur before a woman recognizes that she is pregnant, with clinical signs of miscarriage often mistaken for heavy or late menstruation (Griebel et al., 2005, 625142). Thus, U.S. EPA (1985, 017624) concluded that it was not reasonable to draw conclusions on the possible effect of chloroprene on early fetal losses based on the Sanotskii (1976, 063885) 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, 063885) review with any degree of reliability (U.S. EPA, 1985, 017624). Savitz et al. (1994, 068186) and Schrag and Dixon (1985, 062573) separately reviewed the study and also concluded that insufficient methodological details were available to critically evaluate the observation reported by Sanotskii (1976, 063885).

Sanotskii (1976, 063885) 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. Insufficient data on analytical methods and exposure ascertainment used in the investigation of chromosomal aberrations in chloroprene workers preclude drawing conclusions from the results presented by Sanotskii (1976, 063885).

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, 063885)

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, 075453) 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. Severe congestion of the lungs and kidneys was observed in animals treated with chloroprene that died within the first 23–35 weeks of treatment. Multiple liver necroses were observed in some animals (number not specified) autopsied 80–90 weeks after the onset of treatment.

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 male progeny (intestinal leiomyosarcoma, osteoma, kidney mesenchymal tumor, bone hemangioma, neurinoma of the optic nerve, transition-cell carcinoma of urinary bladder, and forestomach papilloma) and female dams and progeny (uterine squamous cell carcinoma, lung reticulosarcoma, forestomach papilloma, sebaceous basal cell carcinoma) 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. Mammary and ovarian tumors were slightly elevated in chloroprene-treated female 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, 075453)

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 females	1	6.3	6	37.5	2	12.5	---	---	---	---	1	6.3	4[a]	25.0
Treated progeny														
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 females	1	7.1	4	28.6	---	---	---	---	1	7.1	---	---	1[c]	7.1
Control progeny														
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, 075453)

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, 042076). Results of the 13-week study were reported by

Melnick et al. (1996, 625207), while the cancer results of the 2-year study were discussed separately by Melnick et al. (1999, 000297) 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 target concentrations of 0, 32, 80, 200, or 500 ppm chloroprene (NTP, 1998, 042076). Actual chamber concentrations were 0, 31.1 ± 1.9, 80.7 ± 5.0, 198 ± 10, and 503 ± 24 ppm chloroprene. 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, 042076)

Minimal to mild olfactory epithelial degeneration was significantly increased in all exposed groups of males and females compared to 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 (female) and 500 (male and female) 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 (1.0)[b]	10/10** (1.0)	10/10** (1.1)	10/10** (1.9)	10/10** (3.8)
Metaplasia, squamous, olfactory epithelium	0/10	0/10	0/10	1/10 (2.0)	4/10* (1.8)
Metaplasia, respiratory, olfactory epithelium	0/10	2/10 (1.0)	5/10* (1.0)	6/10* (1.0)	1/10 (2.0)
Metaplasia, squamous, respiratory epithelium	1/10 (1.0)	1/10 (1.0)	0/10	0/10	7/10 (1.7)
Liver[a]	10/10	1/10	10/10	10/10	10/10

	CONTROL	32 ppm	80 ppm	200 ppm	500 ppm
Necrosis, centrilobular	0/10	0/10	0/10	1/10 (2.0)	9/10** (3.4)
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** (1.2)	10/10** (1.6)	10/10** (3.4)	10/10** (3.3)
Metaplasia, squamous, olfactory epithelium	0/10	1/10 (1.0)	1/10 (1.01)	4/10* (1.0)	0/10
Metaplasia, respiratory, olfactory epithelium	0/10	7/10** (1.0)	8/10** (1.2)	3/10 (1.0)	7/10** (1.4)
Metaplasia, squamous, respiratory epithelium	1/10 (2.0)	1/10 (1.0)	0/10	0/10	4/10 (1.3)
Liver[a]	10/10	3/10	10/10	10/10	10/10
Necrosis, centrilobular	0/10	0/10	0/10	7/10** (2.6)	3/10 (2.0)
Inflammation, chronic	0/10	0/10	0/10	2/10 (1.0)	5/10* (1.0)

[a] number of animals with tissue examined microscopically.

[b] Average severity grade of lesions in affected rats: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

[*] $p \leq 0.05$.

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

Source: NTP (1998, 042076)

In the mouse portion of the 16-day NTP (1998, 042076) study, target exposure levels were 0, 12, 32, 80, and 200 ppm chloroprene. The actual exposure chamber concentrations were 0, 11.9 ± 0.8, 31.1 ± 2.0, 80.8 ± 5.2, and 301 ± 12 ppm chloroprene. 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. Hematological and clinical chemistry parameters in exposed mice were similar to those in the chamber controls. Increased incidences of multifocal random hepatocellular necrosis and thymic necrosis, characterized by karyorrhexis of thymic lymphocytes, were observed in male and female mice exposed to 200 ppm. No histopathological damage was observed in the lungs of exposed mice.

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**
80	10/10	24.8 ± 0.5	26.1 ± 0.6	+ 1.3 ± 0.2**
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	---	---

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

Source: NTP (1998, 042076)

A range-finding 13-week inhalation study was conducted by NTP (1998, 042076) (reported by Melnick et al (1996, 625207)), using both mice and rats. In the rat, target exposure groups were 0, 5, 12, 32, 80, and 200 ppm chloroprene. The actual chamber concentrations achieved were 0, 5.03 ± 0.18, 12.1 ± 0.4, 31.9 ± 1.0, 80.2 ± 1.7, and 200 ± 5.0 ppm chloroprene. 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
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, 042076)

On day 2, hematocrit values, hemoglobin concentrations, and erythrocyte counts were increased 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, was observed in 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. No effects were observed in the respiratory epithelium of exposed rats. 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 (1.0)[b]	10/10** (1.0)	10/10** (2.0)
Metaplasia, respiratory, olfactory epithelium	0/10	---	0/10	0/10	4/10* (1.3)	4/10* (1.3)
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 (2.0)
Inflammation, chronic	0/10	1/10 (1.0)	0/10	0/10	1/10 (1.0)	2/10 (1.0)
Hemosiderin pigmentation	0/10	0/10	0/10	0/10	0/10	5/10* (1.6)
Female						
Nose[a]	10/10	0/10	10/10	10/10	10/10	10/10
Degeneration, olfactory epithelium	0/10	---	0/10	4/10* (1.0)	9/10** (1.9)	10/10** (1.9)
Metaplasia, respiratory, olfactory epithelium	0/10	---	0/10	0/10	8/10** (2.0)	9/10** (2.0)
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* (1.0)
Inflammation, chronic	2/10 (2.0)	0/10	1/10 (2.0)	0/10	1/10 (2.0)	8/10* (1.3)
Hemosiderin pigmentation	3/10 (1.0)	0/10	1/10 (3.0)	0/10	0/10	9/10** (1.7)

[a] Number of animals with tissue examined microscopically.

[b] Average severity grade of lesions in affected rats: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

* Significantly different ($p \leq 0.05$) the chamber control group by Fisher's exact test.

** Significantly different ($p \leq 0.01$) from the chamber control group by Fisher's exact test.

Source: NTP (1998, 042076)

In the mouse portion of the NTP 13-week inhalation study, the target concentration exposure groups were 0, 5, 12, 32, and 80 ppm chloroprene. Actual chamber concentrations of 0, 5.02 ± 0.2, 12.1 ± 0.3, 31.9 ± 0.9, and 80.2 ± 1.6 ppm chloroprene were achieved. 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*	7.9 ± 0.5*
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

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

Source: NTP (1998, 042076)

Hematology variables were similar to, although more mild than, the 13-week rat study. 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 increased platelet production. No significant organ weight effects were observed. Sperm morphology and vaginal cytology parameters were similar to those of the chamber controls. The incidence of squamous epithelial hyperplasia of the forestomach was significantly increased in male and female mice exposed to 80 ppm (Table 4-21). Preening behavior may have lead to direct gastrointestinal exposure to chloroprene.

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* (1.5)[a]
Female					
Number examined microscopically	10/10	0/10	0/10	10/10	10/10
Squamous epithelial hyperplasia	0/10	---	---	0/10	9/10** (1.9)

[a] Average severity grade of lesions in affected mice: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

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

[**] $p \leq 0.01$.

Source: NTP (1998, 042076)

In the 2-year (NTP, 1998, 042076) inhalation study of chloroprene in male and female rats, groups were exposed to target concentrations of 0, 12.8, 32, and 80 ppm chloroprene. The actual chamber concentrations animals were exposed to were 0, 12.8 ± 0.4, 31.7 ± 1.1, and 79.6 ± 1.6 and 0, 12.7 ± 0.4, 31.9 ± 0.9, and 79.7 ± 1.7 ppm chloroprene for rats and mice, respectively. The high-exposure concentration was chosen based on the observation of anemia and hepatocellular necrosis in rats exposed to 200 ppm for 13 weeks. The range of exposures selected included the NOAEL for degenerative olfactory epithelial lesions in the 13 week study. 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, 624959) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972, 008785) with the chamber controls are in the exposed group columns

Source: NTP (1998, 042076)

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, 042076). Squamous cell papilloma and combined squamous cell papilloma and squamous cell carcinoma of the oral cavity (oral mucosa, tongue, pharynx, and gingiva) was significantly increased in male rats exposed to 32 ppm and male and female rats exposed to 80 ppm compared to those in the chamber controls. The incidences of these tumors 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	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	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] $p \leq 0.01$

Source: NTP (NTP, 1998, 042076)

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, 042076)

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. Renal tubule hyperplasia was distinguished from regenerative epithelial changes commonly observed as a part of nephropathy and was considered a preneoplastic lesion. Hyperplasia was generally a focal, minimal to mild lesion consisting of lesions that were dilated approximately 2 times the normal diameter and were lined by increased numbers of tubule epithelial cells that partially or totally filled the tubule lumen. 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 olfactory 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 of the olfactory epithelium 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. No histopathological effects were observed in the nasal respiratory epithelium of exposed rats.

In the NTP 2-year mouse study, exposure concentrations were 0, 12.8, 32, and 80 ppm. The highest exposure concentration in the 2-year chronic study was chosen based on the observation of mortality in mice exposed to 200 ppm chloroprene in the 16-day study. The observation of squamous epithelial hyperplasia in the forestomach of mice exposed to 80 ppm in the 13-week study was not considered life-threatening. 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

SEX	STATUS	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, 624959) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972, 008785) with the chamber controls are in the exposed group columns

Source: NTP (1998, 042076)

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 males and females exposed to chloroprene. 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)	23/50[c] (2.4)
Kidney (renal tubule) Hyperplasia	2/50	16/49[c] (1.4)	17/50[c] (1.6)	18/50[c] (1.6)	--	--	--	--
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, 042076)

The incidences of olfactory epithelial atrophy, adenomatous hyperplasia, and metaplasia in males and females exposed to 80 ppm were significantly increased compared to 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. Hepatocellular carcinoma was significantly increased relative to control in all exposed female mice as was hepatocellular adenoma or carcinoma combined in females exposed to 32 and 80 ppm.

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	5/50	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] $p < 0.05$, 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, 042076)

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 morphology for renal tubule hyperplasia was similar to that observed in rats exposed to chloroprene. 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 chloroprene, 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 observed in the trachea of one male each exposed to 12.8 ppm or 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, 093236). This adjustment accounts for the effects 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[a] neoplasm rates for mice in the 2-year inhalation study of chloroprene

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

[a] Survival-adjusted neoplasm rates were estimated using the Poly-3 survival-adjusted quantal response method of Portier and Bailer (1989)
In the chamber control column, * indicates a statistically significant trend ($p < 0.05$) across all exposure groups by the Poly-3 quantal response test; ** indicates a statistically significant trend at $p < 0.01$.
In the exposed group columns, * indicates a statistically significant difference ($p < 0.05$) from the chamber control group by pairwise comparison; ** indicates a statistically significant difference at $p < 0.01$.

Source: NTP (1998, 042076)

In another chronic inhalation study, Trochimowicz et al. (1998, 625008) 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 to generate the test atmosphere for each day’s exposure. To generate the test atmospheres, 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 the weeks of exposure, and at 4-week intervals for the remainder of the experiment. During the last 6 months of each study, all animals were examined once a month 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 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 for rats was 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 from an accidental failure of the exposure chamber ventilation system. For hamsters, mortality was negatively correlated with the exposure concentration of chloroprene. At the termination 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.

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. Rats were not affected by exposure to chloroprene in regard to appearance or behavior, 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 and organ 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, $0.1 \le p < 0.005$
[b] Significant, $0.001 \le p < 0.01$
[c] Significant, $p < 0.001$

Source : Trochimowicz et al. (1998, 625008)

Gross pathology revealed that lungs from rats exposed at 10 and 50 ppm had markedly lower incidences of nodular pleural surfaces, consolidation, and atelectasis (gross changes consistent with, and characterized as chronic respiratory disease) than did controls. These 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). 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 of control and high-dose animals were observed to be similar.

The only nonneoplastic effect observed in hamsters was a generalized amyloidosis (in the liver, kidneys, spleen, and adrenals); this effect 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. Only mammary gland tumors and squamous cell carcinomas were observed to demonstrate a statistically significant excess in rats exposed to chloroprene, compared with controls. Mammary tumors were significantly increased ($p < 0.05$) in females in the 50 ppm group. 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 relatively high number of chloroprene-exposed animals bearing benign fibroadenomas was primarily responsible for the increased incidence of mammary tumors. Squamous cell carcinomas involving the nasal cavity, sinus maxillaries, subcutis, and skin were observed in 3 of 100 males of the 50 ppm group and in 1 of 99 females of the control group. The exact origin of these tumors could not be identified through macroscopic or microscopic examination. 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, 063885) 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, 063885), the studies evaluated the systemic effects of chloroprene exposure in rats (strain not specified) 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

SITE AND TYPE OF TUMOR[a]	MALES			FEMALES		
	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	---	---	---

	MALES			FEMALES		
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
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, 625008)

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

SITE AND TYPE OF TUMOR[a]	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

SITE AND TYPE OF TUMOR[a]	MALES			FEMALES		
	0 ppm	10 ppm	50 ppm	0 ppm	10 ppm	50 ppm
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
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, 625008)

Dong et al. (1989, 007520) exposed Kumming albino mice (weaned at 2 weeks age) to 0, 2.9 ± 0.3, 19.2 ± 1.9, or 189 ± 13.3 mg/m^3 chloroprene for 4 hours/day, 6 days/week for 7 months. The purity of the chloroprene used to generate the test atmospheres was stated to be 99.8%. Animals were terminated at the end of the exposure period, or when found moribund. Lung tumors were not observed in treated animals before the 6th month of exposure, and were observed to increase in incidence with increasing concentration. The LOAEL for this study was determined to be 2.9 mg/m^3 (8.1% incidence of lung tumors vs. 1.3% in control animals, $p < 0.05$). Most lung tumors observed were papilloadenomas. Induction of multiple tumors in a single animal was also observed to increase with increasing dose.

4.3 REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Ponomarkov and Tomatis (1980, 075453) 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, 042076) 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, 625205). 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**	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.
[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.
** Significantly different ($p \leq 0.01$) from the control group by Shirley's test.

Source: NTP (1998, 042076)

Sanotskii (1976, 063885) 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, 042076) in F344 rats at much higher concentrations (Table 4-32). Sanotskii (1976, 063885) 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, 063885) 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, 094969) evaluated the embryotoxic, teratogenic, and reproductive toxicity of chloroprene in rats. Culik et al. (1978, 094969) 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 were not significantly different 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) was similar in fetuses from exposed and control dams (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)*	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**
Fetal crown-rump length, mm	32.9 ± 1.4	33.7 ± 1.6	33.8 ± 0.7**	34.1 ± 1.2**
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)

* Significantly different ($p \leq 0.05$) from the control group by Fisher's exact test.
** 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, 094969)

Culik et al. (1978, 094969) 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 (MARTA and MTA, 1996, 625111); 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[1]	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)

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

Source: Culik et al. (1978, 094969)

Mast et al. (1994, 625206) 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, 064938) 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.

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 not affected by exposure. 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, 064942) conducted a study to investigate the acute and subchronic toxicity of chloroprene and to determine the dose range for a 2 year chronic inhalation study (chronic study by Trochimowicz et al. (1998, 625008)) 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, 064942)

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. One 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, 064942) 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, 042076) 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, 042076) 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, 625008) 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, 064942) 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, 063885) 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, 084126). 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, 624893). 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, 084126; Putman et al., 2003, 624893).

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, 625215; Hurst and Ali, 2007, 625159). Other studies have used molecular analysis to study alterations in *ras* proto- oncogenes from lung and Harderian gland tumors identified in the NTP (1998, 042076) chronic bioassay that may indicate events in chloroprene-induced neoplasia (Ton et al., 2007, 625004; Sills et al., 1999, 624952).

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, 010689) 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. (2001, 019012); 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 (100 ppm) 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, 157445) 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. (2004, 625152) 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. Oxidation (V_{max}/K_m) of chloroprene in the liver was slightly faster in the mouse and hamster than in rats or humans. However, 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 the observed carcinogenicity of chloroprene.

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, 064961). 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, 625215) investigated the reactivity of the chloroprene metabolite (1-chloroethenyl)oxirane towards DNA nucleosides and calf thymus DNA *in vitro*. 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) described as an unsaturated aldehyde, yielded 2 major adducts. Reaction of (1-chloroethenyl)oxirane with 2'-deoxy-adenosine, -cytosine, and -thymine individually also resulted in adduct formation. When equimolar quantities of all 4 nucleosides were reacted with (1-chloroethenyl)oxirane simultaneously in a competitive reaction assay, all of the adducts identified from individual nucleoside reactions were observed and were formed at similar rates. 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 (96% on a molar basis), 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, 010173).

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, 625159). 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, 042076) 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, 624952). 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 (59% of all mutations; present in 47% of tumors) 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 (G→T, A, or C transversions), 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, 625004) 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, 010689)
	TA100, TA1535		+	Willems (1980, 625049)
	TA98		–	Willems (1980, 625049)
	TA100, TA1535	10,000-40,000 ppm	+	Willems (1978, 625048)
	TA98, TA1537, TA1538	10,000-40,000 ppm	–	Willems (1980, 625049)
	TA100, TA1535, TA1537, TA98	up to 3,333 µg/plate	–	NTP (1998, 042076)
	TA100	0-5 µmol/plate	–	Westphal et al. (1994, 625047)
	TA100	0-5 µmol/plate[b]	+	Westphal et al. (1994, 625047)
	TA100, TA1535, TA97A, TA98	0 to 69 mM[c]	+	Himmelstein et al. (2001, 019013)
Mammalian cell assays				
Micronucleus	Chinese hamster V79	10% (v/v)	–	Drevon and Kuroki (1979, 010680)
Micronucleus	Chinese hamster V79	0.175 mM[c]	–	Himmelstein et al. (2001, 019013)
In vivo bioassays				
Sex-linked recessive lethal mutation	Drosophila (Canton-S)		–	Foureman et al. (1994, 065173)
Sex-linked recessive lethal mutation	Drosophila (Berlin-K)		+	Vogel (1979, 000948)
Sister chromatid exchange: bone marrow	B6C3F1 mice	12.8, 32, 80 ppm	–	NTP (1998, 042076); Shelby (1990, 624906); Tice (1988, 624981; 1988, 064962)
Chromosomal aberration: bone marrow	B6C3F1 mice	12.8, 32, 80 ppm	–	NTP (1998, 042076)
Chromosomal aberration: bone marrow	C57BL/6 mice	up to 1 ppm	+	Sanotskii (1976, 063885)
Micronucleus: peripheral blood	B6C3F1 mice	12.8, 32, 80 ppm	–	NTP (1998, 042076)
Micronucleus: bone marrow	B6C3F1 mice		–	Shelby and Witt (1995, 624921)

[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, 625047)), 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, 010689) 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, 625048; 1980, 625049) 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, 625047) 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, 625047) 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, 625047) 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, 042076).

Himmelstein et al. (2001, 019013) investigated the mutagenicity of chloroprene monoepoxide, (1-chloroethenyl)oxirane (> 98% purity) in *Salmonella* strains TA100 TA1535, TA97A, and TA98. Exposures were performed with or without S9 activation in airtight capped glass vials in order to prevent the loss of the test substance due to volatilization. Test concentrations were 0-69 mM in DMSO. Cells were preincubated with the test compound for approximately 45 minutes at 37°C and then plated and allowed to incubate for an additional 48 hours. (1chloroethenyl)oxirane was genotoxic in all *Salmonella* strains tested without Aroclor-induced S9 activation (Himmelstein et al., 2001, 019013); inclusion of S9 did not enhance the mutagenic effect in any of the tester strains. 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, 010680). 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. (2001, 019013) 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. Clastogenicity was determined as the presence of a dose-dependent increase in the frequency of micronucleated cells, with at least one concentration producing a 3-fold increase. Cytotoxicity, reported as a reduction in the number of binucleated cells, and altered cell morphology were observed starting at 0.175 mM. Although no clastogenic response (as determined by the above criteria) was noted at concentrations up to 0.175 mM, at least three concentrations induced an increase in the frequency of micronucleated cells over control levels and a dose-dependent (although, not monotonic) increase was apparent over the tested range of concentrations.

4.5.2.3. In Vivo Bioassays

Vogel (1979, 000948) evaluated the *in vivo* genotoxic potential of chloroprene (99% pure with negligible dimer content) to induce recessive lethal mutations on the X chromosome of male *Drosophila melanogaster* (wild-type strain Berlin-K). Storage conditions and the elapsed time between receipt and use were not reported. Chloroprene was dissolved in DMSO and diluted with a 5% sucrose solution to obtain a final concentration of 1% DMSO and the desired experimental concentration. Adult males (2-3 days old) were treated at 25°C for 1-3 days in sealed beakers placed in a dessicator to account for the volatility of chloroprene. 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. Two different samples of chloroprene were used, one that was highly purified and one that contained several impurities (chemical characterization not stated). There were no apparent differences in mutagenic potential between the two samples of chloroprene, suggesting the impurities were not responsible for the observed genotoxicity.

In a study by Foureman et al. (1994, 065173), chloroprene (purity 50%) 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* (strain 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, 000948) 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, 625047; Gahlmann, 1993, 625174).

Cytogenetic tests using chloroprene were nonpositive. In studies performed by Brookhaven National Laboratories for the NTP (1998, 042076), 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, 042076) bioassay. Results were published separately by Shelby (1990, 624906), Tice (1988, 624981), and Tice et al. (1988, 064962). 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, 624981) and Tice et al (1988, 064962) 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, 624981), and Tice et al (1988, 064962) 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. Results of the NTP (1998, 042076) demonstrate that carcinogenic activity can occur at sites distal to the portal-of-entry, so lack of an effect in bone marrow may be due to low metabolic activity in this tissue.

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, 042076; MacGregor et al., 1990, 625184).

Sanotskii (1976, 063885) 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, 624921) 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, 625176). 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 comparison of the carcinogenic potency of butadiene and chloroprene in mice highlights the general quantitative concordance of their tumorigenic effects (Melnick and Sills, 2001, 051506). All of the tumorigenic effects (except for chloroprene induced mammary tumors) exhibited supralinear or linear dose-response curves when fit with a Weibull model. Chloroprene appeared more potent in the induction of forestomach and lung tumors in male mice and liver tumors in female mice, whereas butadiene was more potent in inducing Harderian gland tumors in both male and female mice. However, the female mouse lung was the most sensitive site of carcinogenicity for both chloroprene and butadiene, and both chemicals seemed equally potent in that particular neoplasm's induction (ED10 = 0.3 ppm).

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				
Circulatory	M, F	M	M, F			
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, 042076); Melnick et al. (1994, 625208); Placke et al. (1996, 624891); U.S. EPA (2002, 052153)

Table 4-38 Quantitative comparison of carcinogenic potency of butadiene and chloroprene in mice

Site	Males		Females	
	Butadiene	Chloroprene	Butadiene	Chloroprene
Lung	2.8[a]	0.9	0.3	0.3
Harderian gland	4.4	12	12	23
Forestomach	120	70	62	79
Liver			10	1.9
Mammary gland			13	12

[a] ED10 values (concentration assocated with 10% excess cancer risk) in ppm

Source: Melnick and Sills (2001, 051506)

4.6 SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1 Human Studies

There is a limited body of information on the nonneoplastic toxicological consequences to humans who are exposed to chloroprene. In a summary by Nystrom (1948, 003695), 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, 063885) 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).

4.6.2. Animal Studies

4.6.2.1. *Oral Exposure*

The toxic potential of chloroprene by the oral route has been assessed in only one study (Ponomarkov and Tomatis, 1980, 075453). 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.

4.6.2.2. 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, 000297)]), two chronic inhalation bioassays (NTP (1998, 042076)[also reported by Melnick et al (1999, 000297)]; Trochimowicz et al (1998, 625008)) and four reproductive developmental studies (Mast et al., 1994, 625206; Culik et al., 1978, 094969; Appelman and Dreef-van der Meulen, 1979, 064938; Sanotskii, 1976, 063885). These studies associate chloroprene inhalation exposure with toxicity in multiple organ systems, including respiratory tract, kidney, liver, spleen, and forestomach effects.

Increased mortality was observed in male and female rats exposed to 500 ppm chloroprene for 16 days (NTP, 1998, 042076). In male rats, the mortality reached 90% (9/10), whereas mortality was lower in females exposed to the same concentration (3/10). In mice exposed to chloroprene for 16 days, all of the males and females in the high-exposure group (200 ppm) died. In the 2-year chronic bioassay (NTP, 1998, 042076), mortality was increased over controls in male mice exposed to 32 or 80 ppm chloroprene and in females at all exposure concentrations tested. Decreased body weights were observed in male and female rats exposed for 16 days (≥ 200 ppm), male mice exposed 16 days (32 and 80 ppm), and in female mice exposed for 2 years (80 ppm) (NTP, 1998, 042076).

Hematological and clinical chemistry effects were also reported by the NTP (1998, 042076) study. In rats exposed to chloroprene for 16 days, increases in serum enzyme (ALT, GDH, and SDH) activities, as well as anemia and thrombocytopenia (decreased platelet count), were observed in the 200 and 500 ppm groups on day 4 of exposure only. In rats exposed to chloroprene for 13 weeks, minimal increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts were observed in males exposed to ≥ 32 ppm and in females exposed to 200 ppm on day 2. 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. Transient thrombocytopenia, evidenced by a reduction in circulating platelet numbers, occurred in male and female rats in the 200 ppm group on day 2 and in females at 80 and 200 ppm on day 22. At study termination (13 weeks) increases in platelet numbers were observed at 80 and 200 ppm in exposed males and females. Transient increases in activities of serum enzymes (ALT, GDH, and SDH) were 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. In mice exposed to chloroprene for 13 weeks (NTP, 1998, 042076), hematological changes were similar to those observed in rats; however, they were less severe. Minimal anemia, including decreased hematocrit values, erythrocyte counts, and platelet numbers were observed in female mice exposed to 32 or 80 ppm chloroprene.

Respiratory effects included a number of nasal and pulmonary effects in both rats and mice exposed to chloroprene (NTP, 1998, 042076; Trochimowicz et al., 1998, 625008). In rats exposed to chloroprene for 16 days (NTP, 1998, 042076), minimal to mild olfactory epithelial degeneration was observed in all exposed male and females. Additionally, metaplasia of the olfactory epithelium, characterized as replacement with a simple columnar respiratory-like epithelium, was observed in males at ≥ 80 ppm and females at ≥ 32 ppm. In rats exposed to chloroprene for 13 weeks (NTP, 1998, 042076), increased incidences of minimal to moderate olfactory epithelial degeneration and olfactory metaplasia (characterized as replacement with a simple columnar respiratory-like epithelium) occurred in male and female rats at 80 or 200 ppm. Olfactory epithelial degeneration was observed in female rats exposed to 32 ppm. In rats exposed to chloroprene for 2 years (NTP, 1998, 042076), 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 additionally increased at 12.8 ppm. 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. Increased incidences were observed for chronic inflammation in males (≥ 12.8 ppm) and in females (80 ppm), fibrosis and adenomatous hyperplasia of the olfactory epithelium in males and females (80 ppm), and alveolar/bronchiolar hyperplasia in males and females in every exposure group. No histopathological changes were observed in the respiratory tract of mice exposed to chloroprene for either 16 days or 13 weeks. In mice exposed to chloroprene for 2 years (NTP, 1998, 042076), increases in the incidences of olfactory epithelial atrophy, adenomatous hyperplasia, and metaplasia were observed in males and females at 80 ppm. Atrophy and metaplasia of the olfactory epithelium was similar to lesions observed in rats exposed to chloroprene. Suppurative inflammation was observed in female mice exposed to 32 or 80 ppm. Bronchiolar hyperplasia was increased in males and females in all exposure groups, whereas pulmonary histiocytic cellular infiltration was increased in every dose group in females only. 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. Histiocytic cellular infiltration consisted of histiocytes within alveolar lumens, usually adjacent to alveolar/bronchiolar neoplasms. In a second chronic 2-year bioassay (Trochimowicz et al., 1998, 625008), male and female rats exposed to 50 ppm chloroprene displayed mild respiratory effects such as lymphoid aggregates around bronchi, bronchioles, and blood vessels.

Toxicity was also observed in the kidneys and livers of rats and mice exposed to chloroprene. In rats exposed to chloroprene for 16 days (NTP, 1998, 042076), significant increases in kidney weight (right kidney only) were seen at 80 and 500 ppm. Mild to moderate centrilobular hepatocellular necrosis and increased liver weight was also observed in male and female rats exposed to 200 or 500 ppm chloroprene. In rats exposed to chloroprene for 13 weeks (NTP, 1998, 042076), increases in kidney weight was observed in males at 200 ppm and females at ≥ 80 ppm and the incidence of hepatocellular necrosis was increased in female rats exposed to 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. Increased incidence of kidney (renal tubule) hyperplasia was observed in rats exposed to chloroprene for 2 years when combined single- and step-sections were analyzed; incidence was increased in males at ≥ 32 ppm and in females at 80 ppm. Renal tubule hyperplasia was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy and was considered a preneoplastic lesion. Hyperplasia was generally a focal, minimal to mild lesion consisting of lesions that were dilated approximately 2 times the normal diameter and were lined by increased numbers of tubule epithelial cells that partially or totally filled the tubule lumen. In rats exposed to chloroprene for 2 years (Trochimowicz et al., 1998, 625008), the number of rats with one or more small foci of cellular alteration in the liver was higher in the 50 ppm exposure 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. Increased incidences of multifocal random hepatocellular necrosis were observed in male and female mice exposed to 200 ppm chloroprene for 16 days. In mice exposed to chloroprene for 2 years (NTP, 1998, 042076), the incidence of kidney (renal tubule) hyperplasia was increased in males exposed to 32 or 80 ppm when only single-sections were analyzed, and in all groups of exposed males when single- and step-sections were combined. The morphology of renal tubule hyperplasia in male mice was similar to that observed in rats.

The reproductive and developmental effects of chloroprene exposure are equivocal. In male rats exposed to chloroprene for 13 weeks, sperm motility was decreased at 200 ppm, whereas sperm morphology and vaginal cytology parameters were similar to those in the control groups in exposed male and female mice. In a study by Culik et al. (1978, 094969), rats were exposed on either gestational day 1-12 (embryotoxicity study) or 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 statistical significance relative to controls. An increase in the percentage of litters with resorptions was not observed in the larger embryotoxicity portion of the study which was specifically designed to detect such an effect. 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 and 25 ppm in the teratology study (62% and 59%, respectively). 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 outside of the historical control range for this strain of rat (MARTA and MTA, 1996, 625111). 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 in litters with resorptions at 10 ppm is spurious.

Chloroprene exposure did result in statistically significant increases in average fetal body weight and length. No major compound-induced or dose-related skeletal or soft tissue anomalies were observed. No exposure-related effects on maternal health, number of implantations, live pups, resorptions, fetal body weight or length, organ weights, or malformations were observed in New Zealand white rabbits exposed to chloroprene (Mast et al., 1994, 625206). In a two-generation reproduction study (Appelman and Dreef-van der Meulen, 1979, 064938), effects on body weight were observed in the F_0 and F_1 animals. Exposed F_1 males also had smaller testes and females had larger ovaries, livers, and lungs compared to controls. No histopathological changes were observed in those organs. The general lack of effects in the above reproductive and developmental studies is not consistent with the many positive effects seen in previous Russian studies reviewed by Sanotskii (1976, 063885). However, the Sanotskii review is severely lacking in many important study details, including the purity of the test substance and experimental design, and is therefore difficult to interpret with any confidence.

Chloroprene toxicity was observed in a number of additional organ systems. In mice exposed to chloroprene for 16 days, thymic necrosis, characterized as karyorrhexis of thymic lymphocytes, and hypertrophy of the myocardium was observed at 200 ppm. In rats exposed for 13 weeks, neurobehavioral parameters were affected: horizontal activity was increased in male rats exposed to ≥ 32 ppm compared with chamber control animals and total activity was increased in male rats at 32 and 200 ppm. No exposure-related effects on motor activity, fore/hindlimb grip strength, or startle response were observed. In mice exposed to chloroprene for 13 weeks, increased incidences of squamous epithelial hyperplasia of the forestomach were observed in male and female mice exposed to 80 ppm. Preening behavior may have lead to direct gastrointestinal exposure to chloroprene. In mice exposed for 2 years, the incidence of hyperplasia of the forestomach epithelium was increased in males and females at 80 ppm. The hyperplastic lesions were similar to those seen in the 13-week study and consisted of focal to multifocal changes characterized by an increase in the number of cell layers in the epithelium. The incidence of thyroid follicular cell hyperplasia was increased in male rats exposed to 32 ppm chloroprene for 2 years. Increased splenic hematopoietic cell proliferation was observed in male mice (≥ 12.8 ppm) and female mice (≥ 32 ppm) exposed to chloroprene for 2 years.

4.7 EVALUATION OF CARCINOGENICITY

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), 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, 042076) 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) suggestive 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* (2005, 086237) 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. Quantitative 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. Synthesis of Human, Animal, and Other Supporting Evidence

4.7.1.1. 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., 2007, 625187; Marsh et al., 2007, 625188; Colonna and Laydevant, 2001, 625112; Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Romazini et al., 1992, 624896; Li et al., 1989, 625181; Leet and Selevan, 1982, 094970; Pell, 1978, 064957). 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 339, 240, 242, 571 when compared to external populations (Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Li et al., 1989, 625181; Leet and Selevan, 1982, 094970).

Although sample size and statistical power were limited (thus limiting the precision of risk estimates), Bulbulyan et al. (1999, 157419; 1998, 625105) observed significantly elevated relative risk estimates for liver cancer incidence and mortality among intermediate and highly exposed workers. The study involving four plants (including the Louisville Works plant included in the Leet and Selevan (1982, 094970) study) by Marsh et al. (2007, 625188), 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). Although not statistically significant, these findings are consistent in magnitude with results (RR range: 2.9-7.1) detected in two other studies for high and intermediate cumulative exposures (Bulbulyan et al., 1999, 157419; Bulbulyan et al., 1998, 625105). 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. These data allowed for internal analyses to be conducted which should be less impacted by bias due to the healthy worker effect; however, the potential for healthy survivor effect remains as noted previously. 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, 157419; Marsh et al., 2007, 625188). Among these studies, only Bulbulyan et al. (1999, 157419) 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 precluded its examination as a potential confounder, 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 the possibility of co-exposures which may be confounders, although Bulbulyan et al. (1999, 157419) discussed the known co-exposures at the study facility in Armenia and reported that none were known liver carcinogens. One study with data on a co-exposure (vinyl chloride) reported evidence of negative confounding (Marsh et al., 2007, 625188). 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. Lastly, another concern in some of the occupational cohorts is the low reported expected counts for liver and lung cancer mortality (Li et al., 1989, 625181; Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419). This could be an indication of inaccurately applied population rates or incorrect calculation of expected values based on the selected population mortality rates. Use of very low expected counts of cancer mortality may result in unstable estimates of effect. Regardless, the results of the studies reporting very low expected counts of cancer mortality and increased SMRs should not be discounted from the weight of evidence of the carcinogenicity of chloroprene; these studies do indicate a statistically significant association across heterogeneous populations and exposure scenarios.

It is also important to note that some of the epidemiology studies investigated the same cohort. For example, the Marsh et al. (2007, 625187; 2007, 625188) study investigated a employee cohort from the Louisville Works DuPont plant that was previously investigated in Leet and Selevan (1982, 094970). However, there are a number of differences between the studies that warranted independent analysis of each. Specifically, Leet and Selevan (1982, 094970) reported that the Louisville cohort consisted of 1575 male employees (salaried and female employees excluded due to "minimal or no potential exposure to chloroprene") who were working at the Louisville plant on 6/30/1957. The authors further reported that most of the employees had 15 years of potential exposure to chloroprene (indicating that most had worked at the plant since it's opening in 1942). Also, the cohort was followed until 1974. Marsh et al. (2007, 625187; 2007, 625188) included "all workers (male and female) in each plant with potential exposure to chloroprene from the “start of production” until 2000. For the Louisville plant, this included a total of 5507 workers employed from 1949-1972. The Marsh et al. (2007, 625187; 2007, 625188) analyses started at 1949 to "avoid methodological problems associated with the earlier fifth revision of the ICD" and stopped at 1972 for the Louisville plant as that was when they report chloroprene production stopped at that plant, although chloroprene purification and polymerization still occurred there according to Leet and Selevan (1982, 094970). Also, there are important differences in how each study assessed exposure. Leet and Selevan (1982, 094970) used worker history summaries to classify workers as either "high" or "low" chloroprene exposure, whereas Marsh et al. (2007, 625187; 2007, 625188) used a more sophisticated approach that considered worker history summaries and worker exposure profiles to generate quantitative estimates of chloroprene exposure intensity. Similar differences between Colonna and Laydevant (2001, 625112) and Marsh et al. (2007, 625187; 2007, 625188) relative to the Isere/Grenoble cohort also warrant independent analysis of these studies. Therefore, although these studies investigated members of the same cohort, a number of methodological differences between the studies warrant the independent analysis of each.

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.

4.7.1.1.1. 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. These guidelines advocate the use of "criteria" proposed by Hill (1965, 071664) to assess causality. 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., 2007, 625187; Marsh et al., 2007, 625188; Colonna and Laydevant, 2001, 625112; Bulbulyan et al., 1998, 625105; Pell, 1978, 064957).

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, 625181), Louisville, United States (Leet and Selevan, 1982, 094970), Russia (Bulbulyan et al., 1998, 625105) and Armenia (Bulbulyan et al., 1999, 157419) despite evidence of healthy worker effect bias. Despite relatively small numbers, there were also suggestive data from the re-analysis of the Louisville cohort by Marsh et al. (2007, 625188),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., 1998, 625105; Bulbulyan et al., 1999, 157419) 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, examining four independent cohorts, have shown an association between chloroprene exposure and liver cancer incidence and mortality (Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Li et al., 1989, 625181; Leet and Selevan, 1982, 094970), while a fifth study showed evidence suggesting an association when examined in relation to detailed exposure data (Marsh et al., 2007, 625188). It is important to note that the Marsh et al. (2007, 625188; 2007, 625187) study investigates an employee cohort from the Louisville Works DuPont plant that was previously investigated in Leet and Selevan (1982, 094970). However, there are a number of differences between the studies (e.g., different exposure assessment methodologies) that warrants independent analysis of each. 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.

Biological Gradient – refers to the presence of a dose-response and/or exposure/duration-response between a health outcome and exposure of interest. The aforementioned internal analyses for chloroprene and liver cancer mortality (Bulbulyan et al., 1999, 157419; Leet and Selevan, 1982, 094970) suggest a potential biological gradient by comparing highly exposed workers to low or unexposed workers. In Bulbulyan et al. (1999, 157419), the SIR for intermediate cumulative exposure to chloroprene is 293 (95% CI: 41 – 2080), whereas the SIR for the high cumulative exposure group is 486 (95% CI: 202 – 1170). Although these effect estimates are not statistically significant from one another, the presence of monotonically increasing effects relative to cumulative exposure is apparent. In Leet and Selevan (1982, 094970), there is a dose-response apparent in the author-reported effect of cancer of the liver and biliary passage. However, if liver cancer was considered separately, this dose-response would disappear as only one of the three reported cases was liver cancer. The other studies examining exposure-response relationships do not demonstrate a monotonic increase in risk but have reported consistent elevated risks above 3.3 in the upper exposure categories (Bulbulyan et al., 1998, 625105; Marsh et al., 2007, 625188). Some suggestion of an exposure-response effect has also been observed in comparisons between long-term employees and short-term employees in the Bulbulyan studies.

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 the carcinogenicity of chloroprene in humans.

4.7.1.2. *Laboratory* Animal

According to the NTP (1998, 042076), 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, statistically significantly increased incidences of neoplastic lesions occurred in the oral cavity (papillomas or carcinomas, males and females), kidney (renal tubule adenomas or carcinomas, males), thyroid gland (adenomas or carcinomas, males) and mammary gland (fibroadenomas, females). In mice, increased incidences in neoplasms occurred in the lungs (adenomas or carcinomas, males and females), circulatory system (hemangiomas or hemangiosarcomas, all organs, males and females), Harderian gland (adenomas or carcinomas, males and females), liver (adenomas or carcinomas, females), skin and mesentery (sarcomas, females), mammary gland (carcinomas, females), and kidney (renal tubule adenomas or carcinomas, males). The observation of that chloroprene is more potent in inducing tumors in B6C3F1 mice compared to F344/N rats may be due to species differences in metabolism. The activity of liver or lung microsomal oxidation of chloroprene and the formation of (1-chloroethenyl)oxirane was generally higher in the mouse than the rat (Himmelstein et al. (2004, 625152), see Tables 3-4 and 3-6); additionally, the activity of epoxide hydrolase in liver microsomes was greater in the rat compared to the mouse (epoxide hydrolase activity was approximately equal in lung microsomes). The observation that formation of the reactive epoxide metabolite of chloroprene is greatest in the mouse lung may explain the observation that chloroprene exposure induces lung tumors in mice, but not rats.

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, 625008). 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, 042076) 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, 625008), 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. (2001, 019012) observed that liver microsomes from B6C3F1 mice and the F344 rats, the two species used in the NTP (1998, 042076) study, produced more (1-chloroethenyl)oxirane than those from hamsters or Wistar rats, the two species used in the Trochimowicz et al. (1998, 625008) study. These differences in production of (1-chloroethenyl)oxirane were as great as 12-fold greater (F344 rats vs. hamsters). However, measurements of V_{max}/K_m for liver microsomal oxidation of chloroprene were approximately equal for the mouse and hamster, with both being greater than either strain of rat (Himmelstein et al., 2004, 625152). In lung microsomes, the activity was much greater in the mouse compared to all other species. The activity of epoxide hydrolase in liver microsomes was highest in the hamster, followed by both rat strains with the mouse having the lowest activity. Epoxide hydrolase activity in lung microsomes was highest in hamsters, with rats and mice being approximately equal. The combination of highest rate of oxidation of chloroprene with the slowest rate of epoxide detoxification in mouse microsomes provides some insight on the observation that the mouse is the most sensitive species/strain across both studies.

The inhalation study by Dong et al. (1989, 007520) 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, 042076) 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, 075453). The number of tumor-bearing animals was similar to controls.

4.7.2. Summary of Overall Weight of Evidence

In the current document, a total of nine studies covering eight 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., 1998, 625105;

Bulbulyan et al., 1999, 157419; Li et al., 1989, 625181; Leet and Selevan, 1982, 094970) and lung (Marsh et al., 2007, 625188; Colonna and Laydevant, 2001, 625112; Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Leet and Selevan, 1982, 094970; Pell, 1978, 064957). 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 a 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.

There was a statistically significant excess of liver cancers in four of the cohorts reviewed (Bulbulyan et al., 1999, 157419; Bulbulyan et al., 1998, 625105; Li et al., 1989, 625181; Leet and Selevan, 1982, 094970), 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., 2007, 625188), the observed RR increased with increasing cumulative exposure in the plant with the highest exposure levels, indicating a dose-response trend. 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 4.7.1.1.

According to NTP (1998, 042076), 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). Additionally, metabolites of chloroprene include DNA-reactive epoxides and a mutagenic mode of action is proposed based on suggestive results in *in vitro* bacterial assays and the observation of *in vivo* K- and H-*ras* mutations in animals exposed to chloroprene (see Section 4.7.3.2).

Table 4-39. 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. • 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, 042076) • Humans – Leet and Selevan (1982, 094970), Li et al. (1989, 625181), Bulbulyan et al. (1998, 625105), and Bulbulyan et al. (1999, 157419)
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., lack of data on confounders, 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

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 or distributed systemically throughout the body. 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 (2005, 088823; 2000, 194536)) 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, 625093; U.S. EPA, 2002, 052153; Sills et al., 1999, 624952). 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; 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, 042076) and Melnick et al. (1999, 000297), 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, 010689) showed that 2-chloro-2-ethynyloxirane and/or (1-chloroethenyl)oxirane could be intermediates in the biotransformation of chloroprene. Himmelstein et al. (2001, 019012) confirmed the identity of the volatile metabolite reported by Bartsch et al. (1979, 010689) as the epoxide (1-chloroethenyl)oxirane. Himmelstein et al. (2001, 019012) 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, 157445)confirmed the results of Himmelstein et al. (2001, 019012), 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, 625159) 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 (U.S. EPA, 2002, 052153). 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 (U.S. EPA, 2002, 052153). 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, 625045; U.S. EPA, 2000, 194536).

Metabolites of chloroprene 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, 625215). 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, 010173).

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, 624952; Sills et al., 2001, 624922; Ton et al., 2007, 625004), 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, 042076) were shown to have a higher frequency of mutations in K- and H-*ras* proto-oncogenes than in spontaneous occurring tumors (Sills et al., 2001, 624922; Sills et al., 1999, 624952). 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, 625004). 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, 042076; U.S. EPA, 2000, 194536). Activated K-*ras* oncogenes were observed in lung tumors, hepatocellular carcinomas, and lymphomas in B6C3F1 mice exposed to 1,3-butadiene (U.S. EPA, 2002, 052153). 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 (U.S. EPA, 2002, 052153).

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, 625049; Bartsch et al., 1979, 010689) while the bacterial frame shift (*S. typhimurium* strains TA 97 and TA 98) assays have been nonpositive (NTP, 1998, 042076; Willems, 1978, 625048; Willems, 1980, 625049). 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., 2001, 624922; Sills et al., 1999, 624952). In contrast, other studies (NTP, 1998, 042076) have reported nonpositive results for all bacterial strains. Westphal et al. (1994, 625047) suggested that decomposition products of chloroprene may be responsible for the mutagenicity seen in positive tests. Westphal et al. (1994, 625047) exposed bacteria directly to liquid chloroprene in solution and observed no increase in mutagenicity, whereas positive tests (Willems, 1978, 625048; Willems, 1980, 625049; Bartsch et al., 1979, 010689) 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 the major epoxide metabolite of chloroprene, (1-chloroethenyl)oxirane, in solution (Himmelstein et al., 2001, 019013).

Conflicting results (positive in Vogel (1979, 000948); nonpositive in Foureman et al. (1994, 065173)) 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, 019013; Drevon and Kuroki, 1979, 010680), *in vivo* chromosomal damage (NTP, 1998, 042076) assay, and bone marrow micronucleus assays (NTP, 1998, 042076; Shelby and Witt, 1995, 624921). 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* toxicity 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, 042076), 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., 2001, 624922; Sills et al., 1999, 624952). However, a positive result with all bacterial strains was observed with the epoxide intermediate of chloroprene, (1-chloroethenyl)oxirane (Himmelstein et al., 2001, 019013).

A comparative analysis by Melnick and Sills (2001, 051506) 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, 051506) 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 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 leads 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, although alternative or additional modes of action for chloroprene carcinogenicity may exist in certain situations (i.e., at high exposure levels), these modes of action have not been definitively identified or supported by existing evidence.

Strength, Consistency, Specificity of Association – Data from NTP (1998, 042076) and Sills et al. (2001, 624922; 1999, 624952) 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, 624922; Sills et al., 1999, 624952). 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, 625208). 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, 624922; Sills et al., 1999, 624952; NTP, 1998, 042076). 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, 624922; Sills et al., 1999, 624952) 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, 042076). 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 (U.S. EPA, 2005, 086237).

Early-Life Susceptibility – According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* (U.S. EPA, 2005, 088823) 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, 042076).

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

Bernauer et al. (2003) investigated cytochrome P450 variability in leukapheresed samples from 50 humans as an indication of extrahepatic P450 variability via Western blotting and immunoquantification. CYP2E1 was observed to have a median expression of 0.2 pmol/mg protein and varied between 0.13 and 0.68 pmol/mg protein. The ratio between the 5th and 95th percentile was 3.3, which was the lowest level of variability in the six P450 isoforms investigated. Additionally, Neafsey et al. (2009, 196814) identified, in a review of the open literature, a number of CYP2E1 genotypic and phenotypic polymorphisms in a number of human populations, and postulated that the influence of CYP2E1 polymorphisms on adverse responses in exposed subjects would be expected to be significant. However, the authors further state that the direction and magnitude of enzyme activity changes due to polymorphisms is generally not well delineated and ultimately conclude that “the evidence for particular CYP2E1 polymorphisms having a significant effect on enzyme activity *in vivo* is too limited to support the population distribution of CYP2E1 enzyme activity based upon genotype”. They suggest that dietary, lifestyle, and physiological factors may exert substantial influence on CYP2E1 phenotypes. Additionally, P450 mediated metabolism of chloroprene may be multifactoral, with multiple individual CYPs playing a role. Thus the expression of one single CYP may not adequately describe the possible variations within the human population. No data is currently available on the toxicodynamic variability within the human population.

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, 064961). Additionally, the major metabolite of chloroprene, (1-chloroethenyl)oxirane, is rapidly detoxified via epoxide hydrolase-mediated hydrolysis in mouse liver microsomes (Himmelstein et al., 2001, 019012). The levels of both epoxide hydrolase and glutathione transferase (GST) have been shown to be lower in infants than adults (Ginsberg et al., 2004, 625124). Epoxide hydrolase is active at birth, but only at 50% of adult function for as long as 2 years. Evidence, although limited, suggests that 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, 064961). 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 the observed carcinogenicity of chloroprene (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, 2005, 088823).

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, 075453).

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. A route-to-route extrapolation from available chronic inhalation data to oral data for the purposes of deriving an RfD was not performed due to the inadequacies of the current chloroprene PBPK model (see Section 3.5).

Therefore, an RfD was not derived due to the significant uncertainty associated with the oral database for chloroprene and the lack of a validated PBPK model for route-to-route extrapolation.

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, 042076), and a 2-year chronic study in Wistar rats and Syrian gold hamsters (Trochimowicz et al., 1998, 625008).

The chronic NTP inhalation bioassay (1998, 042076) 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, 042076). 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, 625008) 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, 042076) 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, 042076) 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 the observed toxicity of chloroprene in two species. Trochimowicz et al. (1998, 625008) was not chosen as the principal study due to concerns regarding the high mortality observed in the low dose male and female rats due to the failure in the exposure chamber ventilation system. The high mortality in this dose group prevented histopathological examination of most organ systems (except for liver samples) and precluded any firm conclusions on dose-response characteristics from being drawn. Also, a lack of adverse effects at similar exposure levels as the NTP (1998, 042076) study (Trochimowicz et al. (1998, 625008); see Section 4.7.2.2 for discussion of potential causes of differences in observed toxicity between the NTP and Trochimowicz studies) was observed and influenced the choice to not select the Trochimowicz et al. (1998, 625008) as the principal study.

From the NTP (1998, 042076) study, all portal-of-entry and systemic nonneoplastic lesions that were statistically increased in mice or rats at the low- or mid-exposure concentration (12.8 or 32 ppm) compared to chamber controls, or demonstrated a suggested dose-response relationship in the low- or mid-exposure range in the absence of statistical significance, were considered candidates for the critical effect. Nonneoplastic effects identified as secondary to neoplastic effects (i.e., histiocytic cell proliferation in mice) were not considered candidates for the critical effect. The candidate endpoints included olfactory suppurative inflammation, bronchiolar hyperplasia, kidney (renal tubule) hyperplasia, forestomach epithelial hyperplasia, and splenic hematopoietic cell proliferation in mice, and olfactory atrophy, olfactory basal cell hyperplasia, olfactory metaplasia, olfactory necrosis, olfactory chronic inflammation, alveolar epithelial hyperplasia, and kidney (renal tubule) hyperplasia in rats (Table 5-1).

