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TOXICOLOGICAL REVIEW

OF

HEXACHLOROETHANE

(CAS No. 67-72-1)

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

March 2010

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike's Information Criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosome aberrations
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary
CL	confidence limits
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
$FEV_{1.0}$	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transpeptidase, γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
$H_{b/g-A}$	animal blood:gas partition coefficient
$H_{b/g-H}$	human blood:gas partition coefficient
HCE	hexachloroethane
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocytes
MTD	maximum tolerated dose
NAG	*N*-acetyl-β-D-glucosaminidase
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program

OCT	ornithine carbamoyl transferase
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PCBs	polychlorinated biphenyls
PCNA	proliferating cell nuclear antigen
PERC	tetrachloroethene, tetrachloroethylene, perchloroethylene
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
QSAR	quantitative structure-activity relationship
R&D	research and development
RDS	replicative DNA synthesis
REL	reference exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
S9	supernatant fraction from 9000 × g centrifugal spin
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TCVC	S-1,2,2-trichlorovinyl-L-cysteine
TCVG	S-1,2,2-trichlorovinyl glutathione
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
U.S. EPA	United States Environmental Protection Agency

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

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/AUTHOR

John Cowden, Ph.D.
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

AUTHORS

Samantha Jones, Ph.D.
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

CONTRIBUTORS

Ted Berner, M.S.
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Glinda Cooper, Ph.D.
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Andrew A. Rooney, Ph.D.
Currently at National Toxicology Program
Center for the Evaluation of Risks to Human Reproduction
National Institute of Environmental Health Sciences
Research Triangle Park, NC

CONTRACTOR SUPPORT

James Kim, Ph.D.
Sciences International, Inc.
Alexandria, VA

REVIEWERS

This document has been provided for review to EPA scientists and interagency reviewers from other federal agencies and White House offices.

INTERNAL EPA REVIEWERS

Ambuja Bale, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Ghazi Dannan, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

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

Maureen Gwinn, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

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

Channa Keshava, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Allan Marcus, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

D. Charles Thompson, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

John Whalan
National Center for Environmental Assessment
Office of Research and Development

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

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

The literature search strategy employed for this compound was based on the 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 February 2010.

2. CHEMICAL AND PHYSICAL INFORMATION

Hexachloroethane (HCE) (CASRN 67-72-1) is a halogenated hydrocarbon consisting of six chlorines attached to an ethane backbone (Figure 2-1). Synonyms include 1,1,1,2,2,2-hexachloroethane, ethane hexachloride, ethylene hexachloride, perchloroethane, carbon hexachloride, and carbon trichloride (ChemIDplus Advanced, 2005; ACGIH, 1991). Certain physical and chemical properties are shown below in Table 2-1 (ACGIH, 2001; ATSDR, 1997a; Budavari, 1989; Howard, 1989; Weast, 1986; Spanggord et al., 1985; Verschueren, 1983; U.S. EPA, 1982, 1979).

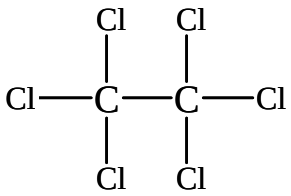


Figure 2-1. Structure of HCE.

Table 2-1. Physical properties of HCE.

Name	HCE
CASRN	67-72-1
Synonyms	1,1,1,2,2,2-hexachloroethane, ethane hexachloride, ethylene hexachloride, perchloroethane, carbon hexachloride, carbon trichloride
Molecular weight	236.74 g/mol
Molecular formula	C_2Cl_6
Melting point	Sublimes without melting
Boiling Point	186.8°C
Density	2.091 g/mL at 20°C
Water solubility[a]	50 mg/L at 22°C; 14 mg/L at 25°C
Log K_{ow}	3.82[a], 3.34[b], 4.14[c]
Log K_{oc}	4.3
Vapor pressure	0.5 mmHg at 20°C; 1.0 mmHg at 32.7°C
Henry's law constant	2.8×10^{-3} atm-m^3/mol at 20°C
Conversion factor	1 ppm = 9.68 mg/m^3; 1 mg/m^3 = 0.10 ppm