Table 5-1. Incidences of nonneoplastic lesions resulting from chronic exposure (ppm) to chloroprene considered for identification of critical effect

SPECIES	TISSUE	ENDPOINT	MALE				FEMALE			
			0	12.8	32	80	0	12.8	32	80
Mice	Nose	Suppurative inflammation	--	--	--	--	0/50	1/49	3/49*	4/50**
	Lung	Bronchiolar hyperplasia	0/50	10/50**	18/50**	23/50**	0/50	15/49**	12/50**	30/50**
	Kidney	Renal tubule hyperplasia	2/50	16/49**	17/50**	18/50**	--	--	--	--
	Fore-stomach	Epithelial hyperplasia	4/50	6/48	7/49	29/50**	4/50	3/49	8/49	27/50**
	Spleen	Hematopoietic cell proliferation	26/50	22/49	35/50[a]	31/50[a]	13/50	25/49[a]	42/49[a]	39/50[a]
Rats	Nose	Atrophy	3/50	12/50*	46/49**	48/49**	0/49	1/50	40/50**	50/50**
		Basal cell hyperplasia	0/50	0/50	38/49**	46/49**	0/49	0/50	17/50**	49/50**
		Metaplasia	6/50	5/50	45/49**	48/49**	0/49	1/50	35/50**	50/50**
		Necrosis	0/50	11/50**	26/49**	19/49**	0/49	0/50	8/50**	12/50**
		Inflammation, chronic	0/50	5/50*	9/49**	49/49**	--	--	--	--
	Lung	Alveolar epithelial hyperplasia	5/50	16/50**	14/49*	25/50**	6/49	22/50**	22/50**	34/50**
	Kidney	Renal tubule hyperplasia	14/50	20/50	28/50**	34/50**	6/49	6/50	11/50	21/50

* $p < 0.05$

** $p < 0.01$

-- Endpoint not considered for selection of critical effect

[a] Reported as statistically significantly greater than controls, but level of significance not reported

Source: NTP (1998, 042076)

5.2.2. Methods of Analysis

This assessment used benchmark dose (BMD) methodology, where possible, to estimate a POD for the derivation of an RfC for chloroprene. The use of the BMD methodology was preferred for the estimation of a POD for many reasons, including consideration of the shape of the entire dose-response curve and estimation of the experimental variability associated with the calculated dose-response relationship. Use of BMD methods involves fitting mathematical models to the observed 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 in lieu of the NOAEL or LOAEL 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. The data for some endpoints were not amenable to BMD modeling for a number of reasons, including the observation of maximal or near-maximal reponse at the lowest dose tested, the failure to achieve an incidence greater than the BMR at any dose level, or equal incidence in all dose groups. Additionally, even when some datasets were deemed adequate for BMD modeling, no model provided adequate model fit. In these cases, the NOAEL/LOAEL approach was used.

A BMR of 10% extra risk is typically chosen as a response level for dichotomous data and is recommended for the BMR when using dichotomous models to facilitate a consistent basis of comparison across assessments and endpoints (U.S. EPA, 2000, 052150). For the data from the NTP (1998, 042076) study, a BMR of 10% extra risk was used initially under the assumption that it represents a minimal biologically significant change. In addition to the incidence of the endpoints, the NTP (1998, 042076) study also reported the severity scores for individual animals in each dose group, thus making it possible to determine whether the endpoints were increasing in severity as well as incidence with dose (see Table B-1). In the case of endpoints that progressed in incidence as well as severity (i.e., progression from mild to moderate lesions) from the control dose to the lowest dose showing reponse, a BMR of 10% was not considerd to be a minimal biologically significant change. Therefore, for these endpoints, a BMR of 5% was used. All available dichotomous models in the EPA BMD software (BMDS version 2.1.1) were fit to the incidence data for lung, nasal, and systemic effects in rats and mice (Table 5-1).

The models selected for each particular endpoint were chosen based on global and local goodness-of-fit criteria (global p-value and chi-square [χ^2] residual values, respectively) and visual inspection. The global goodness-of-fit p-value provides an indication of how well a particular model fits the observed dose-reponse data across the entire range of doses, whereas the χ^2 residual gives an indication of how well the model fits at the dose group closest to the calculated BMD. A global p-value ≥ 0.1 and χ^2 residual $\leq |2|$ is required for a model to be considered as adequately fitting the dose-response data. Finally, a visual inspection of the dose-response curve is necessary in order to determine whether the calculated dose-response curve is appropriate (e.g., monotonically increasing). When multiple appropriately fitting models are identified for a particular endpoint, the “best” model must be selected out of the group. When the calculated BMDLs are within a 3-fold difference of one another for a particular endpoint, indicating a low degree of model-dependence, the model with the lowest Akaike Information Criterion (AIC) is selected as the best model. The AIC awards the most parsimonious model so that models with higher numbers of parameters are only selected as the best fitting model when they significantly improve model fit. When the calculated BMDLs are not within a 3-fold difference, model dependence is assumed and the model returning the lowest BMDL is selected (U.S. EPA, 2000, 052150). Details of the BMD modeling analysis, including all relevant model-fit criteria and final model selection information, are provided in Appendix B1.

The BMDs and BMDLs associated with an extra risk of 10% or 5% for the best-fitting models for each endpoint are shown in Table 5-2. NOAELs and LOAELs were used as potential PODs for the endpoints not deemed appropriate for BMD modeling, or when adequate model fit could not be achieved by any model.

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, 042076) 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 systemic lesions in rats and mice are calculated as follows:

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

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:

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

For example, for olfactory atrophy in the male rat, the POD_{ADJ} would be calculated as follows:

$$POD_{ADJ}\text{ (ppm)} = 3.5\text{ ppm} \times 6\text{ hours/24 hours} \times 5\text{days/7days}$$
$$POD_{ADJ}\text{ (ppm)} = 0.6\text{ ppm}$$
$$POD_{ADJ}\text{ (mg/m}^3\text{)} = 0.6\text{ ppm} \times 3.62\text{ mg/m}^3\text{/1ppm}$$
$$POD_{ADJ}\text{ (mg/m}^3\text{)} = 2.3\text{ mg/m}^3$$

The calculated POD_{ADJ} (mg/m^3) values for all considered endpoints are presented in the last column of Table 5-2.

Table 5-2. Duration adjusted point of departure estimates for best fitting models of the BMD from chronic exposure to chloroprene

Endpoint	Species/ Sex	NOAEL (ppm)	LOAEL (ppm)	Model[a]	BMR	BMD[b] (ppm)	BMDL[b] (ppm)	POD$_{ADJ}$[c] (mg/m^3)
Nasal Effects - Olfactory								
Atrophy	Rat/male	--	12.8	Logistic[d]	5	4.9	**3.5**	2.3
	Rat/female	**12.8**	32	[e]	--	--	--	8.3
Basal cell hyperplasia	Rat/male	**12.8**	32	[e]	--	--	--	8.3
	Rat/female	12.8	32	Log-probit[f]	10	23.5	**19.7**	12.7
Metaplasia	Rat/male	**12.8**	32	[e]	--	--	--	8.3
	Rat/female	**12.8**	32	[e]	--	--	--	8.3
Necrosis	Rat/male	--	12.8	Log-probit[d]	5	5.6	**4.5**	2.9
	Rat/female	12.8	32	Log-probit[f]	5	24.8	**19.7**	12.7
Chronic inflammation	Rat/male	--	12.8	Log-logistic[d]	10	14.6	**9.3**	6.0
Suppurative inflammation	Mouse/female	**12.8**	32	[e]	--	--	--	8.3
Lung Effects								
Alveolar hyperplasia	Rat/male	--	12.8	Log-logistic	5	5.4	**3.3**	2.1
	Rat/female	--	12.8	Log-logistic	10	4.9	**3.3**	2.1
Bronchiolar hyperplasia	Mouse/male	--	12.8	Log-logistic	5	3.6	**2.7**	1.7
	Mouse/female	--	**12.8**	[g]	--	--	--	8.3
Systemic Effects								
Kidney (renal tubules) hyperplasia	Rat/male	12.8	32	Log-logistic	10	6.5	**4.0**	2.6
	Rat/female	32	80	Log-probit	10	32.5	**23.5**	15.2
	Mouse/male	--	**12.8**	[e]	--	--	--	8.3
Forestomach epithelial hyperplasia	Mouse/male	32	80	Multistage	10	24.7	**20.5**	13.3
	Mouse/female	32	80	Multistage	10	31.0	**19.3**	12.5
Splenic hematopoietic proliferation	Mouse/male	**12.8**	32	[e]	--	--	--	8.3
	Mouse/female	--	12.8	Probit[d]	5	2.1	**1.7**	1.1

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

[b] BMR = benchmark dose response

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

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

[e] Did not model endpoint (reasons include: maximal response in lowest dose showing response over controls, response levels did not achieve 10% incidence, incidence equal in all doses with response). Therefore, the NOAEL/LOAEL approach is recommend to determine a POD

[f] Dichotomous hill model had lowest AIC, but model output warned that BMDL estimate was "imprecise at best". Therefore, the model with the next lowest AIC was chosen (see Appendix B for details)

[g] No model fits appropriately according to fit statistics or visual inspection.

Source: NTP (1998, 042076)

The results of BMD modeling indicated that splenic hematopoietic cell proliferation in the female mouse was the most sensitive endpoint, with a POD_{ADJ} value of 1.1 mg/m^3. Several other endpoints (olfactory atrophy and necrosis in the male rat, alveolar hyperplasia in male and female rats, bronchiolar hyperplasia in male mice, and renal tubule hyperplasia in male mice) had somewhat higher POD_{ADJ} values, ranging from 1.7 to 2.9 mg/m^3.

Chloroprene is a relatively water-insoluble, non-reactive gas, with an approximate blood:air partition coefficient of less than 10 (see Table 3-1), that induces a range of nasal, thoracic, and systemic 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, 016157). The observation of systemic (i.e., non-respiratory) effects resultant from chloroprene exposure clearly indicates the compound is absorbed into the bloodstream and distributed throughout the body. 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 mucosa and lower respiratory tract 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 mucosa of rats has been shown to specifically express CYP2E1 at levels more similar to hepatic levels than any other non-hepatic tissue examined (Thornton-Manning and Dahl, 1997, 597688). Himmelstein et al. (2004, 625152) observed that the microsomal fraction of rat lung homogenates was active in the metabolic oxidation of chloroprene into (1-chloroethenyl)oxirane at levels between 10-30% that of liver microsomes. *In situ* conversion of chloroprene into its highly reactive epoxide metabolite in the olfactory epithelia and lower respiratory tract may facilitate its uptake in these tissues and explain a portion of its biological activity in those regions. As it is also observed that chloroprene induces adverse effects in organ systems distal to the portal-of-entry, consistent with the parent compound's water-insoluble and non-reactive chemical properties, it is possible that observed nasal and respiratory effects are due to systemic redistribution of chloroprene to these tissues. Currently, the contribution of either route of delivery (portal-of-entry vs. systemic distribution) to the induction of nonneoplastic respiratory effects is unknown.

However, the selected critical effect, splenic hematopoietic cell proliferation, is clearly a systemic effect and the human equivalent concentration (HEC) for chloroprene was calculated by the applicaton of the appropriate dosimetric adjustment factor (DAF) in accordance with the U.S. EPA RfC methodology (U.S. EPA, 1994, 006488). 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, 1994, 006488). For gases with systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

$$DAF = (H_{b/g})_A/(H_{b/g})_H$$

where:

$(H_{b/g})_A$ = the animal blood:air partition coefficient

$(H_{b/g})_H$ = the human blood:air partition coefficient

$$DAF = 7.8/4.5$$

$$DAF = 1.7$$

In cases where the animal blood:air partition coefficient is higher than the human value (see Table 3-1), resulting in a DAF >1, a default value of 1 is substituted (U.S. EPA, 1994, 006488). Therefore, the HEC for splenic hematopoietic cell proliferation in female mice is calculated as follows:

$$\begin{aligned} POD_{HEC}\ (mg/m^3) &= POD_{ADJ}\ (mg/m^3) \times DAF \\ &= POD_{ADJ}\ (mg/m^3) \times 1.0 \\ &= 1.1\ mg/m^3 \times 1.0 \\ &= 1.1\ mg/m^3 \end{aligned}$$

Therefore, the POD_{HEC} of 1.1 mg/m^3 for the critical effect of splenic hematopoietic cell proliferation in female mice was selected for the derivation of the RfC for chloroprene.

5.2.4. RfC Derivation—Including Application of Uncertainty Factors

A POD_{HEC} value of 1.1 mg/m^3 for increased incidence of splenic hematopoietic cell proliferation in female B6C3F1 mice (NTP, 1998, 042076) was used as the POD to derive the chronic RfC for chloroprene. A total UF of 100 was applied to this POD_{HEC} 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, 1994, 006488). 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 10-fold UF_H was used to account for variation in susceptibility among members of the human population (i.e., interindividual variability). Only limited information is available to predict potential variability in human susceptibility, including some data regarding the human variability in expression of enzymes involved in chloroprene metabolism (e.g., metabolic activation via p450 isoform CYP2E1) (see Section 4.8). Due to this limited data on variations in susceptibility within the human population, default 10-fold UF_H is applied.
- 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 5% change in splenic hematopoietic cell proliferation 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 thorough examination of the observed toxicity of chloroprene 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 full two-generation reproductive toxicity study (the Appelman and Dreef van der Meulen

(1979, 064938) unpublished study exposed F_0 and F_1 rats to chloroprene, but did not allow the F_1 rats to mate).

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

$$RfC = POD_{HEC} \div UF = 1.1\ mg/m^3 \div 100 = 1.1 \times 10^{-2}\ mg/m^3 = 1 \times 10^{-2}\ mg/m^3$$

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, 042076) study that were modeled with BMDS (version 2.1.1), including nasal, pulmonary, and systemic effects in male and female rats and mice. Of the considered studies, the NTP (1998, 042076) 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 based on the BMDL of the best fitting model from BMD modeling and were adjusted for duration and dosimetry before applications of uncertainty factors.

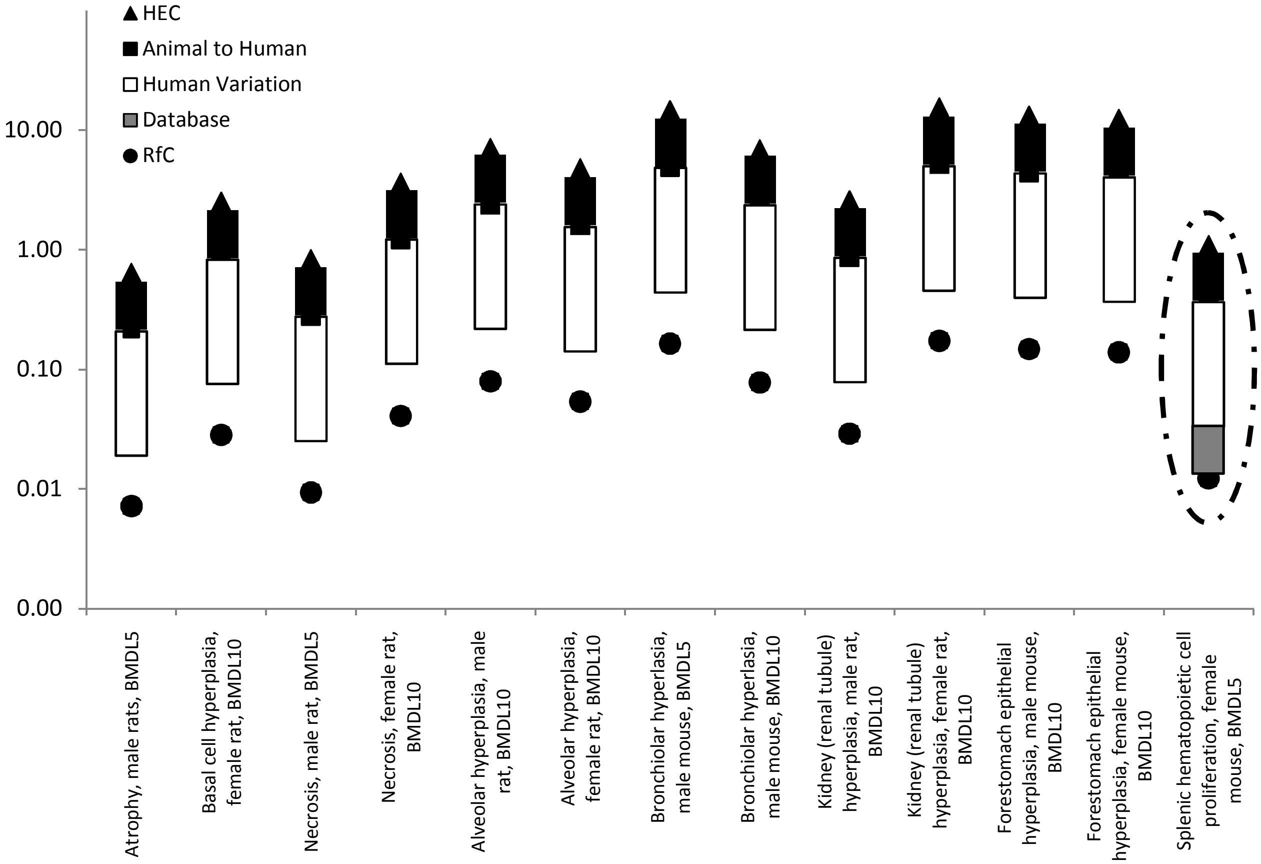


Figure 5-1. Points of departure (in mg/m^3) for selected endpoints with corresponding applied uncertainty factors and derived sample RfCs (chosen RfC value is circled)

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, 1994, 006488), 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 extrapolation from an animal bioassay to human exposure, a diverse population of varying susceptibilities, POD determination methodologies (NOAEL, LOAEL, or BMDL), 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, 042076) chronic inhalation study ranged from 6.0 × 10^{-3} to 2.0 × 10^{-1} mg/m^3. Sample RfC values primarily depended on whether olfactory, pulmonary, or systemic effects were considered, what mode of delivery was assumed (i.e., portal-of-entry vs. systemic delivery), what BMR was chosen, and whether the POD$_{ADJ}$ or POD$_{HEC}$ was used for selection of endpoint. The chosen critical effect, increased incidence of splenic hematopoietic cell proliferation in female mice, was considered to be the most sensitive endpoint because it returned the lowest POD_{ADJ} values compared to all other considered endpoints.

In the case of chloroprene, the POD_{ADJ} was used as the basis for selection of the critical effect because of uncertainties concerning the application of a portion of the DAF procedures used in calculating the POD_{HEC}. HECs are human toxicokinetically equivalent external air concentrations of the animal POD_{ADJ} that have been dosimetrically adjusted through application of DAFs, in accordance with the current RfC methodology (U.S. EPA, 1994, 006488). The DAF for respiratory portal-of-entry effects is the regional gas dose ratio (RGDR), a ratio of animal and human physiological parameters that normalizes inhaled doses (expressed as the minute volume) to surface areas of specific regions in the respiratory tract (i.e., the extrathoracic or nasal, region). The normalization of inhaled dose to respiratory tract surface area outlined in the RfC methodology is based on three major assumptions: uniformity in the flow of gas through the respiratory tract region of interest, uniformity and equivalency (in terms of cell types, relative amount, and distribution) of the surface areas of the respiratory tract regions subtending the gas flow, and uniformity in the deposition of gas over the entire surface area of the respiratory tract region of interest. Application of the default DAFs, based on the ratio of minute volume and respiratory tract surface area between rats and humans, indicates that humans receive approximately five times the dose in the nasal region compared to rats (i.e., DAF = 0.28). Recent advances have been made in inhalation dosimetry, including analyses investigating the nature of gas flow through the upper respiratory tract (via flow-dye cast models and computational fluid dynamics modeling) and the deposition of inhaled toxicants in the respiratory tract (as detailed in U.S. EPA, 2009, 625038). These analyses have shown that gas flow through the upper respiratory tract is not uniform in rats or humans, and that uneven gas flow results in uneven distribution of gas deposition. Further advances in modeling, combining CFD and PBPK models, have allowed for the calculation of target tissue doses for a number of chemicals in both rats and humans, and therefore for calculation of alternative DAFs for interspecies extrapolation. Use of these hybrid CFD-PBPK models generally indicate that the upper respiratory system (i.e., nasal region) of rats and humans receive nearly equivalent doses at equivalent external exposure concentrations for most gases (i.e., DAFs ≈ 1). For some gases, though, CFD-PBPK modeling indicates that rats receive higher doses in the nasal region than humans (i.e., DAFs > 1). No such CFD-PBPK model currently exists for chloroprene. The default DAF for systemic effects is the ratio of animal and human blood:air partition coefficients. When the animal value is greater than the human value the DAF for systemic effects defaults to one. Selection of critical effect based on POD_{HEC} values calculated using default DAFs, would result in olfactory effects (either necrosis or atrophy) being identified as the critical effect. Selection of these two outcomes as critical effects would have lowered the derived RfC approximately 20-40% (8.0×10^{-3} mg/m^3 for necrosis or 6.0×10^{-3} mg/m^3 for atrophy) relative to splenic hematopoietic proliferation.

Uncertainty also surrounded the assumed mode of delivery of chloroprene (or its reactive epoxide metabolite) to the target tissues. The current RfC methodology attempts to group chemicals into one of three discrete categories based on their physio-chemical properties and presumed toxicokinetics (i.e., regional gas uptake). Using this scheme, chloroprene would be best classified as a category 3 gas, being relatively water insoluble and non-reactive, and would be expected to only elicit extrarespiratory effects. Medinsky and Bond (2001, 016157) describe an alternative method of classification that represents the reactivity and water solubility as continuous variables that allow insight to be gained regarding sites of and conditions of toxicity. Additionally, although not explicitly included in this scheme, *in situ* metabolism in the respiratory tract can be considered as a component of reactivity. In this manner, chloroprene can best be described as both a non-reactive, water insoluble chemical that is absorbed into the blood stream and induces systemic toxicity, as well as a chemical that is metabolized into a reactive epoxide within the respiratory tract, inducing portal-of-entry toxicity. This method of classification is consistent with what is proposed for the mode of action of chloroprene: conversion of the parent compound into its epoxide metabolite via P450 isoform CYP2E1, which is expressed in both the olfactory and pulmonary regions of the respiratory tract. *In situ* metabolism in the respiratory tract may thus explain a portion of the biological activity of chloroprene in these regions. It is observed that chloroprene induces adverse effects in organ systems distal to the portal-of-entry, consistent with its water insoluble and non-reactive chemical properties. Therefore, it is possible that observed pulmonary effects, such as alveolar hyperplasia in the female rat, may be explained by blood-borne delivery, rather than air-borne. Currently, the contribution of either mode of delivery to the sites of observed toxicity is not known. However, splenic hematopoietic cell proliferation is unequivocally a systemic lesion, and calculation of the POD_{HEC} uses a DAF = 1 and results in a value of 1.1 mg/m^3.

Choice of pulmonary effects treated as portal-of-entry effects would result in RfCs up to 20-fold higher than the RfC for splenic hematopoietic cell proliferation. For example, bronchiolar hyperplasia in male mice, using a BMR of 5% extra risk results in a POD_{ADJ} = 1.7 mg/m^3. Treating this endpoint as a portal-of-entry effect dictates that a DAF of 4.1 be applied, resulting in a POD_{HEC} of 7.0 mg/m^3 and an ultimate RfC of 7.0×10^{-2} mg/m^3. Choice of other systemic effects (i.e., forestomach epithelial hyperplasia in the male mouse) or pulmonary effects treated as systemic lesions would result in RfCs up to 15-fold greater.

In summary, for chloroprene, the POD_{ADJ} was used as the basis for selection of the critical effect because of uncertainties concerning the application of a portion of the DAF procedures used in calculating the POD_{HEC}, as pointed out by the External Peer Review committee. Based on the POD_{ADJ} of 1.1 mg/m^3, splenic hematopoietic proliferation was chosen as the critical effect, with an ultimate RfC of 1×10^{-2} mg/m^3 being derived.

Choice of model for BMDL derivation. The probit model fit the data for splenic hematopoietic cell proliferation in the female mouse adequately (global goodness of fit p-value = 0..9466). Data points are well-predicted near the BMD (χ^2 residual = 0.033). Use of other models would either increase or decrease the RfC by approximately 50%. However, the probit model was chosen over these models based on current BMD technical guidance (U.S. EPA, 2000, 052150).

Choice of BMR. There is uncertainty in the selection of the benchmark response (BMR) level. For increased incidence of splenic hematopoietic cell proliferation in female mice, definitive data do not exist to further inform the selection of what the appropriate BMR should be. However, the observation was made that the incidence and severity of this lesion increases in low dose animals compared to control animals; therefore a BMR of 5% extra risk was chosen based on the assumption that a 5% increase in incidence of this effect is minimally biologically significant.

Statistical uncertainty at POD. For the probit model applied to splenic hematopoietic cell proliferation in female mice, there is a reasonably small degree of statistical uncertainty at the 5% extra risk level (the point of departure for derivation of the RfC), with the BMDL being about 20% below the BMD.

Choice of bioassay. The NTP (1998, 042076) 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 chronic bioassay (Trochimowicz et al., 1998, 625008) was discounted for use as the principal study due to interpretational difficulties (i.e., high, accidental mortality in low dose animals resulting from the failure of the ventilation system) and a general lack of effects at exposure levels similar to those showing effects in the NTP (1998, 042076) study .

Choice of species. The RfC was based on increased incidence of splenic hematopoietic cell proliferation in female mice exposed to chloroprene via inhalation for 2 years. Use of other effects that occurred in another species, F344/N rats, would result in RfCs approximately 40% lower to 20 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. Expression levels of extrahepatic CYP2E1 have been shown to vary by approximately 3-fold (Bernauer et al., 2003, 625103). Neafsey et al. (2009, 196814) concluded that evidence for particular CYP2E1 polymorphisms having significant effect on enzyme activity *in vivo* is too limited to support generalized statements on populational distribution of CYP2E1 activity based on genotype. A number of issues, including lower enzyme levels and renal clearance in children, potential distribution of chloroprene to breast milk, and the proposed mutagenic mode of action for chloroprene suggest that childhood may represent a potentially susceptible lifestage to chloroprene 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 20-fold or ↓ RfC by up to 40%	RfC is based on the endpoint with the lowest POD_{ADJ} , increased incidence of splenic hematopoietic cell proliferation in female mice	Chosen endpoint is considered to be the most sensitive (based on POD_{ADJ} values). Its observed systemic toxicity is consistent with the physio-chemical properties of chloroprene. Selection of the critical effect was based on the POD_{ADJ} consistent with peer reviewer comments.
Choice of model for BMDL derivation	Other models would ↑ or ↓ RfC	Probit model used	U.S. EPA (2000, 052150) 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 5% extra risk chosen	BMR of 5% extra risk was chosen based on the assumption that a 5% increase in incidence of this effect is minimally biologically significant
Statistical uncertainty at POD	RfC would be ~ 20% higher if BMD (vs. BMDL) were used	BMDL used as POD per U.S. EPA guidance (2000, 052150)	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, 042076) 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 ↑ or ↓ if based on another species	Mice chosen	RfC is based on the most sensitive endpoint (incidence of splenic hematopoietic cell proliferation) in the most sensitive species (mouse), based on POD_{ADJ}
Human population variability	RfC could ↑ or ↓ if a non-default value of UF was used	10-fold uncertainty factor applied to derive the RfC	10-fold UF, the default value, is applied principally because of limited data on human variability or potential susceptible subpopulations

CONSIDERATION	POTENTIAL IMPACT[a]	DECISION	JUSTIFICATION
Completeness of the database	RfC could ↑ or ↓ if a different UF for database limitations was applied	3-fold uncertainty factor applied to derive the RfC	3-fold UF is applied as a major strength of the database is the inclusion of a well-designed chronic inhalation study investigating effects in multiple species. A limitation of the data is the lack of a multi-generational reproductive/developmental study.

[a] ↑ = increase, ↓ = decrease

5.4 CANCER ASSESSMENT

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

Both epidemiological and toxicological investigations of chloroprene carcinogenicity were available. Epidemiological studies of chloroprene provided evidence of associations between liver or lung cancer risk and occupational exposure to chloroprene (see Section 4.7.); however, study limitations precluded developing quantitative risk estimates from these studies. Two chronic bioassays were available, NTP (1998, 042076) and Trochimowicz et al. (1998, 625008). In the NTP (1998, 042076) 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. Examination of appropriate toxicological endpoints in both sexes of rats and mice was included. 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. Trochimowicz et al. (1998, 625008) studied groups of 100 male and femal Wistar and Syrian gold hamsters exposed via inhalation to 0, 10, or 50 ppm chloroprene for 6 hours/day, 5 days/week for up to 18 months (hamsters) or 24 months (rats). This study was not considered for quantification purposes, due to less pronounced sensitivity in the tested animals to neoplastic effects at similar exposure levels as in the NTP (1998, 042076) study, in part associated with high accidental mortality in the low-dose rats (see Section 4.2.2. for study details). The NTP (1998, 042076) study was used for development of an inhalation unit risk.

5.4.2. Dose-Response Data

The NTP (1998, 042076) study incidence data are summarized in Tables 5-4 (mice) and 5-5 (rats). Mice demonstrated statistically significant increases in tumor incidence at multiple sites: hemangiomas or hemangiosarcomas (all organs), alveolar /bronchiolar adenomas or carcinomas, forestomach (squamous cell papillomas or carcinomas), Harderian gland (adenomas and carcinomas), kidney adenomas (males only), skin sarcomas, hepatocellular adenomas or carcinomas, mammary gland (females), and Zymbal's gland carcinomas (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, $p \leq 0.001$, as conducted and reported by NTP). 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, 2005, 086237). The tumors observed in the Harderian and Zymbal's glands, however, were confirmed histopathologically only if observed grossly at necropsy; the corresponding tissues for most mice were not examined histopathologically. Use of the incidence data from these two sites as reported in Table 5-4 for dose-response analysis may underestimate the true incidence because other instances were possibly missed, but the sites were carried through the dose-response analysis in order to consider their relative impact. 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. Individual animal data including the time of observation of tumors is provided in Table C-1.

Rats demonstrated statistically significant increases in tumor incidence at multiple sites as well: oral cavity, squamous cell papillomas or carcinomas; thyroid gland, follicular cell adenomas or carcinomas; renal tubule adenomas or carcinomas; alveolar/bronchiolar adenomas or carcinomas (males); and mammary gland fibroadenomas. Overall, rats were not as sensitive as the mice, and were not considered further for dose-response analysis.

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

TISSUE		ADMINISTERED CHLOROPRENE CONCENTRATION (ppm)			
		Control	12.8	32	80
Females					
All organs: hemangioma or hemangiosarcoma	Unadjusted rate First incidence (days)	4/50 541	6/49 482	18/50 216	8/50 523
Lung: alveolar/bronchiolar adenoma or carcinoma	Unadjusted rate First incidence (days)	4/50 706	28/49 447	34/50 346	42/50 324
Liver: hepatocellular adenoma or carcinoma	Unadjusted rate First incidence (days)	20/50 493	26/49 440	20/50 503	30/50 384
Skin or mesentery: sarcoma	Unadjusted rate First incidence (days)	0/50 -	11/49 285	11/50 524	18/50 462
Mammary gland: adenocarcinoma, carcinoma or adenoacanthoma	Unadjusted rate First incidence (days)	3/50 527	6/49 440	11/50 394	14/50 336
Forestomach: squamous cell papilloma or carcinoma	Unadjusted rate First incidence (days)	1/50 734	0/49 -	0/50 -	4/50 576
Harderian gland[a]: adenoma or carcinoma	Unadjusted rate First incidence (days)	2/50 527	5/50 621	3/50 524	9/50 467
Zymbal's gland[a]: carcinoma	Unadjusted rate First incidence (days)	0/50 -	0/50 -	0/50 -	3/50 565

Males					
All organs: hemangioma or hemangiosarcoma	Unadjusted rate First incidence (days)	3/50 733	14/50 659	23/50 495	21/50 454
Lung: alveolar/bronchiolar adenoma or carcinoma	Unadjusted rate First incidence (days)	13/50 635	28/50 530	36/50 382	43/50 523
Forestomach: squamous cell papilloma or carcinoma	Unadjusted rate First incidence (days)	1/50 733	0/48 -	2/49 733	5/50 587
Harderian gland[a]: adenoma or carcinoma	Unadjusted rate First incidence (days)	2/50 596	5/50 701	10/50 596	12/50 589
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	Unadjusted rate First incidence (days)	0/50 -	2/49 722	3/50 715	9/50 567

[a] Tissues were examined histopathologically only if a lesion was observed grossly at necropy

Source: NTP (1998, 042076).

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

TISSUE		ADMINISTERED CHLOROPRENE CONCENTRATION (ppm)			
		Control	12.8	32	80
Females					
Oral cavity: papillomas or carcinomas	Unadjusted First incidence (days)	1/49 687	3/50 681	5/50 588	11/50 660
Thyroid gland: follicular cell adenomas or carcinomas	Unadjusted First incidence (days)	1/49 733	1/50 721	1/50 733	5/50 617
Mammary gland: fibroadenomas	Unadjusted First incidence (days)	24/49 366	32/50 302	36/50 470	36/50 433
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	Unadjusted First incidence (days)	0/49 -	0/50 -	0/50 -	4/50 609
Males					
Oral cavity: papillomas or carcinomas	Unadjusted First incidence (days)	0/50 -	2/50 701	5/50 609	12/50 539
Thyroid gland: follicular cell adenomas or carcinomas	Unadjusted First incidence (days)	0/50 -	2/50 597	4/49 569	5/50 307
Lung: alveolar/bronchiolar adenoma or carcinoma	Unadjusted First incidence (days)	2/50 616	2/50 702	4/49 505	6/50 540
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	Unadjusted First incidence (days)	1/50 733	8/50 600	6/50 679	8/50 625

[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, 042076).

5.4.3. Dose Adjustments and Extrapolation Methods

The current EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237) 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 for chloroprene is supported by 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, 042076) study.

For these reasons, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with chloroprene exposure.

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:

$$P(d) = 1 - exp[-(b_0 + b_1d + b_2d^2 + ... + b_kd^k) \times (t - t_0)^c]$$

where $P(d)$ represents the lifetime risk (probability) of cancer at dose d (i.e., human equivalent exposure in this case); parameters $b_i \geq 0$, for i = 0, 1, ..., k; t is the time at which the animal's tumor status, either no tumor, tumor, or unknown (e.g., missing or autolyzed) was observed; and c 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, such as interim sacrifice data. Parameters were estimated using the method of maximum likelihood estimation (MLE). Note that animals with unknown tumor status contribute to the model fit through the likelihood function including the respective lengths of time on study without a tumor. The dose-response analyses were conducted using the U.S. EPA's MSW computer software program (http://epa.gov/ncea/bmds/msw.html), which is based on Weibull models drawn from Krewski et al. (1983, 003194).

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 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. Hemangiosarcomas were treated as fatal tumors unless observed at 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 sacrifice data. Tumors at all other sites were treated as incidental.

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, 2002, 052153). 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. In all cases, this was equivalent to selecting the model with the lowest AIC.

PODs for estimating low-dose risk were identified at doses consistent with the lower end of the observed data, generally corresponding to 10% extra risk, defined as the extra risk over the background tumor rate, $[P(d) - P(0)]/[1 - P(0)]$. In some cases the highest observed response was not as high as 10% extra risk. In accordance with the cancer guidelines (U.S. EPA, 2005, 086237), PODs near the lower end of these data ranges were selected. Next, all PODs were converted to equivalent continuous exposure levels by multiplying by (6 hours)/(24 hours) × (5 days)/(7 days), or 0.178, under the assumption of equal cumulative exposures leading to equivalent outcomes ($C \times T = k$).

Additionally, in accordance with the U.S. EPA (1994, 006488) RfC methodology, the HECs for the various tumors were calculated by the application of DAFs (see also section 5.2.3). 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 (see Section 5.2.3 and 5.3 for a additional discussion). However, the contribution of either route of delivery (i.e., inhalation vs. bloodstream) to the induction of lung tumors is currently unknown. *In situ* conversion of chloroprene into its highly reactive epoxide metabolite in the olfactory epithelia and lower respiratory tract may facilitate its uptake in these tissues and explain a portion of its biological activity in those regions. For reactive gases (in this case, the reactive epoxide metabolite of chloroprene) with portal-of-entry effects in the pulmonary region, the DAF is the regional gas dose ratio ($RGDR_{PU}$) and is expressed (in this particular case, for female rats) as follows:

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

where:
MV_r **=** B6C3F1 female mouse minute volume (0.053 L/min)**[6]**
MV_h = human minute volume, (13.8 L/min)
S_{PU_r} = surface area of the pulmonary region in rats (.05 m^2)
S_{PU_h} = surface area of the pulmonary region in humans (54 cm^2),

$RGDR_{PU}$ = (.053/.05)/(13.8/54)

$RGDR_{PU}$ = 4.1

For non-reactive gases (in this case the proportion of inhaled chloroprene not metabolized within the respiratory tract) with systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients (see calculation in Section 5.2.3). In cases where the animal blood:air partition coefficient is higher than the human value, resulting in a DAF >1, a default value of 1 is substituted as for the other systemic effects considered above**.** 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 POD, the lower 95% bound on the exposure associated with a level of extra risk near the low end of the data range. 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 multisite carcinogenic effects of chloroprene observed in mice exposed via the inhalation route.

First, the results of applying the multistage Weibull models to each elevated female and male mouse tumor site were evaluated (see Tables 5-6 and 5-7, respectively). Human equivalent unit risks estimated from the mouse tumor sites with statistically significant increases ranged from 3.4×10^{-6} to 1.8×10^{-4} per μg/m^3, approximately a 50-fold range. The highest unit risk (1.8×10^{-4} per μg/m^3) corresponded to lung tumors treated as systemic lesions in female mice, and the lowest unit risk for female mice corresponded to forestomach tumors, 3.4×10^{-6} per μg/m^3. The highest unit risk in male mice, 8.3×10^{-5} per μg/m^3, also corresponded to lung tumors treated as systemic lesions, and was approximately twofold lower than in female mice. The lowest unit risk in male mice was for renal tubule adenomas or carcinomas, at 9.4×10^{-6} μg/m^3. Note that lung tumors were the most sensitive

---

[6] 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 0.289, kg for female rats

response in mice for the structurally related compound 1,3-butadiene as well (U.S. EPA, 2002, 052153).

Regarding the model fits for hemangiomas or hemangiosarcomas, although there was a statistically significant increasing trend for both female and male mice, a satisfactory model fit was not possible without dropping the highest exposure group in both cases, whether or not all tumors were treated as incidental. The incidences in the highest exposure group (80 ppm) were lower than in the 32 ppm group, even after adjusting for intercurrent mortality. However, given the overall tumor response in both the 32 and 80 ppm groups, fitting the decreased high dose circulatory system tumor response does not appear relevant to estimating low dose risk. The result of treating hemangiosarcomas occurring before final sacrifice as rapidly fatal (in combination with hemangiomas; see Section 5.4.3.) were nearly two-fold higher than site-specific unit risks for both female (1.9-fold) and male mice (1.6-fold). The unit risks for hemangiomas or hemangiosarcomas were approximately an order of magnitude lower than that for lung tumors as systemic lesions in female mice, while for male mice these unit risks were approximately 3-fold lower.

Alternatively, if the lung tumors were strictly portal-of-entry effects, the estimated human equivalent unit risks associated with this site would be four-fold lower, and the highest unit risk estimates would be from female mice liver tumors, at 6.3×10^{-5} per $\mu g/m^3$, and male mice hemangiomas and hemangiosarcomas (early instances considered fatal), at 4.7×10^{-5} per $\mu g/m^3$.

Concerning the unit risks for the two sites without complete histopathologic evaluation, Harderian gland and Zymbal’s gland (see Section 5.4.2); the female mice Zymbal’s gland unit risk was quite low, at 3.5×10^{-6} per $\mu g/m^3$, virtually identical to the forestomach unit risk in both female and male mice. The Harderian gland unit risks were 1.2×10^{-5} and 1.5×10^{-5} per $\mu g/m^3$, for females and males, respectively, and were intermediate in the range of available unit risks, along with skin, mammary gland, and hemangiomas/hemangiosarcomas (all assumed nonfatal) in female mice. For male mice, this unit risk was less than twofold higher than that for renal tumors, the lowest male mouse unit risk.

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 EPA 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* (NRC, 1994, 006424) made a case that this approach would tend to underestimate composite 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, which indicates an association of increasing incidence with time. Fitting a model like the multistage-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 female mouse tumors associated with inhalation exposure to chloroprene

TUMOR TYPE*	Power Parameter c[a]	BMR	POINT OF DEPARTURE[b] Modeled from bioassay (ppm) BMDL	POINT OF DEPARTURE[b] Modeled from bioassay (ppm) BMD	POINT OF DEPARTURE[b] Continuous, Human equivalent[c] (μg/m^3) BMDL	POINT OF DEPARTURE[b] Continuous, Human equivalent[c] (μg/m^3) BMD	UNIT RISK[d] /(μg/m^3)	COMPOSITE UNIT RISK[e] /(μg/m^3)
Lung: alveolar/ bronchiolar adenoma or carcinoma[f]	3.8	0.1	0.88	1.20	*5.69×10^{2}* 2.33×10^{3}	*7.71×10^{2}* 3.16×10^{3}	*1.8×10^{-4}* 4.3×10^{-5}	*2.7×10^{-4}* 1.5×10^{-4}
All organs: hemangiosarcomas, hemangiomas[g, h]	5.9	0.1	5.75	10.1	3.71×10^{3}	6.52×10^{3}	2.7×10^{-5}	
All organs: hemangiosarcomas, hemangiomas[g, i]	1.0	0.1	11.1	14.9	7.13×10^{3}	9.62×10^{3}	1.4×10^{-5}	
Mammary gland: adenocarcinoma, carcinoma or adenoacanthoma	1.0	0.1	14.1	20.4	9.06×10^{3}	1.32×10^{4}	1.1×10^{-5}	
Forestomach: squamous cell papilloma or carcinoma	4.1	0.1	46.3	67.8	2.98×10^{4}	4.37×10^{4}	3.4×10^{-6}	
Liver: hepatocellular adenoma or carcinoma	4.2	0.1	2.45	4.24	1.58×10^{3}	2.73×10^{3}	6.3×10^{-5}	
Harderian gland: adenoma or carcinoma	2.9	0.1	12.6	27.1	8.13×10^{3}	1.75×10^{4}	1.2×10^{-5}	
Skin: sarcoma	1.6	0.1	7.18	9.49	4.63×10^{3}	6.11×10^{3}	2.2×10^{-5}	
Zymbal's gland: carcinoma	1.1	0.05	22.5	80.5	1.45×10^{4}	5.19×10^{4}	3.5×10^{-6}	

[a] Multistage-Weibull model: $P(d) = 1 - \exp[-(b_0 + b_1 d + b_2 d^2 + \ldots + b_k d^k) \times (t-t_0)^c]$, coefficients estimated in terms of ppm as administered in bioassay; lower stage b_i not listed were estimated to be zero. See Appendix C for modeling details.

[b] BMD = Concentration at specified extra risk;
BMDL = 95% lower bound on concentration at specified 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 by the BMDL.

[e] Composite 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.

[g] Highest exposure group dropped in order to better characterize low-dose responses.

[h] Malignancies at early deaths considered fatal

[i] All tumors considered incidental

* Tumor incidence data from NTP (1998, 042076)

Table 5-7. Dose-response modeling summary for male mouse tumor sites associated with inhalation exposure to chloroprene

TUMOR TYPE*	**Power Parameter c**[a]	**BMR**	**POINT OF DEPARTURE**[b]				**UNIT RISK**[d] **($\mu g/m^3)^{-1}$**	**COMPOSITE UNIT RISK**[e] **($\mu g/m^3)^{-1}$**
			Modeled from bioassay (ppm)		**Continuous, human equivalent**[c] **($\mu g/m^3$)**			
			BMDL	**BMD**	**BMDL**	**BMD**		
Lung: alveolar/ bronchiolar adenoma or carcinoma[f]	3.4	0.1	1.86	2.46	*1.20×10^{3}* 4.92×10^{3}	*1.59×10^{3}* 6.50×10^{3}	*8.3×10^{-5}* 2.0×10^{-5}	*1.4×10^{-4}* 8.5×10^{-5}
All organs: hemangio-sarcomas, Hemangiomas[g,h]	13.2	0.1	3.34	5.28	2.15×10^{3}	3.40×10^{3}	4.7×10^{-5}	
All organs: hemangio-sarcomas, Hemangiomas[g,i]	3.9	0.1	5.34	7.75	3.44×10^{3}	4.99×10^{3}	2.9×10^{-5}	
Harderian gland: adenoma or carcinoma	5.6	0.1	10.5	16.7	6.74×10^{3}	1.08×10^{4}	1.5×10^{-5}	
Kidney: renal tubule adenomas or carcinomas (extended and standard evaluations combined)	6.1	0.1	16.5	26.7	1.06×10^{4}	1.72×10^{4}	9.4×10^{-6}	
Forestomach: squamous cell papilloma or carcinoma	1.3	0.05	22.8	45.1	1.47×10^{4}	2.91×10^{4}	3.4×10^{-6}	

[a] Multistage-Weibull model: $P(d) = 1 – \exp[-(b_0 + b_1d + b_2d^2 + ... + b_kd^k) \times (t-t_0)^c]$, coefficients estimated in terms of ppm as administered in bioassay; lower stage b_i not listed were estimated to be zero.
[b] BMD = Concentration at specified extra risk;
BMDL = 95% lower bound on concentration at specified 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 by the BMDL.
[e] Composite 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.
[g] Highest exposure group dropped in order to better characterize low-dose responses.
[h] Malignancies at early deaths considered fatal
[i] All tumors considered incidental

* Tumor incidence data from NTP (1998, 042076)

Following the recommendations of the NRC (1994, 006424) and the current *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237) to consider total risk, an upper bound on the composite risk was estimated for all tumor sites in female and male B6C3F1 mice. Note that this upper bound estimate of composite 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. Summing of individual upper bound would tend to overestimate the composite upper bound. Consequently, this analysis used the same method as in several previous IRIS assessments (e.g., 1,3-butadiene (U.S. EPA, 2002, 052153), 1,2-dibromoethane (U.S. EPA, 2004, 594429), 1,2,3-trichloropropane (2009, 625769)) which involves assuming asymptotic normality for slope factors. Each composite unit 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, 006424) 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, 2005, 086237) 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 composite 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:

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

rearranged to:

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

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 = $\text{variance}^{1/2}$).

The resulting composite unit risk for all tumor types for female mice was 2.7×10^{-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 1.5-fold from the highest unit risk for any individual tumor type, 1.8×10^{-4} per $\mu g/m^3$ for female 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 composite 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 composite unit risk for all tumor types was 1.4×10^{-4} per $\mu g/m^3$ (with lung tumors treated as a systemic lesion), a 1.7-fold increase compared to the highest unit risk for any individual tumor type, 8.3×10^{-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 composite 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 composite risk decreases to 1.5×10^{-4} per $\mu g/m^3$ (females) and 8.5×10^{-5} 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, 006488), 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 composite 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, 2005, 088823). 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 default 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, 2005, 088823). 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, 2005, 088823), 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:

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

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, 2005, 086237) 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 actual carcinogen(s).
Bioassay	Unknown; others unsuitable or 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.

Consideration	**Potential Impact**[a]	**Decision**	**Justification**
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
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. Cause of death information was not available for this model; if available, risk estimates would tend to be slightly higher. For example, treatment of early deaths (prior to final sacrifice) with hemangiosarcomas as fatal, with all other hemangiomas and hemangiosarcomas as incidental to death, led to unit risks up to two-fold higher than unit risks treating all hemangiosarcomas (and hemangiomas) as incidental.

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 composite 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, 2005, 086237), 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 Section 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 (Section 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 (U.S. EPA, 1994, 006488) 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 composite 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 composite 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 (Cottrell et al., 2001, 157445; Himmelstein et al., 2001, 019013; Himmelstein et al., 2001, 019012; Himmelstein et al., 2004, 625152; Hurst and Ali, 2007, 625159; Munter et al., 2003, 625214; Munter et al., 2007, 576501; Munter et al., 2007, 625213; Summer and Greim, 1980, 064961). 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 futher oxidation, hydrolysis and/or glutathione conjugation reactions. Similar to 1,3-butadiene, an epoxide metabolite, (1-chloroethenyl)oxirane 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 the eparenchymal cells). No information is available on the oral toxicity of chloroprene 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, 063885) 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.

The toxic and carcinogenic potential of chloroprene 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, 042076), a subchronic range-finding and a chronic study in rats and hamsters conducted by Trochimowicz et al. (1998, 625008), an embryotoxicity and a teratology study by Culik et al. (1978, 094969), and a series of Russian reproductive and developmental toxicity studies reviewed by Sanotskii (1976, 063885). These studies associate chloroprene inhalation exposure with respiratory, kidney, liver, splenic, and forestomach effects. The pulmonary (alveolar and bronchiolar hyperplasia), nasal (olfactory epithelium), and splenic (hematopoietic cell proliferation) 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, 042076) study of rats and mice. In the chronic study by Trochimowicz et al. (1998, 625008), 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, 094969). 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.

The carcinogenic potential of chloroprene in humans has been assessed in a number of occupational epidemiologic studies among workers exposed to chloroprene monomer and/or polychloroprene latex conducted in eight cohorts from the United States, Russia, Armenia, France, China, and Ireland. Four cohorts with sufficient numbers of liver/biliary passage cancer cases showed evidence of association with occupational chloroprene exposure, and reported significantly elevated SMRs when compared to external populations (Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Leet and Selevan, 1982, 094970; Li et al., 1989, 625181). These measures of association were observed, even 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 (Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Marsh et al., 2007, 625187). Known risk factors for liver cancer (e.g., alcohol consumption, hepatitis B infection, etc.) were not controlled for in the studies observing associations between occupational chloroprene exposure and liver/biliary cancers. Several studies also reported higher SMRs for lung cancer among workers exposed to chloroprene, although few of the associations were significant and none of the studies controlled 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, 042076) 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 (Bartsch et al., 1979, 010689; Willems, 1980, 625049), while the bacterial frame shift (*S. typhimurium* strains TA97 and TA98) mutation assays have been nonpositive (NTP, 1998, 042076; Willems, 1980, 625049). In contrast, other studies (NTP, 1998, 042076) have reported nonpositive results for all bacterial strains. A positive result with all bacterial strains was observed with the epoxide metabolite of chloroprene, (1-chloroethenyl)oxirane (Himmelstein et al., 2001, 019013). Chloroprene has been primarily nonpositive in *in vitro* micronucleus assays (Drevon and Kuroki, 1979, 010680; Himmelstein et al., 2001, 019013), *in vivo* chromosomal damage assays (1998, 042076), and bone marrow micronucleus assays (NTP, 1998, 042076; Shelby and Witt, 1995, 624921). Conflicting results (positive in Vogel (1979, 000948); nonpositive in Foureman et al. (1994, 065173)) have been reported for the *in vivo* Drosophila sex-linked lethal mutation assay. Further *in vivo* evidence for the mutagenicity of chloroprene is the observation that tissues from lung, forestomach, and Harderian gland tumors from mice exposed to chloroprene in the NTP chronic bioassay (1998, 042076) were shown to have a higher frequency of mutations in K- and H-*ras* proto-oncogenes than in spontaneous occurring tumors (Sills et al., 2001, 624922; Sills et al., 1999, 624952).
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, 625004). 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 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, 042076) 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, 042076) study.

6.2 DOSE RESPONSE

The chronic inhalation study conducted by NTP (1998, 042076) was considered as the principal study for both the noncancer and cancer effects of chloroprene exposure.

A range of portal-of-entry and systemic effects from the NTP study (1998, 042076), including alveolar epithelial hyperplasia, bronchiolar hyperplasia, pulmonary histiocytic cell infiltration, olfactory epithelial atrophy, olfactory epithelial necrosis, chronic inflammation, kidney hyperplasia, forestomach hyperplasia, and splenic hematopoietic cell proliferation, 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 (benchmark concentration [BMD]) and it's BMDL at a specified level of response (i.e., BMR) for each selected chloroprene-induced respiratory and systemic effect (see Table 5-2). Splenic hematopoietic cell proliferation in female mice resulted in the lowest POD_{ADJ} value of approximately 1.1 mg/m^3. This POD was then converted into the POD_{HEC} by application of the dosimetric adjustment factor (DAF) for a systemic effect. Application of a 100-fold UF (3 for uncertainty associated with animal to human differences, 10 for consideration of human variability, and 3 for database deficiencies) resulted in 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 lower-bound $BMDL_{10}$ 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 (1.8×10^{-4} per µg/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 composite inhalation unit risk for female mice was 2.7×10^{-4} per µg/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 µg/m^3. Application of the ADAFs to account for early-life susceptibility to the proposed mutagenic mode of action for chloroprene yields an adjusted human lifetime cancer risk of $5 \text{ x } 10^{-4}$ per µg/m^3.

Confidence in the principal study (NTP, 1998, 042076) 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 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 the observed toxicity of chloroprene 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 complete two-generation reproductive toxicity study. Therefore, confidence in the RfC is judged to be medium to high.