Sources: [a]Howard, 1989; [b]U.S. EPA, 1979; [c]Hansch et al., 1995

HCE was produced in the United States (U.S.) for commercial distribution from 1921 to 1967 but is currently not commercially distributed (ATSDR, 1997a; IARC, 1979). In the 1970s, producers of HCE reported that HCE was not distributed, but used in-house or recycled (ATSDR, 1997a); distributors in the 1970s imported HCE from France, Spain and the United Kingdom (ACGIH 2001; ATSDR, 1997a). HCE and tetrachloroethane imports combined were 1.5 million pounds in 1989 and 612,000 pounds in 2000 (NTP, 2005). HCE production in 1977

was between 2 and 20 million pounds; more recent information on production of HCE was not located (NTP, 2005; ATSDR, 1997a). HCE is produced by the chlorination of tetrachloroethylene (PERC) in the presence of ferric chloride at temperatures of 100–140°C (ATSDR, 1997a; U.S. EPA, 1991b; Fishbein, 1979; IARC, 1979). HCE is primarily used in the military for smoke pots, smoke grenades, and pyrotechnic devices (ACGIH, 2001; ATSDR, 1997; U.S. EPA, 1991b; IARC, 1979). HCE was also identified in the headspace of chlorine-bleach-containing household products (Odabasi, 2008). In the past, HCE was used as an antihelminthic for the treatment of sheep flukes but is no longer used for this purpose since the Food and Drug Administration withdrew approval for this use in 1971 (ATSDR, 1997a). HCE has also been used as a polymer additive, moth repellant, plasticizer for cellulose esters, insecticide solvent, and in metallurgy for refining aluminum alloys (ATSDR, 1997a; U.S. EPA, 1991b).

3. TOXICOKINETICS

3.1. ABSORPTION

There are no studies that have systematically evaluated HCE absorption in humans by the oral or inhalation routes of exposure. However, uptake was demonstrated by Younglai et al. (2002) when HCE was identified in follicular fluid during an analysis for environmental contaminants in 21 couples undergoing in vitro fertilization (IVF). These data identify the potential for HCE absorption but not the source or route of exposure. No studies have been reported that assess the inhalation absorption of HCE in humans. The dermal absorption rate of HCE has been described as limited (ATSDR, 1997a). Based on physical properties, the absorption of a saturated HCE solution across human skin was estimated to be 0.023 mg/cm^2/hour (Fiserova-Bergerova et al., 1990).

Studies in animals via the oral route of exposure demonstrated that HCE is absorbed and primarily distributed to fat (Gorzinski et al., 1985; Nolan and Karbowski, 1978; Fowler, 1969). Fowler (1969) orally administered 500 mg/kg HCE to Scottish Blackface or Cheviot sheep and found maximal venous blood concentrations of HCE (10-28 μg/mL) were reached at 24 hours after HCE exposure, indicating slow absorption. Jondorf et al. (1957) reported that rabbits fed [^{14}C]-radiolabeled HCE at 500 mg/kg excreted only 5% of the applied radioactivity in urine over a period of 3 days (fecal measurements were not conducted). During this 3-day period, 14–24% of the applied radioactivity was detected in expired air, and the remainder was present in the tissues and intestinal tract. The amount of HCE absorbed by the rabbits was not determined; however, based on the amount of radioactivity present in urine and expired air, approximately 19–29% of the HCE was absorbed. Studies in rats and mice (Mitoma et al., 1985) using [^{14}C]-radiolabeled HCE (500 mg/kg for rats; 1,000 mg/kg for mice) administered orally, via corn oil, indicated that the amount absorbed was 65–71% and 72–88%, respectively, based on the amount of radiolabel detected in expired air and excreta.

3.2. DISTRIBUTION

There are limited data on the distribution of HCE in humans (Younglai et al., 2002). The animal studies evaluated (Gorzinski et al., 1985; Nolan and Karbowski, 1978; Fowler, 1969) consistently demonstrated that HCE is distributed primarily to fat tissue followed by the kidney and to a lesser extent the liver and the blood (Gorzinski et al., 1985; Nolan and Karbowski, 1978).

Younglai et al. (2002) evaluated the concentrations of various environmental contaminants in follicular fluid, serum, and seminal plasma of 21 couples undergoing IVF. HCE was one of the contaminants identified in >50% of follicular fluid samples, suggesting post-absorptive distribution to reproductive organs. The average HCE concentration in follicular

fluid was 232 ± 27 pg/mL (mean ± standard error [SE]). HCE was not detected in human female serum obtained during oocyte retrieval for IVF. This study focused primarily on chemicals such as pesticides and polychlorinated biphenyls (PCBs), and the authors could not make any conclusions with regards to the level of HCE in follicular fluid and its effect on fertility.

Fowler (1969) evaluated the tissue distribution of HCE in sheep. Two sheep were fasted for 24 hours and then anesthetized with pentobarbitone sodium. An HCE solution (15% w/v in olive oil) was injected for a total dose of 500 mg/kg directly into the rumen and lower duodenum (dose was divided). Anesthesia was maintained for 8.5 hours, after which time the sheep were sacrificed and tissues were taken within 10 minutes of death. Tissues that were evaluated for HCE include the brain, fat, kidney, liver and muscle. Bile and blood were also evaluated. HCE was widely distributed and the highest levels were found in fat of one sheep. Fat from different sites did not show significant variation in HCE concentration. The second sheep had only trace amounts of HCE in tissue (see Table 3-1).

Table 3-1. HCE, PERC, and pentachloroethane tissue concentrations in anesthetized sheep 8.5 hours after injection of 500 mg/kg HCE

	Concentrations (μg/g)					
	Sheep 1			Sheep 2		
Tissue	**HCE**	**PERC**	**Pentachloroethane**	**HCE**	**PERC**	**Pentachloroethane**
Bile (4 hr)	1.7	0.3	Trace	2.2	0.5	Nil
Blood (6 hr)	0.2	0.4	Trace	0.2	0.2	Nil
Brain	0.2	0.9	0.02	Trace	Trace	Trace
Fat	1.1	2.1	0.02	Trace	0.6	Nil
Kidney	0.1	1.2	Trace	Trace	0.6	Trace
Liver	0.2	0.9	0.01	Trace	2.8	Trace
Muscle	0.04	0.5	0.01	Trace	Trace	Trace

Source: Fowler (1969).

Nolan and Karbowski (1978) studied tissue clearance of HCE in rats. Male F344 rats were placed on an HCE-containing diet that was calculated to deliver 100 mg/kg-day (later determined to be 62 mg/kg-day by Gorzinski et al., 1985) for 57 days. After this exposure period, the rats were returned to a HCE-free control diet and sacrificed (groups of 3 or 4 rats) 0, 3, 6, 13, 22, and 31 days after this change in exposure. Samples of fat, liver, kidney, and whole blood were collected for HCE analysis. The time-course related tissue HCE concentrations are presented in Table 3-2. The highest tissue concentrations of HCE were in fat, which were 3-fold greater than the concentration in the kidney and over 100-fold greater than blood and liver concentrations. Fat concentrations decreased from 303 ± 50 μg/g in a first-order manner with a half-life of 2.7 days. Concentrations in blood and kidney also decreased in a first-order manner with half-lives of 2.5 and 2.6 days, respectively. Liver concentrations initially increased in the first 3 days postexposure, but began to decrease by day 6. The half-life for liver HCE was 2.3

days (calculated after peak levels were reached at day 3). These same results were published in a follow-up study by Gorzinski et al. (1985) that included a toxicity assessment.