7. REFERENCES

Acquavella JF; Leonard RC (2001). A review of the epidemiology of 1,3-butadiene and chloroprene. Chem Biol Interact, 135-136: 43-52. doi:10.1016/S0009-2797(01)00169-7 628495

Appelman LM; Dreef-van der Meulen HC (1979). Reproduction study with beta-chloroprene vapour in rats. Central Institute for Nutrition and Food Research (CIVO). Zeist, The Netherlands. 6225. 064938

Arrighi HM; Hertz-Picciotto I (1994). The evolving concept of the healthy worker survivor effect. Epidemiology, 5: 189-196. 625164

Bartsch H; Malaveille C; Barbin A; Planche G (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues. Arch Toxicol, 41: 249-277. 010689

Begemann P; Christova-Georgieva NI; Sangaiah R; Koc H; Zhang D; Golding BT; Gold A; Swenberg JA (2004). Synthesis, characterization, and identification of N7-guanine adducts of isoprene monoepoxides in vitro. Chem Res Toxicol, 17: 929-936. 625093

Bernauer U; Garritsen H; Heinrich-Hirsch B; Gundert-Remy U (2003). Immunochemical analysis of cytochrome P450 variability in human leukapheresed samples and its consequences for the risk assessment process. Regul Toxicol Pharmacol, 37: 318-327. 625103

Bukowski JA (2009). Epidemiologic evidence for chloroprene carcinogenicity: Review of study quality and its application to risk assessment. Risk Anal, 29: 1203-1216. doi:10.1111/j.1539-6924.2009.01254.x 628496

Bulbulyan MA; Changuina OV; Zaridze DG; Astashevsky SV; Colin D; Boffetta P (1998). Cancer mortality among Moscow shoe workers exposed to chloroprene (Russia). Cancer Causes Control, 9: 381-387. 625105

Bulbulyan MA; Margaryan AG; Ilychova SA; Astashevsky SV; Uloyan SM; Cogan VY; Colin D; Boffetta P; Zaridze DG (1999). Cancer Incidence and Mortality in a Cohort of Chloroprene Workers from Armenia. Int J Cancer, 81: 31-33. 157419

Clary JJ; Feron VJ; Reuzel PGJ (1978). Toxicity of "beta"-chloroprene (2-chlorobutadiene-1,3): acute and subacute toxicity. Toxicol Appl Pharmacol, 46: 375-384. 064942

Colonna M; Laydevant G (2001). A cohort study of workers exposed to chloroprene in the department of Isère, France. Chem Biol Interact, 135-136: 505-514. 625112

Cottrell L; Golding BT; Munter T; Watson WP (2001). In Vitro Metabolism of Chloroprene: Species Differences, Epoxide Stereochemistry and a De-chlorination Pathway. Chem Res Toxicol, 14: 1552-1562. 157445

Cox DR (1972). Regression models and life-tables. J Roy Stat Soc B Stat Meth, 34: 187-220. 008785

Culik R; Kelly DP; Clary JJ (1978). Inhalation studies to evaluate the teratogenic and embryotoxic potential of "beta"-chloroprene (2-chlorobutadiene-1,3). Toxicol Appl Pharmacol, 44: 81-88. 094969

DeWoskin RS (2007). PBPK models in risk assessment--A focus on chloroprene. Chem Biol Interact, 166: 352-359. 202141

Dong QA; Xiao BL; Hu YH; Li SQ (1989). Short-term test for the induction of lung tumor in mouse by chloroprene. Biomed Environ Sci, 2: 150-153. 007520

Dourson ML; Knauf LA; Swartout JC (1992). On reference dose (RfD) and its underlying toxicity data base. Toxicol Ind Health, 8: 171-189. 004400

Drevon C; Kuroki T (1979). Mutagenicity of vinyl chloride, vinylidene chloride and chloroprene in V79 Chinese hamster cells. DNA Repair (Amst), 67: 173-182. 010680

Esmen NA; Hall TA; Phillips ML; Jones EP; Basara H; Marsh GM; Buchanich JM (2007). Chemical process-based reconstruction of exposures for an epidemiological study: Part II. Estimated exposures to chloroprene and vinyl chloride. Chem Biol Interact, 166: 264-276. 625118

Esmen NA; Hall TA; Phillips ML; Mars GM (2007). Chemical process based reconstruction of exposures for an epidemiological study: I. Theoretical and methodological issues. Chem Biol Interact, 166: 254-263. 625114

Esmen NA; Kennedy KJ; Hall TA; Phillips ML; Marsh GM (2007). Classification of worker exposures. Chem Biol Interact, 166: 245-253. 625121

Fajen JM; Ungers LJ (1986). Industrial hygiene walk-through survey report of E.I. duPont deNemours and Companry. National Institute for Occupational Safety and Health; Centers for Disease Control. Cincinnati, OH. 628500

Foureman P; Mason JM; Valencia R; Zimmering S (1994). Chemical mutagenesis testing in Drosophila X Results of 70 coded chemicals tested for the National Toxicology Program. Environ Mol Mutagen, 23: 208-227. 065173

Gahlmann R (1993). Pros and cons of transgenic mouse mutagenesis test systems. J Exp Anim Sci, 35: 232-243. 625174

Gargas ML; Burgess RJ; Voisard DE; Cason GH; Andersen ME (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol, 98: 87-99. 063084

Ginsberg G; Slikker W Jr; Bruckner J; Sonawane B (2004). Incorporating children's toxicokinetics into a risk framework. Environ Health Perspect, 112: 272-283. 625124

Gooch JJ; Hawn WF (1981). Biochemical and hematological evaluation of chloroprene workers. J Occup Environ Med, 23: 268-272. 064944

Griebel CP; Halvorsen J; Golemon TB; Day AA (2005). Management of spontaneous abortion. Am Fam Physician, 72: 1243-1250. 625142

Grosjean D (1990). Atmospheric chemistry of toxic contaminants 1. Reaction rates and atmospheric persistence. J Air Waste Manag Assoc, 40: 1397-1402. 625143

Grosjean D (1991). Atmospheric chemistry of toxic contaminants 5. Unsaturated halogenated aliphatics: Allyl chloride, chloroprene, hexachlorocyclopentadiene, vinylidene chloride. J Air Waste Manag Assoc, 41: 182-189. 625149

Haley TJ (1978). Chloroprene (2-chloro-1, 3-butadiene)--what is the evidence for its carcinogenicity? Clin Toxicol, 13: 153-170. 010685

Hall TA; Esmen NA; Jones EP; Basara H; Phillips ML; Marsh GM; Youk AO; Buchanich JM; Leonard RC (2007). Chemical process based reconstruction of exposures for an epidemiological study: III. Analysis of industrial hygiene samples. Chem Biol Interact, 166: 277-284. 625243

Heuser VD; de Andrade VM; da Silva J; Erdtmann B (2005). Comparison of genetic damage in Brazilian footwear-workers exposed to solvent-based or water-based adhesive. Mutat Res Genet Toxicol Environ Mutagen, 583: 85-94. 479853

Hill AB (1965). The environment and disease: Association or causation. J R Soc Med, 58: 295-300. 071664

Himmelstein MW; Carpenter SC; Evans MV; Hinderliter PM; Kenyon EM (2004). Kinetic modeling of beta-chloroprene metabolism: II. The application of physiologically based modeling for cancer dose response analysis. Toxicol Sci, 79: 28-37. 625154

Himmelstein MW; Carpenter SC; Hinderliter PM (2004). Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. Toxicol Sci, 79: 18-27. 625152

Himmelstein MW; Carpenter SC; Hinderliter PM; Snow TA; Valentine R (2001). The metabolism of "beta"-chloroprene: preliminary in-vitro studies using liver microsomes. Chem Biol Interact, 135: 267-284. 019012

Himmelstein MW; Gladnick NL; Donner EM; Snyder RD; Valentine R (2001). In vitro genotoxicity testing of (1-chloroethenyl)oxirane, a metabolite of "beta"-chloroprene. Chem Biol Interact, 135: 703-713. 019013

Hurst HE; Ali MY (2007). Analyses of (1-chloroethenyl)oxirane headspace and hemoglobin N-valine adducts in erythrocytes indicate selective detoxification of (1-chloroethenyl)oxirane enantiomers. Chem Biol Interact, 166: 332-340. 625159

IARC (1999). Re-evaluation of some organic chemicals, hydrazine, and hydrogen peroxide. , 71: 1-1586. 201838

Immels H; Willems M (1978). Initial submission: Dominant lethal assay with beta-chloroprene in male mice with cover letter dated 09/01/92. Central Institute for Nutrition and Food Research (CIVO). The Netherlands. EPA# 88-920008417; NTIS# OTS0570699. 625176

Jemal A; Murray T; Samuels A; Ghafoor A; Ward E; Thun MJ (2003). Cancer statistics, 2003. CA Cancer J Clin, 53: 5-26. 625160

Jones JH; Young RJ; Selevan S; NIOSH (1975). Walk-through survey of E.I. du Pont de Nemours and Company, Inc. Louisville, Kentucky. Division of Field Studies and Clinical Investigations. Cincinnati, OH. IWS-87-10; PB89-129845. 625203

Koskinen M; Calebiro D; Hemminki K (2000). Styrene oxide-induced 2'-deoxycytidine adducts: implications for the mutagenicity of styrene oxide. Chem Biol Interact, 126: 201-213. 010173

Krewski D; Crump KS; Farmer J; Gaylor DW; Howe R; Portier C; Salsburg D; Sielken RL; Van Ryzin J (1983). A comparison of statistical methods for low dose extrapolation utilizing time-to-tumour data. Toxicol Sci, 3: 140-160. 003194

Leet TL; Selevan SG (1982). Mortality analysis of workers exposed to chloroprene. 094970

Lefaux R (1968). Chloroprene. In Practical toxicology of plastics (pp. 69-71). London: Iliffe Books, Ltd. 625192

Leonard RC; Kreckmann KH; Lineker GA; Marsh G; Buchanich J; Youk A (2007). Comparison of standardized mortality ratios (SMRs) obtained from use of reference populations based on company-wide registry cohort to SMRs calculated against local and national rates. Chem Biol Interact, 166: 317-322. 625179

Li SQ; Dong QN; Liu YQ; Liu YG (1989). Epidemiologic study of cancer mortality among chloroprene workers. Biomed Environ Sci, 2: 141-149. 625181

Luster MI; Portier C; Pait DG; White KLJ; Gennings C; Munson AE; Rosenthal GJ (1992). Risk assessment in immunotoxicology I Sensitivity and predictability of immune tests. Toxicol Sci, 18: 200-210. 084126

Lynch M (2001). Manufacture and use of chloroprene monomer. Chem Biol Interact, 135-136: 155-167. 625182

MacGregor JT; Wehr CM; Henika PR; Shelby MD (1990). The in vivo erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. Fundam Appl Toxicol, 14: 513-522. 625184

Marsh GM; Youk AO; Buchanich JM; Cunningham M; Esmen NA; Hall TA; Phillips ML (2007). Mortality patterns among industrial workers exposed to chloroprene and other substances: I. General mortality patterns. Chem Biol Interact, 166: 285-300. 625187

Marsh GM; Youk AO; Buchanich JM; Cunningham M; Esmen NA; Hall TA; Phillips ML (2007). Mortality patterns among industrial workers exposed to chloroprene and other substances: II. Mortality in relation to exposure. Chem Biol Interact, 166: 301-316. 625188

MARTA ; MTA (1996). Historical control data (1992-1994) for developmental and reproductive toxicity studies using the Crl:CD (SD)BR rat. Charles River Laboratories. Malvern, PA.http://www.criver.com/sitecollectiondocuments/rm_rm_r_tox_studies_crlcd_sd_br_rat.pdf. 625111

Mast TJ; Evanhoff JJ; Westerberg RL; Weigel RJ; Rommereim RL (1994). Inhalation developmental toxicology studies: Developmental toxicity of chloroprene vapors in New Zealand white rabbits. U.S. Department of Health and Human Services. Research Triangle Park, NC. PNL-7458; DE94012384. 625206

McGlothlin JD; Meyer C; Leet TL (1984). Health hazard evaluation report HETA-79-027: E.I. du Pont de Nemours and Company, Inc. Louisville, Kentucky. National Institute for Occupational Safety and Health. Cincinnati, OH. HETA 79-027-1459; PB85-186757 . 625204

Medinsky MA; Bond JA (2001). Sites and mechanisms for uptake of gases and vapors in the respiratory tract. Toxicology, 160: 165-172. 016157

Melnick RL; Elwell MR; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1996). Toxicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344 rats and B6C3F(1) mice. Toxicology, 108: 79-91. 625207

Melnick RL; Sills RC (2001). Comparative carcinogenicity of 1,3-butadiene, isoprene, and chloroprene in rats and mice. Chem Biol Interact, 135-136: 27-42. 051506

Melnick RL; Sills RC; Portier CJ; Roycroft JH; Chou BJ; Grumbein SL; Miller RA (1999). Multiple organ carcinogenicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344/N rats and B6C3F1 mice and comparison of dose-response with 1,3-butadiene in mice. Carcinogenesis, 20: 867-878. 000297

Melnick RL; Sills RC; Roycroft JH; Chou BJ; Ragan HA; Miller RA (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res, 54: 5333-5339. 625208

Munter T; Cottrell L; Ghai R; Golding BT; Watson WP (2007). Erratum to "The metabolism and molecular toxicology of chloroprene" [Chem Biol Interact 2007; 166(1-3):323-331]. Chem Biol Interact, 168: 169. 625213

Munter T; Cottrell L; Ghai R; Golding BT; Watson WP (2007). The metabolism and molecular toxicology of chloroprene. Chem Biol Interact, 166: 323-331. 576501

Munter T; Cottrell L; Golding BT; Watson WP (2003). Detoxication pathways involving glutathione and epoxide hydrolase in the in vitroin vitro metabolism of chloroprene. Chem Res Toxicol, 16: 1287-1297. 625214

Munter, T; Cottrell, L; Hill, S; Kronberg L; Watson WP; Golding BT (2002). Identification of adducts derived from reactions of (1-chloroethenyl)oxirane with nucleosides and calf thymus DNA. Chem Res Toxicol, 15: 1549-1560. 625215

Musak L; Soucek P; Vodickova L; Naccarati A; Halasova E; Polakova V; Slyskova J; Susova S; Buchancova J; Smerhovsky Z; Sedikova J; Klimentova G; Osina O; Hemminki K; Vodicka P (2008). Chromosomal aberrations in tire plant workers and interaction with polymorphisms of biotransformation and DNA repair genes. Mutat Res-Fundam Mol Mech Mutagen, 641: 36-42. doi:10.1016/j.mrfmmm.2008.02.007 628501

Neafsey P; Ginsberg G; Hattis D; Johns DO; Guyton KZ; Sonawane B (2009). Genetic polymorphism in CYP2E1: Population distribution of CYP2E1 activity. J Toxicol Environ Health B Crit Rev, 12: 362-388. 196814

NLM (2010). Hazardous Substances Data Bank (HSDB). Retrieved 12-FEB-10, from http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. 594343

NRC (1994). Science and judgment in risk assessment. National Research Council. Washington, DC. 006424

NTP (1985). Technical protocol for sperm morphology and vaginal cytology evaluations in toxicity testing for rats and mice, 10/31/82 version (updated March 1985). Public Health Service, U.S. Department of Health and Human Services. Washington, DC. 625205

NTP (1998). Toxicology and carcinogenesis studies of chloroprene (CAS No 126-99-8) in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program. Research Triangle Park, NC. NTP TR 467; NIH PUB 98-3957. https://ntrl.ntis.gov/search/TRLProductDetail.aspx?ABBR=PB99123671. 042076

NTP (2005). Report on carcinogens. National Toxicology Program. 093207

Nystrom AE (1948). Health hazards in the chloroprene rubber industry and their prevention: A clinical and experimental study with special reference to chloroprene as well as oxidation and polymerization products thereof. J Intern Med, 219: 1-125. 003695

OECD (Organisation for Economic Co-operation and Development) (1998). Screening Information Data Set (SIDS) high production volume chemicals. United Nations Environment Programme (UNEP) Publications. Nairobi, Kenya.http://www.inchem.org/documents/sids/sids/Chloroprene.pdf. 624889

Pell S (1978). Mortality of workers exposed to chloroprene. J Occup Environ Med, 20: 21-29. 064957

Placke ME; Griffis L; Bird M; Bus J; Persing RL; Cox LA, Jr (1996). Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. Toxicology, 113: 253-262. 624891

Ponomarkov V; Tomatis L (1980). Long-term testing of vinylidene chloride and chloroprene for carcinogenicity in rats. Oncology, 37: 136-141. 075453

Portier CJ; Bailer AJ (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. Toxicol Sci, 12: 731-737. 093236

Putman E; van der Laan JW; van Loveren H (2003). Assessing immunotoxicity: guidelines. Fundam Clin Pharmacol, 17: 615-626. 624893

Rice JM; Boffetta P (2001). 1,3-Butadiene, isoprene and chloroprene: reviews by the IARC monographs programme, outstanding issues, and research priorities in epidemiology. Chem Biol Interact, 135-136: 11-26. 624894

Romazini S; Laydevant G; Lutz J; Colonna M; Menegoz F (1992). Mortality study in occupational exposure to chloroprene. , 54: 721-725. 624896

Rothman KJ; Greenland S (1998). Modern epidemiology. Philadelphia, PA: Lippincott-Raven Publishers. 086599

Sanotskii IV (1976). Aspects of the toxicology of chloroprene: immediate and long-term effects. Environ Health Perspect, 17: 85-93. 063885

Savitz DA; Sonnenfeld NL; Olshan AF (1994). Review of epidemiologic studies of paternal occupational exposure and spontaneous abortion. Am J Ind Med, 25: 361-383. 068186

Schrag SD; Dixon RL (1985). Occupational exposures associated with male reproductive dysfunction. Annu Rev Pharmacol Toxicol, 25: 567-592. 062573

Shelby MD (1990). Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. Environ Health Perspect, 86: 71-73. 624906

Shelby MD; Witt KL (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. Environ Mol Mutagen, 25: 302-313. 624921

Sills RC; Hong HL; Boorman GA, Devereux TR; Melnick RL (2001). Point mutations of K-ras and H-ras genes in forestomach neoplasms from control B6C3F1 mice and following exposure to 1,3-butadiene, isoprene or chloroprene for up to 2-years. Chem Biol Interact, 135-136: 376-386. 624922

Sills RC; Hong HL; Melnick RL; Boorman GA; Devereux TR (1999). High frequency of codon 61 K-rasras A-->T transversions in lung and Harderian gland neoplasms of B6C3F1 mice exposed to chloroprene (2-chloro-1,3-butadiene) for 2 years, and comparisons with the structurally related chemicals isoprene and 1,3-butadiene. Carcinogenesis, 20: 657-662. 624952

Stewart CA Jr (1971). Dimerization of chloroprene and related dienes. J Am Chem Soc, 93: 4815-4821. 010705

Stewart CA Jr (1993). Chloroprene. In JI Kroschwitz; M Howe-Grant (Ed.),Kirk-Othmer encyclopedia of chemical technology (pp. 70-78). New York, NY: John Wiley & Sons. 010679

Summer K-H; Greim H (1980). Detoxification of chloroprene (2-chloro-1,3-butadiene) with glutathione in the rat. Biochem Biophys Res Commun, 96: 566-573. 064961

Tarone RE (1975). Tests for trend in life table analysis . Biometrika, 62: 679-690. 624959

Thornton-Manning JR; Dahl AR (1997). Metabolic capacity of nasal tissue: Interspecies comparisons of xenobiotic-metabolizing enzymes. Mutat Res, 380: 43-59. 597688

Tice RR (1988). The cytogenetic evaluation of in vivo genotoxic and cytotoxic activity using rodent somatic cells. Cell Biol Toxicol, 4: 475-486. 624981

Tice RR; Boucher R; Luke CA; Paquette DE; Melnick RL; Shelby MD (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis, 3: 141-146. 064962

Ton TV; Hong HH; Devereux TR; Melnick RL; Sills RC; Kim Y (2007). Evaluation of genetic alterations in cancer-related genes inlung and brain tumors from B6C3F1 mice exposed to1,3-butadiene or chloroprene. Chem Biol Interact, 106: 112-120. 625004

Trochimowicz HJ; Loser E; Feron VJ; Clary JJ; Valentine R (1998). Chronic Inhalation toxicity and carcinogenicity studies on ß-chloroprene in rats and hamsters . Inhal Toxicol, 10: 443-472. 625008

U.S. EPA (1989). Health and environmental effects document for 2-chloro-1,3,-butadiene (chloroprene). U.S. EPA. Cincinnati, OH. 625024

U.S. EPA (2000). Chloroprene (2-chloro-1,3-butadiene). U.S. EPA. Washington, DC.http://www.epa.gov/ttn/atw/hlthef/chloropr.html. 625036

U.S. EPA (1985). Summary review of health effects associated with chloroprene: health issue assessment. 017624

U.S. EPA (1986). Guidelines for mutagenicity risk assessment. Risk Assessment Forum, U.S. Enviornmental Protection Agency. Washington, DC. EPA/630/R-98/003. 001466

U.S. EPA (1986). Guidelines for the health risk assessment of chemical mixtures. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-98/002. 001468

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

U.S. EPA (1991). Guidelines for developmental toxicity risk assessment. U.S. Environmental Protection Agency, Risk Assessment Forum. Washington, DC. EPA/600/FR-91/001. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162. 008567

U.S. EPA (1994). Interim policy for particle size and limit concentration issues in inhalation toxicity studies. U.S. Environmental Protection Agency, Health Effects Division. Washington, DC.http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=186068. 076133

U.S. EPA (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. Research Triangle Park, NC. EPA/600/8-90/066F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993. 006488

U.S. EPA (1995). The use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-94/007. http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=42601. 005992

U.S. EPA (1996). Guidelines for reproductive toxicity risk assessment. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-96/009. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=2838. 030019

U.S. EPA (1998). Guidelines for neurotoxicity risk assessment. National Center for Environmental Assessment; Office of Research and Development; U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-95/001F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12479. 030021

U.S. EPA (2000). Benchmark dose technical guidance document [external review draft]. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-00/001. http://www.epa.gov/raf/publications/benchmark-dose-doc-draft.htm . 052150

U.S. EPA (2000). Science policy council handbook: Risk characterization. Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency. Washington, D.C.. EPA 100-B-00-002. www.epa.gov/spc/pdfs/rchandbk.pdf. 052149

U.S. EPA (2000). Supplemental guidance for conducting for health risk assessment of chemical mixtures. U.S. EPA. Washington, DC. 196144

U.S. EPA (2000). Toxicological review of vinyl chloride. U.S. EPA. Washington, DC. EPA/635R-00/004. http://www.epa.gov/iris. 194536

U.S. EPA (2002). A review of the reference dose and reference concentration processes. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-02/0002F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=55365. 088824

U.S. EPA (2002). Health assessment of 1,3-butadiene. U.S. Environmental Protection Agency. Washington, D.C. . EPA/600/P-98/001F. 052153

U.S. EPA (2004). Toxicological review of 1,2-dibromoethane (CASRN 106-93-4) in support of summary information on the Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency. Washington, D.C..http://www.epa.gov/iris/subst/0361.htm. 594429

U.S. EPA (2005). Guidelines for carcinogen risk assessment, Final Report. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-03/001F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283. 086237

U.S. EPA (2005). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/R-03/003F.

http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283. 088823

U.S. EPA (2006). A framework for assessing health risk of environmental exposures to children (final). National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. Washington, DC. EPA/600/R-05/093F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363. 194567

U.S. EPA (2006). Science Policy Council Handbook: Peer Review. Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency. Washington, DC. EPA/100/B-06/002. http://www.epa.gov/peerreview/pdfs/peer_review_handbook_2006.pdf. 194566

U.S. EPA (2009). Status report: Advances in inhalation dosimetry of gases and vapors with portal of entry effects in the upper respiratory tract. U.S. Environmental Protection Agency. Washington, DC. EPA/600/R-09/072. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=212131 . 625038

U.S. EPA (2009). Toxicological review OF 1,2,3-trichloropropane (CASRN 96-18-4): In support of summary information on the Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency. Washington, DC.http://www.epa.gov/iris/subst/0200.htm. 625769

Vogel E (1979). Mutagenicity of chloroprene, 1-chloro-1,3-trans-butadiene, 1,4-dichlorobutene-2 and 1,4-dichloro-2,3-epoxybutane in drosophila melanogaster. DNA Repair (Amst), 67: 377-381. 000948

Watson WP; Cottrell L; Zhang D; Golding BT (2001). Metabolism and molecular toxicology of isoprene. Chem Biol Interact, 135-136: 223-238. 625045

Westphal GA; Blaszkewicz M; Leutbecher M; Müller A; Hallier E; Bolt HM (1994). Bacterial mutagenicity of 2-chloro-1,3-butadiene (chloroprene) caused by decomposition products. Arch Toxicol, 68: 79-84. 625047

Willems MI (1978). Evaluation of beta-chloroprene and five dimmers in the Salmonella/microsome mutagenicity test. Central Institute for Nutrition and Food Research (CIVO). Zeist, The Netherlands. R 5712. 625048

Willems MI (1980). Evaluation of beta-chloroprene and four chloroprene dimers in the Ames test by atmospheric exposure of the tester strains. Central Institute for Nutrition and Food Research (CIVO) . Zeist, The Netherlands. R 6392; OTS0570703; 88-920008421. https://ntrl.ntis.gov/search/TRLProductDetail.aspx?ABBR=OTS0570703. 625049

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

The Toxicological Review of chloroprene has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006, 194566). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA also received scientific comments from the public. These comments and EPA's responses were included in a separate section of this appendix. There were six external peer reviewers.

EXTERNAL PEER REVIEW PANEL COMMENTS

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

When the external peer reviewers commented on decisions and analyses in the Toxicological Review under multiple charge questions, these comments were organized under the most appropriate charge question. In addition, the external peer reviewers made numerous specific comments that were organized and responded to in a separate section of the section of this appendix. When multiple reviewers provided specific comments on the same subject, or suggested similar revisions to the document, their comments were combined, as appropriate.

General Charge Questions:

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

Comment:

All six reviewers commented that the Toxicological review was generally logical, clear, and concise, although individual reviewers provided suggestions for the improvement of the document with regards to clarity, transparency and thoroughness. One reviewer commented that a more rigorous and transparent evaluation of the epidemiological evidence and how it integrated with the entirety of the chloroprene database should be performed. This reviewer commented that the descriptor of *"likely to be carcinogenic to humans"* was justified based on the animal and genotoxicity data, but this reviewer felt that the human epidemiological data had been overstated. One reviewer commented that it was not clear why a particular dose-response model was chosen in the quantitative analysis of non-cancer effects if more than one model provided adequate fit. This reviewer also commented that the rationale for the benchmark response level was not adequately justified. One reviewer commented that the epidemiology section should have been consolidated (e.g., all studies using a particular cohort, the Louisville DuPont Works for instance, should be discussed together). This reviewer also recommended that additional analyses (by age at onset/death with lags) and substudies (nested case-control) should have been included in the document. This reviewer also commented that additional studies should be included (this issue is addressed in general charge question #2) and that discrepancies in employee populations included in the studies between epidemiology studies and NIOSH walk-throughs should be resolved.

Response:

Additional information and a more thorough evaluation, integration and discussion of the epidemiologic database, including individual study limitations, were included in the document to enhance document completeness, transparency, and clarity (see Sections 4.1.1 and 4.7.2). EPA concluded that the epidemiologic data, considered as a complete database of information with study and methodological issues taken into account, is generally coherent with the animal and genotoxic data, and thus supports the conclusion that the most suitable descriptor was "*likely to be carcinogenic to humans*".

Additional discussion regarding how the benchmark modeling of noncancer endpoints was performed and how and why particular models were selected for each endpoint was included in the text (see Section 5.2.2). Specifically, the criteria that were used to determine adequacy of model fit (global goodness-of-fit p-value, χ^2 residuals, and visual inspection) were discussed, as well as how the EPA chose the best model when multiple models appropriately fit the dose-response data for an individual endpoint (i.e., AIC when no model dependence is assumed, and BMDL otherwise). Additional discussion and rationale for the chosen BMR levels was included.

The basic structure of the epidemiology section (see Section 4.1.1.2; i.e., discussion of earlier studies first) was retained in the document. The recommendation of additional analyses (by age at onset/death with lags) and substudies (nested case-control) of existing cohorts was beyond the purpose and purview of the Toxicological Review and none were included therein. Discrepancies in the study populations in epidemiological studies and the NIOSH walk-through reports were due to study inclusion criteria. The NIOSH reports enumerated all previous and current employees of the Louisville Works plant, whereas Marsh et al. (2007, 625187; 2007, 625188) indicated that the study population was limited to only those employees with a possibility of chloroprene exposure from plant start-up through 2000. A more complete description of inclusion criteria for the Marsh papers was added to the document, but no discussion regarding worker numbers contained in the NIOSH reports was deemed necessary.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of chloroprene.

Comment:

Four reviewers reported that they were not aware of any additional studies whose exclusion would significantly impact the Toxicological review. Two reviewers commented that three NIOSH walk-through studies of DuPont plants involved in chloroprene production (Fajen and Ungers, 1986, 628500; Jones et al., 1975, 625203; McGlothlin et al., 1984, 625204) should be included in Toxicological review. These walk-through studies included medical assessments of worker health and industrial hygienic analyses of ambient chloroprene concentrations in manufacture areas. These two reviewers also commented that two additional health studies, one investigating clinical chemistry and hematological outcomes (Gooch and Hawn, 1981, 064944) and the other a reanalysis of the Louisville cohort compared to an external employee database (Leonard et al., 2007, 625179), be included. One reviewer suggested that two additional reviews of the epidemiology literature at least be considered for inclusion in the Toxicological Review (Acquavella and Leonard, 2001, 628495; Bukowski, 2009, 628496). One reviewer suggested that a study detailing the use of a PBPK model for estimation of rodent vs. human delivered doses be included in the document (DeWoskin, 2007, 202141). One reviewer commented that two recent studies of genetic damage in workers potentially exposed to chloroprene be included (Heuser et al., 2005, 479853; Musak et al., 2008, 628501).

Response:

Two of the three suggested NIOSH walk-through studies were added to the discussion of human health effects of chloroprene exposure (Jones et al., 1975, 625203; McGlothlin et al., 1984, 625204; see Section 4.1.2.1). These studies included both ambient and personal air monitoring of chloroprene exposures within the Louisville Works DuPont plant as well as a qualitative medical examination. Although no health effects were associated with chloroprene exposure, these studies provided information on pre- and post-employment health assessments conducted at the plant, as well as air monitoring information. The third NIOSH walk-through report was not included in the Toxicological Review as it primarily dealt with butadiene air monitoring (Fajen and Ungers, 1986, 628500). The two additional health studies were included in the document (Gooch and Hawn, 1981, 064944; Leonard et al., 2007, 625179; see Sections 4.1.1.2 and 4.1.2.1). The first was an examination of clinical chemistry and hematological effects at the Louisville Works plant and found no significant health outcomes associated with chloroprene exposure. The second study was a re-analysis of cancer mortality data from the Louisville Works plant compared to external DuPont employee mortality databases in order to assess the effects of the healthy worker bias. When mortality data from the Louisville Works plant was compared to employee mortality databases, significant increases in SMRs were observed. These findings possibly indicated that the protective associations observed when comparing Louisville Works mortality data to general population databases may have been due to the healthy worker effect. The two additional reviews of the primary epidemiology literature (Acquavella and Leonard, 2001, 628495; Bukowski, 2009, 628496) were reviews of primary literature that was included in the assessment. Therefore, these reviews were not added to the document as the purpose of the Toxicological Review is to provide information on the EPA's independent review of the epidemiology database. A discussion of the paper detailing the potential use of a PBPK model was added to the document (DeWoskin, 2007, 202141; see Section 3.5). The two recent papers reporting on genetic damage in workers exposed to chloroprene were not added to the document as one was a study of health effects associated with exposure to solvent- vs. water-based adhesives and included multiple coexposures, and the other focused on lymphocyte chromosomal aberrations due to butadiene exposure (Heuser et al., 2005, 479853; Musak et al., 2008, 628501). The second study did provide information on genetic polymorphisms in genes encoding metabolic enzymes, but this was duplicative of background information already provided in the document.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Chloroprene

1. An RfD was not derived for chloroprene. Has the scientific justification for not deriving an RfD been clearly described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study.

Comment:

All six reviewers commented that the rationale for not deriving an RfD, including lack of an adequate multiple-dose oral animal toxicity study and the lack of any human data on oral exposure to chloroprene, was suitably described in the document. The reviewers concluded that the scientific justification was appropriate and the decision to not derive an RfD was well founded. One reviewer commented that an RfD derivation would be supported if a suitable PBPK model were used for a route-to-route extrapolation from inhalation to oral data. One reviewer disagreed, and commented a reliable route-to-route extrapolation via a PBPK model was not supported due to lack of information on the disposition of chloroprene after inhalation or oral exposures.

Response:

A more thorough discussion of the current PBPK model, including its strengths and weaknesses relevant to route-to-route extrapolations, was included in Section 3.5. EPA concluded that, based on the available scientific information and consistent with the conclusions of the External Peer

Review panel, an RfD derivation was not supported. The expanded discussion of the PBPK model was referenced in this decision not to use a route-route extrapolation for the purpose of deriving an RfD.

B) Inhalation Reference Concentration (RfC) for Chloroprene

1. A chronic RfC for chloroprene has been derived from an inhalation toxicity study (NTP, 1998, 042076) investigating non-cancer effects in multiple organ systems. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

   Comment:

   All six reviewers concluded that the selection of the NTP (1998, 042076) inhalation toxicity study as the principal study was scientifically justified as it was a well designed and conducted study that identified multiple non-cancer effects in multiple organ systems in rats and mice exposed to a wide range of chloroprene. Two reviewers noted that not chosing the Trochimowicz et al. (1998, 625008) study for selection as the principal study was justified, although one of these reviewers offered that the specific reason for not considering the study was weak and that a more appropriate and defensible justification would be the high mortality in the low dose animals due to the failure of the ventilation system. One reviewer noted that two human studies conducted at the Louisville plant (Gooch and Hawn, 1981, 064944; McGlothlin et al., 1984, 625204) may contain useful information on subchronic effects in humans. The reviewer also suggested that the limitation of the studies (i.e., lack of quantitative exposure data in Gootch and Hawn (1981, 064944); and lack of quantitative medical data in McGlothlin (1984, 625204)) limit their utility and rule out their selection as the principal study.

   Response:

   Selection of the NTP (1998, 042076) study as the principal study was maintained in the Toxicological Review. Text was added to the document (see Section 5.2.1) clarifying the reasons Trochimowicz et al. (1998, 625008) was not selected as the principal study. Discussion of both the Gootch and Hawn (1981, 064944) and McGlothlin (1984, 625204) studies was added to the appropriate sections of Section 4, including study details, strengths, weaknesses, and findings. Additional text was not necessary in Section 5 detailing why these studies were not selected as the critical study; Section 5 contained text stating “no human studies are available that would allow for the quantification of sub-chronic or chronic non-cancer effects”.

2. An increase in the incidence of degenerative nasal lesions in male rats, characterized by olfactory epithelial atrophy and/or necrosis with increasing severity, was selected as the critical effect. Please

comment on the scientific justification for combining the incidence of atrophy and necrosis and for selecting this endpoint as the critical effect. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comment:

Five reviewers commented that the selection of an increase in the incidence of degenerative nasal lesions (characterized by olfactory epithelial atrophy and/or necrosis) was reasonable and justified. One reviewer disagreed with selection of degenerative nasal lesions as the critical effect for a number of reasons. First, this reviewer commented that the rationale for combining the lesions and the precise way in which they were combined was poorly described. Second, the reviewer stated that the concept that necrosis precedes atrophy is straightforward and has been observed for a number of inhaled toxicants, whereas the draft Toxicological Review suggested that atrophy occurred first. Lastly, the reviewer commented that nasal lesions should not be selected as the critical effect due to the way the HECs were calculated (see comments below in question B3).

One reviewer noted that combination of the two lesion types did not make a large difference in the overall determination as the incidences of each endpoint were equivalent and the calculated POD_{HEC} values were 1.1 mg/m^3 for atrophy and 1.0 mg/m^3 for the combined lesions. This reviewer also commented that the limitation of considering only endpoints that were significantly increased at the low dose for the critical effect was not justified as it could have inappropriately excluded sensitive endpoints that may return lower PODs given the nature of the dose-response relationship. This reviewer commented that kidney (renal tubule) hyperplasia in male mice and rats should be considered, and that these endpoints, as well as olfactory effects in female rats, female mice, and male mice, should be included in Figure 5-1. One reviewer commented Table 5-1 did not include p-values for trend for the dose-response for the various endpoints, but that the relative magnitude of trend appeared to be greater for atrophy and necrosis combined than for splenic hematopoietic cell proliferation. One reviewer commented that issues relating to *in situ* metabolism should be discussed in more detail, specifically in regard to why upper respiratory effects were selected rather than lower respiratory effects.

Response:

Section 5.2 of the document was rewritten significantly in response to reviewer comments regarding question B3 (see below). Specific comments regarding the combination of nasal olfactory atrophy and necrosis (e.g., poorly explained rationale, incorrect conclusion that atrophy preceeds necrosis, and the negligible effect combining the lesions has on the POD_{HEC} values) were no longer relevant as the combination of nasal lesions was not performend for the purposes of deriving the RfC; all text describing the combination of atrophic and necrotic nasal lesions has been deleted. In response to the comment regarding endpoint selection criteria, additional endpoints were considered for selection as the critical effect (see Section 5.2.1). PODs for these endpoints were determined using either BMD modeling or the NOAEL/LOAEL approach and were included in Table 5-2. The additional endpoints considered were nasal olfactory basal cell hyperplasia in male and female rats, nasal olfactory metaplasia in male and female rats, nasal olfactory atrophy in female rat, nasal olfactory necrosis in female rats, nasal olfactory suppurative inflammation in female mice, kidney (renal tubule) hyperplasia in male and female rats and male mice, forestomach epithelial hyperplasia in male and female mice, and splenic hematopoietic cell proliferation in male mice. Histiocytic cell infiltration was excluded from consideration as NTP (1998, 042076) noted that it was an effect secondary to lung neoplasms.

Figure 5-1 was removed from the document due to the extensive rewriting of Section 5.2 (see below). Results for statistical tests of trend were not included for non-cancer effects in the NTP (1998, 042076) study and thus were not added to Table 5-1. However, the global goodness-of-fit p-values for each dose-response model fit to the data for each individual endpoint were included in the modeling results in Appendix B. Discussion of *in situ* metabolism was included in Section 5.2, specifically as it relates to how chloroprene, as a water insoluble and non-reactive gas, can exert portal-of-entry effects in the upper and lower respiratory tract.

3. Benchmark dose (BMD) modeling was used to define the point of departure (POD) for the derivation of the RfC. The POD was based on increased incidence of degenerative nasal lesions in male rats at a benchmark response (BMR) of 10% extra risk. Has the BMD approach been appropriately conducted? Is the BMR selected for use in deriving the POD (i.e., 10% extra risk of degenerative nasal lesions of less than moderate severity) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Comment:

All six reviewers commented that the use of BMD modeling was appropriate to define the POD for derivation of the RfC. Four of the reviewers specifically commented that the BMD approach was justified given a number of reasons, particularly that the database appears sufficiently robust and that BMD modeling is preferred because it takes into consideration all of the dose-response data and is less impacted by group size. One reviewer commented that use of a PBPK model could clarify the saturation of metabolism into active metabolites and that this could facilitate dose-response modeling and lead to a lower POD. One reviewer commented that selection of a BMR of 10% extra risk was appropriate for degenerative nasal lesions, whereas four reviewers commented that a BMR of 10% was too high. Specifically, one reviewer noted that the NTP study did not identify a NOAEL and that the severity of nasal lesions seen in the lowest exposure group was greater than minimal. These four reviewers suggested that a lower BMR be selected for modeling purposes, and specifically suggested BMRs in the range of 2-5% extra risk. One reviewer noted that, because severity data was available for individual animals, EPA's categorical regression (CatReg) software could be used to incorporate severity into the modeling scheme. Two reviewers commented that EPA could be clearer in regard to the derivation of the RfC, and suggested that EPA provide a clearer indication of how and why particular models were selected for the various endpoints and provide a step-by-step derivation of the RfC in the document.

Five reviewers commented that justification for treating chloroprene as a category 1 gas and the impact this had on dosimetric adjustments was not sufficiently justified in the document and that further justification should be added. One reviewer objected strongly to the approach used to derive the $\mathrm{POD_{HEC}}$s for a number of reasons. First, this reviewer stated that the PODs used in the calculation of the HECs are very similar (2.1 – 8.3 $\mathrm{mg/m^3}$) and that nasal lesions were chosen only because the dosimetric adjustment factor (DAF) for nasal effects was so low. Thus, in the reviewer's opinion, the selection of the nasal lesions as the critical effect was an artifact of the DAF (RGDR) calculation and not based on the primary experimental observations. The reviewer then delineated their concerns relative to the RGDR, stating that the RGDR calculation was theoretically flawed and discordant with the inhalation dosimetry database. This reviewer also objected the conclusion that air-borne, rather than blood-borne, chloroprene induces nasal lesions, stating that it was confusing why a discussion of portal-of-entry effects vs. systemic redistribution was discussed for cancer effects, but not for non-cancer effects. This reviewer ultimately provided an alternative scheme for RfC development: selection of the critical effect based on a POD of a parameter closer to the observed data (i.e., $\mathrm{POD_{ADJ}}$) and then applying the DAF calculation (both portal-of-entry and systemic for respiratory effects, similar to what was done for cancer effects) to arrive at the HEC.

<u>Response:</u>

The global BMD modeling approach was maintained in the document where possible (i.e., all endpoints that were considered for the critical effect that were amenable to BMD modeling were modeled using the current version of BMDS software). When endpoints were not amenable to BMD modeling, or no adequate model fit could be obtained, the NOAEL/LOAEL approach was used. A PBPK model was not used in the modeling scheme due to limitations in the currently available, peer-reviewed model (Himmelstein et al., 2004, 625154). A more detailed discussion of the current PBPK model for chloroprene was included in Section 3.5 and covers the model structure, the metabolic and physiological parameters used, and limitations that preclude its use in the Toxicological Review.

The selection of appropriate BMRs for endpoints under consideration for the critical effect was modified. A BMR of 10% extra risk was used initially under the assumption that it represented a minimal biologically significant change. In addition to reporting the incidence of the endpoints, the NTP (1998, 042076) study also reported the severity scores for individual animals in each dose group, thus making it possible to determine whether the endpoints were increasing in severity as well as incidence with dose (see Table B-1). In the case of endpoints that progressed in incidence as well as severity (i.e., progression from mild to moderate lesions) from the control dose to the lowest dose showing response, a BMR of 10% was not considerd to be a biologically minimal effect. Therefore, for these endpoints, a BMR of 5% was used for the estimation of BMDs and BMDLs (see Section 5.2.2). CatReg software was not utilized in the modeling scheme due to considerable uncertainty in assigning consistent severity scores to multiple lesions across organ systems.

Additional discussion regarding how the modeling was performed and how and why particular models were selected for each endpoint was included in the text (see Section 5.2.2). Specifically, the criteria that were used to determine adequacy of model fit (global goodness-of-fit p-value, χ^2 residuals, and visual inspection) were discussed, as well as how the EPA chose the best model when multiple models appropriately fit the dose-response data for an individual endpoint (i.e., AIC when no model dependence is assumed, and BMDL otherwise). EPA has also added step-by-step calculations of the POD_{ADJ} and POD_{HEC} values as well as the final RfC calculation in order to improve clarity in the methods of RfC derivation.

Additional discussion was added to Section 5.2.3 covering the physio-chemical properties of chloroprene as they relate to the observed pattern of adverse respiratory and systemic effects. The current RfC methodology (U.S. EPA, 1994, 006488) attempts to group chemicals into one of three discrete categories based on their physio-chemical properties and presumed toxicokinetics; using this scheme, chloroprene would be best classified as a category 3 gas, being relatively water insoluble and non-reactive, and would be expected to only elicit extrarespiratory effects. Medinsky and Bond (2001, 016157) described an alternative method of classification that represents the reactivity and water solubility as continuous variables that allows insight to be gained regarding sites of and conditions of toxicity. Additionally, although not explicitly included in this scheme, *in situ* metabolism in the respiratory tract can be considered as a component of reactivity. In this manner, chloroprene can best be described as both a non-reactive, water insoluble chemical that is absorbed into the blood stream and induces systemic toxicity, as well as a chemical that is metabolized into a reactive epoxide within the respiratory tract, inducing portal-of-entry toxicity. This method of classification is consistent with what is proposed for the mode of action of chloroprene: conversion of the parent compound into its epoxide metabolite via P450 isoform CYP2E1, which is expressed in both the olfactory and pulmonary regions of the respiratory tract. *In situ* metabolism in the respiratory tract may thus explain a portion of the biological activity of chloroprene in these regions. Alternatively, it is observed that chloroprene induced adverse effects in organ systems distal to the portal-of-entry, consistent with its water insoluble and non-reactive chemical properties. Therefore, it is possible that observed pulmonary effects, such as alveolar hyperplasia in the female rat, may be explained by blood-borne delivery, rather than air-borne. Currently, the contribution of either mode of delivery to the sites of observed toxicity is not known.

This discussion of the uncertainty surrounding the mode of delivery to respiratory tissues, included in Section 5.3, harmonized the non-cancer and cancer derivations.

In order to minimize uncertainty surrounding the application of the default DAFs, the selection of the critical effect was based on the observed experimental data (i.e., the POD_{ADJ}) and DAFs were then applied to the selected critical effect to calculate the HEC.

Given the above changes to the modeling scheme, increased incidence of splenic hematopoietic proliferation in female mice was chosen as the critical effect based on the observation that this endpoint had the lowest POD_{ADJ} (1.1 mg/m^3, based on a $BMDL_{05}$ of 1.7 ppm [6.2 mg/m^3]) (see Section 5.2.3). Using a DAF of 1 (for systemic effects), the calculated POD_{HEC} for increased splenic hematopoietic proliferation was also 1.1 mg/m^3.

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

Comment:

Six reviewers commented that the selection of the uncertainty factors, 10 for human variation, 3 for animal-to-human extrapolation, and 3 for database deficiencies were reasonable and consistent with EPA policy. One reviewer commented that application of the 3-fold database uncertainty could be the source of some contention, in that it seemed justified considering the absence of a two-generational reproductive study, but that negative findings for teratogenesis and dominant lethal effects could be considered an adequate substitute. One reviewer commented that a multi-generational study was available and should be discussed in regard to the selection of the database uncertainty factor. One reviewer noted the lack of data on potential neurodevelopmental toxicity or long-term effects following perinatal exposure. One reviewer suggested discussion of the uncertainty surrounding application of the DAFs for effects resulting from airborne delivery (i.e., portal-of-entry effects) should be discussed. Two reviewers commented that there is probably considerable human variability in the metabolism of chloroprene due to genetic polymorphisms in the genes coding metabolizing enzymes and the activity of enzymes. One reviewer suggested that an additional uncertainty factor of 3-10 be added if the RfC was derived from a $BMDL_{10}$ in the presence of moderately severe lesions in the low dose.

Response:

The current selection and application of uncertainty factors was maintained in the document (see Section 5.2.4). A two-generational reproductive study was not available in the database for chloroprene. The Appelman and Dreef van der Meulen (1979, 064938) study was an unpublished report in which F_0 and F_1 rats were exposed to chloroprene. However, this study did not involve the mating of the F_1 generation, so developmental effects to the F_2 generation could not be assessed. Lack of a developmental neurotoxicity study was not considered a sufficient reason to increase the database uncertainty factor, as there was limited data indicating the neurotoxic or developmental effects of chloroprene. Therefore, EPA concluded that the application of a database uncertainty factor of 3 be retained for deriving the RfC. A discussion of the uncertainty surrounding the application of the default DAFs for portal-of-entry effects was including in Section 5.3 (Uncertainties in the Inhalation Reference Concentration), but not in the section outlining the application of the actual uncertainty factors (Section 5.2.4). A concise discussion of the observed variation in CYP2E1 in human populations was included in Section 5.2.3 supporting the human variation uncertainty factor of 10. The uncertainty factor of 10 was maintained as it was presumed to account for variations in susceptibility within the human population. An additional uncertainty factor to account for derivation of an RfC based on a BMR of 10% for effects showing an increase in severity in the low dose was not supported by EPA guidance covering uncertainty factors, and further, was not needed as a BMR of 5% was ultimately used in the document for derivation of the RfC based on increased incidence of splenic hematopoietic proliferation in female mice.

(C) Carcinogenicity of Chloroprene

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.htm), the Agency concluded that chloroprene is *likely to be carcinogenic to humans* by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

Comment:

Six reviewers commented that the characterization of chloroprene as "*likely to be carcinogenic to humans*" was appropriate and clearly justified based on the animal and genotoxicity data. Three reviewers commented that the animal data provided ample evidence of carcinogenesis in both sexes of two rodent species (mouse and rat) at multiple organ sites, many of which were distal to the point-of-contact. One reviewer commented that there was clear information on the formation of mutagenic metabolites of chloroprene and analogies to related chemical carcinogens with analogous metabolic pathways that made the determination of "*like to be carcinogenic*" unequivocal. One reviewer commented that chloroprene was likely to be carcinogenic by all routes of exposure because its carcinogenicity is likely due to formation of epoxide metabolites, and because P450-mediated epoxidation of chloroprene can occur in several organs. Another reviewer noted that if there is a critical role for blood-borne chloroprene, as was assumed for the induction of pulmonary neoplasms, the possibility of carcinogenicity from multiple routes of exposure is elevated.

One reviewer commented that the mode of action for chloroprene is such that it may not be carcinogenic via dermal exposure as the parent compound is non-reactive and insoluble in water.

One reviewer noted that there were potential increases in liver tumors in occupationally exposed cohorts that supported the determination that chloroprene may represent a carcinogenic hazard to humans. Two reviewers suggested that the strength of the epidemiological data was sufficient to change the descriptor to "*carcinogenic to humans*", with one reviewer citing the multiple tumor responses in animals, the metabolic activation of chloroprene by rat, mouse, and human liver microsomes, the finding of K-*ras* mutations in lung neoplasms in mice, and the relatively consistent finding of increased risk of liver cancer mortality in occupational cohorts. This reviewer felt that the EPA did not sufficiently justify the "*likely to be carcinogenic*" over "*carcinogenic*" descriptor given that many of the limitations in the epidemiology database (healthy worker effect, etc.) result in underestimations of risk. This reviewer also commented that EPA's cancer guidelines allow for the determination of "*carcinogenic*" when there is less than convincing epidemiologic evidence, but there is strong animal carcinogenicity and when the mode of action identified in animals is anticipated to occur in humans.

One reviewer commented that, while the animal and genotoxicity data backed up the current cancer determination, the epidemiology data did not support that determination and was overstated in the document. This reviewer commented that the document reported on the evidence of dose-response for liver cancer in the Marsh et al. (2007, 625188) study, but did not provide the relative risks (and confidence limits) in each of the exposure categories. This reviewer also commented that the EPA misrepresented the evidence regarding the presence of dose-response trends in other studies – responses in the low and high exposure groups are not statistically different (Bulbulyan et al., 1999, 157419), and there is no dose response for liver cancers in the high dose because only one cancer case was liver cancer (the remaining two cancers were of the gall bladder) (Leet and Selevan, 1982, 094970). This reviewer also commented that known risk factors for liver cancer (hepatitis infection, alcohol consumption, etc.) were not discussed in sufficient detail given the level of discussion included for risk factors for lung cancer. This reviewer commented further that discussion of co-exposures and potential confounding was inadequate. The reviewer provided a list of suggestions in order to increase the transparency of the presentation of the data on liver cancer in humans, including: discussion on whether the cohorts that studies investigated (i.e., the Louisville Works cohort investigated by Leet and Selevan (1982, 094970) and Marsh et al. (2007, 625188) were adequately independent; more complete presentation of results from Marsh et al. (2007, 625188); and increased discussion regarding the variability around central effect measurements based on small numbers of cases in the Bulbulyan et al. (1998, 625105; 1999, 157419), Li et al. (1989, 625181), and Leet and Selevan (1982, 094970). Lastly, this reviewer commented that, given the various study limitations in the studies that observed increased incidence of liver cancer mortality, it is unclear whether an association exists between chloroprene exposure and liver cancer, especially considered that the best conducted study, Marsh et al. (Marsh et al., 2007, 625188), failed to observed an increased risk.

Response:

The determination that chloroprene is "*likely to be carcinogenic to humans*" by all routes of exposure was maintained in the document based on a weight of evidence approach that considered human epidemiology, animal toxicology, and genotoxicity data (see Section 4.7). U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (2005, 086237) 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. Although there are no toxicity studies involving dermal exposure, carcinogenicity by this route of exposure may be inferred as there is no convincing toxicokinetic data to preclude absorbtion by this route of exposure, and that rapid absorption of chloroprene through the skin occurs (NLM, 2010, 594343).

Although there was evidence of increased risk of liver cancer mortality in occupational cohort studies, EPA concluded that the strength of evidence did not support the cancer descriptor of "*carcinogenic*". In order for a chemical to be found to be "*carcinogenic*", there either must be convincing epidemiologic evidence of a causal association or a lesser weight of epidemiologic evidence that is strengthened by all of the following: (1) strong evidence of an association between human exposure and cancer, (2) there is extensive evidence of carcinogenicity in animals, (3) the mode of action has been identified in animals, and (4) the key precursor events that precede the cancer response in animals are anticipated to occur in humans. EPA: (1) demonstrated throughout the document that there exists unequivocal evidence of carcinogenicity in animals, (2) provided a plausible mode of action based on animal and human *in vitro* metabolic and toxicokinetic studies, and (3) discussed that the precursor events that occur in animals are reasonably anticipated to occur in humans. However, EPA concluded that the epidemiologic data, while providing a fairly consistent evidence of liver cancer mortality (4 studies report statistically significant associations in 4 separate cohorts), did not support changing the cancer determination to "*carcinogenic*". This was due to 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) that made it difficult to draw firm conclusions regarding the findings of these studies. The most recent and comprehensive studies (Marsh et al., 2007, 625187; Marsh et al., 2007, 625188) used quantitative exposure ascertainment, and failed to observe statistically significant relationships between exposure and outcome. These findings did not diminish the observations of the four studies that did observe statistically significant associations, but rather indicated that the epidemiologic database is somewhat equivocal, and did not support changing the cancer determination from "*likely to be carcinogenic*".