Table 3-2. Time course of HCE concentrations in male rat tissues after 57 days of dietary exposure to 62 mg/kg-day

	HCE tissue concentrations (n = 3 or 4) (mean ± SD µg/g tissue)			
Days after cessation of HCE exposure	**Blood**	**Liver**	**Kidney**	**Fat**
0	0.834 ± 0.223	0.143 ± 0.040	81.8 ± 5.3	303 ± 50
3	0.279 ± 0.048	0.399 ± 0.188	41.0 ± 1.4	107.8 ± 10.5
6	0.0835 ± 0.006[a]	0.303 ± 0.156[a]	18.5[b]	62.45 ± 3.04[a]
13	0.015 ± 0.005	0.039 ± 0.023	2.53 ± 1.02	6.56 ± 0.52
22	0.002 ± 0.001	0.001 ± 0.001	0.194 ± 0.171	0.472 ± 0.232
31	ND[c]	ND[c]	0.026 ± 0.006	0.125 ± 0.020

[a]Values from one of the three rats was consistently low and not used to obtain the mean ± SD.
[b]One sample was lost and a mean ± SD could not be calculated.
[c]ND: not detected (detection limit of 0.001 µg/g)

Sources: Gorzinski et al. (1985); Nolan and Karbowski (1978).

Nolan and Karbowski (1978) also evaluated tissue concentrations of HCE in both male and female rats after an exposure period of 110–111 days (16 weeks) to doses of 3, 30, and 100 mg/kg-day via the diet. The actual doses were approximated as 1, 15, and 62 mg/kg-day after factoring in volatility of the test material from the food and based on linear nighttime food consumption rates (Gorzinski et al., 1985). The tissue concentrations are presented in Table 3-3. Kidney concentrations of HCE were much higher in male rats compared with female rats, particularly at the highest dose (47-fold greater in males) (Nolan and Karbowski, 1978). Kidney concentrations of HCE proportionately increased with the doses in males, whereas the increase in females was dose-dependent but not proportionate. The authors noted that the HCE kidney concentrations and kidney toxicity were consistently different for the male and female rats. Consequently, they speculated that the male rats would be 10-30 times more sensitive than female rats to HCE toxicity, based on the relative HCE concentration measured in the rat kidney (assuming that toxicity is due to HCE and not a metabolite). Both sexes exhibited comparable levels (although levels in males were slightly greater) of HCE in blood, liver and fat; concentrations in fat were the highest for both sexes. Blood levels of HCE did not correlate well to either the exposure dose or the dose at the major target organ, the kidney, indicating that blood levels of HCE may not be a suitable metric for the estimation of exposure to HCE in rats.

Table 3-3. HCE concentrations in male and female rat tissues after 110 or 111 days of dietary exposure

Dose (mg/kg-day)		HCE tissue concentration (n = 3 or 4) (mean ± SD, µg/g tissue)			
		Blood	Liver	Kidney	Fat
1	Male	0.079 ± 0.057	0.291 ± 0.213	1.356 ± 0.286	3.09 ± 0.33
	Female	0.067 ± 0.039	0.260 ± 0.035	0.369 ± 0.505	2.59 ± 0.72
15	Male	0.596 ± 0.653	1.736 ± 1.100	24.33 ± 5.73	37.90 ± 6.10
	Female	0.162 ± 0.049	0.472 ± 0.204	0.688 ± 0.165	45.27 ± 11.33
62	Male	0.742 ± 0.111	0.713 ± 0.343	95.12 ± 11.56	176.1 ± 14.5
	Female	0.613 ± 0.231	0.631 ± 0.262	2.01 ± 0.66	162.1 ± 7.1

Sources: Gorzinski et al. (1985); Nolan and Karbowski (1978).

3.3. METABOLISM

In vitro studies using liver microsomes indicated that the major enzymes involved in HCE metabolism are phenobarbital-inducible cytochrome P450 (CYP450) isoforms (Salmon et al., 1985; Town and Leibman, 1984; Nastainczyk et al., 1982; Nastainczyk et al., 1981; Salmon et al. 1981); however, no specific (phenobarbital-inducible) isoforms have been identified. The isoforms induced by phenobarbital include those from the 2A, 2B, 2C, and 3A subfamilies. One study (Yanagita et al., 1997) found some evidence for CYP1A2 involvement in the metabolism of HCE, although this was not supported by the results from in vitro studies with 3-methylcholanthrene, an inducer of the CYP450 1 subfamily (Nastainczyk et al., 1982; Nastainczyk et al., 1981; Van Dyke and Wineman, 1971). Information regarding the roles of aroclor 1254-inducible enzymes other than 1A2 (including CYP 2A6, 2E1, 2C9, 2C19, 2D6, and 3A4) is not available for HCE.

The metabolism data for HCE are limited because there are only three in vivo studies available that provide information on metabolites: Mitoma et al. (1985) in rats and mice, Jondorf et al. (1957) in rabbits, and Fowler (1969) in sheep. Each of these studies tends to support limited metabolism for HCE. The data from the in vivo and in vitro studies support a conclusion that metabolism of HCE is incomplete, with excretion of unmetabolized HCE in exhaled air and possibly in urine. A variety of intermediary metabolites have also been identified in exhaled air and urine (Fowler, 1969; Jondorf et al., 1957). Figure 3-1 provides a possible metabolic pathway for HCE derived from the in vivo and in vitro data with ordering of metabolites based on sequential dechlorination and oxidation state. The HCE metabolism information was supplemented with data on the metabolism of the PERC (ATSDR, 1997b), trichloroethylene (TCE; ATSDR, 1997c), and 1,1,2,2-tetrachloroethane (ATSDR, 2008) intermediary metabolites.

Mitoma et al. (1985) examined the distribution of HCE in male Osborne-Mendel rats and male B6C3F_1 mice to evaluate the extent to which radiolabeled compound is metabolized in the 48 hours after administration of 125 or 500 mg/kg to the rats and 250 or 1,000 mg/kg to the mice. These doses were selected based on the maximum tolerated dose (MTD) and ¼ MTD of HCE; the MTD in rats and mice is 500 mg/kg (2.11 mmol/kg) and 1,000 mg/kg (4.22 mmol/kg),

respectively. The animals, 4/dose, were orally administered unlabeled HCE as a solution in corn oil 5 days/week for 4 weeks, followed by a single dose of [^{14}C]-radiolabeled HCE. The 48 hour observation period began after administration of the radiolabeled HCE. The animals were then sacrificed, and urine and feces were collected from the cages. Table 3-4 summarizes the metabolic disposition data (based on the detection of radiolabel) at the high dose in rats and mice. The comparable data for the lower doses were not reported.

Table 3-4. Disposition of HCE in male rats and mice during 48 hours following administration of an MTD for 4 weeks

	Rat (500 mg/kg-day)	Mouse (1,000 mg/kg-day)
	Percent of administered dose	
Expired air	64.55 ± 6.67	71.51 ± 5.09
CO_2	2.37 ± 0.76	1.84 ± 0.94
Excreta	6.33 ± 2.39	16.21 ± 3.76
Carcass	20.02 ± 3.70	5.90 ± 1.60
Recovery	93.28 ± 6.23	95.47 ± 23.95
Total metabolism (CO_2 + excreta + carcass)	28.72	23.95

Source: Mitoma et al. (1985).

Recovery of the radiolabel was >90% for both rats and mice. Total metabolism was calculated by the authors as the sum of the radiolabel present in carbon dioxide, excreta, and the carcass. This is an assumption by the authors and is not an accurate estimate of metabolism since actual metabolites were not quantified. Data on the extent of metabolism for the radiolabeled material are presented in Table 3-5. Based on the mass balance between dose and the estimate for the sum of the metabolites, 30% of the parent compound was metabolized by both the rats and mice. This is consistent with the 60-70% of the high dose that was reported to be present unchanged in exhaled air. However, this assumes that all of the exhaled radiolabel that was not identified as carbon dioxide was the unmetabolized parent compound. The major urinary metabolites, determined qualitatively by high performance liquid chromatography, were trichloroethanol and trichloroacetic acid (TCA) for both rats and mice. Trichloroethanol and TCA were also qualitatively considered the major urinary metabolites for other halogenated hydrocarbon compounds, including PERC, that were evaluated by Mitoma et al. (1985).

Table 3-5. Metabolism of HCE measured in rats and mice

Species	Dose (mmol/kg)	Metabolism (mmol/kg)	Percent metabolized[a]
Rat	0.53	0.16	30
	2.11	0.60	28
Mouse	1.05	0.32	30
	4.22	1.01	24

[a]Percent metabolism was calculated from the dose and the reported sum of the metabolites. This calculation is likely an underestimation of metabolism since the exhaled air was likely to include some volatile metabolites based on the data from Jondorf et al. (1957).