Additional text regarding the relative risks and confidence limits for each of the exposure categories for liver cancer in the Louisville cohort from (Marsh et al., 2007, 625188) was added to the document in Section 4.1.1.2. A more thorough discussion of the suggested dose-response relationships observed in Bulbulyan et al. (1999, 157419) and Leet and Selevan (1982, 094970) was added to Section 4.7.1.1. This discussion highlights issues surrounding the determination that there exists a suggestive dose-response relationship in these two studies, even though the responses in the two exposure categories are not statistically significantly different from one another (Bulbulyan et al., 1999, 157419) and that a dose response only exists when liver and biliary/gall bladder cancers are grouped together (Leet and Selevan, 1982, 094970). Additional text and discussion was added throughout the document regarding known risk factors for liver cancer (including hepatitis B infection, alcohol consumption, and aflatoxin ingestion), and the lack of control for these factors in the epidemiologic studies observing a statistically significant association between liver cancer and occupational exposure to chloroprene. Also, a more complete discussion regarding potential co-exposures to industrial chemicals and the possibility of confounding was added to numerous sections of the Toxicological Review. A complete evaluation of the independence of Leet and Selevan (1982, 094970) and Marsh et al. (2007, 625188) studies was added to Section 4.7.1.1. This evaluation highlights differences in the methodologies employed by the two studies as well as differences in the demographics of the sub-sets of the Louisville cohort that were investigated in the studies. EPA concluded that there exist sufficient differences between these two studies investigating the Louisville cohort to warrants the independent analysis of each. Additional text was added to Section 4.7.1.1 regarding the variability of the central effect measures based on low reported expected counts for liver and lung cancer mortality in Li et al. (1989, 625181), Bulbulyan et al. (1998, 625105), and Bulbulyan et al. (1999, 157419).

Additional text and discussion was added throughout the Toxicological Review regarding individual study limitations in those studies that observe a statistically significant association between chloroprene exposure and increased liver cancer mortality. Although limitations that need to be considered carefully exist in these studies, EPA concluded there is evidence of an association between liver cancer risk and occupational exposure to chloroprene based on the observation of increased liver cancer mortality across multiple studies investigating the outcome in heterogeneous populations and exposure scenarios. This conclusion was based on a consistent two- to more than five-fold increased risk of liver cancer mortality in the SMRs observed 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., 2007, 625188), the observed RR increased with increasing cumulative exposure in the plant with the highest exposure levels, indicating a dose-response trend. 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 4.7.1.1.

2. A two-year inhalation cancer bioassay in B6C3F1 mice (NTP, 1998, 042076) was selected as the basis for derivation of an inhalation unit risk (IUR). Please comment on whether the selection of this study for quantification is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the basis for quantification.

Comment:

Five reviewers commented that the selection of the NTP 2-year inhalation carcinogenicity bioassay was scientifically justified based on the fact that the study was well-designed and conducted, the study identified carcinogenic effects in multiple organ systems in rats and mice exposed to a wide range of chloroprene concentrations, and the study was peer-reviewed. One reviewer noted that a major strength of this study was the multiple histopathological reviews of lesions identified in rats and mice. One reviewer commented that a stronger reason than presented in the draft Toxicological Review for not selecting the Trochimowicz et al. (1998, 625008) study as the principal study was the high mortality in the low dose animals due to the failure of the ventilation system. One reviewer commented the dosimetry in terms of an active metabolite may be informed by the application of a PBPK model. Two reviewers commented that inclusion of lung tumors observed in mice may be problematic due to greatly increased metabolic activation rate in mice compared to humans or rats and one of these reviewers commented that a discussion of this should be included in the document. One reviewer did not comment on the choice of the NTP (1998, 042076) study as justified, but commented that selection of the mouse as the most appropriate species over the rat was not adequately explained.

Response:

Choice of the NTP (1998, 042076) 2-year inhalation carcinogenicity bioassay as the basis for derivation of an inhalation unit risk was maintained. Text was added to the document clarifying the reasons the Trochimowicz et al. (1998, 625008) was not chosen for selection as the principal study; the high mortality in the low dose group was identifed as the main reason for not selecting the study as the principal study (see Section 5.2.1). A more thorough discussion of the current PBPK model, including its inadequacies relevant to use in the current Toxicological Review, was included in Section 3.5. Specifically, the current PBPK model was concluded to be inadequate for use to inform dosimetry in terms of an active metabolite. A more complete and detailed discussion of metabolism and toxicokinetic differences between species was added to Section 3.3, to indicate that differences in epoxide production in the lungs of mice and humans are not 50-fold, but may be as little as 2- to 10-fold. These additional data also indicated that in some cases (i.e., glutathione transferase activity) detoxification of the epoxide metabolite may be faster in mice than humans. Additionally, the evidence for further oxidation of (1-chloroethenyl)oxirane in mice, but not in humans, rats, or hamsters was characterized. The mouse was chosen over the rat as the most appropriate species for the inhalation unit risk derivation based on the observation that it was more sensitive to the carcinogenic effects of chloroprene exposure.

3. A mutagenic mode of carcinogenic action is proposed for chloroprene. Please comment on whether the weight of evidence supports this conclusion. Please comment on whether this determination is scientifically justified. Please comment on data available for chloroprene that may support an alternative mode(s) of action.

Comment:

Six reviewers commented that a mutagenic mode of carcinogenic action for chloroprene was appropriate based on the evidence that chloroprene metabolism operates via P450-mediated oxidation to a DNA-reactive epoxide metabolite, which is mutagenic in multiple strains of *Salmonella*, and the observation of K- and H-*ras* mutations in tumors obtained from mice exposed to chloroprene. One reviewer specifically noted that the proposed mode of action was consistent with other epoxide-forming carcinogens (i.e., 1,3-butadiene). Three reviewers commented that they were not aware of any scientific data that would support an alternative mode of action. One reviewer commented that while a mutagenic mode of action may not be the only mode of action, it was clearly one possibility. One reviewer commented that if it was concluded that a metabolite represented the ultimate toxic species, the quantitative risk assessment should be discussed in regard to the large differences observed between mice, rats, and humans.

Response:

The proposed mutagenic mode of carcinogenic action for chloroprene was maintained in the document. A more complete discussion of the metabolic and toxicokinetic differences between mice, rats, and humans was included in Section 3.3.

4. Data on hemangiomas/hemangiosarcomas (in all organs) and tumors of the lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas), skin and mesentery, mammary gland and liver in B6C3F1 mice were used to estimate the inhalation unit risk. Please comment on the scientific justification and transparency of this analysis. Has the modeling approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the inhalation unit risk and discuss whether such approaches are preferred to EPA's approach.

Comment:

Two reviewers supported the use of a dose-response model which accounted for differences in survival such as the multistage-Weibull model. One of these reviewers suggested an alternative modeling approach whereby the assumption of saturating metabolism was incorporated in the model structure, and provided an extensive example using the mice data. The other reviewers did not comment on the dose-response model specifically, with one of these commenting only that the derivation of the inhalation unit risk could be made clearer in the text.

Four reviewers commented that the scientific justification of combining unit risks for all tumor types was scientifically justified and conducted, with one noting further,that basing the unit risk derivation on one tumor type would underestimate the carcinogenic potential of chloroprene. One of these reviewers suggested further that the results of the animal study should be evaluated to determine if there are genetic or other factors between animals that determine which get one tumor vs. those that get more than one tumor type.

One reviewer commented that the quantitative importance of the mouse lung tumors was questionable given the differences in metabolic activation between mice and humans. One reviewer commented that a discussion of site concordance/discordance between mice and humans and human relevance of observed rodent tumors should be included in the document. Two reviewers commented that a useful analysis would be to compare the unit risk calculated from the animal study to unit risks calculated from the human epidemiology studies, with one reviewer specifically suggesting that the Marsh et al. (2007, 625187; 2007, 625188) Louisville cohort be used because it has the most quantitative exposure information. The other reviewer asked whether it was possible to project human occupational risks from the unit risk to consider consistency with epidemiologic observations.

A reviewer also commented that discussion should be included why an uncertainty factor for human variability (other than the application of the ADAFs) was not applied to the cancer risk estimate.

Response:

The assessment’s modeling approach, use of a time-to-tumor model and subsequent estimation of a composite unit risk for all tumor types in female mice, was maintained and more thoroughly explained and discussed in the document (see Sections 5.4.3 and 5.4.4). The suggested alternative modeling approach incorporating saturating metabolism was a constructive approach that EPA will consider with regards to future methods developed for human health risk assessment. However, as noted by the reviewers this model did not currently incorporate time-to-tumor information, and it appeared complex to do so and was beyond the scope of the Toxicological Review. Also, the saturating metabolism parameters were not derived from pharmacokinetic data but from empirical fits to dose and tumor incidence data, so it was as much an empirical model as the multistage-Weibull. Further, the saturating behavior observed, especially at the two higher doses, reflected to a large degree the limiting condition that only 100% of the animals can develop tumors. The multistage-Weibull model did adequately fit the monotonic, supralinear dose-response relationships seen in the NTP study; EPA retains the analysis in the assessment..

Additional mouse and human metabolic and toxicokinetic data (Himmelstein et al., 2004, 625152; Himmelstein et al., 2004, 625154) added the document indicated that the metabolic differences between humans and mice are not as great as previously represented in the document (see Section 3). Therefore, the mouse lung tumor data was considered relevant for human risk estimation and was retained in the modeling approach. The composite risk analysis addressed the risk of developing any combination of tumors in animals in order to estimate the risk of developing any combination of tumors in humans. It was reasonable to assume, given the observed multi-site carcinogenicity of chloroprene, that induction in tissues specific to humans was possible.

It was unclear what the reviewer was suggesting in regard to evaluating genetic factors that may influence which animals get more than one tumor type. Given that the animal species used in the 2-year cancer bioassay was an inbred strain of mouse and that all conditions except exposure concentration were maintained across dose groups, it is unlikely that genetic or other factors other than dose influenced whether an animal developed one or multiple tumors.

One reviewer suggested and another reviewer concurred that a comparison of the inhalation unit risk estimates derived in this Toxicological Review (Table 5-7) to unit risks calculated from human epidemiology studies should be conducted. EPA maintains that unit risk estimates could not be derived from human epidemiology studies because the available quantitative exposure assessments were not sufficient for this purpose. . However, a comparison of the number of cancer cases predicted by the mice tumors with those observed in the study with the most thorough exposure assessment (the Marsh et al. Louisville cohort) was considered in a sensitivity analysis context. Briefly, the unit risk for composite cancer risk derived from male mice (1.4×10^{-4} per $\mu g/m^3$) was applied to the median cumulative exposure for the Louisville plant, converted to a lifetime equivalent continuous concentration (18.35 ppm-yr ⁄ 70 yr $\times 3.62 \times 10^3$ ($\mu g/m^3$)/ppm ≈ 950 $\mu g/m^3$), yielding an upper bound predicted risk of 0.13 for composite cancer risk. When this risk estimate is applied to the 2282 subjects with known cause of death, the predicted upper bound on the number of cancer cases is ~300. In Louisville, 266+17=283 deaths due to either respiratory or liver cancer—the cancers of a priori concern—were reported. Note that the unit risk is an upper bound estimate, and also includes incident cases as well as deaths.

For the above quantitative comparison, several considerations must be acknowledged with regards to interpretation of the results. These considerations are 1) the quantitative exposure assessment (i.e., cumulative of chloroprene exposure) for the Louisville cohort spanned approximately 3 orders of magnitude, 2) insufficient information regarding whether sufficient latency for subjects to develop cancer existed,, 3) exposure estimates were for the full cohort and likely not applicable to the subset with known cause of death equally well, 4) concerns already elaborated in the Toxicological Review regarding incomplete ascertainment of incident cases and other deaths possibly involving cancer, and 5) a quantitative comparison could only be made for Marsh et al. studies (2007, 625187; 2007, 625188) because of the partial availability of exposure information and not for the addititional epidemiological studies that observed significant associations between chloroprene exposure and cancer mortalitiy (Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Leet and Selevan, 1982, 094970; Li et al., 1989, 625181). Given these considerations, the comparison carried out here does not demonstrate a striking disagreement between the animal and human data.

EPA has not developed an Agency-wide policy to apply uncertainty factors to cancer risk estimates. Therefore, no uncertainty factor to take into account human variability was applied to the inhalation unit risk.

5. Lung tumors have been alternatively treated as systemic or portal-of-entry effects in the modeling of cancer endpoints. Please comment on the scientific justification for this modeling approach. Please comment on whether the rationale for this decision has been transparently and objectively described. Please comment on data available for chloroprene that may support an alternative method for modeling the observed lung tumors in mice.

Comment:

Four reviewers agreed that alternatively treating lung tumors as portal-of-entry or systemic effects was appropriate given the absence of data suggesting which route of exposure is more relevant to the carcinogenic effects of chloroprene. However, three of these reviewers also noted that the application of this approach was not sufficiently discussed in the Toxicological Review and that the text should provide more elaboration in that regard. One reviewer commented that lung tumors for both male and female mice appeared to be compatible with systemic saturable metabolic activation and therefore lung tumors should not be treated as portal-of-entry effects. One reviewer commented that treating chloroprene-induced lung tumors as either portal-of-entry or systemic effects would be appropriate given the lack of information only if chloroprene were a gas expected to elicit portal-of-entry effects. However, this reviewer further comments that the justification for treating chloroprene as a category 1 gas and the impact this had on dosimetric adjustments was not sufficiently justified in the document and that further justification should be added. This reviewer suggested that chloroprene is a category 3 gas (i.e., a non-reactive gas expected to elicit its toxicity systemically) and that the DAF should equal 1 for all observed tumor types. Finally, this reviewer noted that the pattern of respiratory injury is suggestive of local metabolic activation but that it was possible active metabolites are formed in and then escape the liver.

Response:

The current modeling approach of treating observed lung tumors as either portal-of-entry or systemic lesions was maintained in the Toxicological review. Additional discussion regarding the justification for and application of this approach as it relates to the observed pattern of adverse respiratory and systemic effects was added to the document (see Sections 5.2.3 and 5.4.3; see response to question 3 comments above as well). Chloroprene is a water insoluble, non-reactive chemical, and is expected to be absorbed into the bloodstream deep in the respiratory tract and exert its toxic effect systemically. Indeed, multiple adverse effects were observed distal to the respiratory tract that supports this assumption. However, lung tissue fractions have been shown to actively produce the reactive epoxide metabolite of chloroprene when exposed. Therefore, toxic effects observed in the respiratory tract of exposed animals may be due to either portal-of-entry effects due to *in situ* chloroprene metabolism or due to systemic redistribution of chloroprene back to the respiratory tissues. This additional discussion is consistent with the final reviewer's comments that the pattern of respiratory injury is suggestive of local metabolic activation, but that systemically distributed metabolites may be a factor in the observed carcinogenicity of chloroprene.

6. An oral slope factor (OSF) for cancer was not derived for chloroprene. Is the determination that the available data for chloroprene do not support derivation of an OSF scientifically justified?

Comment:

Five reviewers commented that the determination that there are no available data to support derivation of an oral slope factor for chloroprene was appropriate. One reviewer commented that an appropriate PBPK model would allow for a route-to-route extrapolation. One reviewer noted that the current PBPK model did not seem to be adequate to allow for route-to-route extrapolation. One reviewer commented that the lack of information on disposition of chloroprene, including the AUC for the DNA-reactive epoxide metabolite, after oral exposure did not support a route-to-route exposure. This reviewer noted that a likely large first-pass liver effect after oral exposure could significantly alter the systemic distribution of chloroprene and its metabolites compared to inhalation exposures.

Response:

The determination that the chloroprene database did not support the derivation of an oral slope factor was maintained in the Toxicological Review (see Section 5.4.4). A more complete discussion of the current PBPK model (Himmelstein et al., 2004, 625154), including its strengths and weaknesses for use in a route-to-route extrapolation in the current assessement, was included in Section 3.3.

Specific Comments

This section contains specific comments received from the external peer reviewers and has been organized so that comments and responses appear sequentially as they relate to the Toxicological Review.

Comment: The data on partition coefficient should be discussed more completely. It is possible to infer information on tissue distribution from such data. It is also possible to make inferences on regional respiratory tract absorption from these numbers. A vapor with a blood:air partition coefficient less than 10 is not likely to be scrubbed efficiently from the airstream in the upper airways.

Response: Additional language was added to the document regarding the partition coefficients for chloroprene and what inferences could be made regarding the magnitude of those partition coefficients (see Sections 3.2 and 3.4).

Comment: More detail should be provided on the metabolism kinetics for chloroprene. The information on elucidation of putative metabolites was clear and concise, but the data on kinetics was incompletely presented data and was very difficult to interpret fully. The meaning of the metabolic and toxicokinetic data, particularly with respect to rodent-human extrapolations, should be synthesized into a coherent explanation of species differences in response. Specific areas that need more attention include species differences in glutathione conjugation with respect to (1-chloroethenyl)oxirane detoxification and differences in chloroprene clearance among species. Factors that can influence the clearance of chloroprene include fat:air partition coefficients and percentage of body weight as fat.

Response: Extensive additional text regarding the metabolism of chloroprene and the toxicokinetic differences that exist among species was added to section 3.3. These additional discussions indicate that differences in epoxide production in the lungs of mice and humans are not 50-fold, but may be as little as 2- to 10-fold. These additional data also indicate that in some cases (i.e., glutathione transferase activity) detoxification of the epoxide metabolite may be faster in mice than humans. Additionally, there appears to be a further detoxification pathway, further oxidation of (1-chloroethenyl)oxirane, active in mice, but not in humans, rats, or hamsters. A discussion of fat:air partition coefficients and body fat percentage was added to the document.

Comment: The text in Section 3.3 should precisely indicate how the estimates for $V_{max}/K_{m,}$ reported in Tables 3-4 and 3-5, for lung metabolism were obtained. The mouse-human comparison for lung metabolism is a particularly important subject; this is a fact that was not adequately considered in the risk evaluation.

Response: Additional text was added to Section 3.3 clarifying how the estimates of V_{mas}/K_m were calculated. A detailed discussion of chloroprene metabolism in the mouse and human lung was also added to the document, as well as extensive discussion on how these differences impacted the risk evaluation.

Comment: The meaning of the ranges given for V_{max}/K_m for the oxidation of chloroprene should be described. If these were in fact the ranges of all observations, then the number of observations should be given.

Response: The ranges previously given in the text were removed and presented only in the corresponding tables. The ranges that were given were the ranges of values observed across the species investigated. These values were calculated from pooled microsomal preparations, authors did not report the number of observations made.

Comment: In table 3-2, results should be expressed as fraction of total metabolites rather than relative to butanol standard. Or it could be expressed in terms of absolute rates per unit time per unit microsomal protein.

Response: The authors reported the formation of (1-chloroethenyl)oxirane relative to butanol standard, and did not present data on the formation of total metabolites or on absolute rates per unit time per unit microsomal protein. Therefore, reporting the formation of (1-chloroethenyl)oxirane formation relative to butanol standard was maintained in the document.

Comment: Presentation of metabolic data in Table 3-4 was inadequate. No error bars or statements of how many animals tested independently (or pooled?), or more crucially, how many humans and how they differ in V_{max}/K_m for various organs.

Response: The data presented in Table 3-4 is how the data was presented by the authors in the original reference. Additional text was added indicating the results were from pooled microsomal preparations, and how many human samples were pooled. No other information was available for human variability in V_{max}/K_m in other organ systems.

Comment: Values for the major physiological parameters (body weight, cardiac output, and alveolar ventilation) should be provided.

Response: Those values were added to Table 3-9.

Comment: While suitable discussions of the epidemiological data regarding the healthy worker effect were included in the document, there were no suitable caveats for the “internal” comparisons by mentioning the distortions expected from the healthy worker survivor effect — that longer exposed workers with higher cumulative exposures have lower mortality than shorter term workers. This must be incorporated into the analysis.

Response: The discussion of the healthy worker survivor effect was expanded in the document (see Section 4.1.1.3).

Comment: SMRs and SIRs should consistently use $base_1$ or $base_{100}$.

Response: The document was revised so that SMRs and SIRs consistently use $base_{100}$ throughout the document.

Comment: It would be useful if as much information on occupational exposure levels would be presented in the text. Information on exposure concentrations in addition to cumulative (ppm-year) exposures would be useful.

Response: Information on the median average intensity of occupational chloroprene (in ppm) was added to the text (see Section 4.1.1.2).

Comment: The discussions of both liver and lung cancer would benefit from some attempt at integrative meta-analysis, combining the effects of multiple studies for reasonably comparable levels of exposure. This, however, likely depends on obtaining some disaggregated data from the individual investigators.

Response: Performance of a meta-analysis on liver and lung cancer data was beyond the scope of this document.

Comment: The document indicates that a limitation of the Li et al. (1989, 625181) paper was that only three years of local area data were used to estimate the expected numbers of deaths which may not be representative with regard to the period of follow-up of the cohort. An issue not considered is the stability of the expected rates based on local data. Also, the discussion of how the calculated SMRs would be biased if the local data for those three years was not representative of the entire period of follow-up is not clear.

Response: A discussion of the stability of the results reported by studies using low expected counts of cancer mortality was added to the document (see Section 4.7.1.1). Also, the text regarding how the SMRs may be biased due to the potential non-representativeness of the available local data was clarified.

Comment: In Colonna and Laydevant (2001, 625112), if there was any indication of how many workers died or left the study area prior to 1979, this should be included in the document. Did the authors have an idea of how much impact this would have on the results?

Response: No such data were available on how many workers left the study area prior to 1979.

Comment: It seems odd that of the 652 cancer cases in the Louisville facility, only 1 case was unexposed (Table 4-8). This might suggest that a large percentage of individuals classified as exposed were essentially unexposed. The document should provide greater emphasis on the potential impact of exposure misclassifications.

Response: The results in Table 4-8 reflect the analysis presented in Marsh et al. (2007, 625187). Text was added to the document highlighting the small number of unexposed workers across the four cohorts and limitations to the ability to draw conclusions based on the exposure classification approach in Marsh et al. (2007, 625187)

Comment: It is not difficult to understand why Marsh et al. (2007, 625188) concluded that their study provided no evidence of cancer risk associated with chloroprene exposures. Table 4-9 on page 4-14 shows little evidence of a dose response. It is inappropriate to conclude, as is done in lines 1-3 on page 4-15, that Marsh et al.'s (2007, 625188) explanations were "not entirely consistent with the data presented." The authors of this document have chosen one interpretation; the authors of the study have chosen another interpretation.

Response: The language regarding the interpretation Marsh et al.'s (2007, 625188) findings was revised in the document. Also, discussion of Leonard et al. (2007, 625179) has been included that adds to the weight of evidence that chloroprene exposure may be associated with cancer mortality, especially when comparions are based on internal populations or other regional/national DuPont workers.

Comment: Some of the criticisms of the occupational cohort studies are too harsh. For example, how often are causes of death verified by histological confirmation or review of medical records? Incomplete enumeration of incident cases is a criticism that could be leveled at many incident studies. The statement "that despite the lack of quantitative exposure information, occupational studies are still able to contribute to the overall qualitative weight of the evidence considerations" states the obvious. There are numerous examples of studies that have limited or no quantitative exposure information that have nevertheless contributed to weight of evidence considerations

Response: It is important to sometimes state the obvious for a broad audience so that readers that are not experts in epidemiology understand that there is still valuable information that can be gleaned from the epidemiology literature (i.e., with regard to lack of quantitative exposure information).

Comment: In Section 4.1.2.1, the statement "no workers experienced hair loss" is made. This is the first place where loss of hair is mentioned. Since that is an unusual effect, it would be better to report the results of the distillation workers after the results of the polymerization workers.

Response: The text was changed so that the results for distillation workers were presented after those for the polymerization workers.

Comment: For later modeling, EPA should report integrated average exposures that were measured, rather than the nominal target exposures. The difference be small, as indicated in the discussion, but the measurements should be used in preference to the target levels in the dose response modeling which appears later in the document.

Response: The actual average exposure concentrations achieved in the NTP (1998, 042076) study were added to the document (see Section 4.2.2). However, the differences between the target and actual chamber concentrations were very small. For the 2-year inhalation exposure, the greatest difference observed between target and actual exposure concentration was 0.9% for rats in the 32 ppm exposure group (target concentration of 32 ppm vs. actual concentration of 31.7 ± 1.1 ppm). Therefore, it was deemed unnecessary to redo the benchmark modeling with the actual exposure concentrations as the difference in results would be negligible.

Comment: Clarity could be improved in the document if the following were included in the document: with regard to Table 4-16, the magnitude of injury should be included (i.e., the average severity score could be added parenthetically in each column); with regard to the lack of histopathological damage in the lungs of mice in the 16-day study, the text should explicitly state as such; with regard to the lack of nasal lesions in the respiratory mucosa of rats in the 13-week study, the text should explicitly state as such (text should differentiate between effects, or lack thereof, observed in the olfactory and respiratory mucosa throughout the document as necessary); with regard to the incidence of forestomach lesions in mice in the 13-week study, text should state that preening behavior might have lead to direct gastrointestinal exposure to chloroprene.

Response: Language regarding these issues was added to the document text and tables where necessary.

Comment: Portions of the text in Section 4.2.2 refer to time to tumor data. Where are these data and derivation described? Should some discussion of maximum tolerated dose and whether it was exceeded be included in the text?

Response: The time-to-tumor analysis was detailed in Section 5.4 and complete time-to-tumor data was added to Appendix C. A discussion regarding maximum tolerated dose and selection of the dose groups for the chronic 2-year inhalation exposure was added to the text.

Comment: Information should be included in the document on how the survival-adjusted neoplasm rates reported in Table 4-28 were calculated.

Response: Text was added as a footnote in Table 4-28 detailing how survival-adjusted neoplasm rates were calculated.

Comment: Additional analyses are needed before dismissing the findings of increased resorptions in the 10 and 25 ppm exposure groups in Culik et al. (1978, 094969).

Response: The uncertainties surrounding these findings, including observation that the control group in the teratology study falls far outside of the historic control range for this strain of rat leading to potentially spurious statistical significance, was discussed fully and appropriately. The interpretation that these data are unreliable was maintained in the document (see Section 4.3).

Comment: Text in Section 4.5.2.1 alternatively stated that genotoxic activity was observed only in strains TA97A and TA98 or in all strains tested.

Response: The text was clarified to state that there was evidence of genotoxicity observed in all *Salmonella* strains tested, without Aroclor-induced S9 activation.

Comment: In Section 4.5.2.3, the hypothesis that chloroprene would only produce tumors in directly exposed tissues has been disproved by the NTP (1998, 042076) studies which demonstrated the multiple organ carcinogenicity of this chemical. This statement needs to be removed.

Response: The statement referenced above was taken from Tice (1988, 624981) and Tice et al. (1988, 064962). A clarifying sentence stating that chloroprene has been demonstrated to produce tumors distal to the portal-of-entry was added, and thus the observed lack of effect in bone marrow may be due instead to low metabolic activity in this tissue.

Comment: With regard to the comparison of carcinogenic potency of chloroprene vs. butadiene, it would be useful to have some quantitative comparison of cancer potency in rodents for these compounds. A more comprehensive summary of potencies for other and/or all tumors would provide important background for the quantitative cancer risk analysis. Table 4-37 should be supplemented with a table giving quantification of the indicated potency for multiple- and all sites.

Response: Table 4-38, which details the relative cancer potencies of chloroprene and butadiene for a number of tumor sites, was added to the document.

Comment: Table 4-37 is very confusing. What was the basis for including data from the rat relative to "sites of increased incidence" of neoplasms? Listed are many sites in which statistically significant results were not enumerated in previous portions of the text.

Response: Table 4-37 compares the incidence of tumors in multiple organ systems in both mice and rats that were exposed to butadiene, isoprene, or chloroprene. Its purpose is to show the similarity in tumor profiles for the three structurally related compounds. All of the tumor types listed for chloroprene have been previously discussed in the text. The lack of previous discussion for the butadiene and isoprene tumors is logical as this document focuses on chloroprene. Any discussion of tumor types induced by butadiene and isoprene is appropriately limited to this section, and Section 4.7.3.2, for the sole purpose of comparing tumor profiles as it contributes to the weight of evidence of the carcinogenic potency of chloroprene operating via a mutagenic mode of action.

Comment: In general, the "synthesis" of the inhalation exposure data (Section 4.6) is not a synthesis but merely a reiteration of the results. Rather than repeat the results study by study, it might be much preferable to organize this section on the basis of target organ. It could, for example, discuss the olfactory lesion data *in toto*, followed by the liver, etc. In this section, it is stated that chloroprene is associated with reproductive and developmental effects, yet the earlier portions of the text concluded otherwise.

Response: This section was extensively reorganized according to organ system and the observed toxicity therein. The discussion on the reproductive and developmental effects of chloroprene exposure was rewritten to emphasize the interpretation that those effects are equivocal.

Comment: Section 4.7 could be better organized. The summary in section 4.7.1 should probably be moved to the end of the entire section on carcinogenicity. The human data are discussed separately in an Evidence for Causality section, yet this is not provided for the animal studies. A true synthesis would discuss Evidence for Causality across studies in all species. This could be integrated with the discussion in Section 4.7.3.3 on Mode of Action to provide a stronger rationale for effects of chloroprene

Response: Section 4.7.1 was moved to the end of the section and serves as the summary for the Evaluation of Carcinogenicity section. While an Evidence for Causality section is included for the epidemiology data, no such section was needed for the animal data. The new Section 4.7.2 (previously

Section 4.7.1) now serves to summarize all of the cancer data across studies in all species. Section 4.7.3.3 was a summary on the mode of action of chloroprene and the weight of evidence supporting a mutagenic mode of action, and was thus limited to discussion of the observations that support this determination.

Comment: In Section 4.7.1.1, the statement "Although not statistically significant, these findings [increased relative risks of liver cancer observed by Marsh et al. (2007, 625188)] 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, 157419; Bulbulyan et al., 1998, 625105)" is made. Given that there could have been considerable differences in exposure, follow-up, duration of exposure, etc. between the studies, such a statement is probably not justified.

Response: This statement provides perspective on carcinogenic potential across studies. There are differences between studies, but this comparison reinforces the fact that the results are consistently elevated across studies.

Comment: In Section 4.7.1.1, the statement "only Bulbulyan (1999, 157419) observed a statistically significant association between chloroprene exposure and liver cancer mortality" suggests that this was done by an internal analysis, but the increase in liver cancer mortality was observed from an external analysis.

Response: Bulbulyan (1999, 157419) observed statistically significant associations between chloroprene exposure and liver cancer mortality based on both external and internal analyses.

Comment: Section 4.7.1.1 states "….although there is no direct evidence that alcohol is related to the exposure of interest (i.e., chloroprene)…" Alcohol may not be related to the exposure of interest, but that doesn't mean it could not have been a significant confounder. More convincing that alcohol did not play a confounding role would have been clear evidence of a dose response to chloroprene since it would be unlikely that alcohol consumption would correlate with chloroprene exposure. Evidence of a dose response, however, seems equivocal (see Table 4-11 on page 4-17).

Response: Alcohol cannot be a confounder if it is not both related to the exposure of interest (chloroprene) and the outcome of interest (liver cancer). There was suggestive evidence of a dose-response, or in consistent elevated risks in the upper exposure categories, in multiple studies (Bulbulyan et al., 1999, 157419; Leet and Selevan, 1982, 094970; Bulbulyan et al., 1998, 625105; Marsh et al., 2007, 625188)

Comment: What "current understanding" allows for the statement that specificity is "one of the weaker Hill criteria [*sic*]"?

Response: The criterion of specificity has many requirements and caveats that have been refuted and deemed invalid by many authors. In particular, Rothman and Greenland (086599) state "Specificity requires that a cause lead to a single effect, not multiple effects. This argument has often been advanced to refute casual interpretations of exposures that appear to relate to myriad effects, especially by those seeking to exonerate smoking as a cause of lung cancer. Unfortunately the criterion is wholly invalid. Causes of a given effect cannot be expected to lack other effects on any logical ground. To summarize specificity does not confer greater validity to any causal inference regarding the exposure effect."

Comment: Section 4.7.1.2 included a listing of increased incidences of tumors, yet the basis for inclusion in this listing is unclear. Some organs are listed in which the tumor incidence was not significantly increased. The discussion of species differences (lines 27-31) should include reference to possible species differences in epoxide hydrolysis rates. Such data are presented earlier and its absence here is confusing. This section failed to include the most important species difference – the appearance of lung tumors in mice but not rats. A clear metabolic basis might be provided, given that the metabolic activation rate in mice appears to be 50-fold higher than the rat. This would also serve to emphasize the potential role of metabolism relative to carcinogenicity. Epoxide formation is thought to be important relative to the respiratory tract toxicity/carcinogenicity of naphthalene and styrene and the same species differences (lung tumors in mice but not in rats) is seen for these vapors. Line 32 includes a reference to Dong et al 1989; this study was not described previously.

Response: This section was rewritten to include discussion of only tumors that were statistically significantly increased in rats and mice exposed to chloroprene for 2-years ((NTP, 1998, 042076). A discussion of species differences in metabolism was also included, as was the fact that lung tumors were induced in mice but not rats. A discussion of Dong (Dong et al., 1989, 007520) has been included in Section 4.2.1.

Comment: Table 4-39 is somewhat confusing. Why was lung cancer mortality listed under "rare tumors?" The table includes a reference to time to tumor, yet such data were not presented earlier in the text.

Response: Primary lung cancer in humans is a rare cancer type. Time to tumor information (presented as survival time) was previously presented in Section 4.2.1, including in the text and in Table 4-25. Time to tumor data was presented more exhaustively in Tables 5-4 and 5-5, as well as in Appendix C.

Comment: In Section 4.7.3.1, the document specifies a mutagenic MOA involving the reaction of epoxide metabolites formed at target sites. Until studies are conducted evaluating blood levels of epoxide intermediates, it would be inappropriate to impose this target site limitation. It is not known if epoxide formation occurs in all of the tumor target sites identified in the rodent carcinogenicity studies.

Response: The sentence was changed to read "…chloroprene acts via a mutagenic mode of action involving reactive epoxide metabolites formed at target sites or distributed systemically throughout the body."

Comment: In section 4.7.3.2, the statement that *in vivo* uptake of chloroprene involved the balance between epoxide formation and detoxification was confusing. Certainly the toxicity depends on the balance, but it is unlikely that uptake does. Uptake rates depend on the blood and tissue concentration of parent, downstream conversion of metabolite is not necessarily important in diffusion-based uptake.

Response: The text was changed to reflect that the toxicity of chloroprene involves a balance of reactive epoxide formation and detoxification.

Comment: In Section 4.7.3.2, it was stated that there is remarkable similarities in the potency and shape of the dose response between butadiene and chloroprene. Such data were not presented in earlier portions of the text.

Response: A discussion of the similarities between the carcinogenic potency and shape of the dose-response curve of butadiene and chloroprene was added to Section 4.5.3 and Table 4-38 was added to summarize that data.

Comment: In Section 4.7.3.3, it was stated that Melnick et al. (1994, 625208) performed a 6 month exposure-6 month follow-up study. Where were these data presented?

Response: This study is used in support of the proposed mutagenic mode of action for chloroprene. It is a study on a structurally related chemical, isoprene, and as such was not previously reported in the document. It was reported in Section 4.7.3.2 to strengthen the argument that *ras* mutations observed in chloroprene-exposed animals were most likely early mutagenic events in the development of neoplasia.

Comment: In Section 5.2.1, the text needs to clearly describe how the atrophy and necrotic data were combined. It is not certain there are any data indicating nasal olfactory atrophy leads to necrosis (as stated on lines 5-6). The concept that necrosis may lead to atrophy is quite straightforward however.

Response: This text was removed as atrophic and necrotic olfactory lesions were no longer combined into one endpoint for the purposes of benchmark modeling.

Comment: In Table 5-2, DAFs greater than 1 for lung and less than 1 for nasal epithelium deserve specific discussion.

Response: The application of DAFs was removed from Table 5-2 and moved to the text where a more complete and in-depth discussion of their calculation and application was included.

Comment: Regarding Section 5.2.3, chloroprene is not a category 1 gas. Its partition coefficient is only 10; clearly backpressure in nasal tissues controls the uptake process. The presence of non-respiratory tract tumors clearly indicates it is absorbed into the bloodstream. This vapor does not possess the physical chemical characteristics required of category 1 gases; in my view, it is a category 3 gas. The text needs to rigorously support this conclusion with respect to the physical chemical characteristics of chloroprene relative to those required of category 1 gases. The presence of olfactory lesions is not evidence that the toxicant is delivered via the airstream. Numerous compounds produce selective olfactory injury after parenteral administration. Indeed, the presence of olfactory but not respiratory nasal mucosal injury might be considered to provide data in support of a blood-borne mechanism. Naphthalene is one example of this phenomenon. Importantly, the subsequent text describes in great detail how the lung lesions may be due to blood-delivered rather than air-delivered chloroprene. The text needs to be consistent.

The RfC methodology is fatally flawed with respect to RGDR calculation. The derivations of these equations are based on the faulty assumption that the mass transfer coefficient is uniform throughout the nose. Dosimetry predictions from RGDR-based evaluations are totally discordant with the data. While application of a flawed methodology may be consistent with EPA policy, it certainly is not consistent with the scientific state-of-the-art. The mode of action is assumed to include metabolic activation to the epoxide. The RGDR of 0.28 indicates the humans will receive roughly 4-fold more toxicant (1/0.28) than the rat. Is it meant to imply that the metabolic activation rate in the human nose is 4-fold higher than the rat? The use of the RGDR needs to be discussed in light of the metabolically-based mode of action.

Response: In response to this reviewer's previous comments (see Charge Question B3), the application of the default DAFs was performed after the critical effect was chosen based on the observed experimental data (i.e., the POD_{ADJ}). Given this new basis for choosing the critical effect, increases in splenic hematopoietic proliferation was chosen over any nasal or pulmonary effect. This approach reduced the influence that application of a DAF has on the selection of a critical effect as this effect is unequivocally systemic in nature.

Increased discussion was included in the document regarding the physio-chemical properties of chloroprene, including how those properties can impact the determination of which dosimetric adjustments should be applied in calculating the human equivalent dose. Information regarding the metabolism of chloroprene into the reactive epoxide and potential for this metabolism in the respiratory tract (expression of CYP2E1 in the olfactory mucosa and microsomal oxidation of chloroprene in mouse lung homogenates) was also included. Additional discussion was also included that posits that the observed toxicity of chloroprene in the respiratory tract may be due systemic redistribution of chloroprene.

Comment: With regard to the application of uncertainty factors, it may be policy to include a database limitation factor due to the lack of a two generation study, but it was not scientifically justified in this case. A multi-generation study does exist. The rationale for the selection of this uncertainty factor should include this study.

Response: A true multi-generational study for chloroprene does not exist. Appelman and Dreef van der Meulen (1979, 064938) study is an unpublished report in which F_0 and F_1 rats were exposed to chloroprene. However, this study did not involve the mating of the F_1 generation, so developmental effects to the F_2 generation could not be assessed.

Comment: Table 5-3 does not include a row in the consideration column for database limitation.

Response: Discussion of uncertainty regarding the completeness of the database was added to Table 5-3.

Comment: In view of the saturation of the generation of an active metabolite, and the need to drop high doses in some cases, there should be an investigation of a Michaelis-Menten transformation of dose, in lieu of a full PBPK model.

Response: The suggested alternative modeling approach incorporating saturating metabolism was a constructive approach that EPA will consider with regards to future methods developed for human health risk assessment. However, as noted by the reviewers this model did not incorporate time-to-tumor information and it was beyond the scope of the Toxicological Review to modify the model to do so. Also, the saturating metabolism parameters were not derived from pharmacokinetic data but from empirical fits to dose and tumor incidence data, so it was as much an empirical model as the multistage-Weibull. Further, the saturating behavior observed, especially at the two higher doses, reflected to a large degree the limiting condition that only 100% of the animals can develop tumors. The multistage-Weibull model did adequately fit the monotonic, supralinear dose-response relationships seen in the NTP study; EPA retained the multistage-Weibull analysis in the assessment.

Comment: If variability or uncertainty in slope factors follows a normal distribution, a lognormal distribution could be used.

Response: The statement referring to variability in slope factors was removed and replaced with a statement that asymptotic normality was assumed for the slope factors (see Section 5.4.4).

PUBLIC COMMENTS

A. Interpretation of Epidemiological Studies

Comment: The Draft Review did not follow the USEPA approved method to assess epidemiological data quality, as detailed in the guidelines for the assessment of human cancer risk (U.S. EPA, 2005, 086237). The Draft Review did not assign a study-specific weight to each study cohort to reflect the quality of the study with regard to the relative strengths and limitations of each study.

Response: The 2005 US EPA Guidelines for Carcinogen Risk Assessment document (U.S. EPA, 2005, 086237) does provide criteria by which epidemiologic studies, whether providing positive or negative evidence of association, can be judged in regards to study quality. Specifically, the guidelines offer a list of characteristics that "are generally desirable in epidemiologic studies". The guidelines also state that "conclusions about the overall evidence for carcinogenicity from available studies in humans should be summarized along with a discussion of uncertainties and gaps in knowledge". However, the guidelines do not support using the suggested criteria as a basis to score studies an individual weight for use in a comparison of study quality across multiple studies. As such, a weighting and comparison scheme as suggested above is not supported by Agency guidance and was not used in the Toxicological Review. Individual studies were assessed on the basis of study quality in the document and extensive discussions of study limitations (individually and as part of the overall weight-of-evidence discussion) were included in the document, in accordance with the 2005 Cancer Guidelines.

Comment: One of the key studies cited by the US EPA as the basis for linking chloroprene exposure with cancer (Leet and Selevan, 1982, 094970) was superceded by the Marsh et al. (2007, 625187; 2007, 625188) study. The Marsh et al. study of cohorts in the United States, Ireland, and France did not report an association between exposure to chloroprene and the incidence of either total cancers or cancers of the lung or liver.

Response: The Marsh et al. (2007, 625187; 2007, 625188) study investigated a employee cohort from the Louisville Works DuPont plant that was previously investigated in Leet and Selevan (1982, 094970). However, there are a number of differences between the studies that warranted independent analysis of each. Specifically, Leet and Selevan (1982, 094970) reported that the Louisville cohort consisted of 1575 male employees (salaried and female employees excluded due to "minimal or no potential exposure to chloroprene") who were working at the Louisville plant on 6/30/1957. The authors further reported that most of the employees had 15 years of potential exposure to chloroprene (indicating that most had worked at the plant since it's opening in 1942). Also, the cohort was followed until 1974. Marsh et al. (2007, 625187; 2007, 625188) included "all workers (male and female) in each plant with potential exposure to chloroprene from the “start of production” until 2000. For the Louisville plant, this included a total of 5507 workers employed from 1949-1972. The Marsh et al. (2007, 625187; 2007, 625188) analyses started at 1949 to "avoid methodological problems associated with the earlier fifth revision of the ICD" and stopped at 1972 for the Louisville plant as that was when they report chloroprene production stopped at that plant, although chloroprene purification and polymerization still occurred there according to Leet and Selevan (1982, 094970). Also, there are important differences in how each study assessed exposure. Leet and Selevan (1982, 094970) used worker history summaries to classify workers as either “high” or “low” chloroprene exposure, whereas Marsh et al. (2007, 625187; 2007, 625188) used a more sophisticated approach that considered worker history summaries and worker exposure profiles to generate quantitative estimates of chloroprene exposure intensity. Therefore, although the two studies investigated members of the same cohort, a number of methodological differences between the studies warrant the independent analysis of each.

Comment: Interpretations of the Chinese, Russian, and Armenian cohorts (Bulbulyan et al., 1998, 625105; Bulbulyan et al., 1999, 157419; Li et al., 1989, 625181) failed to acknowledge the imprecise and unstable estimates of mortality and incidence ratios due to very low expected counts for liver and lung cancer mortality.

Response: Although some cohorts did report very low expected counts for liver and lung cancer, some of these same studies demonstrated statistically significant associations that are fairly precise (e.g., Bulbulyan et al. (1999, 157419))(e.g., Bulbulyan et al., 1999, 157419). Naturally, studies with a limited number of outcomes and those that examine exposure-response relationships with few deaths in each cell will have wider confidence bounds. Given the rarity of the outcomes that were examined (especially in the general population), the expectation would be a low expected number of deaths. This was demonstrated for outcomes (e.g., liver cancer mortality) in many studies including several of the DuPont plants as were statistical power limitations when examining cancer-specific effects and exposure-response relationships. Regardless of the study, the Toxicological Review has highlighted the issue of imprecision by presenting confidence intervals and discussions of small sample size throughout the document. Although, on an individual study basis, there may exist some concern over the potential role of chance for isolated outcomes that were not replicated in later studies, the consistency of the results indicate chance is an unlikely explanation of the results across heterogeneous study populations and exposure scenarios in several countries. As such, these studies contribute to the weight-of-evidence characterization of the carcinogenic potential of chloroprene.

Comment: The Chinese, Russian, and Armenian studies have limitations and confounders that limit the interpretation and conclusions of their reported findings

Response: The limitations of each study, including potential confounding, has been discussed individually and together in multiple sections of the Toxicological Review.

Comment: The Draft Review currently gives limited consideration to the Marsh et al. (2007, 625187; 2007, 625188) studies in regard to the overall weight-of-evidence for the association between chloroprene and cancer mortality.

Response: All studies were judged independently on their individual merits and given full consideration in the overall weight-of-evidence characterization. The Marsh et al. (2007, 625187; 2007, 625188) studies are discussed in detail in Sections 4.1 and 4.7. In regard to study strengths and findings, the studies have been characterized and considered in the overall weight-of-evidence. It is important to note that, although the Marsh et al. (2007, 625187; 2007, 625188) did not observe statistically significant associations between chloroprene exposure and cancer mortality, they did observe elevated risks when internal comparisons were performed (see Section 4.1.1.2). Some of these results were similar in magnitude to findings in other studies which reported more consistent associations between chloroprene exposure and cancer mortality.

Comment: Assessing causality failed to apply methods recommended by the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005, 086237). Specifically, the Draft Assessment does not explicitly evaluate available epidemiologic quantitative results for potential bias due to systematic errors (i.e., bias, misclassification, and confounding) and random errors (i.e., the role of chance). There has been consistent agreement among previous reviews of the epidemiology database for chloroprene that studies indicating a positive association are of insufficient quality to infer a causal relationship between chloroprene and cancer mortality.

Response: The Toxicological Review has exhaustively discussed individual study limitations including a thorough examination of the potential for bias in multiple sections of the document. In regard to inferring a causal relationship between chloroprene exposure and cancer mortality, no such definitive determination is made in the Toxicological Review based on the epidemiologic data. In the discussion of the Evidence of Causality (Section 4.7.1.1.1.), the document explicitly states:

> *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… 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 the carcinogenicity of chloroprene in humans.*

Thus, the document makes no definitive claim of a causal relationship between chloroprene exposure and cancer mortality, but rather explicitly states that there is evidence of an association across the body of scientific literature.

Comment: US EPA interpretation of the potential for lung and liver cancer risks of chloroprene based on the Marsh et al. (2007, 625187; 2007, 625188) study did not fully consider the impact of inordinately low death rates for lung and liver cancer among workers in the baseline categories.

Response: Although the authors highlight some “exceedingly” low mortality figures in the “baseline” exposure levels (i.e., lowest exposure category), comparable numbers of deaths are found in low-, intermediate-, and some high-exposure groups across different outcomes (those RRs ≤ 1.00 for all cancers, respiratory and liver cancer mortality). It is unclear why the authors consider any RRs in excess of 1.00 to be due to an “exceedingly” low baseline mortality rate. There is little evidence to suggest that this is not a valid population in which to base comparisons on, and the results of the internal analyses are preferred given the strong evidence of the healthy worker effect in the SMR analyses. In addition, given the fact that such strong RRs were detected in healthy workers, one would be more concerned about potential risk among less healthy populations under similar circumstances.

Comment: Vinyl chloride exposure as a potential confounder of the association with chlorprene exposure and liver cancer in the Marsh et al. (2007, 625187; 2007, 625188) study is not supported given the lack of correlation between chloroprene and vinyl chloride exposure.

Response: The Toxicological Review has discussed the potential for vinyl chloride to act as a confounder in detail in Section 4.1.1.2. As noted, since there was no association between cumulative exposures to vinyl chloride and chloroprene among these workers, vinyl chloride does not meet the definition of a confounder, and thus any association between chloroprene exposure and cancer mortality is highly unlikely to be modified by vinyl chloride exposure. The internal analyses of Marsh et al. (2007, 625187; 2007, 625188) also indicated that there is an inverse association between vinyl chloride exposure and risk of both respiratory and liver cancers based on limited numbers of cancer deaths in the vinyl chloride-exposed groups. Therefore, even if vinyl chloride exposures were positively correlated with chloroprene exposures among workers, any resulting negative confound would result in attenuation of unadjusted relative risk estimates. That is, associations stronger in magnitude would be expected if the relative risk estimates for chloroprene and cancer were adjusted for vinyl chloride exposures.

B. Interpretation of Mode of Action Based on the Mutagenicity and Genotoxicity Data

Comment: Standard *in vivo* tests for genotoxicity were negative: chloroprene, unlike butadiene and isoprene, does not exert genetic toxicity to somatic cells *in vivo.*

Response: The Toxicological Review describes numerous *in vivo* genotoxicity tests that return non-positive results, including lack of sister chromatid exchange or chromosomal aberrations in bone marrow and no evidence of micronuclei formation in peripheral blood erythrocytes. However, when *Drosophila melanogaster* were exposed to chloroprene (99% pure with neglible dimer content), an increase in recessive lethal mutations on the X chromosome of male flies was observed (Vogel, 1979, 000948). Similar results were not observed in a similar experiment by Foureman et al. (1994, 065173). However, there were significant differences between the two experiments that may explain different findings: (1) differences in purity of the chloroprene sample (99% pure in Vogel (1979, 000948) and only 50% pure in Foureman et al. (1994, 065173)), (2) differences between the Berlin-K (Vogel, 1979, 000948) and Canton-S (Foureman et al., 1994, 065173) strains, (3) differences in sample sizes, and (4) possible genetic drift within the female populations used by the two groups of investigators. Regardless, the strongest evidence of *in vivo* genotoxicity is the observation of genetic alteration of cancer genes including the *ras* proto-oncogenes (Sills et al., 1999, 624952; Sills et al., 2001, 624922; Ton et al., 2007, 625004), 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, 042076) 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, 624952; Sills et al., 2001, 624922). 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, 625004). 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, 042076; U.S. EPA, 2000, 194536). Activated K-*ras* oncogenes were observed in lung tumors, hepatocellular carcinomas, and lymphomas in B6C3F1 mice exposed to 1,3-butadiene (U.S. EPA, 2002, 052153).

Comment: There is a general lack of consistent data for chloroprene-induced point mutations. The ability of chloroprene to induce point mutations in bacteria is equivocal at best and chloroprene did not induce mutations in cultured mammalian cells. Conflicting specificities between *in vitro* bacterial point mutations (GGG) and DNA adduct induction (preferentially forming guanine adducts when incubated with calf thymus DNA) and *in vivo ras* mutations found at tumor sites (A to T transversions) indicate that *in vivo* mutations may be of a non-chloroprene origin.

Response: The Toxicological Review presented the bacterial genotoxicity data as returning conflicting results, but did note that when positive results were observed they occured in *Salmonella* strains that test for point mutations. Assays with *Salmonella* strains that tested for frameshift mutations were consistently negative. A guanine adduct was the major adduct observed (approximately 96% of adducts formed) when the epoxide metabolite of chloroprene is reacted with calf thymus DNA in a cell-free environment. However, when equimolar quantities of all four nucleosides were reacted with (1-chloroethenyl)oxirane simultaneously in a competitive reaction assay, all of the adducts identified from individual nucleoside reactions were observed and were formed at similar rates. As stated above, the strongest line of evidence indicating that chloroprene induced point mutations leading to a carcinogenic response was the observation that tissues from chloroprene-induced lung, forestomach, and Harderian gland tumors in mice demonstrated a higher frequency of mutations in K- and H-*ras* proto-oncogenes than in spontaneous occurring tumors (NTP, 1998, 042076). Although the majority of these point mutations were A to T transversions, a number of G transversions were also observed in lung and forestomach tumors. Another strong indication that the A to T transversion at codon 61 in mouse lung tumors is chloroprene-induced is that it was not observed in spontaneously occurring tumors in NTP historic controls.

Comment: A non-genotoxic mode of action for chloroprene should be considered. An alternative mode of action is that chloroprene induces localized cytotoxicity with subsequent induction of hyperplasia and cell regeneration followed by promotion of pre-existing proto-oncogene mutations.

Response: The document states that there may be alternative modes of action operant in certain situations (i.e., high dose exposures) that may explain why lung tumors are observed at high doses when the frequency of *ras* mutations is less than is observed at lower doses (see Section 5.4.1). However, the scientific evidence indicates that a mutagenic mode of action is a plausible mode of action with regard to the carcinogenicity of chloroprene. The observation that the majority of *ras* mutations in the lungs of chloroprene exposed mice consisted of A to T transversions at codon 61 (22/37) is inconsistent with the proposed alternative mode of action. If chloroprene exposure was initiating cytotoxicity with subsequent hyperplasia/regeneration leading to promotion of pre-existing proto-oncogene mutations, the expectation would be that no A to T transversions at codon 61 would be observed as this mutation is not seen in spontaneously occurring lung tumors in historic controls. The current proposed mutagenic mode of action was unamimously accepted by the External Peer Review panel. Additionally, the mutagenicity of chloroprene is proposed in numerous studies cited in the Toxicological Review, including but not limited to: Munter et al. (2003, 625214); Summer and Greim (1980, 064961), Himmelstein et al. (2001, 019013; 2004, 625154; 2004, 625152); Melnick et al. (1999, 000297); Ponomarkov and Tomatis (1980, 075453).

C. Consideration of Species Differences in Toxicokinetics and Target Tissue Dosimetry

Comment: Significant species differences in metabolism are documented and the peer reviewed literature (Cottrell et al., 2001, 157445; Himmelstein et al., 2004, 625152; Munter et al., 2007, 625213; Munter et al., 2007, 576501) demonstrates that there are significant differences in the metabolism of chloroprene across species that can impact target tissue dose.