Source: Mitoma et al. (1985).

Jondorf et al. (1957) reported that rabbits fed [^{14}C]-radiolabeled HCE at 500 mg/kg (route of administration not reported by study authors) excreted only 5% of the applied radioactivity in urine over 3 days (72 hours), indicating slow metabolism. This is consistent with the results in mice and rats reported by Mitoma et al. (1985) in which approximately 2-4% of the label was found in urine after 48 hours. During this 3-day period, 14–24% of the radioactivity was detected in expired air (a lower percentage than seen for rats at a comparable dose by Mitoma et al., 1985), and the remainder was present in tissues and the intestinal tract. However, the authors did not have the capability of quantifying HCE in tissues. Reported urinary metabolites include trichloroethanol (1.3%), dichloroethanol (0.4%), TCA (1.3%), dichloroacetic acid (0.8%), monochloroacetic acid (0.7%), and oxalic acid (0.1%). The expired air contained HCE, carbon dioxide, PERC, and 1,1,2,2-tetrachloroethane (TCE was not found). Quantitative data on the volatile metabolites in exhaled air were not reported.

The only other metabolite data come from the work of Fowler (1969) in sheep. HCE was administered to four Scottish Blackface and six Cheviot cross sheep at three dose levels: 0 (two sheep), 500 (six sheep), 750 (one sheep), and 1,000 (one sheep) mg/kg. Two HCE metabolites, PERC and pentachloroethane, were detected in sheep blood 24 hours after oral HCE administration by drenching bottle. Following administration of 500 mg/kg, blood measurements were 10–28 μg/mL for HCE, 0.6–1.1 μg/mL for PERC, and 0.06–0.5 μg/mL for pentachloroethane. Blood concentrations of HCE, PERC, and pentachloroethane were 2.3-2.6 times greater than the corresponding concentrations in erythrocytes. Data were not reported for the 750 and 1,000 mg/kg doses. In vitro experiments using fresh liver slices suspended in an olive oil emulsion confirmed the presence of the metabolites PERC and pentachloroethane.

The metabolites identified in the in vivo studies (Mitoma et al., 1985; Fowler, 1969; Jondorf et al., 1957) along with the in vitro studies (Town and Leibman, 1984; Nastainczyk et al., 1982), and ATSDR (1997a) were used in derivation of Figure 3-1. The proposed metabolic pathway is based on limited information; therefore, it is likely that intermediate chemical reactions are not captured in the figure, which presents the formation of the various metabolites as single step reactions.