Response: The observed species differences in metabolism were acknowledged and extensively discussed in the Toxicological Review. While differences in metabolism do exist across species that could substantially impact target tissue dose, additional discussion added to Section 3.3 indicate that differences in epoxide production in the lungs of mice and humans are not as great as 50-fold (as once indicated in a prior draft of the Toxicological Review), but may be as little as 2- to 10-fold. These additional data also indicate that in some cases (i.e., glutathione transferase activity) detoxification of the epoxide metabolite may be faster in mice than humans. Also, there appears to be an additional detoxification pathway, oxidation of (1-chloroethenyl)oxirane, that is active in mice, but not in humans, rats, or hamsters. Therefore, the document clearly and transparently presents data that do indicate that species differences exist in the metabolic activation of chloroprene; however, these differences are not so great as to preclude using animal data to estimate the non-cancer and carcinogentic toxicity of chloroprene in humans.

Comment: Previous analyses (Himmelstein et al., 2004, 625154) support the use of the physiologically based pharmacokinetic (PBPK) model.

Response: The use of the PBPK model described in Himmelstein et al. (2004, 625154) in the Toxicological Review was not supported for a number of reasons discussed throughout the document. Specifically, the model predicted blood chloroprene and delivery of chloroprene to metabolizing tissues based on metabolic constants and partition coefficients based on *in vitro* data. Loss of chamber chloroprene was attributed to uptake and metabolism by test animals and was used to test the metabolic parameters and validate the model. However, Himmelstein et al. (2004, 625154) did not provide results of sensitivity analyses indicating whether chamber loss was sensitive to metabolism, and therefore it is uncertain whether chamber loss was useful for testing the metabolic parameters used in the model. Also, the chamber data were fit by varying alveolar ventilation and cardiac output. This method did not result in adequate testing of the model and did not validate the scaled *in vitro* metabolic parameters. Additionally, there were currently no blood or tissue time-course concentration data available for model validation.

Comment: New data supplied by DuPont at the External Peer Review Meeting 1) support the use of the quantitative PBPK model, 2) increase confidence in the PBPK model parameters (through refined liver and lung microsomal metabolic parameters and new kidney microsomal metabolic parameters), and 3) provide genomic evidence that kinetic differences alone do not influence the production and retention of reactive metabolites.

Response: At the time of the External Peer Review meeting, the data provided by DuPont had not been peer-reviewed and as such could not be used as the basis for the use of the PBPK model and the derivation of the RfC or inhalation unit risk.

D. US EPA Decision Points in the Determination of the Inhalation Unit Risk

Comment: The presentation of datasets to be used to determine the RfC, including the dataset ultimately selected (i.e., nasal lesions in the male rat) needs additional information. Table 5-1 is potentially misleading, in that it suggests by omission that nasal effects are only observed in male rats. Table entries for nasal effects in female rats are listed “not observed”, which is incorrect. Also missing from Table 5-1 are the data for nasal atrophy in male and female mice.

Response: Additional endpoints were added to Table 5-1, including nasal effects observed in female rats. The criteria for what endpoints were considered for selection of the critical effect were changed such that all portal-of-entry and systemic nonneoplastic lesions that were statistically increased in mice or rats at the low- or mid-exposure concentration (12.8 or 32 ppm) compared to chamber controls, or demonstrated a suggested dose-response relationship in the low- or mid-exposure range in the absence of statistical signficance, were considered candidates for the critical effect. Table 5-1 was edited to reflect this. Also, nasal atrophy in male and female mice was not included in Table 5-1 as that endpoint fails to satisfy the criteria listed above.

Comment: A value of 3 for database deficiencies for chloroprene is incorporated in the derivation of the RfC. However, several lines of evidence suggest that this value may not be needed. First, chloroprene is not expected to accumulate in tissues such that in a multigenerational study, exposure to the second generation (F_2) would be greater than experienced by the first generation (F_1). Second, the NOAEL for reproductive toxicity of 100 ppm in the unpublished report by Appelman and Dreef van der Meulen (1979, 064938) is higher than NOAELs/LOAELs for nasal and systemic effects observed in the NTP (1998, 042076) study. Based on this comparison of NOAELs/LOAELs, US EPA should reconsider the application of an UF for database uncertainties due to the lack of a multigenerational study.

Response: A database uncertainty factor of 3 was maintained in the document due to the lack of a multigenerational developmental/reproductive study. The lack of a multigenerational precludes the ability to assess the effects of chloroprene on postnatal maturation and reproductive capacity of the the F_1 offspring, and any cumulative effects that may manifest throughout multiple generations. Additionally, Dourson et al. (1992, 004400) demonstrated that, when comparing ratios of chronic dog, rat, and mouse studies and reproductive/developmental studies in rats, the reproductive/developmental studies were useful in establishing the lowest NOAEL. If one or more bioassays are missing, Dourson et al. (1992, 004400) recommended that an uncertainty factor should be used to account for this uncertainty. Therefore, due to the lack of a multigenerational study, there exists residual uncertainty in the chloroprene database that is accounted for by the current database uncertainty factor of 3.

Comment: In the Draft Review, a proprietary software program (TOX_RISK version 5.3) was relied upon for the time-to-tumor dose-response modeling. This software is no longer available to the general public, and adversely affected the transparency of the dose-response model. Simpler models provided in BMDS should be used instead.

Response: The time-to-tumor dose-response modeling was redone using EPA's Multistage Weibull (MSW) time-to-tumor model. This model is free and available to the general public at: www.epa.gov/ncea/bmds/dwnldu.html. Use of this model removed any previous issues with the transparency of the modeling approach.

Comment: EPA's assumption that hemangiosarcomas were the only fatal tumor type did not appear to be consistent with the data, in that the pattern of responses should have been different if hemangiosarcomas had impacted the occurrence of other tumors. Incidence of these tumors dropped at the high dose, suggesting that other tumors caused deaths before the hemangiosarcomas could have developed. This modeling approach was not viable without considering lethality assumptions further.

Response: EPA agrees that earlier deaths likely impacted the incidence of circulatory system tumors; that is why the multistage-Weibull model was used. However, the designation of some tumors as fatal did not automatically imply that they occurred earlier than the rest of the tumors. The multistage-Weibull model addressed the time of death for each animal as recorded; the fatal designation impacted only the magnitude of the risk estimate for that tumor type and is not a data input for the analysis of other tumor types. Designation of individual tumor occurrences as fatal (as appropriate) will tend to increase unit risk estimates. As shown in the document, analyses of fatal and incidental circulatory system tumors showed a roughly twofold range in unit risks between treating all tumors as incidental or all as fatal; the more representative value is likely between those two extremes. However, without specific causes of death for each animal in this study, it is difficult to consider the impact of this issue more thoroughly. The uncertainty discussion was expanded to include these points.

Comment: Model selection (goodness-of-fit for arriving at final number of stages) was not well characterized.

Response: A summary of the model selection decisions was added (see Section 5.4.3).

Comment: Unit risks from multiple tumor types should not be summed in the determination of the composite unit risk for carcinogenicity. Given the considerable overlap in tumor incidence data among animals, EPA's assumption that the tumors are independent leads to an overstatement of the carcinogenic potential of chloroprene. EPA's method has no precedent in final IRIS assessments, and is statistically flawed. The most appropriate approach for derivation of the unit risk for chloroprene if animal data are used is to rely upon the most sensitive tumor endpoint (i.e., lung tumors) in the most sensitive species.

Response: Basing the inhalation unit risk on only one tumor type when chloroprene has been shown to induce tumors in multiple organ systems in two species of rodents would most likely result in an underestimation of the human carcinogenic potential of chloroprene. The basis for considering the tumor types statistically independent was clarified. Briefly, the commenter's demonstration of the overlap of tumors focused on the overlap of tumors at the high doses, where there is insufficient information to determine whether the tumors are independent or not, since high rates of response have to overlap regardless of their independence. Note that at the lowest exposure, only 9 of 36 female mice with tumors had more than one tumor. The composite unit risk describes the risk for much lower exposures where the risk of multiple tumors is trivial.

Concerning the statistical method, the document was revised to clarify that it is an approximate approach. The document cited two final IRIS assessments that have used this method, and a third has been added; all were externally peer reviewed.

Comment: Because the mode of action proposed for chloroprene in the Draft Review is dependent upon target tissue dose, it is critical that the HEC values take into consideration important species differences in metabolism.

Response: Additional discussion of toxicokinetics included throughout the Toxicological Review clearly and transparently present data that do indicate that species differences exist in the metabolic activation of chloroprene. However, these differences are not so great as to preclude using animal data to estimate the non-cancer and carcinogentic toxicity of chloroprene in humans.

Comment: The points of departure for two tumor types (lung and liver) in the female mice appear to fall considerably below the range of observation (i.e., by more than a factor of 3), and therefore are inconsistent with US EPA guidelines for benchmark modeling.

Response: The selected PODs are in fact consistent with the cited guidance. The BMRs are within the *observable* range, “the range of doses for which toxicity studies have reasonable power to detect effects” (US EPA, 2000), since 10% is within the sensitivity of typical cancer bioassays, such as this one. Use of a BMR which falls within the actual range of responses observed in this study leads to a trivial difference in the estimated PODs for these two derivations.

Comment: The lung tumor response data was assessed in the Draft Review assuming the responses were either portal-of-entry effects or systemic effects. This approach is internally inconsistent with the non-cancer assessment in which the nasal atrophy/necrosis and lung hyperplasia in rodents were attributed as portal-of-entry effects.

Response: In the current derivation of the RfC, a discussion of how assumptions regarding the route-of-exposure can effect HECs was added. Therefore, the non-cancer and cancer quantitative sections have been made consistent in this regard.

E. US EPA's Quality Control in Reporting Chloroprene Data

Comment: In comparing information provided in the Draft Review to that in the primary literature, a number of inconsistencies were noted. In particular, commenters interpreted dose-response modeling to have inappropriately included animals without histopathologic evaluation for particular sites. Commenters also noted inconsistencies within the document. In addition, information on the production of chloroprene in the Draft Review is not current and there are issues in attempting to duplicate some of the quantitative analyses.

Response:

All editorial corrections regarding data reporting were made where needed. The majority of data discrepancies noted suggested that risks may have been underestimated:

- One tumor response, a forestomach tumor in a high dose male mouse, had been inadvertantly omitted from dose-response modeling; all relevant analyses have been revised.
- Animals noted with missing tissues, but included in dose-response analyses were included correctly; time-to-tumor modeling takes into account time on study without appearance of a tumor. If they had been included in a simpler dichotomous model, such as the multistage model, an underestimate of risk would have resulted. In the instance of an animal on study for 3 days, EPA concluded there was likely little impact including or excluding that animal. For purposes of accountability, these animals were included in the analyses.

Other discrepancies noted:

- Number of animals considered at risk for dose-response analysis of Zymbal gland and Harderian gland tumors, which were not evaluated histopathologically in all animals—Denominators were corrected.
- Differences in ToxRisk output suggesting different time value inputs—Time values had been input as week of study, not weeks on study. Since this was done consistently throughout the data sets, no substantive difference wass expected. The input data were included in the assessment.

Information on the physical/chemical properties of chloroprene was corrected. Information provided to EPA by DuPont regarding current production and manufacturing levels and processes was added to the Toxicological Review. Information previously in the Toxicological Review was retained in the document to give a complete description of historical and current production levels and processes. The Sanotskii (1976, 063885) reference was retained in the Toxicological Review: although there are concerns with the methodologies used by the studies cited in the Sanotskii review, these concerns have been detailed appropriately in the

Toxicological Review. The Sanotskii review does not serve as the basis for any quantitiative analysis, and only provides data and results that are qualitatively useful in comparison to other study reports included in the Toxicological Review. The limitations of the Sanotskii review are appropriately detailed when the paper is first referenced; it was not necessary to exhaustively delineate the study limitations at every instance the paper is cited in the Toxicological Review.

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 2.1.1 (BMDS). The BMDS model outputs for the derivation of the chronic RfC are attached. The doses used in modeling the individual endpoints, and reported as BMDs and BMDLs, are in ppm.

The following critical effects were modeled using BMDS: alveolar epithelial hyperplasia (male and female rats), bronchiolar hyperplasia (male and female mice), olfactory epithelial chronic inflammation (male rats), olfactory epithelium atrophy (male and female rats), olfactory epithelial necrosis (male and female rats), olfactory basal cell hyperplasia (female rats), kidney (renal tubule) hyperplasia (male and female rats), forestomach epithelial hyperplasia (male and female mice) and splenic hematopoietic cell proliferation (female mice). A BMR of 10% extra risk was used for each endpoint, unless severity scores (see Table B-1) indicated a progression of severity from the control dose to the lowest dose showing response, in which case a BMR of 5% extra risk was used. A BMR of 5% extra risk was also used in cases involving endpoints with no control response but the presence of moderate or marked lesions in the lowest dose showing response. The endpoint being modeled specified which set of models, either 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 $\alpha = 0.1$ (i.e., p-value > 0.1), χ^2 scaled residuals, visual fit, and consideration of model parameter estimates. Once all appropriately fitting models were identified, final model selection was based on either the Akaike Information Criterion (AIC) when the BMDL estimates for all appropriately fitting models were sufficiently close (i.e., within 3-fold difference of one another) or the lowest BMDL when they were not within 3-fold difference of each other.

The critical endpoint selected for the derivation of the chronic RfC was increased incidence of splenic hematopoietic proliferation in female mice and alveolar hyperplasia in the female rat. The probit model provided the best fit for this data set. The following tables (B-1 through B-21) 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 (figures B-1 through B-16) and output are included immediately after the table.

Table B-1. Severity scores at control dose and lowest dose showing response for endpoints considered for critical non-cancer effect

Endpoint	0 ppm				12.8 ppm				32 ppm			
	I[a]	II	III	IV	I	II	III	IV	I	II	III	IV
Male Rats												
Alveolar hyperplasia	3	2	0	0	10	5	1	0	--[b]	--	--	--
Kidney hyperplasia	--	--	--	--	--	--	--	--	--	--	--	--
Olfactory atrophy	1	2	0	0	6	3	3	0	--	--	--	--
Olfactory basal cell hyperplasia	0	0	0	0	0	0	0	0	18	18	2	0
Olfactory metaplasia	2	4	0	0	5	0	0	0	--	--	--	--
Olfactory necrosis	0	0	0	0	5	1	5	0	--	--	--	--
Olfactory chronic inflammation	0	0	0	0	5	0	0	0	--	--	--	--
Female Rats												
Alveolar hyperplasia	3	2	0	1	15	6	1	0	--	--	--	--
Kidney hyperplasia[c]	--	--	--	--	--	--	--	--	--	--	--	--
Olfactory atrophy	0	0	0	0	1	0	0	0	31	7	2	0
Olfactory basal cell hyperplasia	0	0	0	0	0	0	0	0	16	1	0	0
Olfactory metaplasia	0	0	0	0	1	0	0	0	34	1	0	0
Olfactory necrosis	0	0	0	0	0	0	0	0	3	2	3	0
Male Mice												
Bronchiolar hyperplasia	0	0	0	0	3	5	1	1	--	--	--	--
Kidney hyperplasia	--	--	--	--	--	--	--	--	--	--	--	--
Forestomach epithelial hyperplasia	0	2	0	2	2	3	1	0	--	--	--	--
Splenic hematopoietic cell proliferation	2	12	10	2	2	15	5	0	--	--	--	--
Female Mice												
Bronchiolar hyperplasia	0	0	0	0	4	8	2	1	--	--	--	--
Forestomach epithelial hyperplasia	1	2	0	1	0	0	2	1	--	--	--	--
Splenic hematopoietic cell proliferation	0	8	4	1	3	13	6	3	--	--	--	--

[a] Severity scores – I = minimal, II – mild, III – moderate, IV – marked

[b] Only severity scores in control dose and lowest dose with response used to make determination of severity progression with increasing dose

[c] Severity for single sections and step sections combined not available

Source: NTP (1998)

Table B-2. Benchmark modeling results for alvelolar epithelial hyperplasia in male F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	231.042	0.1317	1.698	14.8657	10.0883
Logistic	232.34	0.0775	-0.092	24.4838	19.1571
Log-logistic[a]	**230.479**	**0.1753**	**1.566**	**11.4228**	**7.06934**
Log-probit	233.859	0.0363	-0.087	28.604	19.5927
Multistage	231.042	0.1317	1.698	14.8657	10.0883
Probit	232.209	0.0813	-0.126	23.3986	18.2584
Weibull	231.042	0.1317	1.698	14.866	10.0883
Quantal-linear	231.042	0.1317	1.698	14.866	10.0883
Dichotomous hill	231.705	0.1112	-0.1356	5.87477	3.85444

[a] model choice based on lowest AIC

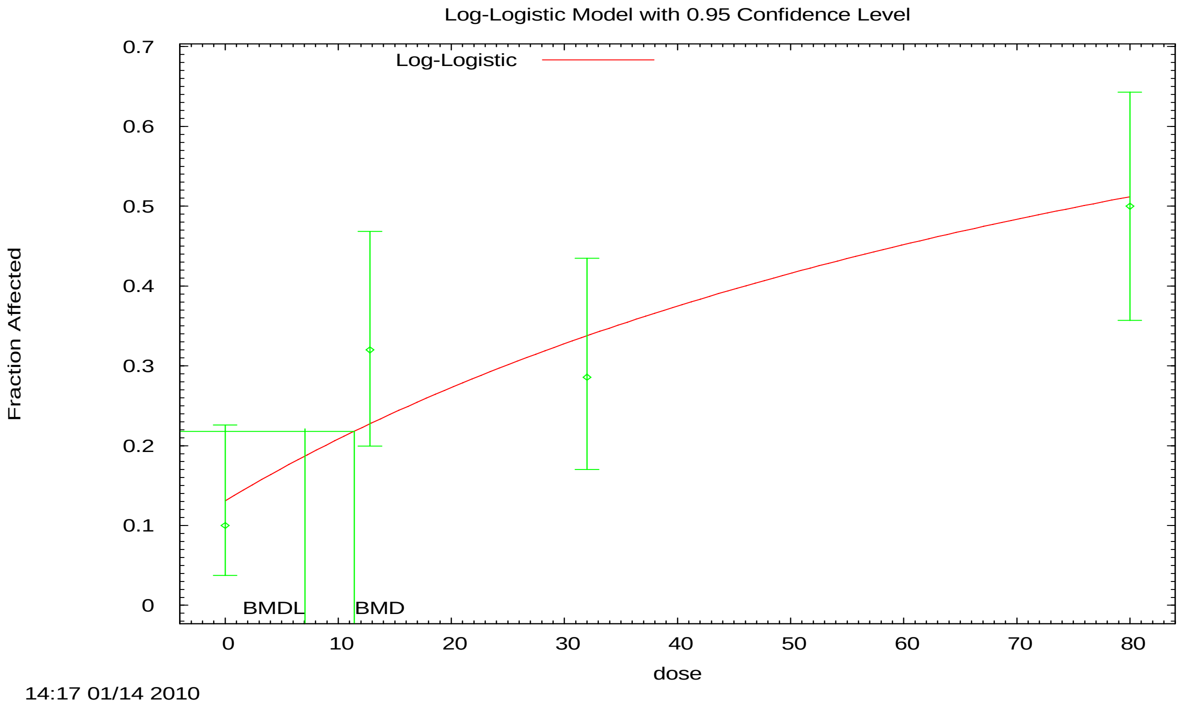


Figure B-1. Log-logistic model fit for alveolar epitheliala hyperplasia in male F344/N rats (BMR = 10% extra risk)

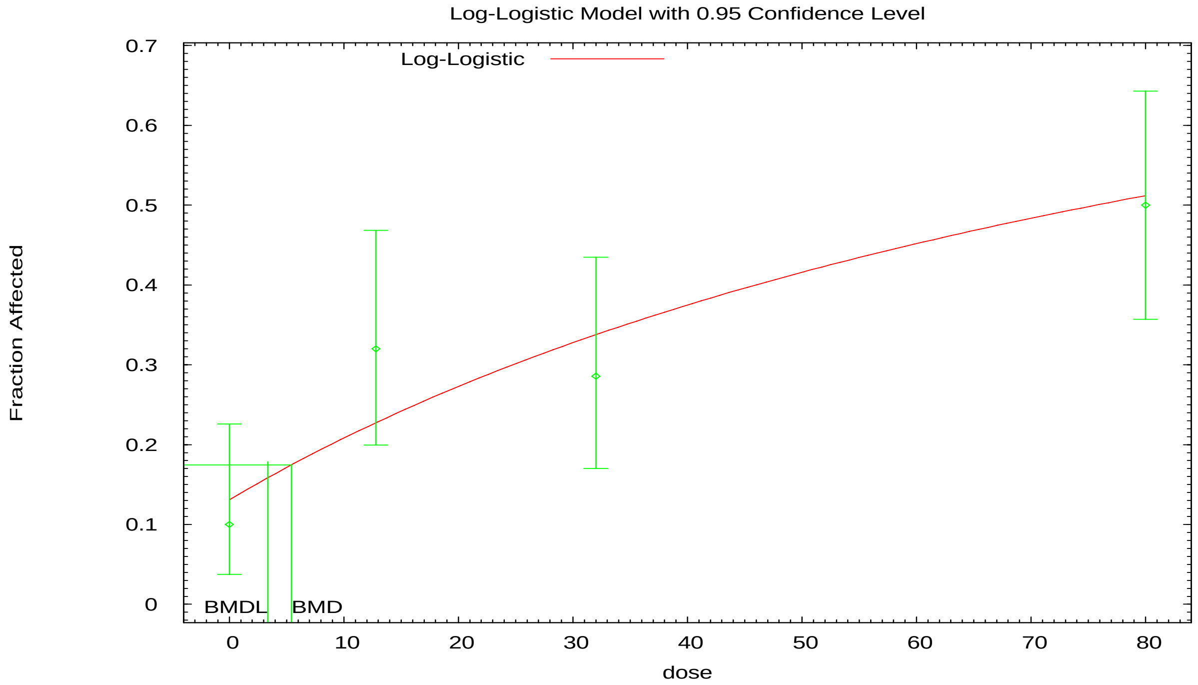
```
 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnl_rat_m_alv_hyper_Lnl-BMR10-
 Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnl_rat_m_alv_hyper_Lnl-BMR10-
 Restrict.plt
 Thu Jan 14 14:17:54 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   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 =          0.1
                      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        Std. 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
                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.1310         6.549     5.000          50       -0.649
   12.8000     0.2272        11.360    16.000          50        1.566
   32.0000     0.3373        16.526    14.000          49       -0.763
   80.0000     0.5113        25.564    25.000          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-3. Benchmark modeling results for alvelolar epithelial hyperplasia in male F344/N rats (BMR = 5% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	231.042	0.1317	1.698	7.23716	4.91134
Logistic	232.34	0.0775	1.701	13.0617	10.1972
Log-logistic[a]	**230.479**	**0.1753**	**-0.649**	**5.41078**	**3.34864**
Log-probit	233.859	0.0363	1.939	19.8906	13.6243
Multistage	231.042	0.1317	1.698	7.23716	4.91134
Probit	232.209	0.0813	1.711	12.3417	9.6301
Weibull	231.042	0.1317	1.698	7.23729	4.91134
Quantal-linear	231.042	0.1317	1.698	7.23729	4.91134
Dichotomous hill	231.705	0.1112	-0.1356	2.63097	1.72618

[a] model choice based on lowest AIC



08:09 02/03 2010

Figure B-2. Log-logistic model fit for alveolar epitheliala hyperplasia in male F344/N rats (BMR = 5% extra risk)

```
 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnl_rat_m_alv_hyper_Lnl-BMR05-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnl_rat_m_alv_hyper_Lnl-BMR05-Restrict.plt
 Wed Feb 03 08:09:27 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   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 =          0.1
                      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        Std. 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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.1310         6.549     5.000          50       -0.649
   12.8000     0.2272        11.360    16.000          50        1.566
   32.0000     0.3373        16.526    14.000          49       -0.763
   80.0000     0.5113        25.564    25.000          50       -0.160

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


Benchmark Dose Computation
Specified effect =          0.05
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =       5.41078
            BMDL =       3.34864
```

Table B-4. Benchmark modeling results for Alveolar epithelial hyperplasia in female F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	245.78	0.0612	1.998	8.0322	5.89582
Logistic	248.949	0.0163	1.99	14.8564	11.9857
Log-logistic[a]	**243.677**	**0.1779**	**-0.453**	**4.90719**	**3.27097**
Log-probit	249.954	0.0079	2.446	15.342	10.7468
Multistage	245.78	0.0612	1.998	8.03223	5.89582
Probit	248.806	0.0171	2.006	14.4844	11.8082
Weibull	245.78	0.0612	1.998	8.03223	5.89582
Quantal-linear	245.78	0.0612	1.998	8.03223	5.89582
Dichotomous hill	244.808	0.113	-0.1096	3.08661	2.02512

[a] model choice based on lowest AIC

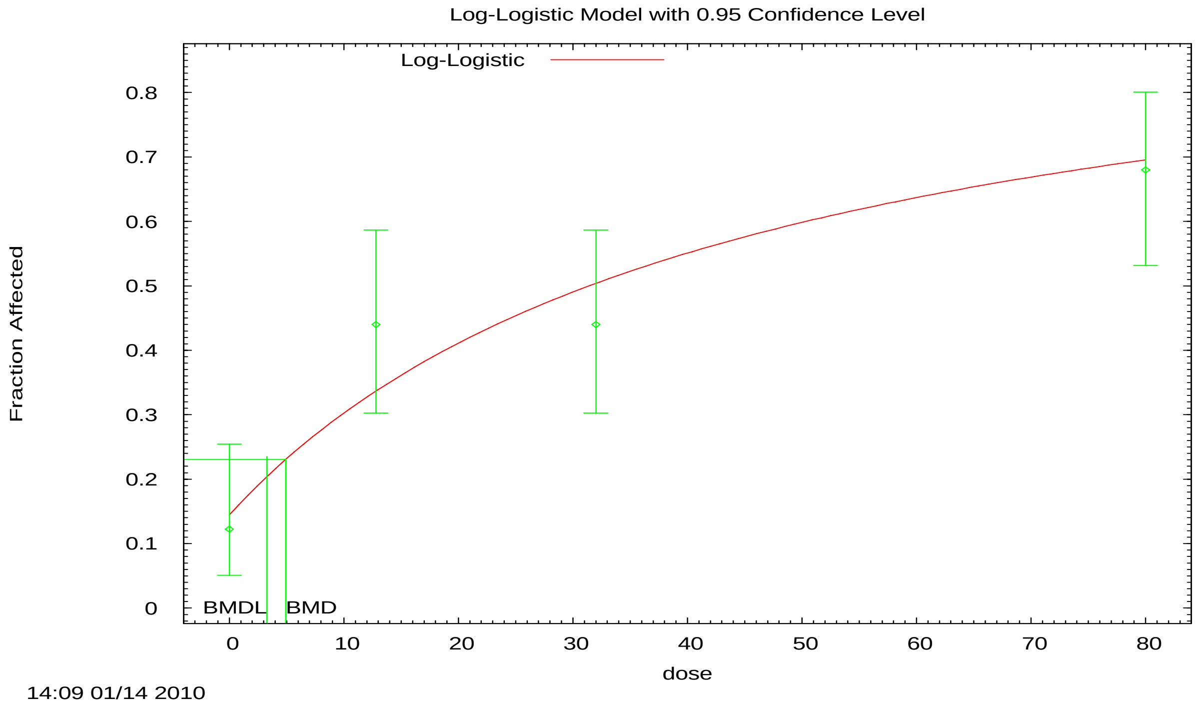


Figure B-3. Log-logistic model fit for alveolar epithelial hyperplasia in female F344/N rats (BMR = 10% extra risk)

```
 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnl_rat_f_alv_hyper_Lnl-BMR10-
 Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnl_rat_f_alv_hyper_Lnl-BMR10-
 Restrict.plt
 Thu Jan 14 14:09:02 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   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
 intercept        -0.62            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.1453         7.117     6.000          49       -0.453
   12.8000     0.3373        16.866    22.000          50        1.536
   32.0000     0.5044        25.218    22.000          50       -0.910
   80.0000     0.6960        34.799    34.000          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-5. Benchmark modeling results for bronchiolar hyperplasia in male B6C3F1 mice (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	192.219	0.1003	1.561	9.962	7.95025
Logistic	206.147	0.0019	2.136	25.582	20.9208
Log-logistic[a]	**188.645**	**0.5085**	**0.8**	**7.54241**	**5.60381**
Log-probit	203.779	0.0009	2.278	18.0076	12.7086
Multistage	192.219	0.1003	1.561	9.962	7.95025
Probit	205.312	0.0023	2.094	23.8731	19.6205
Weibull	192.219	0.1003	1.561	9.962	7.95025
Quantal-linear	192.219	0.1003	1.561	9.962	7.95025
Dichotomous hill	190.376	1	-3.22E-06	6.4695	4.24464

[a] model choice based on lowest AIC

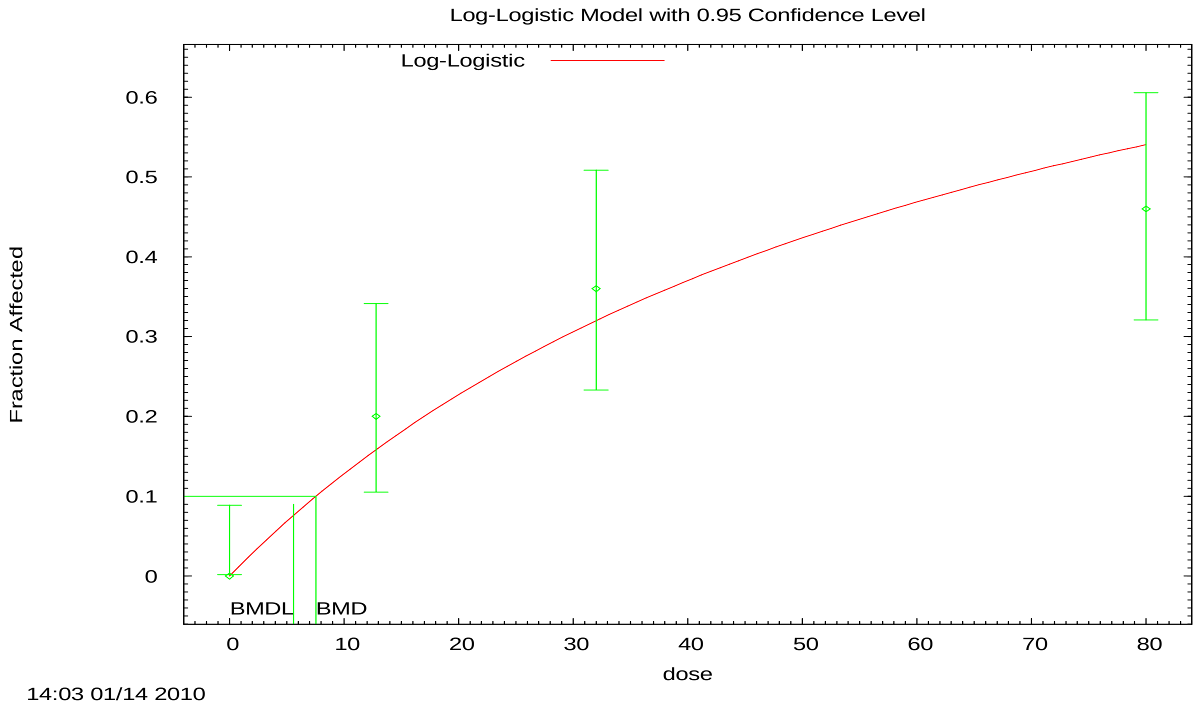


Figure B-4. Log-logistic model fit for bronchiolar hyperplasia in male B6C3F1 mice (BMR = 10% extra risk)

```
 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnl_mouse_m_bronch_hyper_Lnl-BMR10-
 Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnl_mouse_m_bronch_hyper_Lnl-
 BMR10-Restrict.plt
 Thu Jan 14 14:03:40 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   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
                      intercept =     -4.24694
                          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         -4.21777            *                *                   *
          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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          50        0.000
   12.8000     0.1586         7.932    10.000          50        0.800
   32.0000     0.3204        16.019    18.000          50        0.600
   80.0000     0.5410        27.049    23.000          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-6. Benchmark modeling results for bronchiolar hyperplasia in male B6C3F1 mice (BMR = 5% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	192.219	0.1003	0	4.84986	3.87047
Logistic	206.147	0.0019	0.897	14.2582	11.4862
Log-logistic[a]	**188.645**	**0.5085**	**0**	**3.57272**	**2.65444**
Log-probit	203.779	0.0009	2.278	12.522	8.83728
Multistage	192.219	0.1003	0	4.84986	3.87047
Probit	205.312	0.0023	0.992	13.136	10.6641
Weibull	192.219	0.1003	0	4.84986	3.87047
Quantal-linear	190.376	1	0	3.6667	0.932026
Dichotomous hill	192.219	0.1003	0	4.84986	3.87047

[a] model choice based on lowest AIC

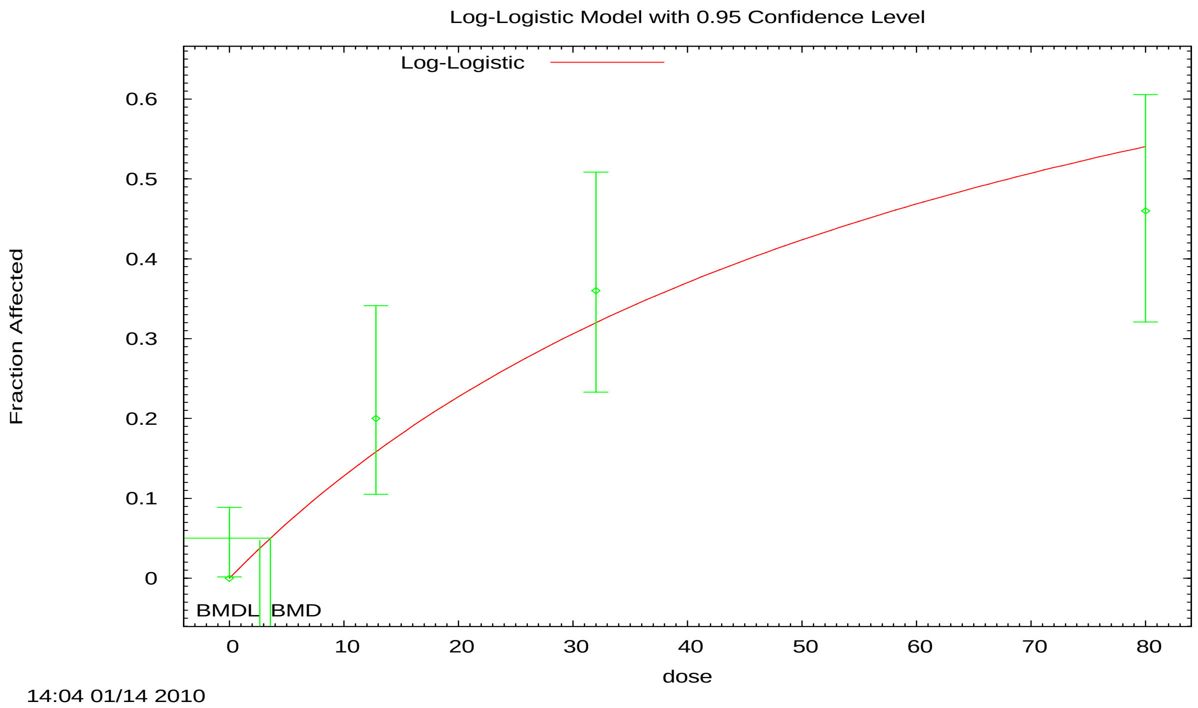


Figure B-5. Log-logistic model fit for bronchiolar hyperplasia in male B6C3F1 mice (BMR = 5% extra risk)

```

 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnl_mouse_m_bronch_hyper_Lnl-BMR05-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnl_mouse_m_bronch_hyper_Lnl-BMR05-
 Restrict.plt
 Thu Jan 14 14:04:34 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   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
                      intercept =     -4.24694
                          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         -4.21777            *                *                   *
          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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          50        0.000
   12.8000     0.1586         7.932    10.000          50        0.800
   32.0000     0.3204        16.019    18.000          50        0.600
   80.0000     0.5410        27.049    23.000          50       -1.149

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


Benchmark Dose Computation
Specified effect =           0.05
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        3.57272
            BMDL =        2.65444
```

Table B-7. Benchmark modeling results for chronic inflammatioin in male F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	81.4586	0.8964	0.39	15.2489	10.1164
Logistic	87.0594	0.0925	-0.291	23.8087	18.9473
Log-logistic[a]	**81.3682**	**0.9398**	**0.286**	**14.6428**	**9.27776**
Log-probit	83.9766	0.2144	1.458	17.7991	13.7362
Multistage	81.4586	0.8964	0.39	15.2489	10.1164
Probit	86.6596	0.1067	-0.345	22.6768	17.7855
Weibull	81.4586	0.8964	0.39	15.2489	10.1164
Quantal-linear	81.4586	0.8964	0.39	15.2489	10.1164
Dichotomous hill					

[a] model choice based on lowest AIC

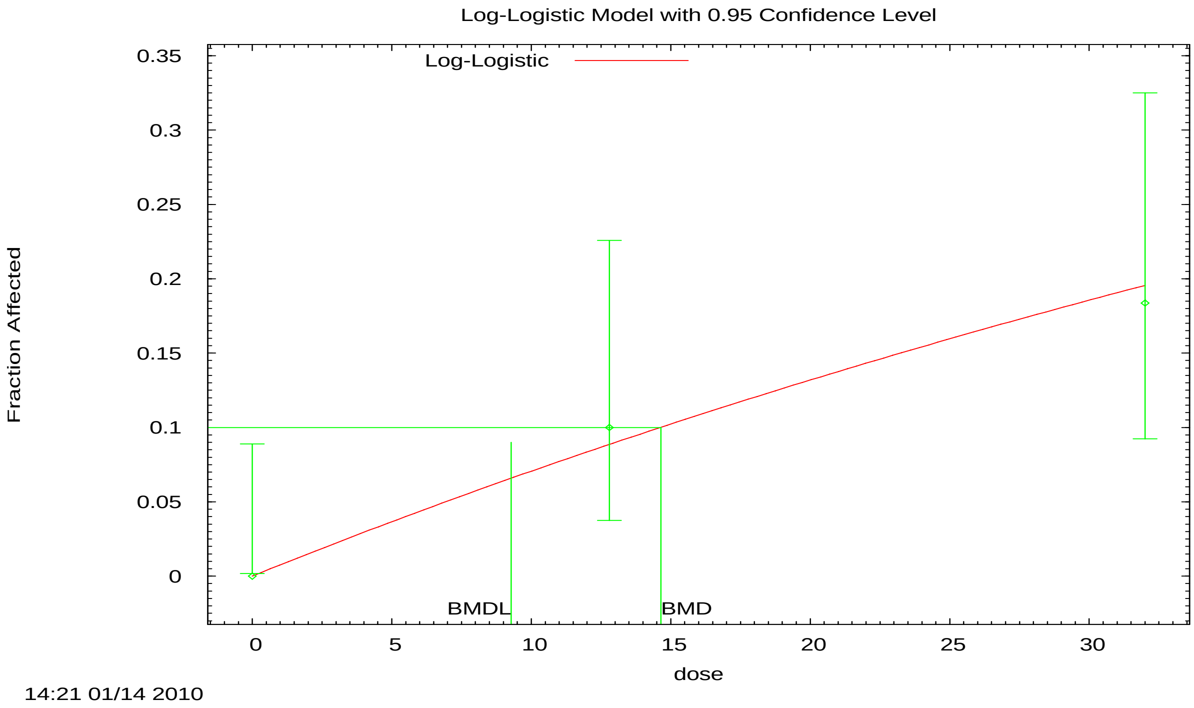


Figure B-6. Log-logistic model fit for olfactory chronic inflammation in male F344/N rats (BMR = 10% extra risk)

```
 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnl_rat_m_inflammation_hdd_Lnl-BMR10-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnl_rat_m_inflammation_hdd_Lnl-BMR10-
 Restrict.plt
 Thu Jan 14 14:21:54 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   Independent variable = Dose
   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 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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       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-8. Benchmark modeling results for olfactory atrophy in male F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	106.376	NA	0	10.6003	7.99938
Logistic[a]	**105.53**	**0.2655**	**-0.597**	**7.70048**	**5.97454**
Log-logistic	106.376	NA	0	10.81	8.62799
Log-probit	106.376	NA	0	10.9386	8.79455
Multistage	107.65	0.0817	-1.408	6.95763	5.20262
Probit	106.283	0.1555	-0.901	6.91725	5.40111
Weibull	106.376	NA	0	9.95012	7.06875
Quantal-linear	125.166	0	0.459	2.28431	1.80011
Dichotomous hill			0		

[a] model choice based on lowest AIC

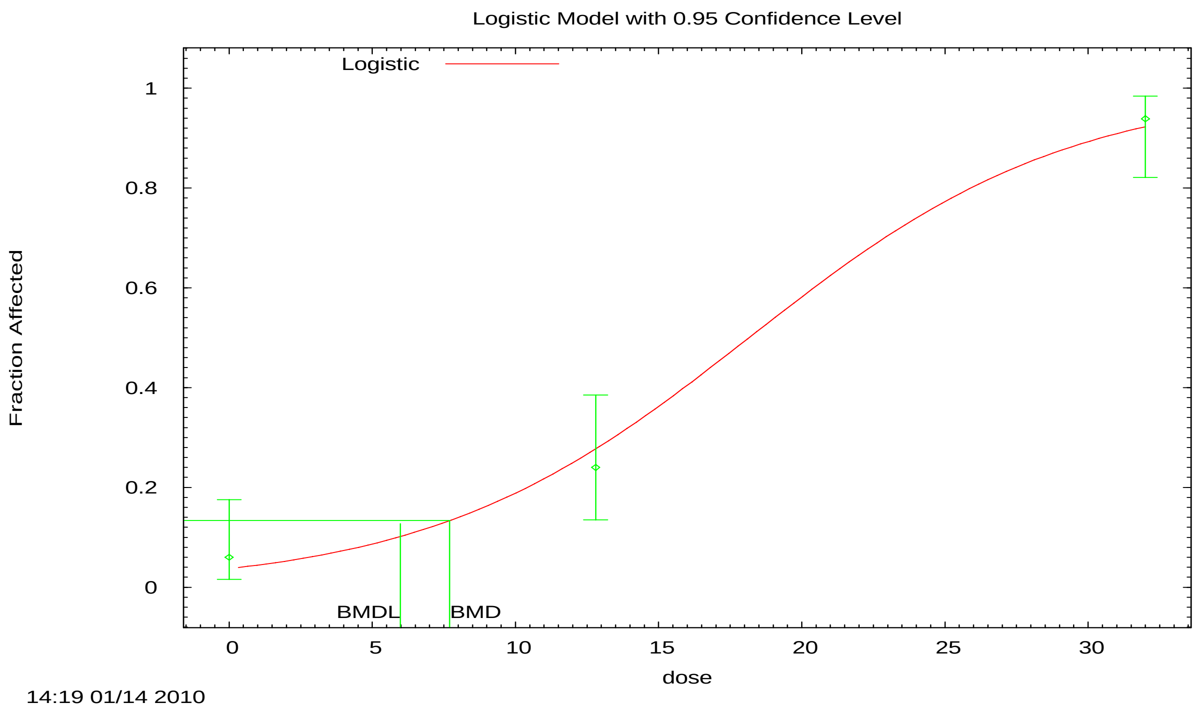


Figure B-7. Logistic model fit for olfactory atrophy in male F344/N rats (BMR = 10% extra risk)

```
====================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File: M:\Chloroprene\NTP_BMDS\log_rat_m_atrophy_hdd_Log-BMR10.(d)
Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\log_rat_m_atrophy_hdd_Log-BMR10.plt
Thu Jan 14 14:19:23 2010
====================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

  P[response] = 1/[1+EXP(-intercept-slope*dose)]

  Dependent variable = Effect
  Independent variable = Dose
  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
                                                                Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
 ------------------------------------------------------------------------
   0.0000     0.0373         1.865     3.000          50        0.847
  12.8000     0.2778        13.892    12.000          50       -0.597
  32.0000     0.9233        45.243    46.000          49        0.406

Chi^2 = 1.24      d.f. = 1        P-value = 0.2655

Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        7.70048
            BMDL =        5.97454
```

1

Table B-9. Benchmark modeling results for olfactory atrophy in male F344/N rats (BMR = 5% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	106.376	NA	0	8.88228	6.24061
Logistic[a]	**105.53**	**0.2655**	**0.847**	**4.90734**	**3.52532**
Log-logistic	106.376	NA	0	9.14915	6.92292
Log-probit	106.376	NA	0	9.51381	7.35396
Multistage	107.65	0.0817	0.377	4.85459	3.12143
Probit	106.283	0.1555	0.977	4.28231	3.10069
Weibull	106.376	NA	0	7.68497	5.01204
Quantal-linear	125.166	0	0.459	1.11208	0.87636
Dichotomous hill			0		

[a] model choice based on lowest AIC

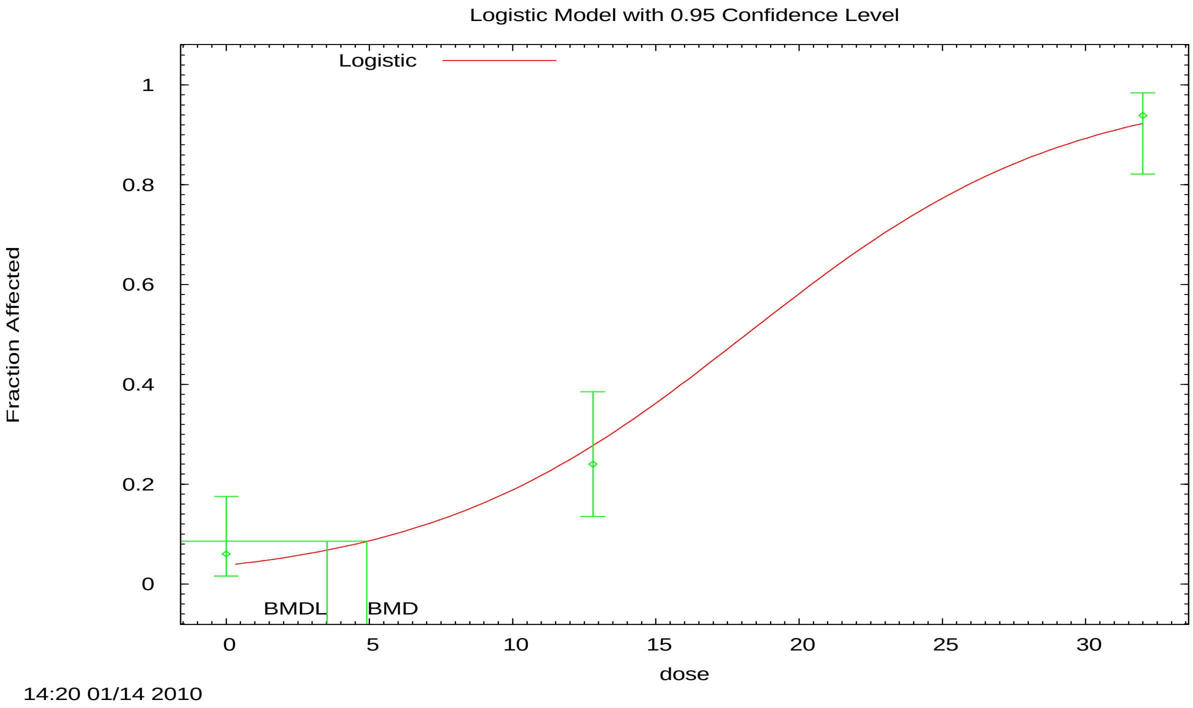


Figure B-8. Logistic model fit for olfactory atrophy in male F344/N rats (BMR = 5% extra risk)

```
 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\log_rat_m_atrophy_hdd_Log-BMR05.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\log_rat_m_atrophy_hdd_Log-BMR05.plt
 Thu Jan 14 14:20:15 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]

   Dependent variable = Effect
   Independent variable = Dose
   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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0373         1.865     3.000          50        0.847
   12.8000     0.2778        13.892    12.000          50       -0.597
   32.0000     0.9233        45.243    46.000          49        0.406

 Chi^2 = 1.24      d.f. = 1        P-value = 0.2655


Benchmark Dose Computation
Specified effect =          0.05
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        4.90734
            BMDL =        3.52532
```

Table B-10. Benchmark modeling results for olfactory necrosis in male F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	124.435	1	0	6.46561	3.70666
Logistic	130.942	0.0328	1.45	12.1684	9.77545
Log-logistic	124.435	1	0	6.92124	2.96263
Log-probit[a]	**122.499**	**0.9686**	**0.188**	**7.98173**	**6.41755**
Multistage	124.435	1	0	5.8893	3.70666
Probit	129.762	0.0494	1.387	11.3581	9.13936
Weibull	124.435	1	0	6.31726	3.70666
Quantal-linear	122.737	0.8622	0	4.75407	3.65317
Dichotomous hill			0		

[a] model choice based on lowest AIC

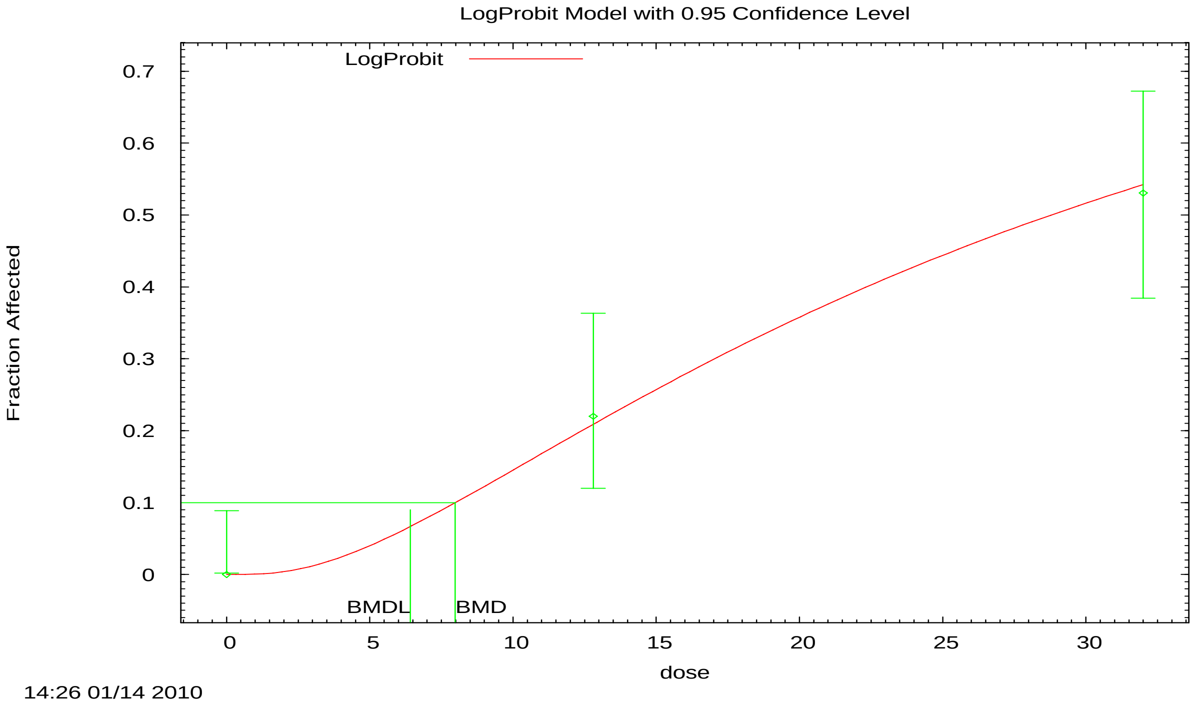


Figure B-9. Log-probit model fit for olfactory necrosis in male F344/N rats (BMR = 10% extra risk)

```

 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnp_rat_m_necrosis_hdd_Lnp-BMR10.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnp_rat_m_necrosis_hdd_Lnp-BMR10.plt
 Thu Jan 14 14:26:59 2010
 ====================================================================

 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 = Effect
   Independent variable = Dose
   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
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       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-11. Benchmark modeling results for olfactory necrosis in male F344/N rats (BMR = 5% extra risk)

Model	**AIC**	**Goodness-of-fit p-value**	**χ^2 residual**	**BMD**	**BMDL**
Gamma	124.435	1	0	3.68522	1.80454
Logistic	130.942	0.0328	1.45	7.60632	5.7094
Log-logistic	124.435	1	0	4.22667	1.40335
Log-probit[a]	**122.499**	**0.9686**	**0**	**5.55031**	**4.46261**
Multistage	124.435	1	0	2.97375	1.80454
Probit	129.762	0.0494	1.387	7.0625	5.28703
Weibull	124.435	1	0	3.49306	1.80454
Quantal-linear	122.737	0.8622	0	2.31445	1.7785
Dichotomous hill			0		

[a] model choice based on lowest AIC

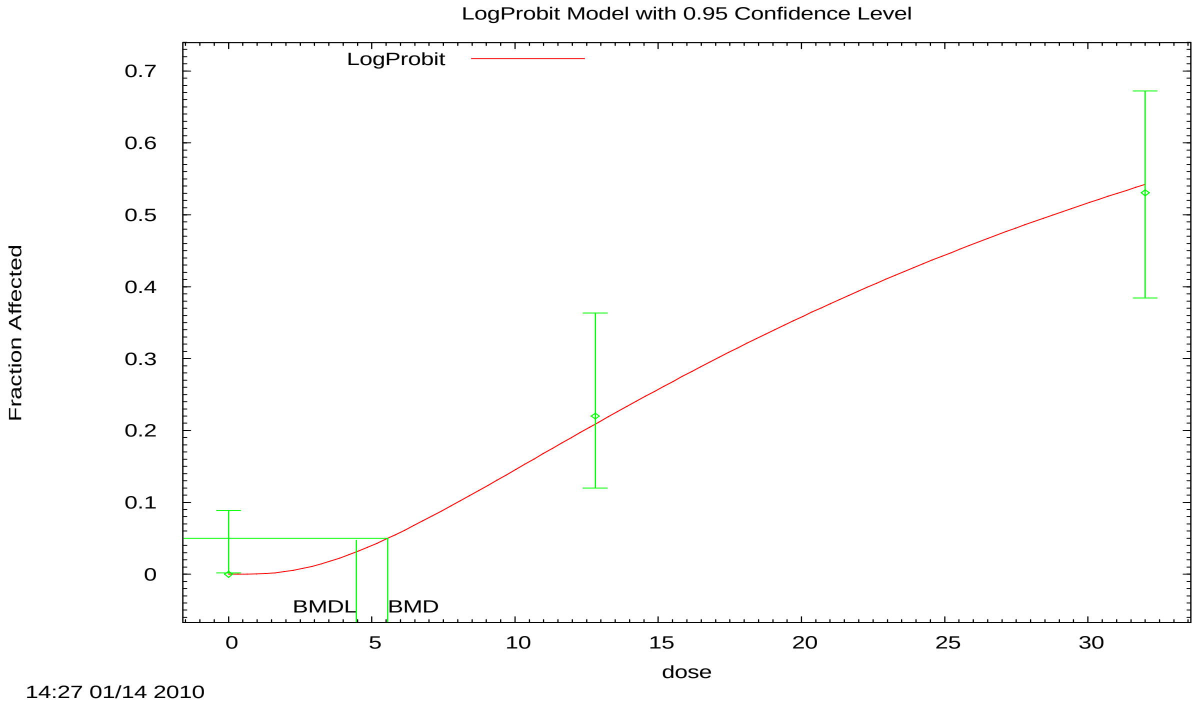


Figure B-10. Log-probit model fit for olfactory necrosis in male F344/N rats (BMR = 5% extra risk)

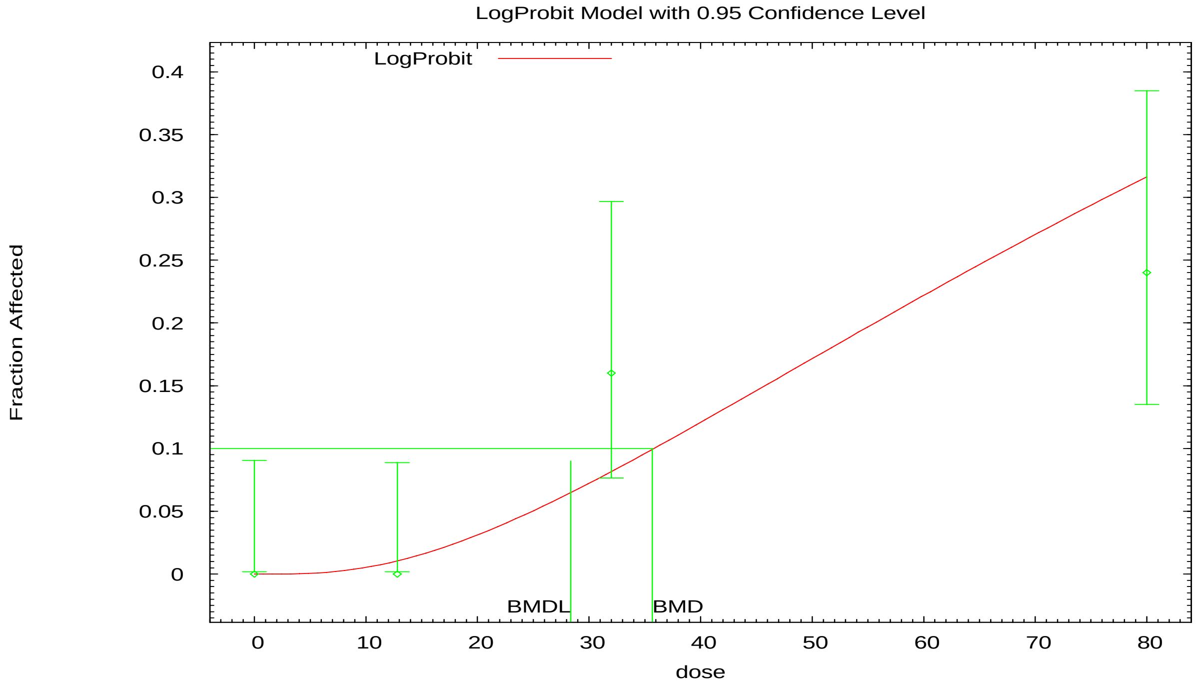
```
 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnp_rat_m_necrosis_hdd_Lnp-BMR05.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnp_rat_m_necrosis_hdd_Lnp-BMR05.plt
 Thu Jan 14 14:27:40 2010
 ====================================================================

 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 = Effect
   Independent variable = Dose
   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
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       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 =           0.05
Risk Type        =      Extra risk
Confidence level =           0.95
```

```
             BMD =          5.55031
            BMDL =          4.46261
```

Table B-12. Benchmark modeling results for olfactory necrosis in female F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	108.455	0.1223	1.54	33.1378	21.4781
Logistic	114.403	0.0069	2.564	50.8598	41.8856
Log-logistic	108.312	0.1357	1.469	32.4911	20.1388
Log-probit[a]	**106.815**	**0.115**	**2**	**35.6629**	**28.3477**
Multistage	108.87	0.1361	1.339	31.2054	20.9166
Probit	113.454	0.0095	2.504	47.668	38.935
Weibull	108.549	0.1241	1.509	32.8886	21.3452
Quantal-linear	106.909	0.2879	1.188	29.5366	20.8661
Dichotomous hill	103.075	1	1.13E-05	30.221	27.5059

[a] Dichotomous hill model has lowest AIC value, but 2 of its parameters were estimated at their respective bounds and the resulting model fit was highly suspect upon visual inspection. The model output warned that the BMDL calculation was "at best imprecise for these data". Therefore, the model with the next lowest AIC (i.e., the log-probit) model was selected.

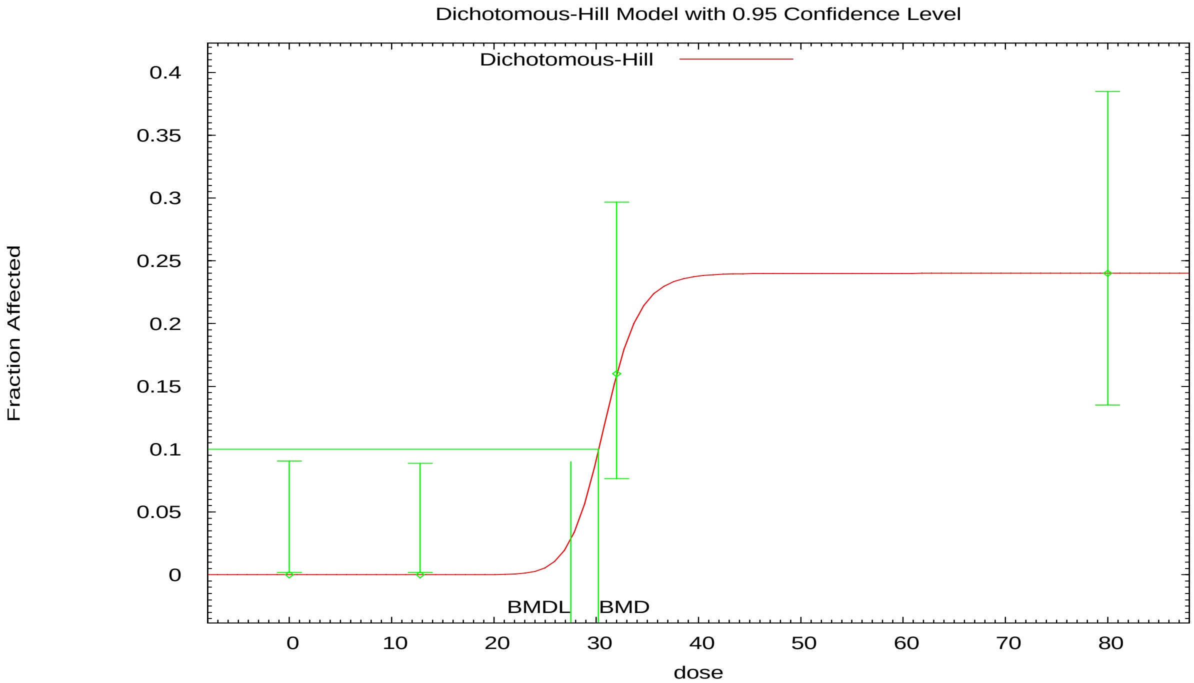


14:16 01/14 2010

Figure B-11. Log-probit model fit for olfactory necrosis in female F344/N rats (BMR = 10% extra risk)

```
 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnp_rat_f_necrosis_Lnp-BMR10.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnp_rat_f_necrosis_Lnp-BMR10.plt
 Thu Jan 14 14:16:20 2010
 ====================================================================

 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 = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
   User has chosen the log transformed model

                  Default Initial (and Specified) Parameter Values
                     background =            0
                      intercept =     -4.83555
                          slope =            1

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

              intercept

 intercept            1

                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background                0               NA
     intercept         -4.85566         0.142346            -5.13466            -4.57667
         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        -49.5375         4
  Fitted model        -52.4076         1      5.74025      3           0.125
 Reduced model         -64.911         1      30.7469      3         <.0001
          AIC:         106.815

                                 Goodness  of  Fit
                                                                Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          49         0.000
   12.8000     0.0105         0.527     0.000          50        -0.730
   32.0000     0.0823         4.114     8.000          50         2.000
   80.0000     0.3179        15.894    12.000          50        -1.183

 Chi^2 = 5.93      d.f. = 3        P-value = 0.1150

Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
```

```
                BMD =          35.6629
               BMDL =          28.3477
```



14:16 01/14 2010

Figure B-12. Dichotomous hill model fit for olfactory necrosis in female F344/N rats (BMR = 10% extra risk)

```
====================================================================
 Dichotomous Hill Model. (Version: 1.0; Date: 09/24/2006)
 Input Data File: M:\Chloroprene\NTP_BMDS\dhl_rat_f_necrosis_Dhl-BMR10-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\dhl_rat_f_necrosis_Dhl-BMR10-Restrict.plt
 Thu Jan 14 14:16:22 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

        where: 0 <= g < 1, 0 < v <= 1

               v is the maximum probability of response predicted by the model,
               and v*g is the background estimate of that probability.


   Dependent variable = Effect
   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


                  Default Initial Parameter Values
```

```
                              v =        -9999
                              g =        -9999
                      intercept =     -9.02343
                          slope =      1.88938


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

                      v    intercept

         v            1        -0.61
 intercept        -0.61            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
              v             0.24        0.0603988            0.121621             0.35838
              g                0               NA
      intercept         -61.6901          1.23084            -64.1025            -59.2777
          slope               18               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)  Deviance  Test d.f.   P-value
     Full model        -49.5375
   Fitted model        -49.5375  3.29863e-006      2               1
  Reduced model         -64.911       30.7469      3          <.0001

           AIC:         103.075

                     Goodness  of  Fit
                                                                Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000          0.000         0          49            0
   12.8000     0.0000          0.000         0          50    -0.001284
   32.0000     0.1600          8.000         8          50   1.131e-005
   80.0000     0.2400         12.000        12          50  -2.788e-006

 Chi^2 = 0.000002     d.f. = 2        P-value = 1.0000


Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        30.221
           Warning: BMDL computation is at best imprecise for these data
            BMDL =       27.5059
```

Table B-13. Benchmark modeling results for olfactory necrosis in female F344/N rats (BMR = 5% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	108.455	0.1223	-1.256	18.8703	10.4563
Logistic	114.403	0.0069	2.564	34.1134	26.6532
Log-logistic	108.312	0.1357	-1.263	18.6016	9.53944
Log-probit[a]	**106.815**	**0.115**	**2**	**24.7991**	**19.7123**
Multistage	108.87	0.1361	-1.458	15.6751	10.1829
Probit	113.454	0.0095	2.504	31.4159	24.4034
Weibull	108.549	0.1241	-1.299	18.2634	10.3916
Quantal-linear	106.909	0.2879	-1.528	14.3795	10.1584
Dichotomous hill	103.075	1	1.13E-05	28.5901	26.0761

[a] Dichotomous hill model has lowest AIC value, but 2 of its parameters were estimated at their respective bounds and the resulting model fit was highly suspect upon visual inspection. The model output warned that the BMDL calculation was “at best imprecise for these data”. Therefore, the model with the next lowest AIC (i.e., the log-probit) model was selected.

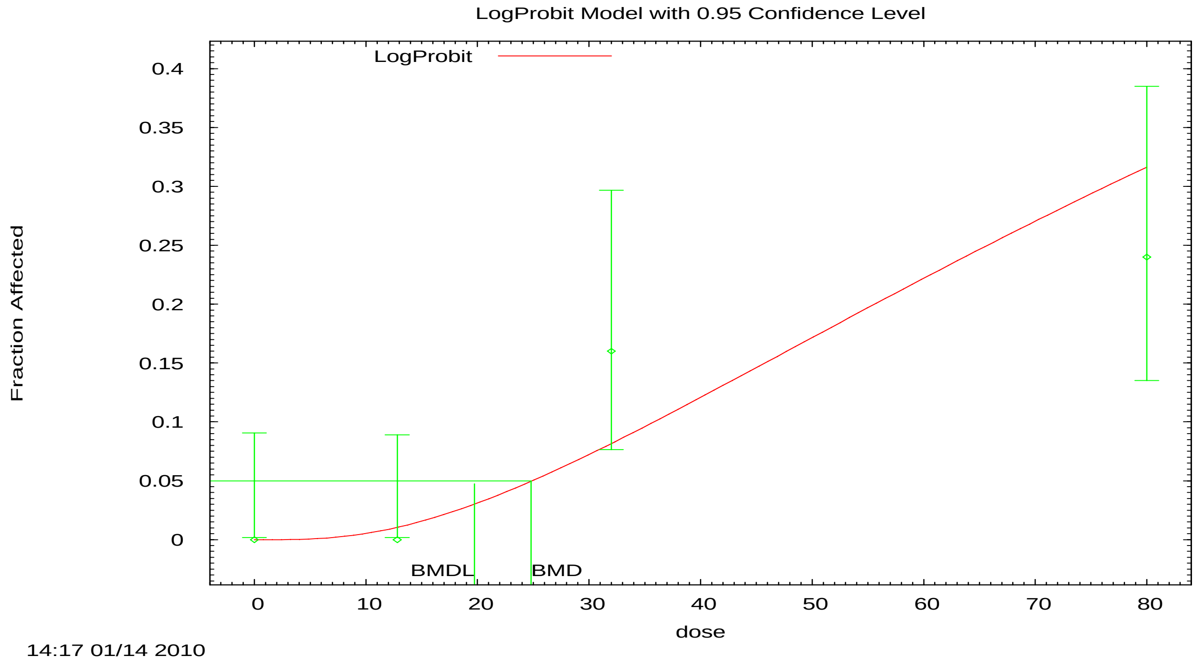


Figure B-13. Log-probit model fit for olfactory necrosis in female F344/N rats (BMR = 5% extra risk)

```
 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnp_rat_f_necrosis_Lnp-BMR05.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnp_rat_f_necrosis_Lnp-BMR05.plt
 Thu Jan 14 14:17:01 2010
 ====================================================================

 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 = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
   User has chosen the log transformed model

                  Default Initial (and Specified) Parameter Values
                     background =            0
                      intercept =     -4.83555
                          slope =            1

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

              intercept

 intercept           1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background                0              NA
      intercept         -4.85566         0.142346            -5.13466            -4.57667
          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        -49.5375         4
   Fitted model        -52.4076         1      5.74025      3           0.125
  Reduced model         -64.911         1      30.7469      3         <.0001
           AIC:         106.815

                                  Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          49        0.000
   12.8000     0.0105         0.527     0.000          50       -0.730
   32.0000     0.0823         4.114     8.000          50        2.000
   80.0000     0.3179        15.894    12.000          50       -1.183

 Chi^2 = 5.93      d.f. = 3        P-value = 0.1150

Benchmark Dose Computation
Specified effect =          0.05
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        24.7991
```

```
BMDL =        19.7123
```

Table B-14. Benchmark modeling results for olfactory basal cell hyperplasia in female F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	78.8231	0.7502	0.324	22.6607	18.6776
Logistic	83.7749	0.0615	0.81	22.7096	18.8101
Log-logistic	78.3698	0.8754	0.12	24.0671	20.2672
Log-probit[a]	**78.083**	**0.9521**	**0.093**	**23.4933**	**19.7198**
Multistage	85.0835	0.155	-1.956	15.3009	13.2469
Probit	83.6185	0.1092	-1.283	22.0007	17.8681
Weibull	81.3562	0.3487	-1.19	20.7516	16.5638
Quantal-linear	120.402	0	0	5.59788	4.52837
Dichotomous hill	77.9075	1	5.70E-07	29.3724	23.7917

[a] Dichotomous hill model has lowest AIC value, but 2 of its parameters were estimated at their respective bounds and the resulting model fit was highly suspect upon visual inspection. The model output warned that the BMDL calculation was “at best imprecise for these data”. Therefore, the model with the next lowest AIC (i.e., the log-probit) model was selected.

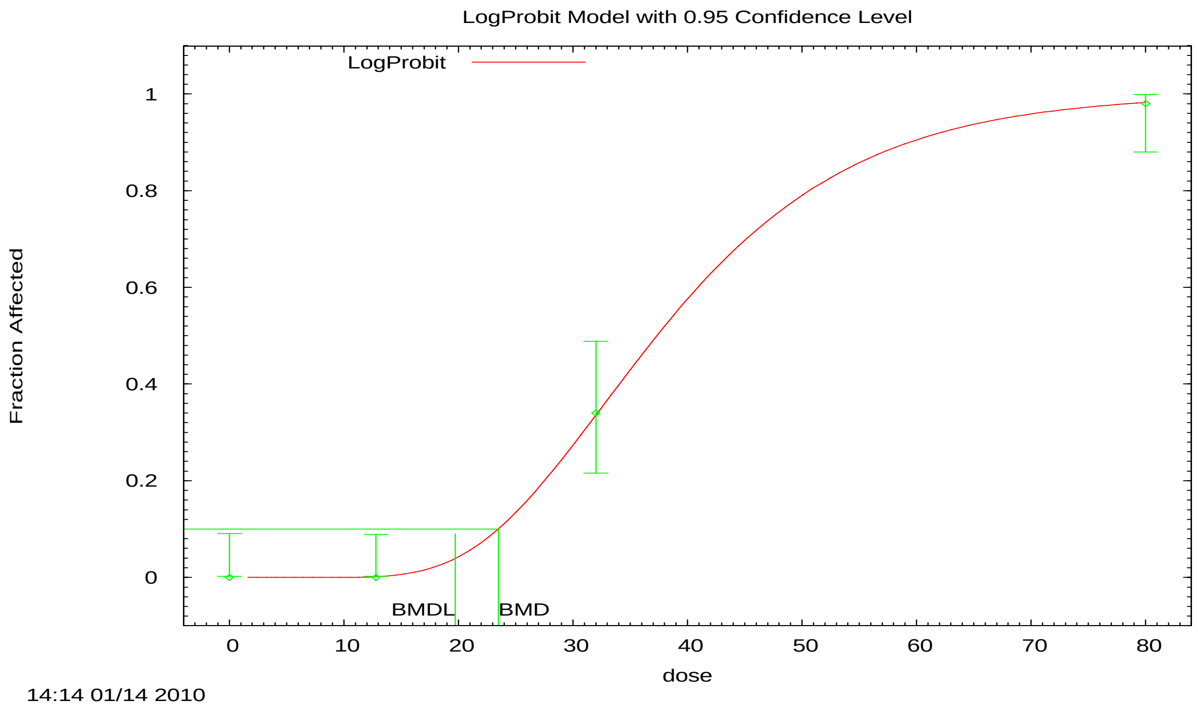


Figure B-14. Log-probit model fit for olfactory basal cell hyperplasia in female F344/N rats (BMR = 10% extra risk)

```
 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnp_rat_f_basal_hyper_Lnp-BMR10.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnp_rat_f_basal_hyper_Lnp-BMR10.plt
 Thu Jan 14 14:14:05 2010
 ====================================================================
 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 = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
   User has chosen the log transformed model

                  Default Initial (and Specified) Parameter Values
                     background =            0
                      intercept =      -8.5284
                          slope =      2.39417

           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        -0.99
     slope        -0.99            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background                0               NA
      intercept          -9.9865          1.64723            -13.215              -6.758
          slope           2.7576         0.456613            1.86265             3.65254

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        -36.9537         4
   Fitted model        -37.0415         2      0.175584      2           0.916
  Reduced model        -126.434         1       178.961      3         <.0001
           AIC:          78.083

                                  Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          49        0.000
   12.8000     0.0016         0.078     0.000          50       -0.279
   32.0000     0.3338        16.691    17.000          50        0.093
   80.0000     0.9820        49.101    49.000          50       -0.107

 Chi^2 = 0.10      d.f. = 2        P-value = 0.9521

Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
```

```
             BMD =         23.4933
            BMDL =         19.7198
```

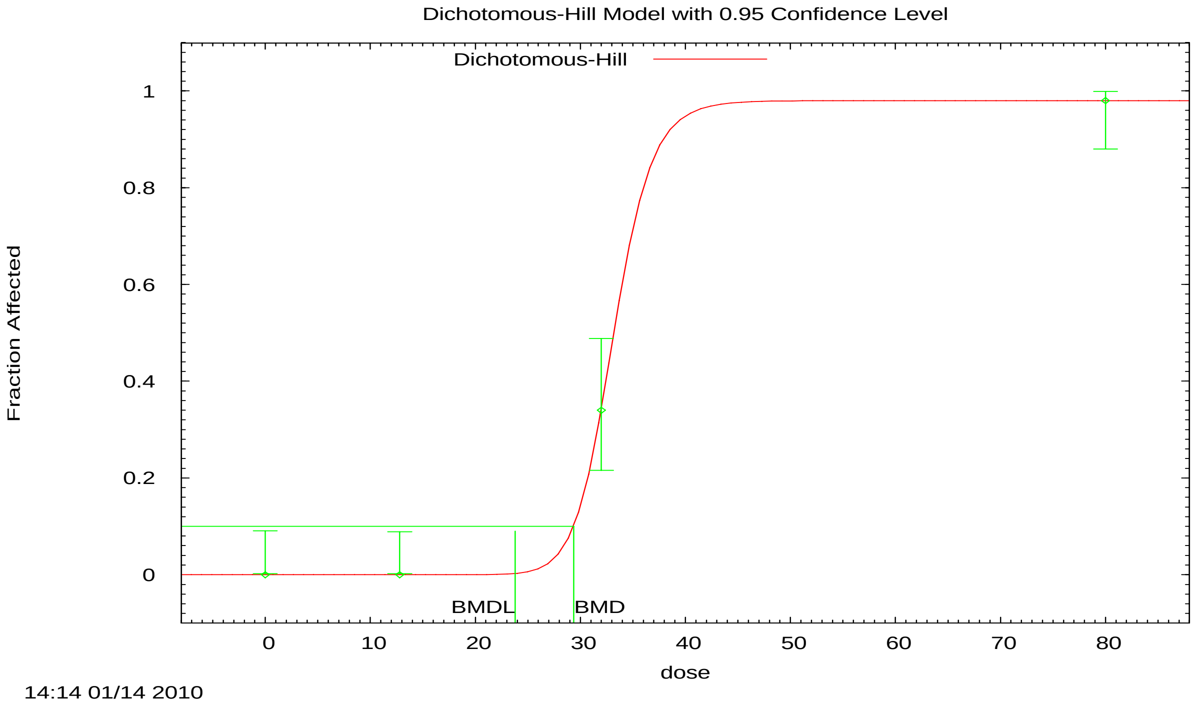


Figure B-15. Dichotomous model fit for olfactory basal cell hyperplasia in female F344/N rats (BMR = 10% extra risk)

```
 ====================================================================
 Dichotomous Hill Model. (Version: 1.0; Date: 09/24/2006)
 Input Data File: M:\Chloroprene\NTP_BMDS\dhl_rat_f_basal_hyper_Dhl-BMR10-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\dhl_rat_f_basal_hyper_Dhl-BMR10-
 Restrict.plt
 Thu Jan 14 14:14:07 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

        where: 0 <= g < 1, 0 < v <= 1

               v is the maximum probability of response predicted by the model,
               and v*g is the background estimate of that probability.

   Dependent variable = Effect
   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

                  Default Initial Parameter Values
                              v =        -9999
```

```
                    g =        -9999
            intercept =     -16.5503
                slope =      4.64205


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

                      v    intercept

         v            1         -0.1
 intercept         -0.1            1

                                 Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
              v             0.98         0.019799            0.941195             1.01881
              g                0               NA
      intercept         -63.0158         0.303295            -63.6102            -62.4213
          slope               18               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)  Deviance  Test d.f.   P-value
     Full model        -36.9537
   Fitted model        -36.9537  3.57771e-006      2            1
  Reduced model        -126.434       178.961      3        <.0001
           AIC:         77.9075

                     Goodness  of  Fit

                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000          0          49           0
   12.8000     0.0000         0.000          0          50   -0.001337
   32.0000     0.3400        17.000         17          50   5.704e-007
   80.0000     0.9800        49.000         49          50  -1.192e-007

 Chi^2 = 0.000002     d.f. = 2        P-value = 1.0000

Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =       29.3724
           Warning: BMDL computation is at best imprecise for these data
            BMDL =       23.7917
```

Table B-15. Benchmark modeling results for olfactory basal cell hyperplasia in female F344/N rats (BMR = 5% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	78.8231	0.7502	-0.597	19.1684	15.0243
Logistic	83.7749	0.0615	-1.253	17.4935	13.1784
Log-logistic	78.3698	0.8754	-0.448	20.879	16.769
Log-probit[a]	**78.083**	**0.9521**	**-0.279**	**20.5934**	**16.7154**
Multistage	85.0835	0.155	-1.956	10.676	8.74893
Probit	83.6185	0.1092	-1.283	16.5716	12.2502
Weibull	81.3562	0.3487	-1.19	15.9699	12.0527
Quantal-linear	120.402	0	0	2.72525	2.20457
Dichotomous hill	77.9075	1	5.70E-07	28.1762	22.8227

[a] Dichotomous hill model has lowest AIC value, but 2 of its parameters were estimated at their respective bounds and the resulting model fit was highly suspect upon visual inspection. The model output warned that the BMDL calculation was “at best imprecise for these data”. Therefore, the model with the next lowest AIC (i.e., the log-probit) model was selected.

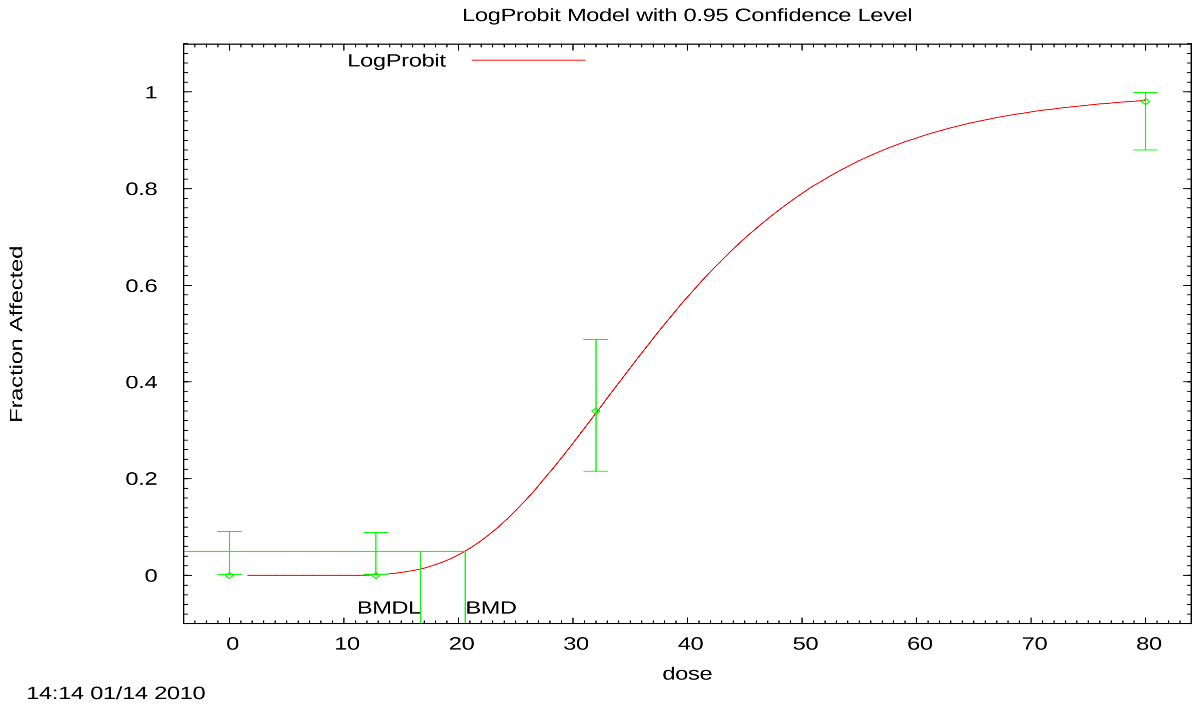


Figure B-16. Log-probit model fit for olfactory basal cell hyperplasia in female F344/N rats (BMR = 5% extra risk)

```
 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnp_rat_f_basal_hyper_Lnp-BMR05.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnp_rat_f_basal_hyper_Lnp-BMR05.plt
 Thu Jan 14 14:14:47 2010
 ====================================================================

 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 = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
   User has chosen the log transformed model

                  Default Initial (and Specified) Parameter Values
                     background =            0
                      intercept =      -8.5284
                          slope =      2.39417

           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        -0.99
     slope        -0.99            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background                0               NA
      intercept          -9.9865          1.64723             -13.215              -6.758
          slope           2.7576         0.456613             1.86265             3.65254

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        -36.9537         4
   Fitted model        -37.0415         2      0.175584      2           0.916
  Reduced model        -126.434         1       178.961      3         <.0001
           AIC:          78.083

                                  Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          49        0.000
   12.8000     0.0016         0.078     0.000          50       -0.279
   32.0000     0.3338        16.691    17.000          50        0.093
   80.0000     0.9820        49.101    49.000          50       -0.107

 Chi^2 = 0.10      d.f. = 2        P-value = 0.9521

   Benchmark Dose Computation
Specified effect =           0.05
Risk Type        =      Extra risk
Confidence level =           0.95
```

```
          BMD =        20.5934
         BMDL =        16.7154
```

Table B-16. Benchmark modeling results for kidney (renal tubule) hyperplasia in male F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	262.742	0.6482	0.091	9.58982	6.61749
Logistic	263.873	0.3712	0.128	14.4291	11.0906
Log-logistic[a]	**262.083**	**0.9017**	**-0.136**	**6.52869**	**3.95681**
Log-probit	264.054	0.3356	0.487	17.4209	11.9381
Multistage	262.742	0.6482	0.091	9.58986	6.61749
Probit	263.882	0.3695	0.131	14.3921	11.164
Weibull	262.742	0.6482	0.091	9.58986	6.61749
Quantal-linear	262.742	0.6482	0.091	9.58986	6.61749
Dichotomous hill	20090.6		0		

[a] model choice based on lowest BMDL

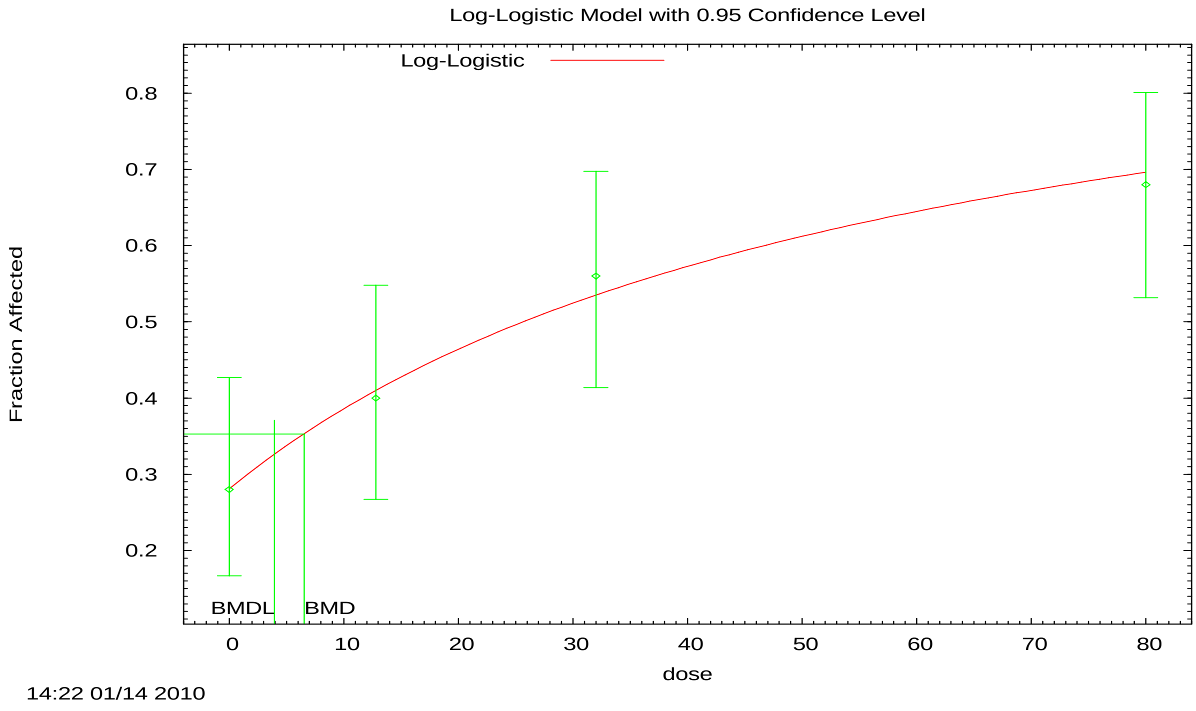


Figure B-17. Log-logistic model fit for kidney (renal tubule) hyperplasia in male F344/N rats (BMR = 10% extra risk)

```

 ====================================================================
 Logistic Model. (Version: 2.12; Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnl_rat_m_kid_hyper_Lnl-BMR10-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnl_rat_m_kid_hyper_Lnl-BMR10-Restrict.plt
 Thu Jan 14 14:22:37 2010
 ====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   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 =         0.28
                      intercept =      -4.0785
                          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        -0.65
 intercept        -0.65            1

                                 Parameter Estimates

                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.280771            *                *                   *
      intercept         -4.07343            *                *                   *
          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        -128.938         4
   Fitted model        -129.042         2      0.206766      2          0.9018
  Reduced model        -138.469         1       19.0624      3       0.0002654
           AIC:         262.083

                                  Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.2808        14.039    14.000          50       -0.012
   12.8000     0.4094        20.471    20.000          50       -0.136
   32.0000     0.5344        26.718    28.000          50        0.363
   80.0000     0.6954        34.772    34.000          50       -0.237

 Chi^2 = 0.21      d.f. = 2        P-value = 0.9017

Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        6.52869
            BMDL =        3.95681
```

Table B-17. Benchmark modeling results for kidney (renal tubule) hyperplasia in female F344/N rats (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	200.059	0.6468	0.236	31.181	14.892
Logistic	198.217	0.8346	0.29	31.33	24.9474
Log-logistic	200.048	0.656	0.211	30.79	13.2994
Log-probit[a]	**197.991**	**0.9302**	**0.269**	**32.5323**	**23.5182**
Multistage	200.173	0.5712	0.302	31.846	14.7635
Probit	198.217	0.8345	0.222	29.6902	23.4384
Weibull	200.09	0.6245	0.244	31.13	14.8568
Quantal-linear	198.787	0.6299	-0.692	21.0465	14.1492
Dichotomous hill	202.036	NA	0.1984	30.4841	12.4518

[a] model choice based on lowest AIC

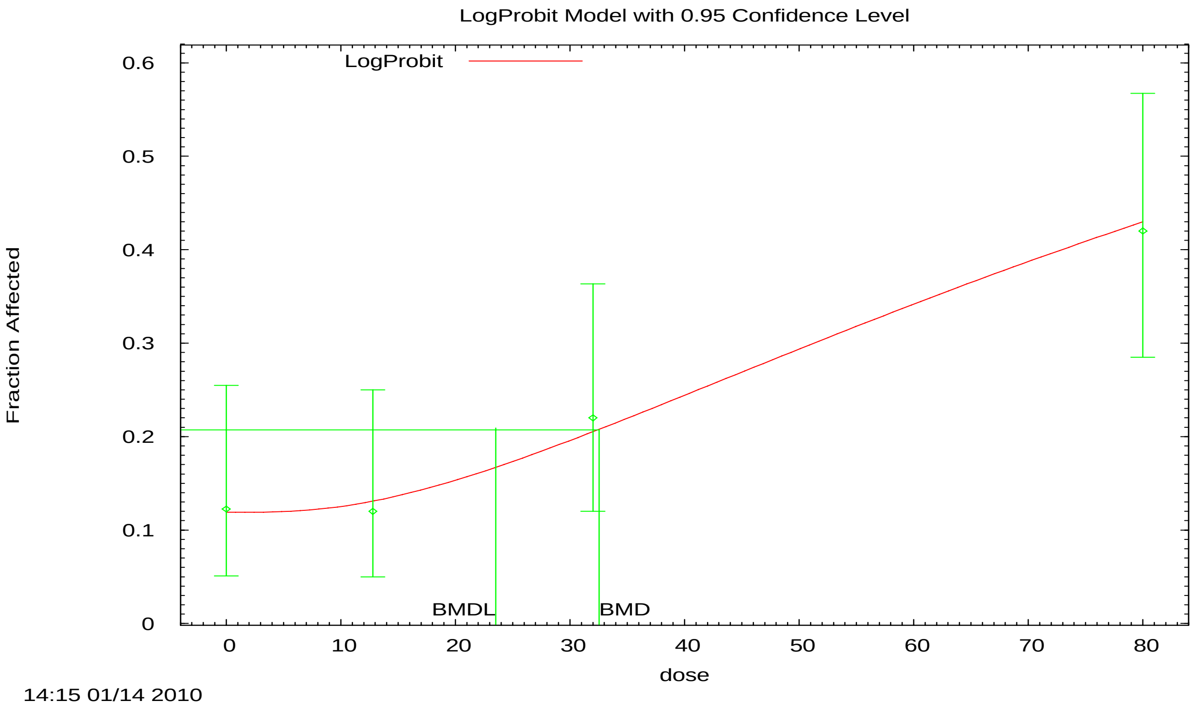


Figure B-18. Log-probit model fit for kidney (renal tubule) hyperplasia in female F344/N rats (BMR = 10% extra risk)

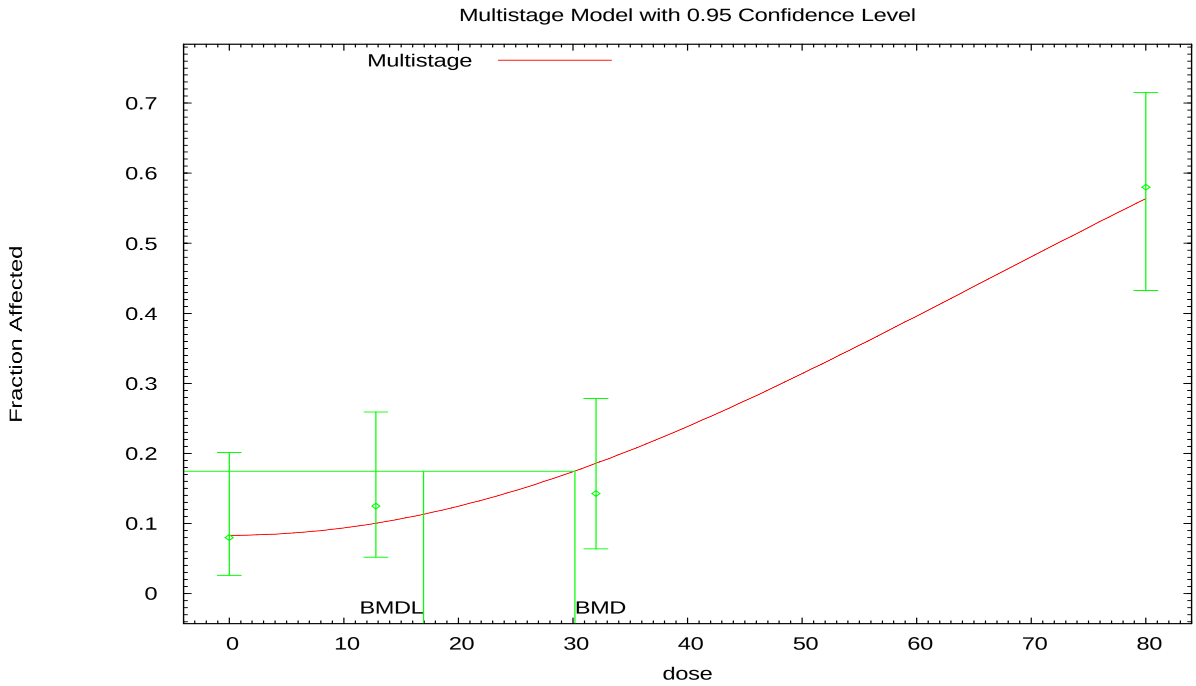
```

 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\lnp_rat_f_kid_hyper_Lnp-BMR10.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\lnp_rat_f_kid_hyper_Lnp-BMR10.plt
 Thu Jan 14 14:15:37 2010
 ====================================================================
 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 = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
   User has chosen the log transformed model

                  Default Initial (and Specified) Parameter Values
                     background =     0.122449
                      intercept =     -4.95177
                          slope =      1.04703

           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        -0.53
 intercept        -0.53            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     background         0.119059        0.0336048           0.0531949            0.184924
      intercept         -4.76379         0.218134            -5.19132            -4.33625
          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        -96.9233         4
   Fitted model        -96.9957         2      0.144742      2          0.9302
  Reduced model        -105.132         1       16.4183      3       0.0009307
           AIC:         197.991

                                  Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.1191         5.834     6.000          49        0.073
   12.8000     0.1309         6.543     6.000          50       -0.228
   32.0000     0.2046        10.231    11.000          50        0.269
   80.0000     0.4286        21.428    21.000          50       -0.122

 Chi^2 = 0.14      d.f. = 2        P-value = 0.9302

Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
```

```
            BMD =          32.5323
           BMDL =          23.5182
```

Table B-18. Benchmark modeling results for forestomach epithelial hyperplasia in male B6C3F1 mice (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	178.784	0.4716	-0.049	39.6884	20.1391
Logistic	177.328	0.5986	-0.825	26.8011	22.1839
Log-logistic	178.762	0.4806	-0.065	39.6607	21.1117
Log-probit[a]	178.805	0.464	-0.013	39.3506	22.7348
Multistage	**177.268**	**0.6124**	**-0.771**	**30.167**	**16.9463**
Probit	177.716	0.5004	-0.984	24.635	20.4757
Weibull	178.737	0.491	-0.095	39.8723	19.6367
Quantal-linear	182.602	0.0523	-0.389	13.9921	10.3765
Dichotomous hill	5853.85		0		

[a] model choice based on lowest AIC



14:05 01/14 2010

Figure B-19. Multistage model fit for forestomach epithelial hyperplasia in male B6C3F1 mice (BMR = 10% extra risk)

```
 ====================================================================
 Multistage Model. (Version: 3.0;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\mst_mouse_m_fore_hyper_Mst-BMR10-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\mst_mouse_m_fore_hyper_Mst-BMR10-
 Restrict.plt
 Thu Jan 14 14:05:47 2010
 ====================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

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

   The parameter betas are restricted to be positive
   Dependent variable = Effect
   Independent variable = Dose
   Total number of observations = 4
   Total number of records with missing values = 0
   Total number of parameters in model = 3
   Total number of specified parameters = 0
   Degree of polynomial = 2

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

                  Default Initial Parameter Values
                     Background =    0.0745999
                        Beta(1) =            0
                        Beta(2) =   0.00012236

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

             Background      Beta(2)
Background           1        -0.48
   Beta(2)        -0.48           1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0832204            *                *                   *
        Beta(1)                0            *                *                   *
        Beta(2)      0.000115775            *                *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -86.1337         4
   Fitted model        -86.6341         2       1.00079      2          0.6063
  Reduced model        -107.064         1       41.8613      3         <.0001
           AIC:         177.268

                                  Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0832         4.161     4.000          50       -0.082
   12.8000     0.1004         4.821     6.000          48        0.566
   32.0000     0.1857         9.100     7.000          49       -0.771
   80.0000     0.5630        28.151    29.000          50        0.242

 Chi^2 = 0.98      d.f. = 2        P-value = 0.6124

Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =         30.167
            BMDL =        16.9463
```

```
               BMDU =          36.6564
Taken together, (16.9463, 36.6564) is a 90 % two-sided confidenceinterval for the BMD
```

Table B-19. Benchmark modeling results for forestomach epithelial hyperplasia in female B6C3F1 mice (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	169.362	0.5864	0.147	33.02	19.9556
Logistic	167.998	0.6241	-0.02	29.3493	24.2933
Log-logistic	169.384	0.5729	0.142	32.8973	20.1355
Log-probit[a]	169.261	0.6545	0.071	32.4471	20.7798
Multistage	**167.53**	**0.7935**	**-0.013**	**30.9965**	**19.3466**
Probit	168.273	0.5353	-0.198	26.9397	22.3632
Weibull	169.457	0.5344	0.19	33.3943	19.6657
Quantal-linear	173.528	0.0476	-1.415	15.4655	11.4268
Dichotomous hill	5845.73		0		

[a] model choice based on lowest AIC

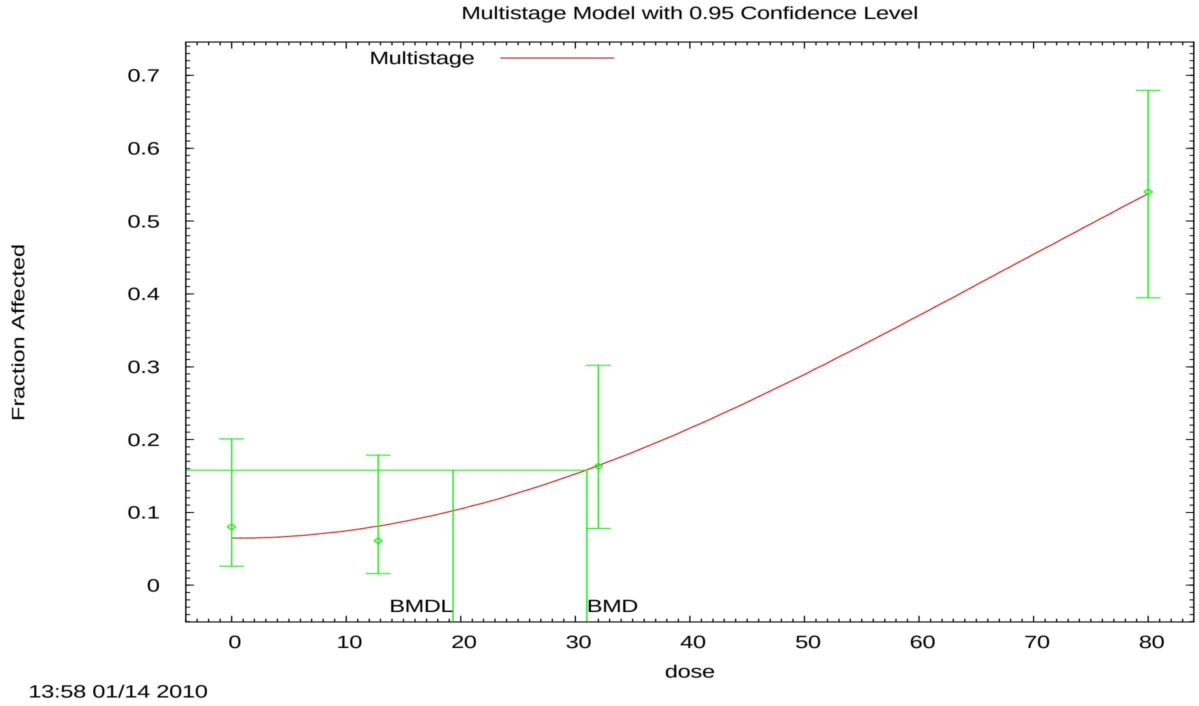


Figure B-20. Multistage model fit for forestomach epithelial hyperplasia in female B6C3F1 mice (BMR = 10% extra risk)

```
 ====================================================================
 Multistage Model. (Version: 3.0;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\mst_mouse_f_fore_hyper_Mst-BMR10-Restrict.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\mst_mouse_f_fore_hyper_Mst-BMR10-
 Restrict.plt
 Thu Jan 14 13:58:16 2010
 ====================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

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

   The parameter betas are restricted to be positive
   Dependent variable = Effect
   Independent variable = Dose
   Total number of observations = 4
   Total number of records with missing values = 0
   Total number of parameters in model = 3
   Total number of specified parameters = 0
   Degree of polynomial = 2

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

                  Default Initial Parameter Values
                     Background =    0.0623808
                        Beta(1) =            0
                        Beta(2) =   0.00011119

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

             Background      Beta(2)
Background            1         -0.5
   Beta(2)         -0.5            1

                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0645849            *                *                   *
        Beta(1)                0            *                *                   *
        Beta(2)      0.000109661            *                *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -81.5287         4
   Fitted model        -81.7648         2      0.472098      2          0.7897
  Reduced model        -102.317         1        41.577      3          <.0001
           AIC:          167.53

                                  Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ------------------------------------------------------------------------
    0.0000     0.0646         3.229     4.000          50        0.443
   12.8000     0.0812         3.981     3.000          49       -0.513
   32.0000     0.1639         8.033     8.000          49       -0.013
   80.0000     0.5363        26.817    27.000          50        0.052

 Chi^2 = 0.46      d.f. = 2        P-value = 0.7935

Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =        30.9965
            BMDL =        19.3466
```

```
             BMDU =          37.6172
Taken together, (19.3466, 37.6172) is a 90% two-sided confidence interval for the BMD
```

Table B-20. Benchmark modeling results for splenic hematopoietic cell proliferation in female B6C3F1 mice (BMR = 10% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	171.405	NA	0	5.73584	1.90919
Logistic	169.421	0.8993	0.064	4.06642	3.28512
Log-logistic	171.405	NA	0	6.5828	2.43228
Log-probit[a]	171.405	NA	0	6.91076	3.48982
Multistage	171.405	NA	0	4.41391	1.90919
Probit	**169.41**	**0.9466**	**0.033**	**4.03306**	**3.33147**
Weibull	171.405	NA	0	5.17994	1.90919
Quantal-linear	170.771	0.2455	0.264	2.34557	1.7616
Dichotomous hill			0		

[a] model choice based on lowest AIC

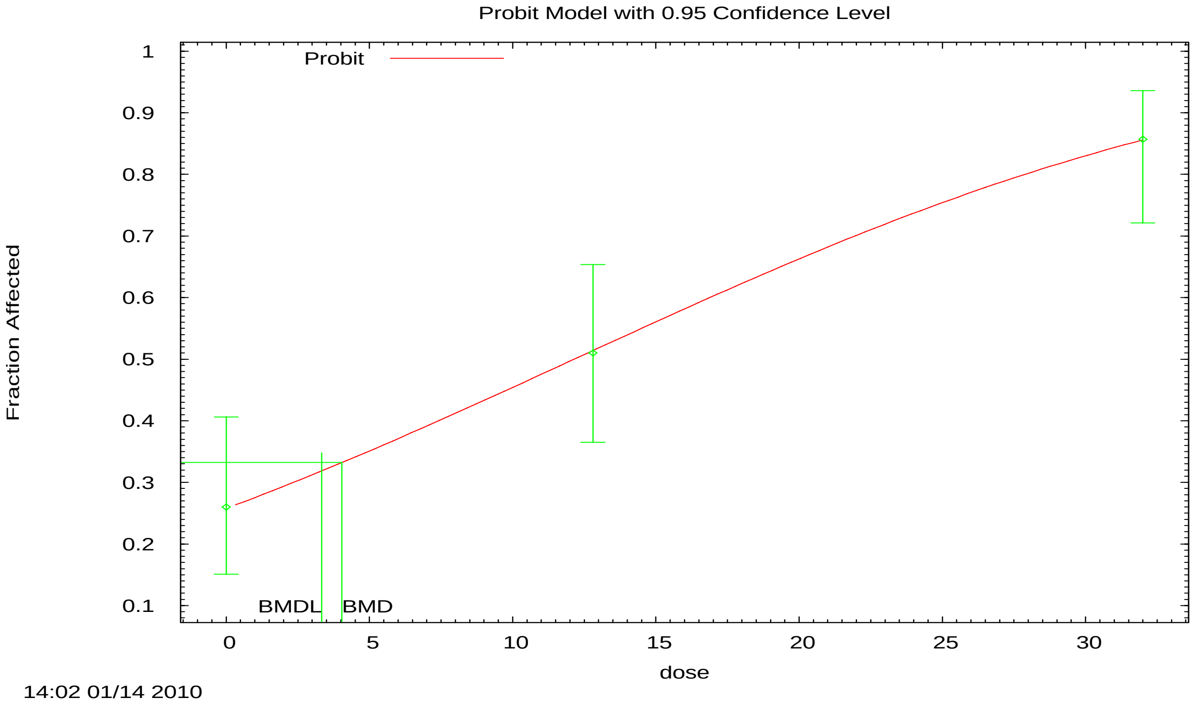


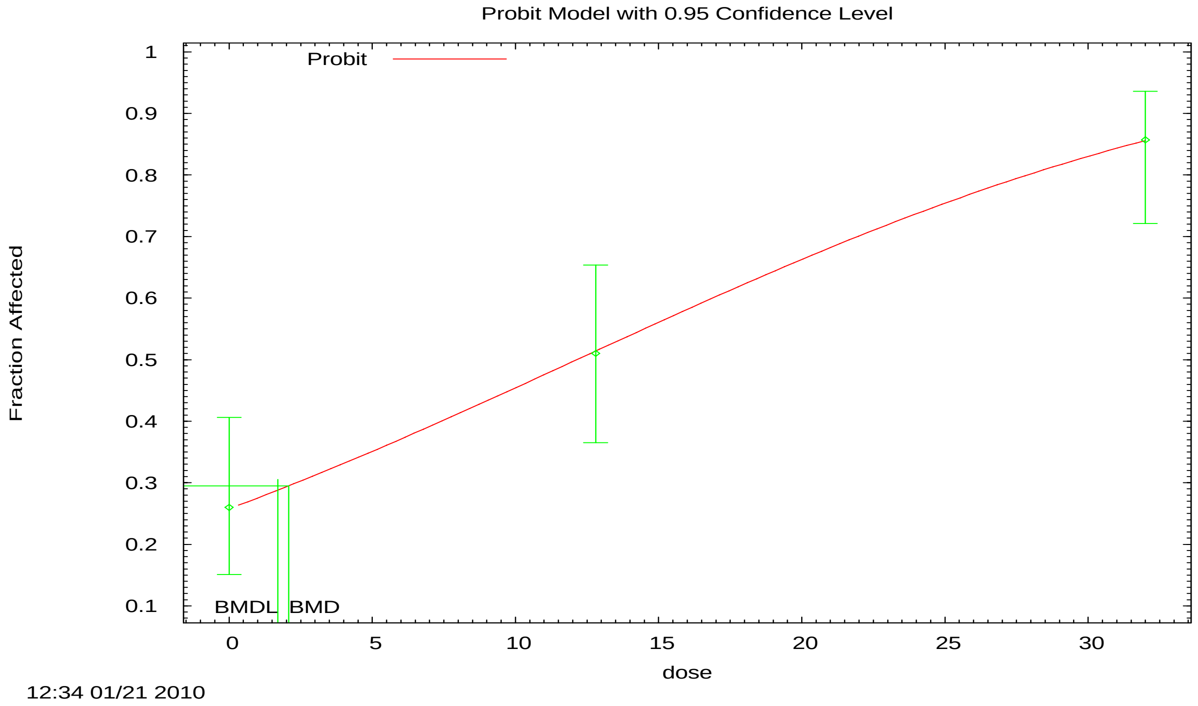
Figure B-21. Probit model fit for splenic hematopoietic cell proliferation in female B6C3F1 mice (BMR = 10% extra risk)