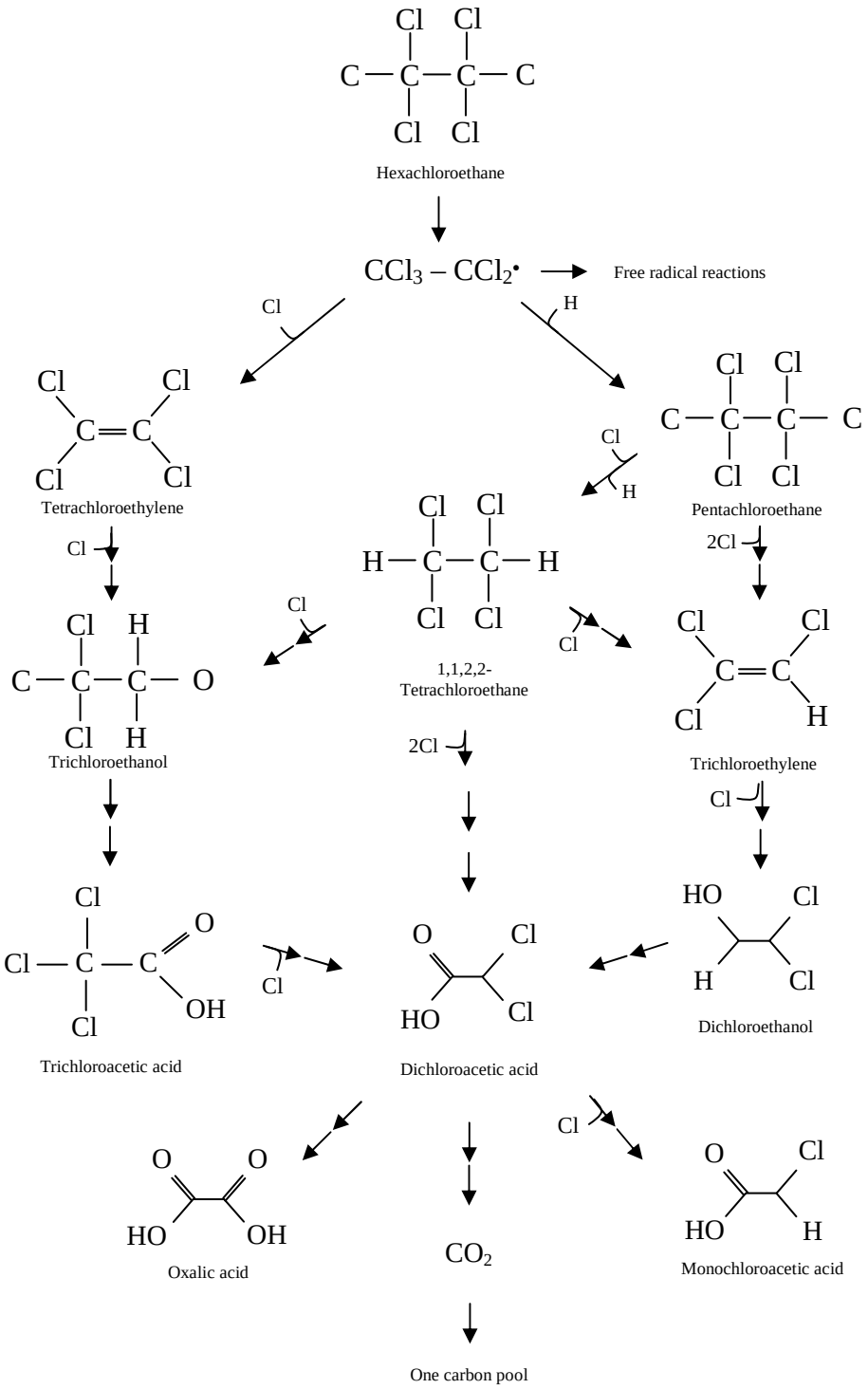


Figure 3-1. Possible metabolic pathway of HCE.

Sources: Adapted from ATSDR (1997a); Mitoma et al. (1985); Town and Leibman, 1984; Nastainczyk et al. (1982, 1981); Bonse and Henschler (1976); Fowler (1969); Jondorf et al. (1957).

The in vivo data on HCE metabolism are supported by in vitro studies of hepatic metabolism using liver microsomes. Nastainczyk et al. (1982, 1981) reported two studies that provide evidence that HCE is metabolized by phenobarbital-inducible CYP450 isoforms that catalyze their reductive dechlorination with NADPH, cytochrome b_5, and NADH as electron donors. HCE metabolism was measured using liver microsomes from male Sprague-Dawley rats that were either pretreated with phenobarbital or 3-methylcholanthrene, or were not pretreated. Only phenobarbital-induced rat liver microsomes demonstrated an increase in HCE metabolism (27.0 ± 1.1 nmol/mg protein/minute [mean + standard deviation or SD] compared with 8.0 ± 1.2 nmol/mg protein/minute for controls). Oxidation of NADPH (under anaerobic conditions) with an oxidation rate of 35 ± 2 nmol/mg protein/minute (mean ± SD) provided support for reductive dehalogenation mediated by cytochrome CYP450. Carbon monoxide inhibited the NADPH oxidation rate, further indicating that CYP450 enzymes were involved in the reaction. The major HCE metabolite of this reductive process was PERC. Nastainzcyk et al. (1982) determined that the stoichiometry of the reaction was represented by the following equation:

$$\underset{}{\text{NADPH} + \text{H}^+ + \underset{\text{(HCE)}}{\text{Cl}_3\text{C—CCl}_3}} \longrightarrow \underset{}{\text{NADP}^+ + \underset{\text{(PERC)}}{\text{Cl}_2\text{C=CCl}_2} + 2\,\text{H}^+ + 2\,\text{Cl}^-}$$