```
 ====================================================================
 Probit Model. (Version: 3.1;  Date: 05/16/2008)
 Input Data File: M:\Chloroprene\NTP_BMDS\pro_mouse_f_spleen_hemato_hdd_Pro-BMR10.(d)
 Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\pro_mouse_f_spleen_hemato_hdd_Pro-
 BMR10.plt
 Thu Jan 14 14:02:47 2010
 ====================================================================
 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 = Effect
   Independent variable = Dose
   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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       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
```

Table B-21. Benchmark modeling results for splenic hematopoietic cell proliferation in female B6C3F1 mice (BMR = 5% extra risk)

Model	AIC	Goodness-of-fit p-value	χ^2 residual	BMD	BMDL
Gamma	171.405	NA	0	3.86036	0.929461
Logistic	169.421	0.8993	0.064	2.10908	1.68891
Log-logistic	171.405	NA	0	4.75284	1.34665
Log-probit[a]	171.405	NA	0	5.33285	2.42674
Multistage	171.405	NA	0	2.35161	0.929461
Probit	**169.41**	**0.9466**	**0.033**	**2.07526**	**1.70339**
Weibull	171.405	NA	0	3.21493	0.929461
Quantal-linear	170.771	0.2455	0.264	1.14191	0.85761
Dichotomous hill			0		

[a] model choice based on lowest AIC



12:34 01/21 2010

Figure B-22. Probit model fit for splenic hematopoietic cell proliferation in female B6C3F1 mice (BMR = 5% extra risk)

```
 ====================================================================
Probit Model. (Version: 3.1;  Date: 05/16/2008)
Input Data File: M:\Chloroprene\NTP_BMDS\pro_mouse_f_spleen_hemato_hdd_Pro-BMR05.(d)
Gnuplot Plotting File:  M:\Chloroprene\NTP_BMDS\pro_mouse_f_spleen_hemato_hdd_Pro-BMR05.plt
Thu Jan 21 12:34:25 2010
 ====================================================================
 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 = Effect
   Independent variable = Dose
   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
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       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 =          0.05
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =       2.07526
            BMDL =       1.70339
```

APPENDIX C: CANCER DOSE-RESPONSE MODELING

Table C-1: Tumor incidence, with time to death with tumor: female mice exposed to chloroprene via inhalation (NTP, 1998)

Dose Group	Week of Study	Total examined	Number of animals with tumors at each site, at specified week of study								
			Lung	Hemangiomas, Hemangiosarcomas		Harderian Gland[c]	Mammary	Forestomach	Liver	Skin	Zymbal Gland[c]
				Incid.[a]	Fatal[a]						
0	5	1	0	0	0	0	0	0	0	0	0
	69	1	0	0	0	0	0	0	0	0	0
	70	1	0	0	0	0	0	0	0	0[b]	0
	71	1	0	0	0	0	0	0	1	0	0
	76	1	0	0	0	1	1	0	0	0	0
	78	1	0	0	1	0	0	0	0	0	0
	88	1	0	0	0	0	0	0	0	0	0
	91	2	0	0	0	0	0	0	1	0	0
	95	1	0	0	0	0	0	0	0	0	0
	97	1	0	0	0	0	0	0	0	0	0
	98	1	0	0	0	0	1	0	0	0	0
	101	2	1	0	0	0	0	0	0	0	0
	105	36	3	3	0	1	1[b]	1	18	0	0
12.8	41	1	0	0	0	0	0	0	0	1	0
	46	2	0[b]	0[b]	0	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]
	63	1	0	0	0	0	1	0	1	1	0
	64	1	1	0	0	0	1	0	0	0	0
	69	1	0	0	1	0	0	0	0	0	0
	75	1	1	0	0	0	0	0	1	0	0
	76	1	0	0	0	0	0	0	1	0	0
	78	1	0	0	0	0	1	0	0	0	0
	79	3	0	0	0	0	0	0	1	0	0
	87	1	0	0	0	0	0	0	1	1	0
	89	2	2	0	0	1	0	0	1	0	0
	90	1	0	0	0	0	0	0	1	1	0
	91	3	2	0	0	0	0	0	0	1	0
	97	3	2	0	0	0	1	0	2	1	0
	98	1	1	0	0	1	0	0	0	0	0
	99	5	4	0	1	0	0	0	2	2	0
	100	1	0	0	0	0	0	0	0	0	0
	101	1	1	0	1	0	0	0	1	0	0
	102	2	2	0	1	0	0	0	1	0	0
	103	2	1	0	0	0	1	0	2	1	0
	105	16	11	2	0	3	1	0	11	2	0
32	31	1	0	0	1	0	0	0	0	0	0
	50	1	1	0	0	0	0	0	0	0	0
	54	1	0	0	0	0	0	0	0	0	0
	56	1	0	0	1	0	0	0	0	0	0
	57	1	0	0	0	0	1	0	0	0	0
	61	1	0	0	0	0	1	0	0	0	0
	63	1	1	0	1	0	0	0	0	0	0
	65	1	1	0	0	0	0	0[b]	0	0	0
	67	1	0	0	0	0	0	0	0	0	0
	68	1	0	0	0	0	0	0	0	0	0
	70	1	0	0	1	0	0	0	0	0	0
	72	2	2	0	1	0	1	0	1	0	0
	73	1	0	0	0	0	0	0	1	0	0
	74	1	1	0	1	0	0	0	1	0	0
	75	2	1	0	0	1	1	0	0	1	0
	76	2	2	0	1	0	1	0	1	1	0
	77	2	1	0	1	0	0	0	0	0	0
	78	1	0	0	0	0	0	0	1	0	0
	79	2	2	0	2	0	1	0	0	0	0
	82	1	1	0	0	0	1	0	0	0	0
	84	2	1	0	1	0	0	0	0	1	0

Table C-1: Tumor incidence, with time to death with tumor: female mice exposed to chloroprene via inhalation (NTP, 1998)

Dose Group	Week of Study	Total examined	Number of animals with tumors at each site, at specified week of study									
			Lung	Hemangiomas, Hemangiosarcomas		Harderian Gland[c]	Mammary	Forestomach	Liver	Skin	Zymbal Gland[c]
				Incid.[a]	Fatal[a]						
	86	1	1	0	0	0	0	0	1	0	0
	87	3	2	1	1	2	1	0	1	1	0
	89	2	2	0	2	0	1	0	1	0	0
	90	3	3	0	0	0	0	0	0	1	0
	91	3	3	0	1	0	0	0	3	1	0
	92	1	1	0	0	0	1	0	0	0	0
	93	1	1	0	0	0	0	0	1	1	0
	94	3	3	0	0	0	1	0	2	1	0
	96	2	1	0	0	0	0	0	2	1	0
	97	1	1	0	0	0	0	0	1	1	0
	99	1	0	0	1	0	0	0	1	0	0
	103	1	1	0	0	0	0	0	1	0	0
	105	1	1	1	0	0	0	0	1	1	0
80	1	1	0	0	0	0	0	0	0	0	0
	36	1	0	0	0	0	0	0	0	0	0
	47	1	1	0	0	0	0	0	0	0	0
	48	1	0	0	0	0	1	0	0	0	0
	55	1	0	0	0	0	1	0	1	0	0
	64	1	0	0	0	0	0	0	0	0	0
	65	1	1	0	0	0	1	0	1	0	0
	66	1	1	0	0	0	0	0	0	1	0
	67	2	1	0	0	1	0	0	2	2	1
	70	1	1	0	0	0	1	0	0	0	0
	75	4	4	0	1	0	1	0	1	2	0
	76	2	2	0	0	0	0	0	1	1	0
	77	1	0	0	1	1	0	0	1	0	0
	79	1	1	0	0	0	1	0	1	0	0
	81	1	1	0	0	0	0	0	0	0	0
	83	3	3	0	1	0	0	1	1	2	1
	84	1	1	0	0	0	1	0	1	0	0
	86	1	1	0	0	0	1	0	1	0	0
	87	1	0	0	0	1	0	0	0	1	0
	88	2	2	0	0	1	1	1	2	1	0
	90	2	2	1	0	0	0	0	1	1	1
	91	7	7	1	2	2	4	1	3	4	0
	92	1	1	0	0	0	0	1	1	0	0
	93	2	2	0	0	0	0	0	2	1	0
	94	1	1	0	0	1	0	0	1	0	0
	95	2	2	0	0	0	0	0	2	0	0
	96	1	1	0	0	0	0	0	1	0	0
	97	2	2	0	0	0	0	0	2	1	0
	98	1	1	0	0	0	0	0	1	1	0
	105	3	3	1	0	2	1	0	3	0	0

[a] "Incid." , or Incidental, denotes tumors not concluded to have caused the death of the animal. Fatal denotes tumors considered to have caused the death of the animal.

[b] Tissue for one animal of total examined was missing or unsuitable for histopathologic examination.

[c] Harderian gland and Zymbal's gland were examined histopathologically only if a lesion was observed grossly at necropsy; instances of "0" for these tissues indicate only that no tumor was seen grossly, for dose-response modeling purposes.

Table C-2: Tumor incidence, with time to death with tumor: male mice exposed to chloroprene via inhalation (NTP, 1998)

Dose Group	Week of Study	Total examined	Number of animals with tumors at each site, at specified week of study					
			Hemangiomas, Hemangiosarcomas		Lung	Forestomach	Harderian gland[c]	Kidney
			Incid.[a]	Fatal[a]				
0	65	1	0	0	0	0	0	0
	77	1	0	0	0	0	0	0
	79	1	0	0	0	0	0	0
	82	1	0	0	0	0	0	0
	86	1	0	0	0	0	1	0
	87	3	0	0	0	0	0	0
	90	2	0	0	0	0	0	0
	91	2	0	0	1	0	0	0
	92	1	0	0	0	0	0	0
	95	1	0	0	1	0	0	0
	96	1	0	0	0	0	0	0
	97	1	0	0	1	0	0	0
	98	3	0	0	1	0	0	0
	103	1	0	0	0	0	0	0
	104	1	0	0	0	0	0	0
	105	29	3	0	9	1	1	0
12.8	63	1	0	0	0	0	0	0
	75	1	0	0	0	0[b]	0	0
	76	1	0	0	1	0[b]	0	0[b]
	78	1	0	0	0	0	0	0
	83	1	0	0	0	0	0	0
	84	2	0	0	0	0	0	0
	86	1	0	0	0	0	0	0
	87	1	0	0	1	0	0	0
	88	1	0	0	0	0	0	0
	90	1	0	0	0	0	0	0
	91	2	0	0	1	0	0	0
	92	1	0	0	1	0	0	0
	95	1	0	1	0	0	0	0
	96	1	0	0	0	0	0	0
	98	1	0	1	1	0	0	0
	99	3	0	3	1	0	0	0
	101	1	0	0	1	0	1	1
	102	1	0	1	0	0	0	0
	104	1	0	0	1	0	0	0
	105	27	8	0	20	0	4	1

Table C-2: Tumor incidence, with time to death with tumor: male mice exposed to chloroprene via inhalation (NTP, 1998)

			Number of animals with tumors at each site, at specified week of study					
			Hemangiomas, Hemangiosarcomas					
30	55	1	0	0	1	0	0	0
	63	1	0	0	0	0	0	0
	68	1	0	0	1	0[b]	0	0
	71	2	0	1	1	0	0	0
	72	1	0	1	1	0	0	0
	78	1	0	0	0	0	0	0
	79	1	0	0	0	0	0	0
	81	2	0	0	1	0	0	0
	83	1	0	0	1	0	0	0
	86	2	0	1	1	0	1	0
	87	4	1	1	4	0	2	0
	89	2	0	0	1	0	0	0
	90	1	0	0	1	0	0	0
	91	1	0	1	1	0	1	0
	93	1	0	1	1	0	0	0
	95	1	0	1	1	0	0	0
	96	2	0	1	1	0	0	0
	97	2	0	2	2	0	1	0
	98	1	0	1	1	0	0	0
	99	3	0	2	2	0	0	0
	101	2	0	2	1	0	1	0
	102	1	0	1	1	0	0	0
	103	2	0	0	2	0	0	1
	105	14	6	0	10	2	4	2
80	56	1	0	0	0	0	0	0
	61	1	0	0	0	0	0	0
	65	1	0	1	0	0	0	0
	75	2	0	0	2	0	0	0
	81	1	0	0	1	0	0	1
	83	1	0	1	1	0	0	0
	84	1	0	0	1	1	0	1
	85	2	0	0	2	1	1	0
	86	1	0	0	1	0	0	0
	87	2	0	1	1	0	0	0
	89	1	0	0	1	0	0	0
	90	3	0	1	2	1	0	0
	91	3	0	1	3	0	1	0
	92	2	0	2	1	1	1	0
	93	3	0	0	3	0	0	1
	94	2	0	1	2	1	0	0
	95	3	0	2	3	0	1	1
	96	1	0	0	1	0	0	0
	97	2	1	1	2	1	1	0
	98	2	0	1	1	0	0	0
	99	0	0	0	0	0	1	1
	101	2	0	1	2	0	0	1
	105	13	7	0	13	0	6	3

[a] "Incid." , or Incidental, denotes tumors not concluded to have caused the death of the animal. Fatal denotes tumors considered to have caused the death of the animal.

[b] Tissue for one animal of total examined was missing or unsuitable for histopathologic examination.

[c] Harderian gland was examined histopathologically only if a lesion was observed grossly at necropsy; instances of "0" for these tissues indicate only that no tumor was seen grossly, for dose-response modeling purposes.

Table C-3. Summary of Model Selection and Modeling Results for best-fitting multistage-Weibull models, using time-to-tumor data for female mice (NTP, 1998)

Site	stages	LL[a]	Para-meters	AIC	$\chi 2$[b]	BMD$_{10}$	Responses @ppm levels[c]				Multistage-Weibull model parameters					
							0	12.8	32	80	c	t$_0$	b0	b1	b2	b3
Lung[d]							*4*	*28*	*34*	*42*						
	3	-83.02	5	176.04	NA	1.20	4.1	27.4	32.4	43.1	3.78	0	2.24 E-09	2.04E -09		
Hemangiomas, hemangio-sarcomas (fatal)[e]							*4*	*6*	*18*							
	2	-135.848	4	279.70	NA	10.11	3.45	7.4	13.5		5.91	0	1.01 E-13	0	1.27 E-15	
	1	-138.519	3	283.04	5.342	4.60	3.25	10.6	11.1		5.42	0	9.04 E-13	2.69 E-13		
Hemangiomas, hemangio-sarcomas (incidental)[f]							*4*	*6*	*18*							
	2	**-65.8122**	**4**	**139.62**	**NA**	**14.94**	**3.7**	**6.6**	**17.6**		1.00	0	7.9 E-04	0	4.54 E-06	
	1	-66.9531	3	139.91	2.2818	8.32	3.3	9.3	15.3		1.00	0	7.0 E-04	1.2 E-04	NA	
Harderian gland[e]							*2*	*5*	*3*	*9*						
	3	-58.2559	5	126.51	NA	30.09	2.4	3.6	4.1	8.9	2.91	0	7.59 E-08	4.69 E-09	0	1.09 E-13
	2	-58.2661	4	124.53	0.0204	27.47	2.3	3.7	4.2	8.8	2.94	0	6.41 E-08	4.57 E-09	1.42 E-12	
	1	**-58.2663**	**3**	**122.53**	**0.0004**	**27.08**	**2.3**	**3.7**	**4.3**	**8.7**	2.94	0	6.26 E-08	4.60 E-09		
Mammary gland adenomas, carcinomas, adena-canthomas[d]							*3*	*6*	*11*	*14*						
	3	-87.9599	5	185.92	NA	20.42	3.5	5.9	8.5	15.7	1.00	0	7.4 E-04	4.96 E-05		
Forestomach[f]							*1*	*0*	*0*	*4*						
	3	-19.1743	5	48.35	NA	68.44	0.4	0.4	0.4	3.7	4.23	0	3.05 E-11	0	0	9.76 E-16
	2	**-19.5963**	**4**	**47.19**	**0.844**	**67.81**	**0.5**	**0.5**	**0.7**	**3.4**	4.13	0	5.02 E-11	0	1.09 E-13	
	1	-20.7578	3	47.52	2.323	90.02	0.5	1	1.1	2.5	3.54	0	8.24 E-10	8.51 E-11		
Hepatocellular adenomas, carcinomas[d]							*20*	*26*	*20*	*30*						
	3	-119.227	5	248.45	NA	4.24	21.6	23	20.7	30.8	4.16	0	2.70 E-09	1.01 E-10		
Skin[d]							*0*	*11*	*11*	*18*						
	3	-87.4625	5	184.93	NA	9.50	0	5.6	10.6	22.4	1.56	0	0	7.91 E-06		
Zymbal's gland[f]							*0*	*0*	*0*	*3*						
	3	-11.4018	5	32.80	NA	87.88	0	0	0.2	2.8	1.30	0	0	0	0	3.74 E-10
	2	-11.7264	4	31.45	0.6492	97.41	0	0.1	0.4	2.5	1.28	0	1.58 E-27	0	2.91 E-08	
	1	**-12.5984**	**3**	**31.20**	**1.744**	**166.16**	**0**	**0.4**	**0.8**	**1.9**	1.06	0	0	4.72 E-06		

[a] LL=log-likelihood.

[b] χ^2 = chi-squared statistic for testing the difference between 2 model fits. Calculated from 2 × $|(LL_i – LL_j)|$ and evaluated for i-j degrees of freedom. In all cases the difference was evaluated for consecutive numbers of stages; i-j = 1, and the critical chi-squared value was 3.84.

[c] **"**Responses" describes the number of animals with each tumor type; observed responses are in italics, and expected responses (predicted by each model fit) are given to one decimal place for comparison with the observed data.

[d] Model selection rationale was First-order, only available fit

[e] Model selection rationale was $\chi 2$ and AIC

[f] Model selection rationale was AIC

Table C-4. Summary of Model Selection and Modeling Results for best-fitting multistage-Weibull models, using time-to-tumor data for male mice (NTP, 1998)

	stages	LL[a]	Para-meters	AIC	$\chi2$ [b]	BMD_{10}	Responses @ppm levels[c]				Multistage-Weibull model parameters					
Site							0	12.8	32	80	c	t_0	b0	b1	b2	b3
Lung[d]							*13*	*28*	*36*	*43*						
	3	**-104.927**	**5**	**219.86**	**NA**	**2.46**	**14**	**26.6**	**33.9**	**44.6**	3.46	0	4.01 E-08	4.46 E-09		
Hemangiomas, hemangio-sarcomas (fatal)[d]							*3*	*14*	*23*	*21*						
	3	**-201.96**	**5**	**413.92**	**NA**	**6.05**	**4.5**	**11**	**13.8**	**22.3**	10.20	0	3.29 E-22	4.65 E-23		
Hemangiomas, hemangio-sarcomas (incidental)d							*3*	*14*	*23*	*21*						
	3	**-109.463**	**5**	**228.93**	**NA**	**7.75**	**5.3**	**11.1**	**15.9**	**27.2**	3.87	0	2.09 E-10			
Harderian gland[d]							*2*	*5*	*10*	*12*						
	3	**-73.6639**	**5**	**157.33**	**NA**	**NA**	**2.3**	**5.2**	**7.4**	**14**	5.57	0	3.26 E-13	3.60 E-14		
Kidney[e]							*0*	*2*	*3*	*9*						
	3	-40.9478	5	91.90	NA	29.51	0	1.7	3.4	8.9	6.24	0	0	8.87 E-16	0	3.23 E-20
	2	-40.96	4	89.92	0.0244	28.87	0	1.7	3.5	8.8	6.23	0	0	8.88 E-16	2.99 E-18	
	1	**-41.0033**	**3**	**88.01**	**0.0866**	**26.70**	**0**	**2**	**3.7**	**8.3**	6.09	0	0	2.03 E-15		
Forestomach[e]							*1*	*0*	*2*	*4*						
	3	-28.0952	5	66.19	NA	84.00	0.7	0.8	1.2	4.3	1.91	0	2.05 E-06	5.62 E-09	2.01 E-09	
	2	-28.0952	4	64.19	0	84.00	0.7	0.8	1.2	4.3	1.91	0	2.05 E-06	5.62 E-09	2.01 E-09	
	1	**-28.3188**	**3**	**62.64**	**0.4472**	**108.76**	**0.6**	**1.1**	**1.8**	**3.6**	1.79	0	3.03 E-06	2.34 E-07		

[a] LL=log-likelihood.

[b] χ^2 = chi-squared statistic for testing the difference between 2 model fits. Calculated from 2 × $|(LL_i - LL_j)|$ and evaluated for i-j degrees of freedom. In all cases the difference was evaluated for consecutive numbers of stages; i-j = 1, and the critical chi-squared value was 3.84.

[c] **"**Responses" describes the number of animals with each tumor type; observed responses are in italics, and expected responses (predicted by each model fit) are given to one decimal place for comparison with the observed data.

[d] Model selection rationale was First-order, only available fit

[e] Model selection rationale was $\chi2$ and AIC

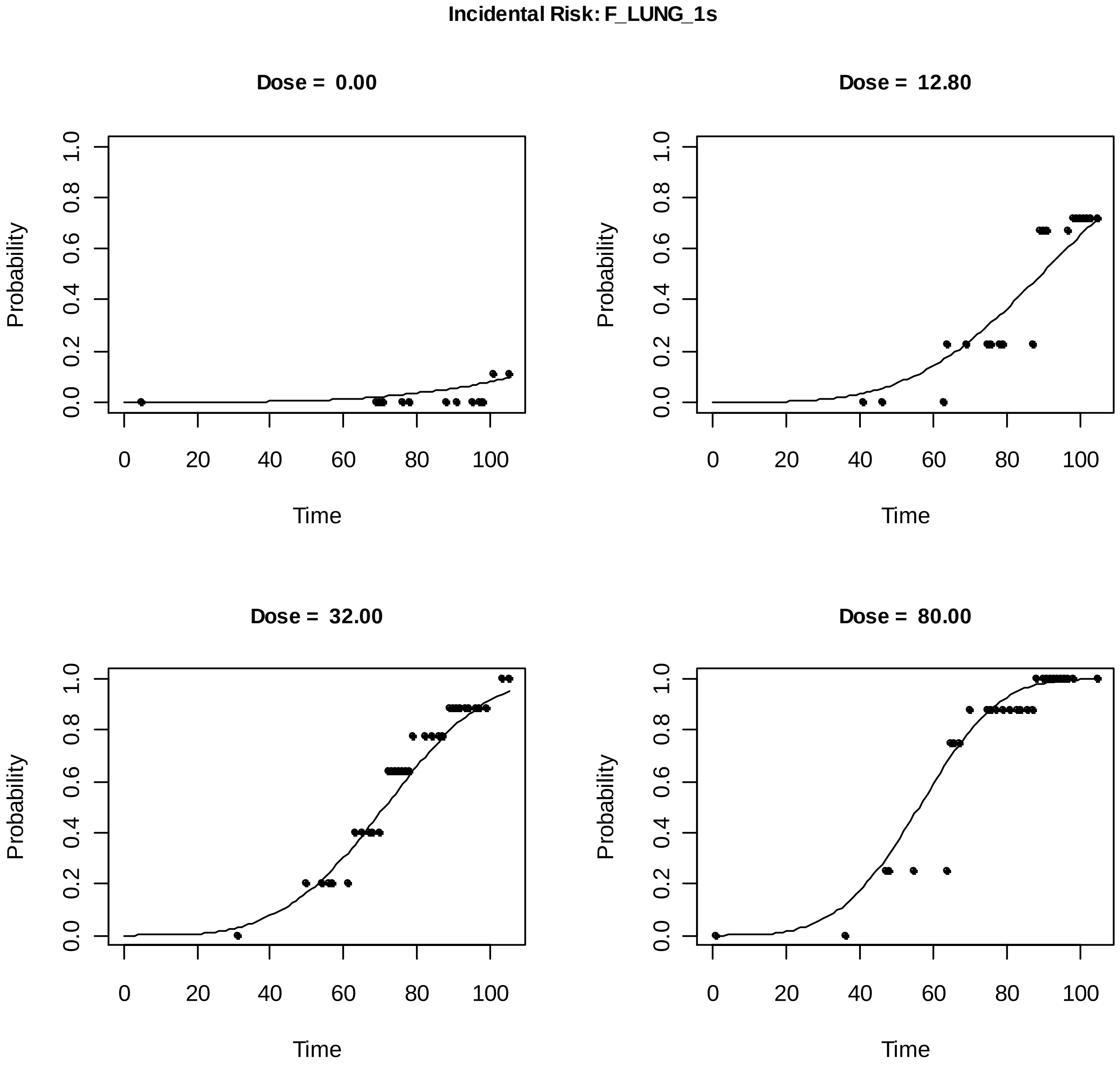


Figure C-1. Female mice, alveolar/bronchiolar tumors. Details below.

```
=======================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M:\_chemicals\chloroprene\msw\F_LUNG_1s.(d)
=======================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}
   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 112
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =         0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =      3.77778
                         t_0    =            0   Specified
                         beta_0 = 2.32179e-009
                         beta_1 = 2.11013e-009

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 c            beta_0       beta_1
    c                 1        -0.99           -1
    beta_0        -0.99            1         0.99
    beta_1           -1         0.99            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               3.78542         0.978326             1.86793              5.7029
         beta_0     2.24014e-009     1.03745e-008       -1.80935e-008        2.25738e-008
         beta_1     2.03972e-009     8.87049e-009       -1.53461e-008        1.94256e-008


                Log(likelihood)   # Param             AIC
   Fitted Model          -83.02         3          172.04


                    Data Summary
                        CLASS
               C      F      I      U  Total  Expected Response
    DOSE
        0     46      0      4      0     50     4.10
       13     21      0     28      1     50    27.45
       32     16      0     34      0     50    32.39
       80      8      0     42      0     50    43.13
```

```
     BENCHMARK DOSE COMPUTATION                    BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL             RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA             RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1             SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9             CONFIDENCE LEVEL =            0.9

TIME             =            104             TIME             =            104

             BMD =        1.19617                          BMD =       0.114103
            BMDL =       0.883475                         BMDL =      0.0865258
            BMDU =        1.60092                         BMDU =       0.148645
```

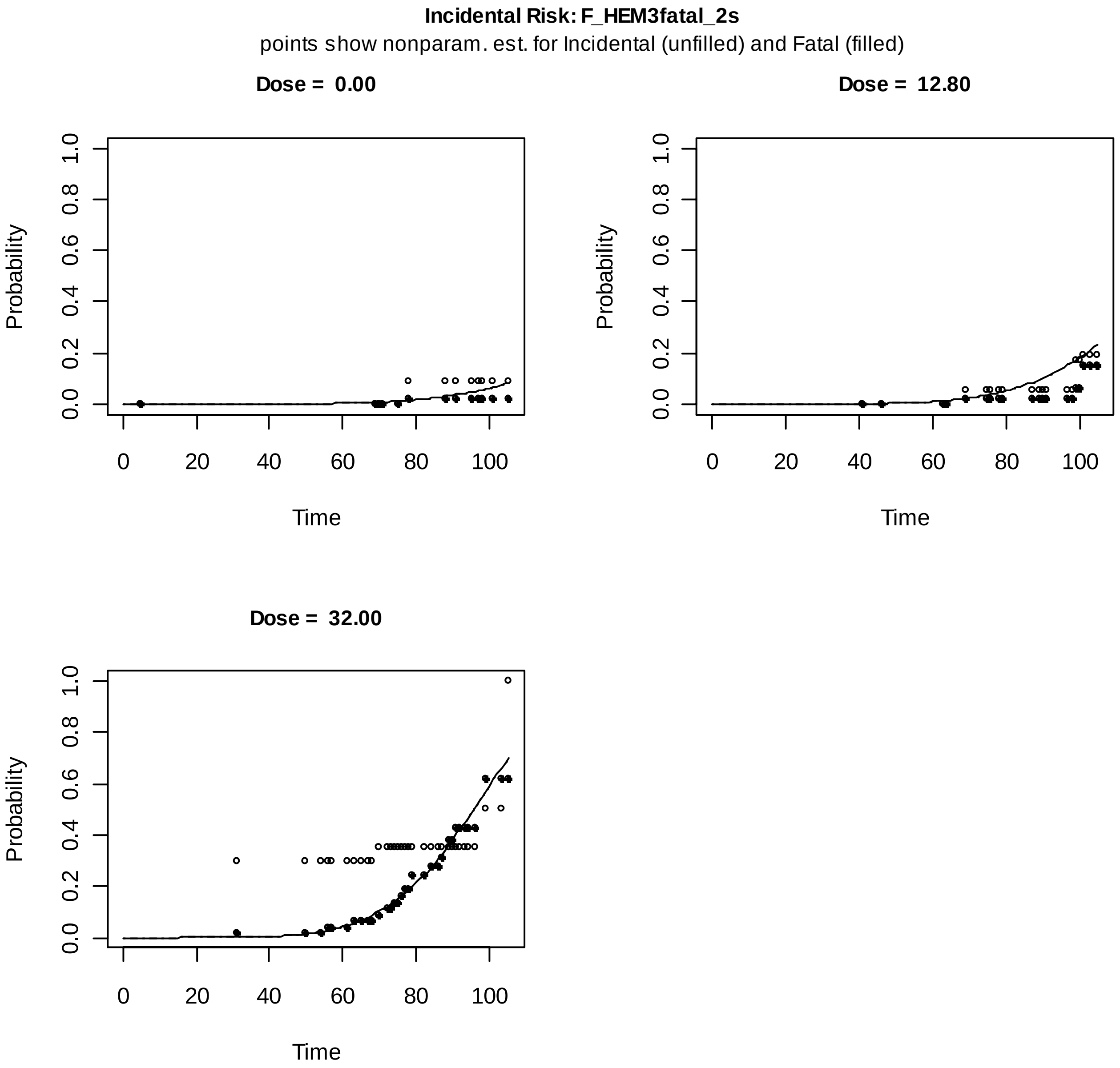


Figure C-2. Female mice, hemangiomas and hemangiosarcomas in all organs; high dose dropped, hemangiosarcomas occurring before termination considered fatal

```
 =======================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: M:\_chemicals\chloroprene\msw\F_HEM3fatal_2s.(d)
 =======================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2)}
   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 84
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 1
 Degree of polynomial = 2

   User specifies the following parameters:
          t_0    =         0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =         4.25
                         t_0    =            0   Specified
                         beta_0 =  2.2479e-010
                         beta_1 = 2.06502e-034
                         beta_2 = 2.12137e-012


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_1
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 c             beta_0       beta_2
    c                     1           -1           -1
    beta_0               -1            1         0.99
    beta_2               -1         0.99            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate         Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               5.90503          1.49573           2.97346              8.8366
         beta_0     1.01175e-013     7.08031e-013       -1.28654e-012        1.48889e-012
         beta_1                0               NA
         beta_2     1.26539e-015     8.53103e-015       -1.54551e-014        1.79859e-014

NA - Indicates that this parameter has hit a
     bound implied by some inequality constraint
     and thus has no standard error.

                Log(likelihood)   # Param            AIC
   Fitted Model        -135.848         4         279.697

                    Data Summary
                        CLASS
              C      F      I      U  Total  Expected Response
    DOSE
        0     46      1      3      0     50     3.45
       13     43      4      2      1     50     7.40
       32     32     16      2      0     50    13.53

    Minimum observation time for F tumor context =         31
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        10.1137
            BMDL =        5.75142
            BMDU =        13.1199
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        3.12363
            BMDL =       0.640904
            BMDU =        4.05212
```

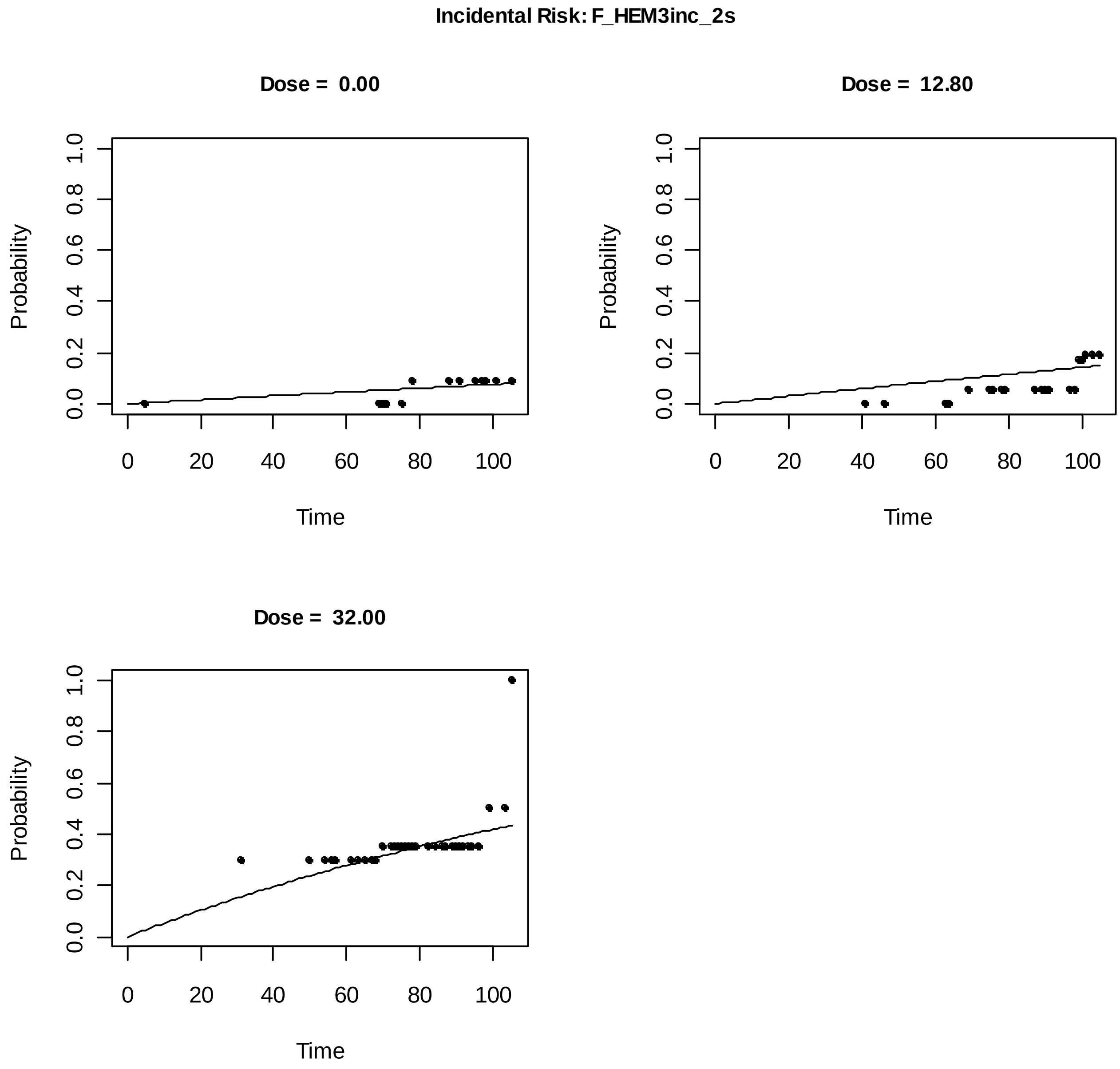


Figure C-3. Female mice, hemangiomas and hemangiosarcomas in all organs; high dose dropped, all tumors considered incidental. Details below.

```
=======================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: F_HEM3inc_2s.(d)
 =======================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 84
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 1
 Degree of polynomial = 2

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =      1.13333
                         t_0    =            0   Specified
                         beta_0 =  0.000428228
                         beta_1 =            0
                         beta_2 = 2.52747e-006

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -c         -t_0       -beta_1
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 beta_0       beta_2
    beta_0            1         -0.4
    beta_2         -0.4            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c                     1               NA
         beta_0      0.000792254      0.000500484       -0.000188678          0.00177319
         beta_1                0               NA
         beta_2     4.54142e-006     1.85042e-006       9.14653e-007        8.16818e-006

NA - Indicates that this parameter has hit a
     bound implied by some inequality constraint
     and thus has no standard error.

                Log(likelihood)   # Param           AIC
   Fitted Model        -65.8122         4         139.624

                    Data Summary
                        CLASS
               C      F      I      U  Total  Expected Response
    DOSE
       0     46      0      4      0     50     3.74
      13     43      0      6      1     50     6.57
      32     32      0     18      0     50    17.56
```

```
    BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        14.9357
            BMDL =        11.0629
            BMDU =       19.8583
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        4.61294
            BMDL =         2.0194
            BMDU =       6.12873
```

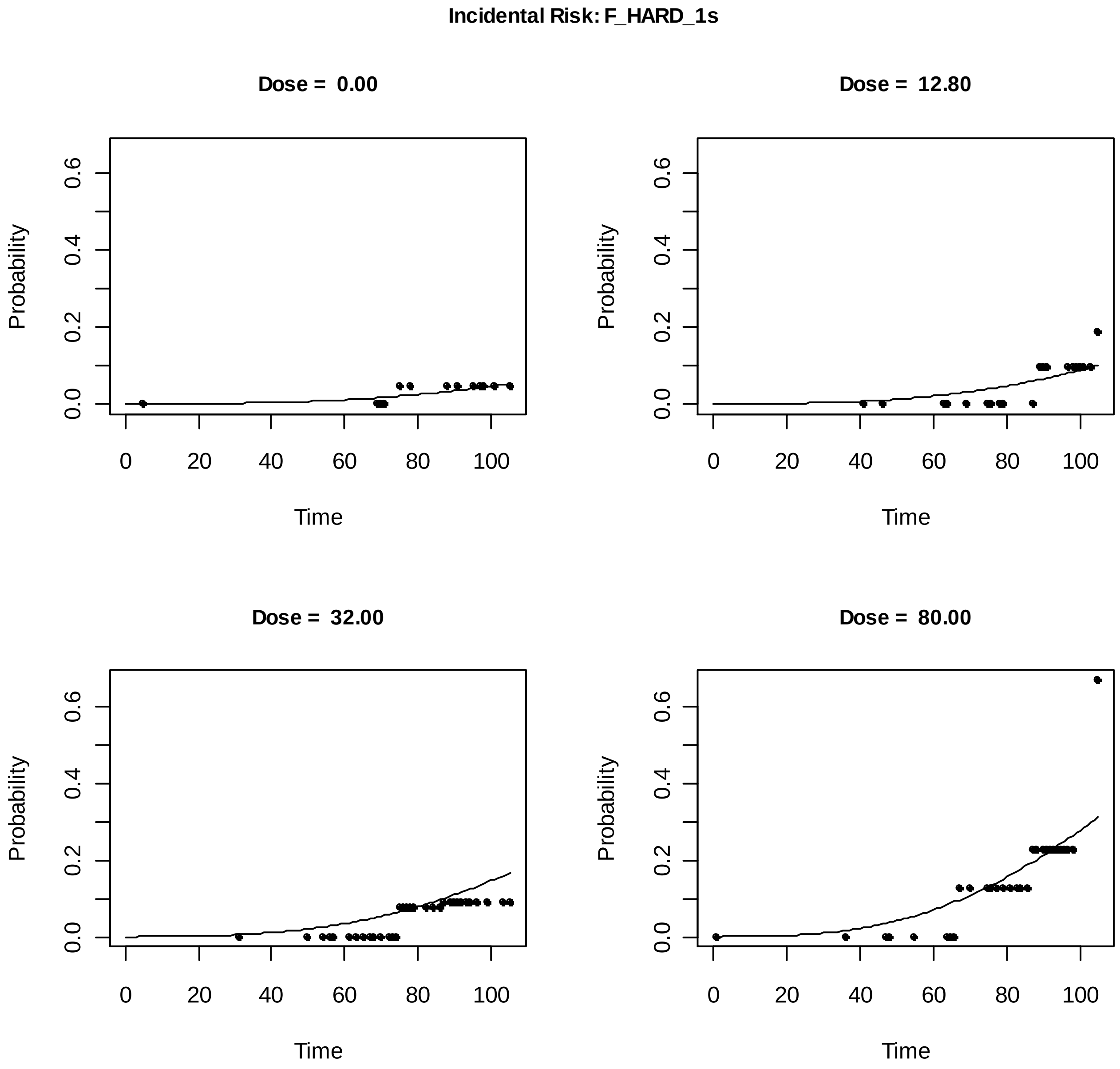


Figure C-4. Female mice, Harderian gland tumors. Details below.

```
 ====================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M:\_chemicals\chloroprene\msw\F_HARD_1s.(d)
 ====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 120
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =         0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =      2.83333
                         t_0    =            0   Specified
                         beta_0 = 1.02152e-007
                         beta_1 =  7.3281e-009

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 c            beta_0       beta_1
    c                 1           -1           -1
    beta_0           -1            1         0.99
    beta_1           -1         0.99            1

                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        c               2.93861          2.46009          -1.88307              7.7603
        beta_0     6.26114e-008     7.18253e-007       -1.34514e-006        1.47036e-006
        beta_1     4.59946e-009     5.01418e-008       -9.36766e-008        1.02876e-007


                Log(likelihood)   # Param            AIC
   Fitted Model        -58.2663         3         122.533


                   Data Summary
                       CLASS
              C      F      I      U  Total  Expected Response
    DOSE
       0     48      0      2      0     50     2.32
      13     45      0      5      0     50     3.71
      32     47      0      3      0     50     4.26
      80     41      0      9      0     50     8.73
```

```
     BENCHMARK DOSE COMPUTATION                    BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL             RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA             RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1             SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9             CONFIDENCE LEVEL =            0.9

TIME             =            104             TIME             =            104

             BMD =        27.0825                          BMD =         2.5834
            BMDL =         12.614                         BMDL =        1.20327
            BMDU =        85.8726                         BMDU =        8.04772
```

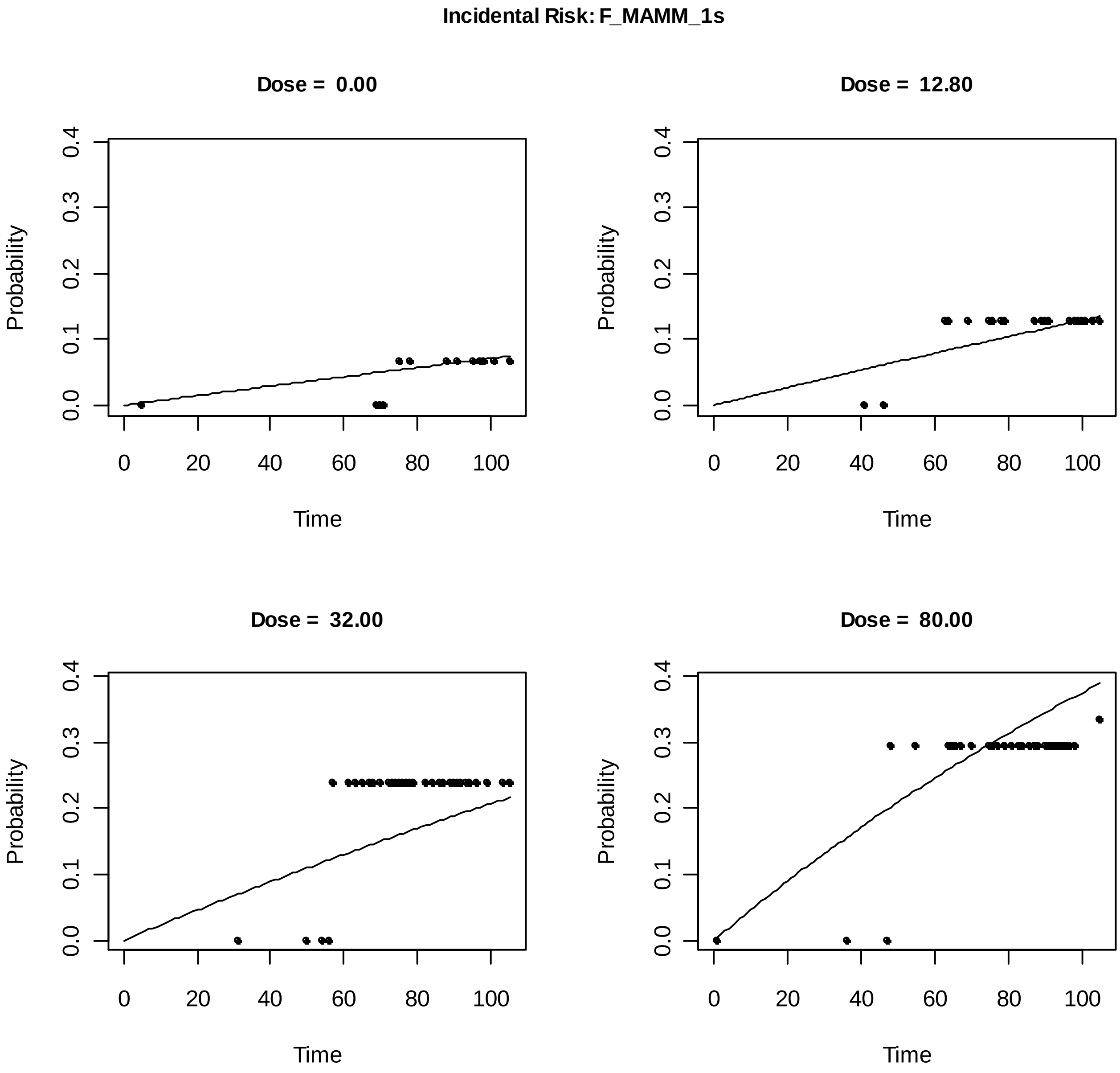


Figure C-5. Female mice, mammary gland tumors. Details below.

```
 ====================================================================
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: M:\_chemicals\chloroprene\msw\F_MAMM_1s.(d)
 ====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 126
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =       1.0303
                         t_0    =            0   Specified
                         beta_0 =  0.000643678
                         beta_1 = 4.34581e-005

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -c         -t_0
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 beta_0      beta_1
    beta_0            1       -0.57
    beta_1        -0.57           1

                                Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c                     1               NA
         beta_0      0.000740811      0.000512345       -0.000263368          0.00174499
         beta_1     4.96148e-005     2.12095e-005       8.04497e-006        9.11846e-005

NA - Indicates that this parameter has hit a bound implied by some inequality constraint
     and thus has no standard error.


                Log(likelihood)   # Param            AIC
   Fitted Model        -87.9599         3          181.92

                    Data Summary
                        CLASS
              C      F      I      U  Total  Expected Response
    DOSE
       0     46      0      3      1     50     3.50
      13     43      0      6      1     50     5.93
      32     39      0     11      0     50     8.48
      80     36      0     14      0     50    15.68
```

```
     BENCHMARK DOSE COMPUTATION                  BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL           RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA           RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1           SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9           CONFIDENCE LEVEL =            0.9

TIME             =            104           TIME             =            104

             BMD =         20.419                        BMD =        1.94776
            BMDL =        14.0543                       BMDL =        1.34101
             MDU =        38.5881                       BMDU =        3.71557
```

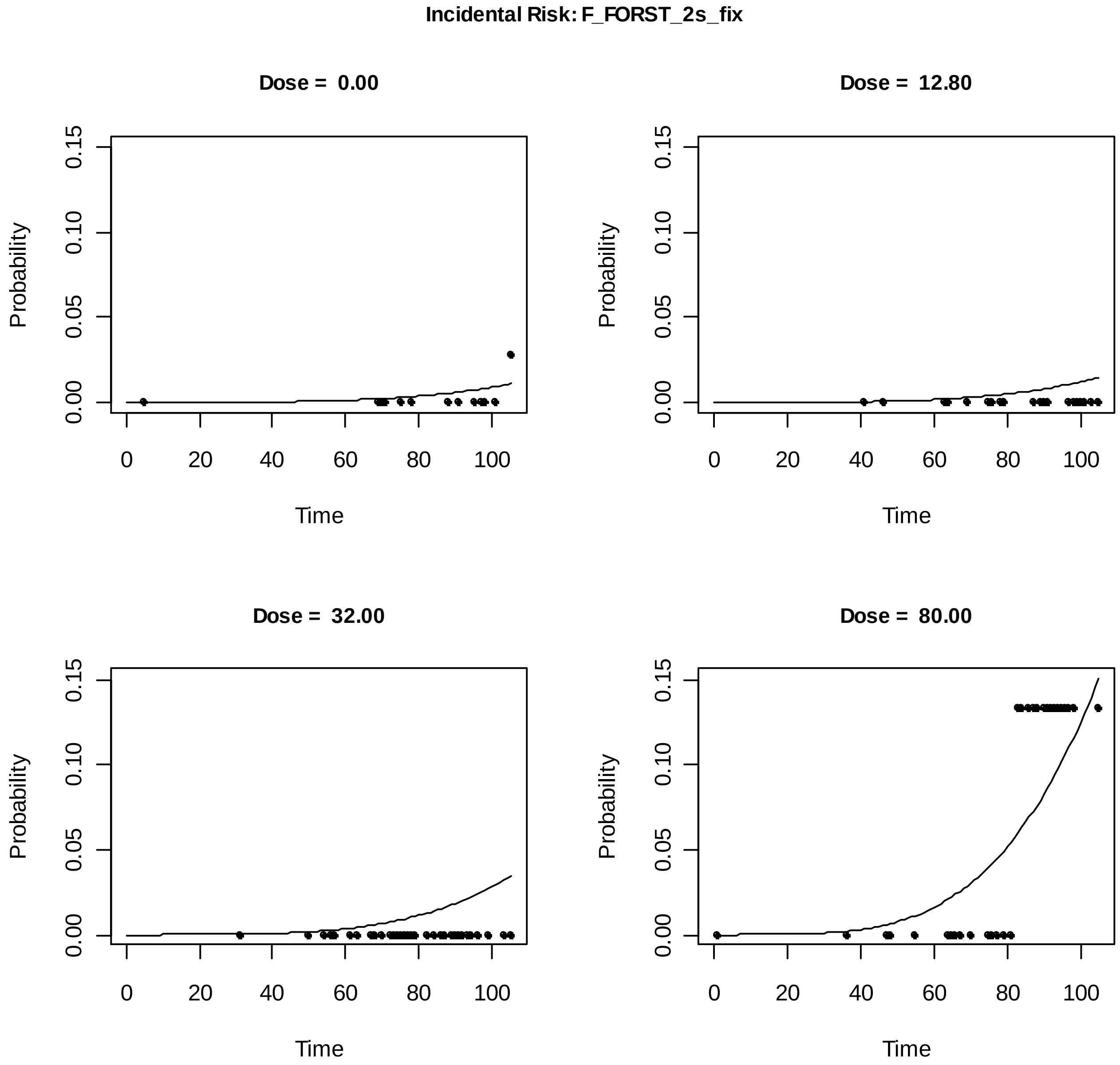


Figure C-6. Female mice, forestomacFigure C-6: Female mice, forestomach tumors. Details below.

```
 ====================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: F_FORST_2s_fix.(d)
 ====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 118
 Total number of records with missing values = 0
 Total number of parameters in model = 5
 Total number of specified parameters = 2
 Degree of polynomial = 2

   User specifies the following parameters:
          c      =     4.1253
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                        c      =      4.12533   Specified
                        t_0    =            0   Specified
                        beta_0 = 5.01708e-011
                        beta_1 =            0
                        beta_2 = 1.09429e-013

          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -c         -t_0       -beta_1
                have been estimated at a boundary point, or have been specified by the
                user,
                and do not appear in the correlation matrix )

                beta_0       beta_2

    beta_0           1        -0.13
    beta_2       -0.13            1

                                Parameter Estimates
                                                      95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         beta_0     5.01701e-011     7.09515e-011       -8.88924e-011        1.89233e-010
         beta_1                0               NA
         beta_2      1.0943e-013     8.36829e-014       -5.45854e-014        2.73445e-013

NA - Indicates that this parameter has hit a
     bound implied by some inequality constraint
     and thus has no standard error.


               Log(likelihood)   # Param           AIC
   Fitted Model        -19.5963         3        45.1926

                   Data Summary
                       CLASS
              C      F      I      U  Total  Expected Response
    DOSE
        0     49      0      1      0     50     0.46
       13     49      0      0      1     50     0.50
       32     49      0      0      0     49     0.68
       80     46      0      4      0     50     3.35
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =         67.812
            BMDL =         46.323
            BMDU =        122.222
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        20.9439
            BMDL =        5.69172
            BMDU =        36.9312
```

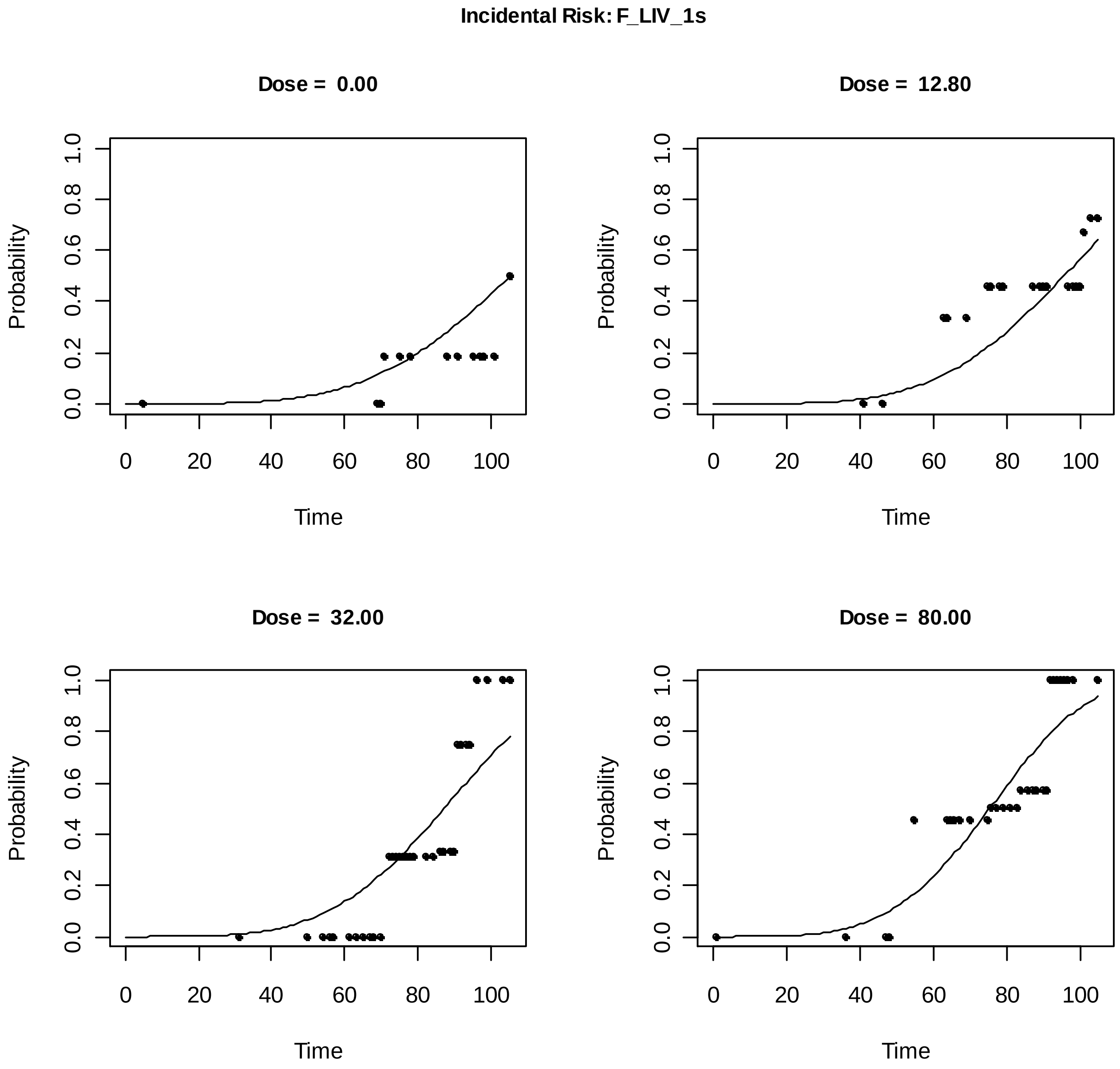


Figure C-7. Female mice, hepatocellular adenomas and carcinomas. Details below.

```
 ====================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: F_LIV_1s.(d)
 ====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 129
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =         4.25
                         t_0    =            0   Specified
                         beta_0 = 1.77794e-009
                         beta_1 = 6.82109e-011


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 c            beta_0       beta_1
    c                 1           -1           -1
    beta_0           -1            1            1
    beta_1           -1            1            1

                                Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        c               4.15974           1.3308            1.55141             6.76806
        beta_0     2.70373e-009     1.67272e-008       -3.00809e-008        3.54884e-008
        beta_1     1.01083e-010      5.8692e-010       -1.04926e-009        1.25142e-009


               Log(likelihood)   # Param            AIC
   Fitted Model        -119.227         3        244.454

                     Data Summary
                         CLASS
              C      F      I      U  Total  Expected Response
    DOSE
        0    30      0     20      0     50    21.63
       13    22      0     26      1     49    22.96
       32    30      0     20      0     50    20.68
       80    20      0     30      0     50    30.79
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
 SPECIFIED EFFECT =           0.1
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =       4.24297
            BMDL =       2.44688
            BMDU =       8.51315
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =       0.404737
            BMDL =       0.233408
            BMDU =       0.818725
```

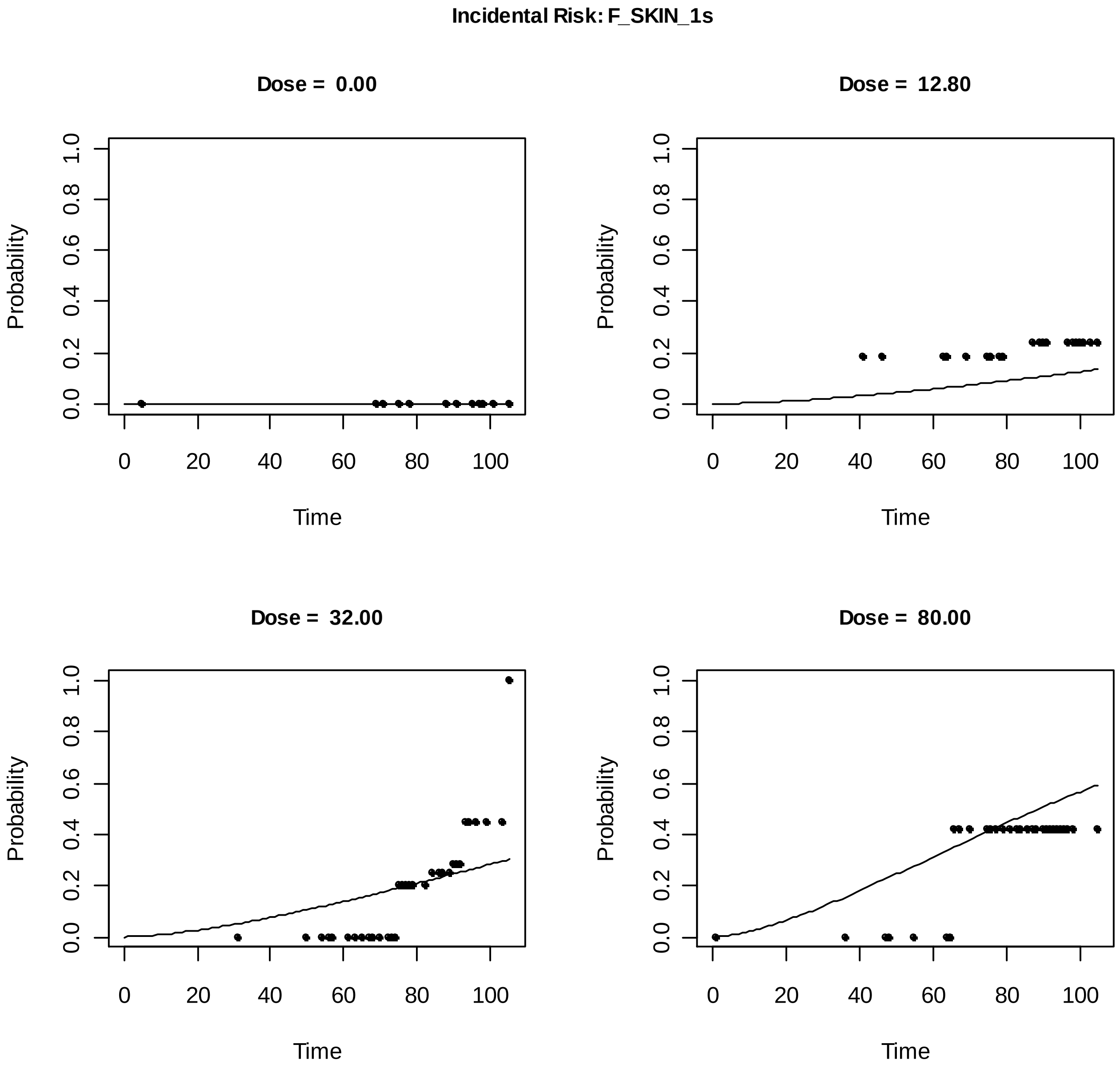


Figure C-8. Female mice, skin sarcomas. Details below.

```
=======================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: F_SKIN.(d)
        Wed Feb 17 15:09:24 2010
 =======================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 121
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =      1.61905
                         t_0    =            0   Specified
                         beta_0 = 4.01488e-023
                         beta_1 = 6.08721e-006

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0
                 have been estimated at a boundary point, or have been specified by the
user,
                 and do not appear in the correlation matrix )

                 c            beta_1
    c                 1           -1
    beta_1           -1            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c              1.56405          1.25364          -0.893041             4.02115
         beta_0               0               NA
         beta_1     7.77467e-006     4.34097e-005       -7.73067e-005        9.28561e-005

NA - Indicates that this parameter has hit a
     bound implied by some inequality constraint
     and thus has no standard error.


                Log(likelihood)   # Param            AIC
   Fitted Model        -87.4625         3         180.925


                    Data Summary
                        CLASS
               C      F      I      U  Total  Expected Response
    DOSE
        0     50      0      0      0     50     0.00
       13     38      0     11      1     50     5.59
       32     39      0     11      0     50    10.58
       80     32      0     18      0     50    22.43
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
 SPECIFIED EFFECT =           0.1
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =       9.48956
           BMDL =         7.1844
            BMDU =        14.5757
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

            BMD =        0.905208
           BMDL =        0.665324
           BMDU =          1.4015
```

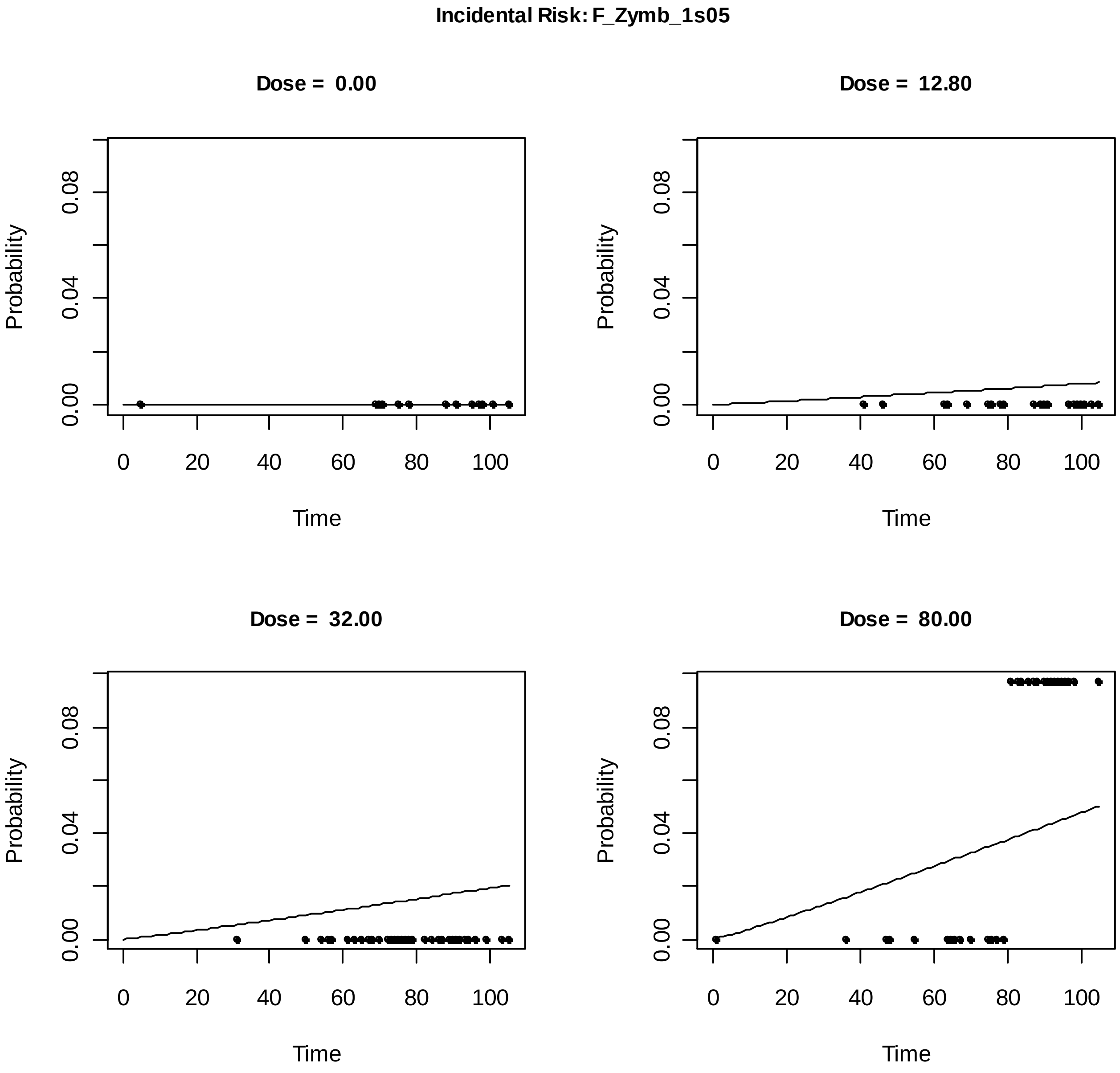


Figure C-9. Female mice, Zymbal's gland tumors. Details below.

```
 ======================================================================= 
         Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009) 
         Solutions are obtained using donlp2-intv, (c) by P. Spellucci
         Input Data File: M:\_chemicals\chloroprene\msw\F_Zymb_1s05.(d)  
 ======================================================================= 

   The form of the probability function is: 
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 119
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1


   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values  
                         c      =      1.09677
                         t_0    =            0   Specified
                         beta_0 = 3.72225e-028
                         beta_1 = 3.90719e-006


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0   
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 c           beta_1
    c               1           -1
    beta_1         -1            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               1.09674         4.17394          -7.08402             9.27751
         beta_0                0              NA
         beta_1     3.90733e-006     7.24422e-005       -0.000138077         0.000145891

NA - Indicates that this parameter has hit a bound implied by some inequality constraint
     and thus has no standard error.


                Log(likelihood)   # Param           AIC
   Fitted Model        -12.6107         3         31.2214


                    Data Summary
                        CLASS
              C      F      I      U  Total  Expected Response
    DOSE
       0     50      0      0      0     50     0.00
      13     50      0      0      0     50     0.36
      32     50      0      0      0     50     0.76
      80     47      0      3      0     50     1.90
```

```
    BENCHMARK DOSE COMPUTATION                        BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL                 RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA                 RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.05                 SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9                 CONFIDENCE LEVEL =            0.9

TIME             =            104                 TIME             =            104

             BMD =        80.5411                              BMD =        15.7811
            BMDL =        22.4657                             BMDL =        5.75828
            BMDU =        255.715                             BMDU =        50.0819
```

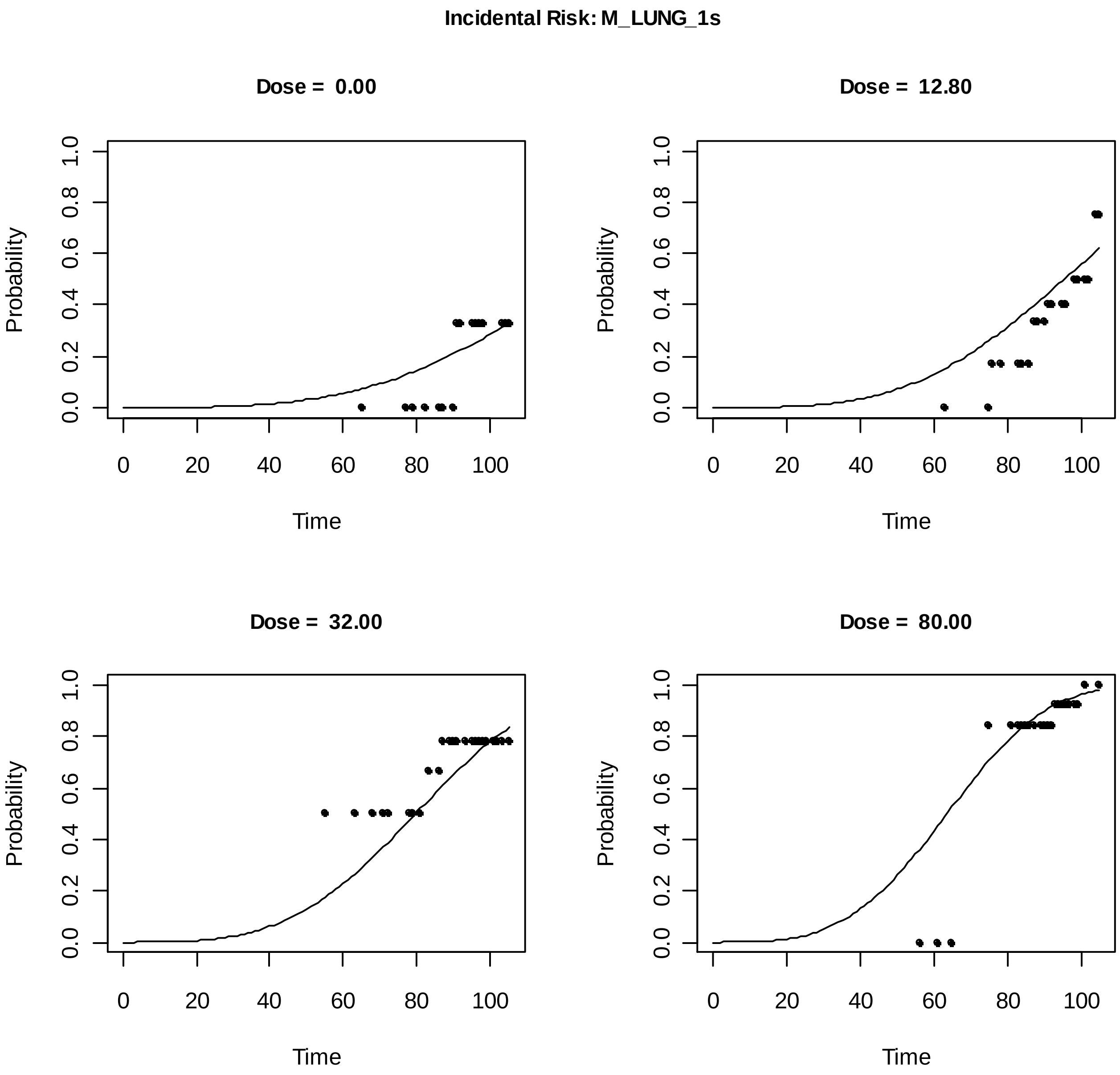


Figure C-10. Male mice, alveolar/bronchiolar tumors. Details below.

```
====================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M:\_chemicals\chloroprene\msw\M_LUNG_1s.(d)
====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 100
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1


   User specifies the following parameters:
          t_0    =         0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =          3.4
                         t_0    =            0   Specified
                         beta_0 =  5.3339e-008
                         beta_1 = 5.89044e-009


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0
                 have been estimated at a boundary point, or have been specified by the
user,
                 and do not appear in the correlation matrix )

                 c           beta_0       beta_1
    c               1           -1           -1
    beta_0         -1            1            1
    beta_1         -1            1            1

                                     Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
          c              3.46155         1.29734           0.918807             6.0043
         beta_0      4.00939e-008     2.41701e-007       -4.33631e-007        5.13819e-007
         beta_1      4.46048e-009     2.61655e-008        -4.6823e-008         5.5744e-008


                Log(likelihood)   # Param            AIC
   Fitted Model        -104.927         3         215.855


                    Data Summary
                        CLASS
              C      F      I      U  Total  Expected Response
    DOSE
       0     37      0     13      0     50    14.02
      13     22      0     28      0     50    26.63
      32     14      0     36      0     50    33.92
      80      7      0     43      0     50    44.57
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1
CONFIDENCE LEVEL =            0.9
TIME             =            104

             BMD =        2.46168
            BMDL =        1.86129
            BMDU =        3.46534
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9
TIME             =            104

             BMD =        0.23482
            BMDL =       0.178411
            BMDU =        0.321837
```

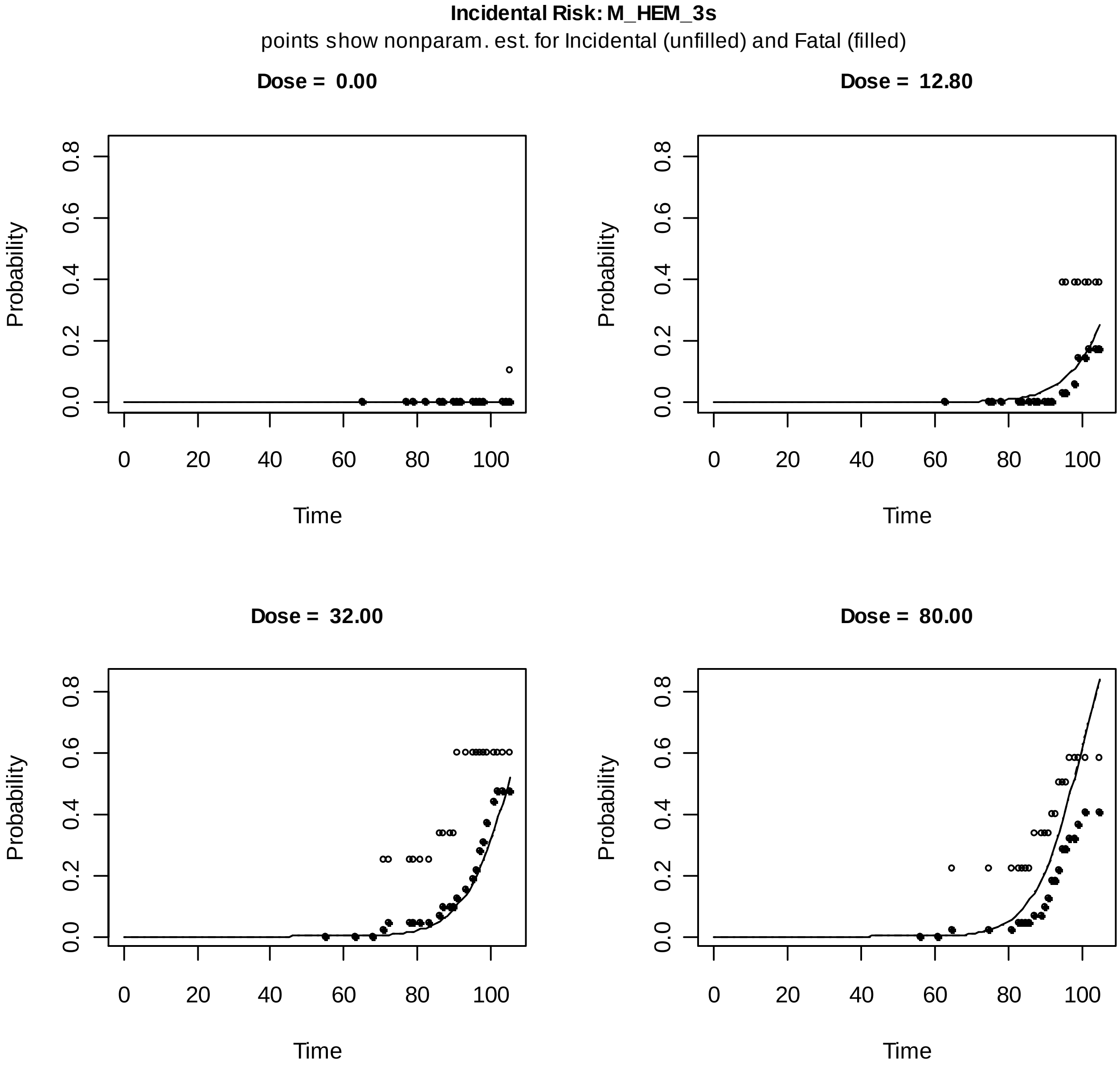


Figure C-11. Male mice, hemangiomas and hemangiosarcomas; hemangiosarcomas occurring before termination considered fatal. Details below.

```
 ====================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M_HEM_3s.(d)
 ====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 103
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         c      =      7.33333
                         t_0    =            0   Specified
                         beta_0 = 2.92735e-016
                         beta_1 = 1.24661e-017
                         beta_2 = 5.74518e-040
                         beta_3 = 1.93026e-021

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_0    -beta_2    -beta_3
                 have been estimated at a boundary point, or have been specified by the
                 user,
                 and do not appear in the correlation matrix )

                 c            beta_1
    c               nan          nan
    beta_1          nan          nan

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               13.2483             nan                 nan                 nan
         beta_0                0              NA
         beta_1     3.78184e-029             nan                 nan                 nan
         beta_2                0              NA
         beta_3                0              NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint
     and thus has no standard error.


                Log(likelihood)   # Param             AIC
   Fitted Model        -537.427         5         1084.85


                    Data Summary
                        CLASS
            C      F      I      U  Total  Expected Response
    DOSE
       0     47      0      3      0     50     0.00
      13     36      6      8      0     50     8.30
      32     27     16      7      0     50    12.19
      80     29     13      8      0     50    20.33

    Minimum observation time for F tumor context =        65
```

```
   BENCHMARK DOSE COMPUTATION                      BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL             RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA             RISK TYPE        =          EXTRA
 SPECIFIED EFFECT =          0.1              SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9             CONFIDENCE LEVEL =            0.9

TIME             =            104             TIME             =            104

            BMD =       2.58208                            BMD =       0.503858
           BMDL =       3.34052                           BMDL =       0.318652
           BMDU =       5.94514                           BMDU =       0.567106
```

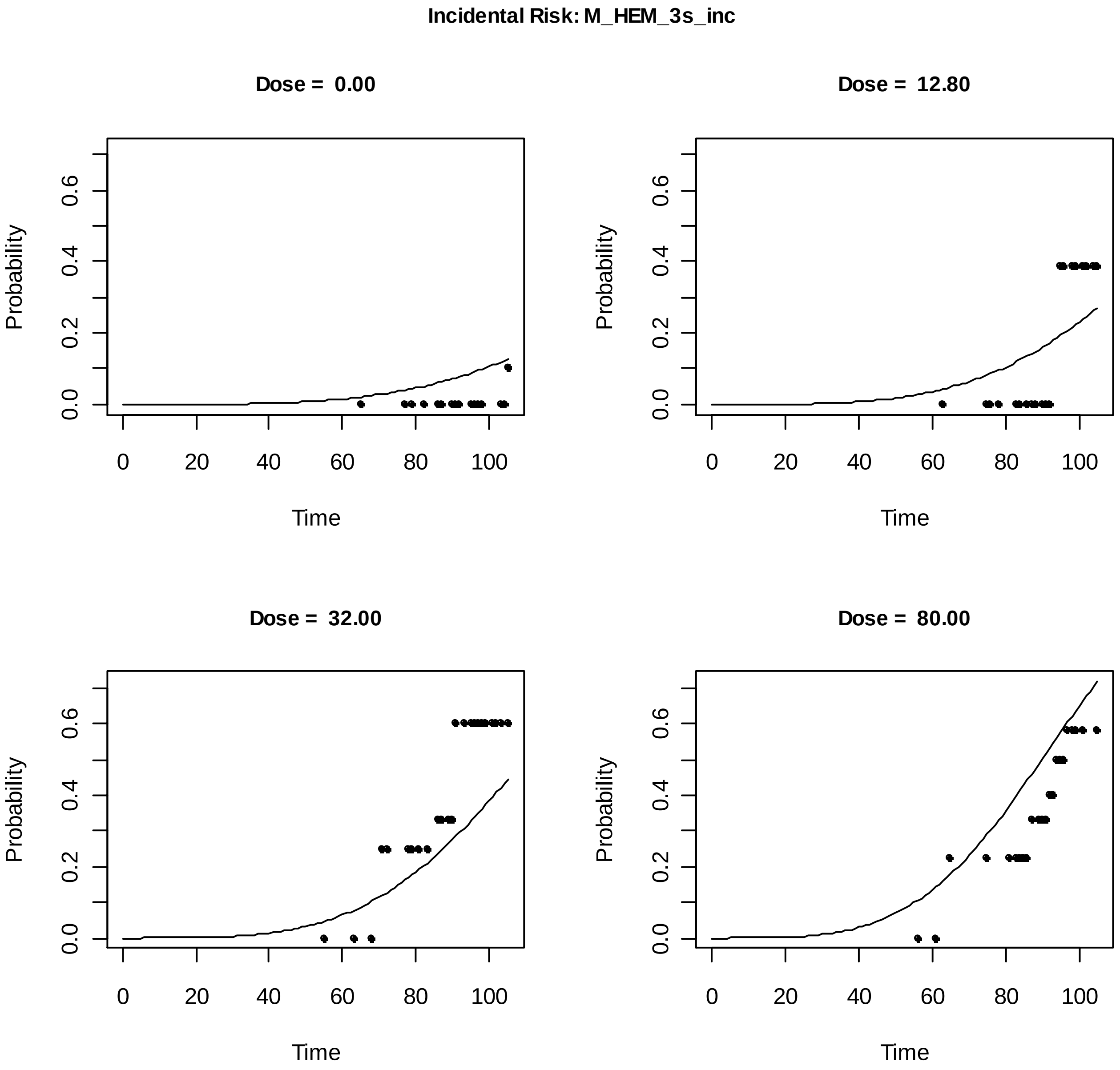


Figure C-12. Male mice, hemangiomas and hemangiosarcomas; all tumors considered incidental. Details below.

```
 ======================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M:\_chemicals\chloroprene\msw\M_HEM_3s_inc.(d)
 ======================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 103
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         c      =      3.88235
                         t_0    =            0   Specified
                         beta_0 = 1.93573e-009
                         beta_1 = 2.00936e-010
                         beta_2 =            0
                         beta_3 =            0

          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -t_0       -beta_2    -beta_3
                have been estimated at a boundary point, or have been specified by the
user,
                and do not appear in the correlation matrix )

                c            beta_0       beta_1
    c                1           -1           -1
    beta_0          -1            1         0.99
    beta_1          -1         0.99            1


                              Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate         Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               3.87398           1.89771          0.154536             7.59343
         beta_0     2.01294e-009      1.78623e-008       -3.29965e-008        3.70224e-008
         beta_1     2.08717e-010      1.80083e-009       -3.32084e-009        3.73828e-009
         beta_2                0                NA
         beta_3                0                NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint
     and thus has no standard error.


                Log(likelihood)   # Param            AIC
   Fitted Model        -109.463         5         228.926


                      Data Summary
                          CLASS
              C      F      I      U  Total  Expected Response
    DOSE
       0     47      0      3      0     50     5.28
      13     36      0     14      0     50    11.12
      32     27      0     23      0     50    15.86
      80     29      0     21      0     50    27.21
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        7.74767
            BMDL =        5.33823
           BMDU =        12.7663
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        0.73905
            BMDL =       0.509228
                BMDU =        1.21647
```

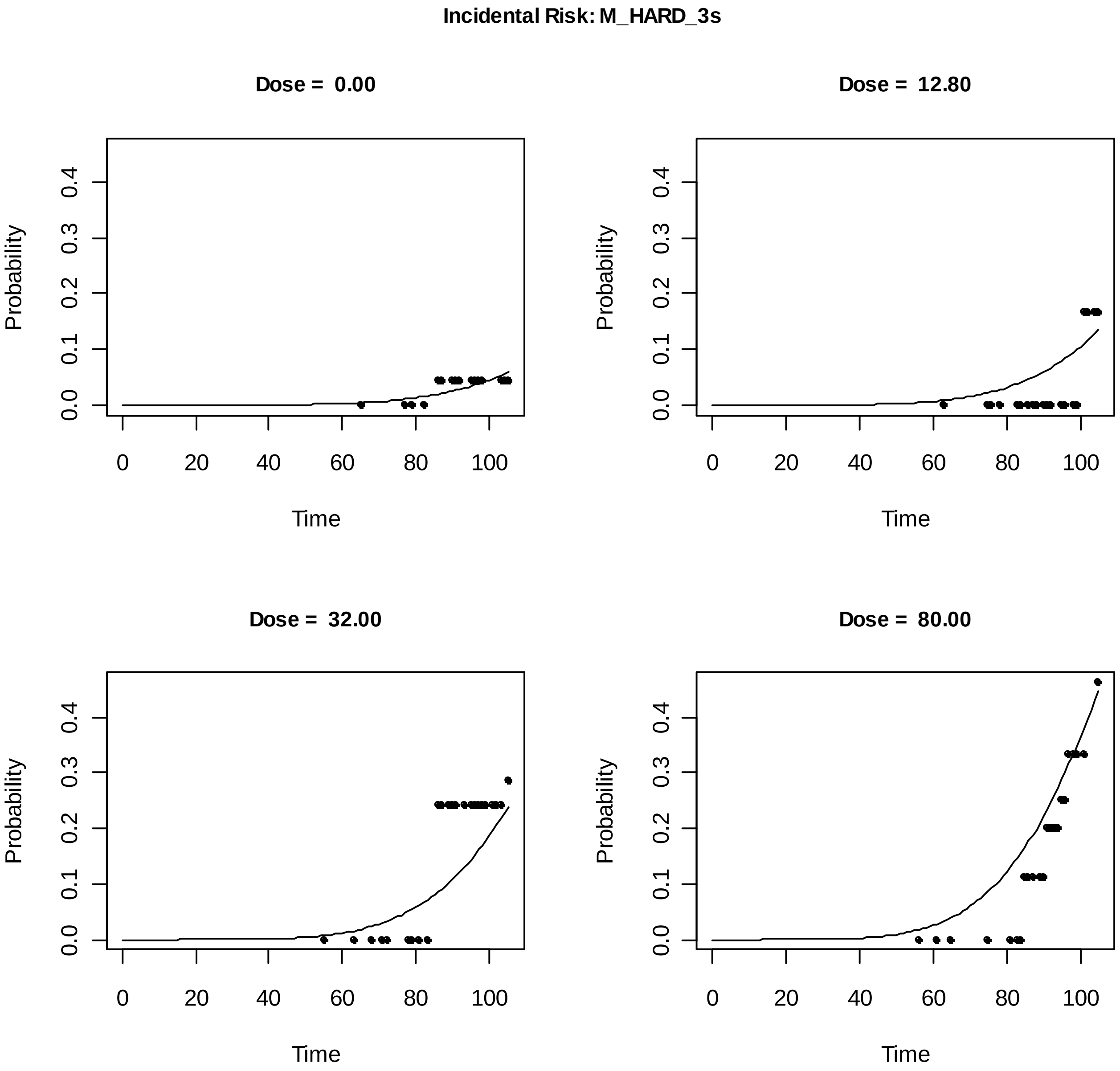


Figure C-13. Male mice, Harderian gland tumors. Details below.

```
 =======================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M_HARD_3s.(d)
        Wed Feb 24 14:48:16 2010
 =======================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 106
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3


   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =         4.25
                         t_0    =            0   Specified
                         beta_0 = 1.53577e-010
                         beta_1 = 1.52041e-011
                         beta_2 =            0
                         beta_3 =            0

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0       -beta_2    -beta_3
                 have been estimated at a boundary point, or have been specified by the
user,
                 and do not appear in the correlation matrix )

                 c            beta_0       beta_1
    c                 1           -1           -1
    beta_0           -1            1            1
    beta_1           -1            1            1

                                 Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               5.57459          3.19215           -0.681904             11.8311
         beta_0     3.25883e-013     4.84471e-012       -9.16957e-012        9.82133e-012
         beta_1       3.598e-014     5.25235e-013       -9.93463e-013        1.06542e-012
         beta_2                0               NA
         beta_3                0               NA

NA - Indicates that this parameter has hit a
     bound implied by some inequality constraint
     and thus has no standard error.


                Log(likelihood)   # Param             AIC
   Fitted Model        -73.6639         5         157.328

                    Data Summary
                        CLASS
               C      F      I      U  Total  Expected Response
    DOSE
        0     48      0      2      0     50     2.29
       13     45      0      5      0     50     5.18
       32     40      0     10      0     50     7.40
       80     38      0     12      0     50    14.04
```

```
   BENCHMARK DOSE COMPUTATION
RISK RESPONSE     =     INCIDENTAL
RISK TYPE         =          EXTRA
 SPECIFIED EFFECT =            0.1
CONFIDENCE LEVEL  =            0.9
TIME              =            104

      BMD =       16.6911
      BMDL =      10.4645
      BMDU =       35.082
```

```
   BENCHMARK DOSE COMPUTATION
RISK RESPONSE     =     INCIDENTAL
RISK TYPE         =          EXTRA
SPECIFIED EFFECT  =           0.01
CONFIDENCE LEVEL  =            0.9
TIME              =            104

      BMD =       1.59216
      BMDL =     0.998471
      BMDU =      5.03875
```

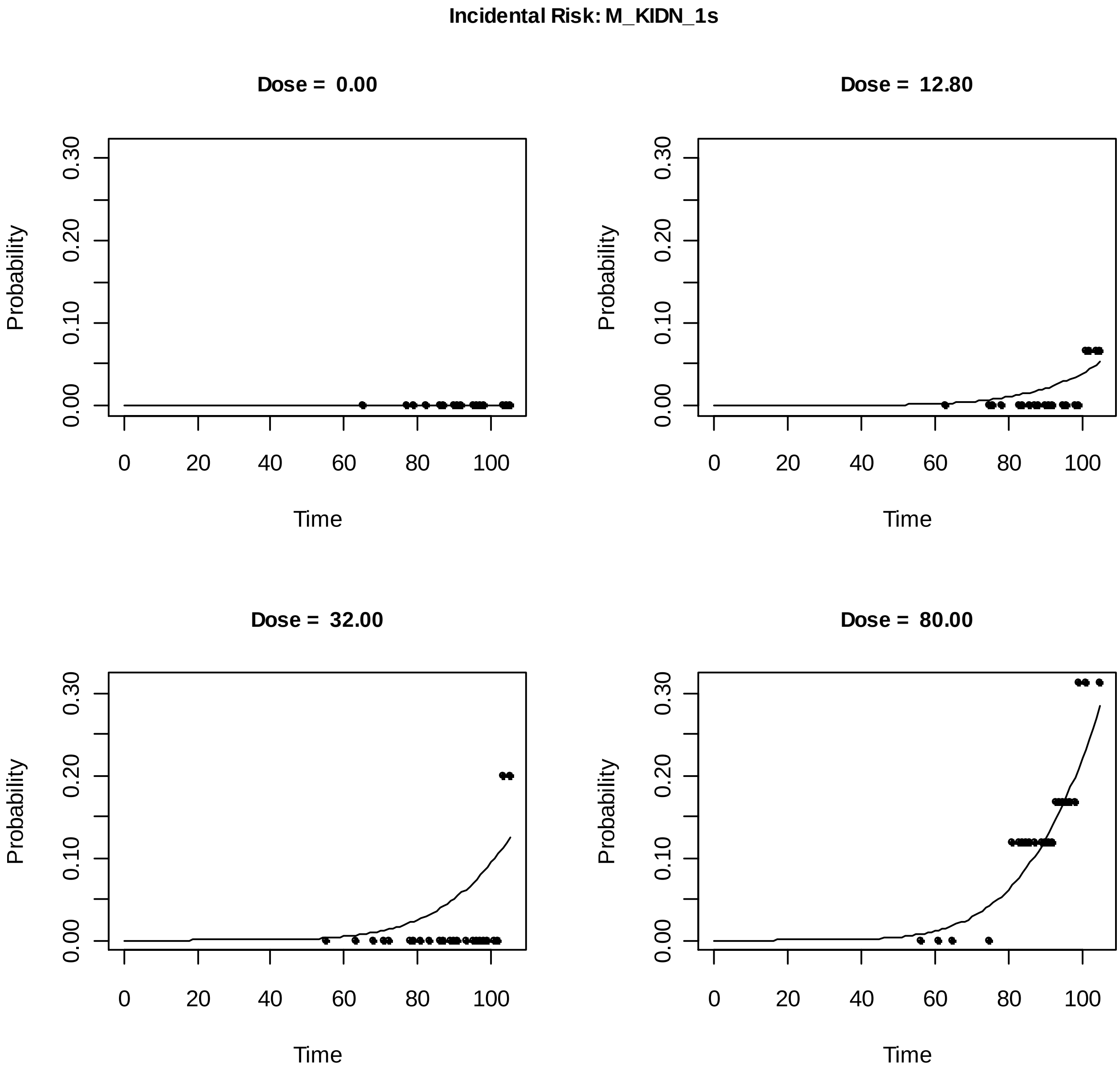


Figure C-14. Male mice, renal tubule tumors. Details below.

```
 ======================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M:\_chemicals\chloroprene\msw\M_KIDN_1s.(d)
        Tue May 11 10:57:53 2010
 ======================================================================

 title = Chloroprene: Male mice, kidney adenomas, source = NTP 1998, chemical =
CHLOROPRENE, mol.wgt = 88.5, route = AIR  (ppm), expt.length = 104, life.length = 104,
dose.avg.factor = 1

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 106
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                         c      =      4.85714
                         t_0    =            0   Specified
                         beta_0 =            0
                         beta_1 = 5.87389e-013

          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -t_0       -beta_0
                have been estimated at a boundary point, or have been specified by the
user,
                and do not appear in the correlation matrix )

                c            beta_1
    c                1           -1
    beta_1          -1            1

                                Parameter Estimates
                                                         95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c               6.09231          4.64814          -3.01787            15.2025
         beta_0                0               NA
         beta_1     2.03124e-015      4.3366e-014       -8.29646e-014        8.70271e-014

NA - Indicates that this parameter has hit a
     bound implied by some inequality constraint
     and thus has no standard error.


               Log(likelihood)   # Param           AIC
   Fitted Model        -41.0033         3         88.0066

                   Data Summary
                       CLASS
             C      F      I      U  Total  Expected Response
    DOSE
       0     50     0      0      0     50     0.00
      13     47     0      2      1     50     1.95
      32     47     0      3      0     50     3.70
      80     41     0      9      0     50     8.34
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =            0.1
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        26.7011
            BMDL =        16.4536
            BMDU =        47.1278
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        2.54702
            BMDL =        1.56959
            BMDU =        4.49547
```

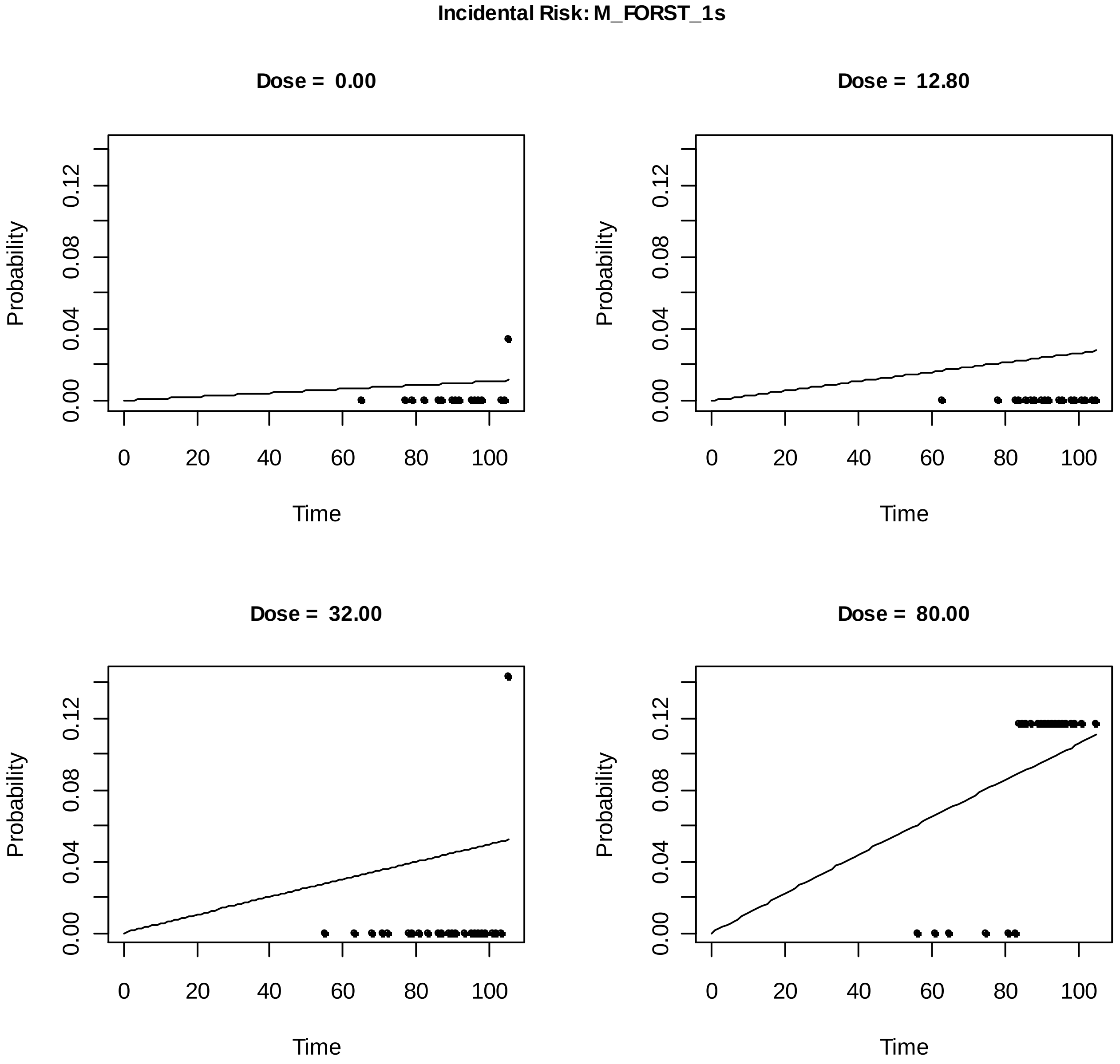


Figure C-15. Male mice, forestomach tumors. Details below.

```
 ====================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: M:\_chemicals\chloroprene\msw\M_FORST_1s.(d)
 ====================================================================

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 106
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =         0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         c      =         1.36
                         t_0    =            0   Specified
                         beta_0 = 2.11777e-005
                         beta_1 = 2.06761e-006


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0
                 have been estimated at a boundary point, or have been specified by the
user,
                 and do not appear in the correlation matrix )

                 c          beta_0       beta_1
    c            1             -1           -1
    beta_0      -1              1            1
    beta_1      -1              1            1


                                Parameter Estimates
                                                       95.0% Wald Confidence Interval
       Variable         Estimate         Std. Err.     Lower Conf. Limit   Upper Conf. Limit
         c                1.2938           4.09082           -6.72406             9.31165
         beta_0      2.8702e-005       0.000540721        -0.00103109           0.0010885
         beta_1     2.79278e-006      5.19088e-005       -9.89466e-005         0.000104532


                Log(likelihood)   # Param           AIC
   Fitted Model        -30.8413         3         67.6827


                    Data Summary
                        CLASS
              C      F      I      U  Total  Expected Response
    DOSE
       0     49      0      1      0     50     0.54
      13     48      0      0      2     50     1.20
      32     47      0      2      1     50     2.03
      80     45      0      5      0     50     4.24
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.05
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        45.1225
            BMDL =        22.7599
            BMDU =        157.031
```

```
     BENCHMARK DOSE COMPUTATION
RISK RESPONSE    =     INCIDENTAL
RISK TYPE        =          EXTRA
SPECIFIED EFFECT =           0.01
CONFIDENCE LEVEL =            0.9

TIME             =            104

             BMD =        8.84123
            BMDL =        4.46001
            BMDU =        30.7684
```

Table C-4. Summary of human equivalent composite cancer risk values estimated by R/BMDR, based on male and female mouse tumor incidence (NTP, 1998)

Tumor Site	BMDL$_{01}$ (ppm)	BMD$_{01}$ (ppm)	Risk value[a] at:		sd	sd^2	Proportion of total variance	
			BMD$_{01}$ (/ppm)	BMDL$_{01}$ (/ppm)				
Female mice								
Lung (systemic dosimetry)	**8.65E-02[b]**	**1.14E-01**	**8.76E-02**	**1.16E-01**	**1.70E-02**	**2.88E-04**	**0.60**	
Lung (portal-of-entry dosimetry)	3.57E-01	4.68E-01	2.14E-02	2.80E-02	4.04E-03	1.63E-05		0.08
Hemangiomas, hemangiosarcomas (fatal)	6.41E-01	3.12E+00	3.20E-03	1.56E-02	7.54E-03	5.68E-05	**0.12**	0.27
Harderian gland	1.20E+00	2.58E+00	3.87E-03	8.31E-03	2.70E-03	7.28E-06	**0.02**	0.03
Mammary gland adenomas, carcinomas, adenacanthomas	1.34E+00	1.95E+00	5.13E-03	7.46E-03	1.41E-03	1.99E-06	**0.00**	0.01
Forestomach	5.69E+00	2.09E+01	4.77E-04	1.76E-03	7.78E-04	6.05E-07	**0.00**	0.00
Hepatocellular adenomas, carcinomas	2.33E-01	4.05E-01	2.47E-02	4.28E-02	1.10E-02	1.22E-04	**0.25**	0.58
Skin	6.65E-01	9.05E-01	1.10E-02	1.50E-02	2.42E-03	5.86E-06	**0.01**	0.03
Zymbal's gland	5.76E+00	1.58E+01	6.34E-04	1.74E-03	6.70E-04	4.50E-07	**0.00**	0.00
Sum, risk values at BMD$_{01}$ (/ppm):			**1.367E-01** 7.043E-02		Sum, sd^2:	**4.829E-04** 2.109E-04		
Human equivalent sum of risk values (/(μg/m^3))[c]:			**2.122E-04** 1.093E-04		Composite sd[d]:	**2.198E-02** 1.452E-02		
Upper bound on sum of risk estimates (/ppm)[e]:				**1.729E-01** 9.432E-02				
Human equivalent upper bound on sum of risk estimates (/(μg/m^3)):				**2.683E-04** 1.464E-04				
Male mice								
Lung (systemic dosimetry)	**1.78E-01**	**2.35E-01**	**5.61E-02**	**4.26E-02**	**8.19E-03**	**6.70E-05**	**0.54**	
lung (portal-of-entry)	7.31E-01	9.63E-01	1.37E-02	1.04E-02	2.00E-03	3.99E-06		0.07
Hemangiomas, hemangiosarcomas (fatal)	3.19E-01	5.04E-01	3.14E-02	1.98E-02	7.01E-03	4.92E-05	**0.40**	0.81
Forestomach	4.46E+00	8.84E+00	2.24E-03	1.13E-03	6.75E-04	4.56E-07	**0.00**	0.01
Harderian gland	9.98E-01	1.59E+00	1.00E-02	6.28E-03	2.27E-03	5.15E-06	**0.04**	0.08
Kidney	1.57E+00	2.55E+00	6.37E-03	3.93E-03	1.49E-03	2.21E-06	**0.02**	0.04
Sum, risk values at BMD$_{01}$ (/ppm):			**7.377E-02** 4.157E-02		Sum, sd^2:	**1.240E-04** 6.098E-05		
Human equivalent sum of risk values (/(μg/m^3)):			**1.145E-04** 6.452E-05		Copmposite sd:	**1.114E-02** 7.809E-03		
Upper bound on sum of risk estimates (/ppm):				**9.209E-02** 5.442E-02				
Human equivalent upper bound on sum of risk estimates (/(μg/m^3)):				**1.429E-04** 8.445E-05				

[a] Risk value=0.01/POD

[b] Summary statistics in bold 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.

[c] Human equivalent estimates were adjusted for continuous exposure and converted to μg/m^3, by dividing by 6/24 (hours) and 3.62 mg/m^3.

[d] Composite SD = (Sum, SD^2)$^{0.5}$

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