Nastainczyk et al. (1982, 1981) proposed that since CYP450 is a one electron donor, the two electrons would be transferred sequentially. The first electron reduction would result in a carbon radical; the second electron reduction would result in a carbanion. From the carbanion, three possible stabilization reactions are possible: (1) protonation by a hydrogen atom from the milieu, forming pentachloroethane, (2) α-elimination of chloride, to form the carbene which could be stabilized by the reduced CYP450, or (3) β-elimination of chloride to form PERC, which is the major HCE metabolite. Nastainczyk et al. (1982) found that the products of reductive dechlorination of HCE were 99.5% PERC and 0.5% pentachloroethane at physiological pHs. At a higher pH (8.4–8.8), the ratio of pentachloroethane (one electron reduction) to PERC (two electron reduction) increased since transfer of the second electron can occur via cytochrome b_5, which is influenced by pH. These reaction outcomes were proposed by the authors to also apply to other polyhalogenated hydrocarbons.

To provide additional support for the reaction being catalyzed by CYP450, Nastainczyk et al. (1982, 1981) inhibited CYP450 using carbon monoxide, metyrapone (CYP450 3A inhibitor), or α-naphthoflavone (CYP450 1A and CYP450 1B inhibitor) (see Omiecinski et al., 1999 for review). In vitro metabolism of HCE by phenobarbital-induced rat liver microsomes was inhibited >99% when carbon monoxide was added to the incubation mixture. Metyrapone at a concentration of 10^{-4} M inhibited PERC formation by $46 \pm 10\%$ (mean ± SD) and pentachloroethane formation by $41 \pm 8\%$. Treatment with 10^{-3} M metyrapone inhibited HCE metabolism to a greater extent, reducing PERC and pentachloroethane formation $66 \pm 8\%$ and

79 ± 10%, respectively. α-Naphthoflavone (10^{-4} M) was not as effective inhibiting HCE metabolism as metyrapone, inhibiting PERC formation 13 ± 2% and inhibiting pentachloroethane formation by 26 ± 4%. These data indicate that CYP450 3A isoforms are involved in HCE metabolism and α-naphthoflavone does not inhibit the primary CYP450 involved in the metabolism of HCE. Since metyrapone did not completely inhibit HCE metabolism by phenobarbital-induced liver microsomes, the remainder of HCE metabolism may be accounted for by the CYP450 2A and 2B subfamilies whose inhibition was not evaluated in this study.

Town and Leibman (1984) prepared liver microsomes from phenobarbital-induced male Holtzman rats to study the rate of metabolism of HCE to PERC. The formation of PERC was favored in a low oxygen environment as observed metabolism rates of 50.2 ± 0.45, 1.25 ± 0.25, and 0 nmol/minute/mg protein in atmospheres of N_2, air, and O_2, respectively. When any part of the NADPH-generating system, such as $NADP^+$, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase, was omitted from the experiment, the metabolism of HCE to PERC was inhibited (≥91%). In addition, the use of carbon monoxide as a monooxygenase inhibitor arrested HCE metabolism. Enzymes responsible for metabolism of HCE to PERC were located in the microsomes, rather than the cytosol, of phenobarbital-treated rat livers. Formation of malondialdehyde and conjugated dienes was statistically, significantly increased following treatment with HCE (8 mM), indicating lipid peroxidation. The authors suggested the involvement of a free radical. The K_m and V_{max} for the enzymatic formation of PERC from HCE were 1.20 mM and 52.0 nmol/minute/mg, respectively. Phenobarbital-induced liver microsomes from ICR mice were also studied and yielded K_m and V_{max} values of 3.34 mM and 30.2 nmol/minute/mg, respectively. PERC formation was not detected in liver microsomes from phenobarbital-induced New Zealand White rabbits, suggesting that HCE metabolism resulting in the formation of PERC did not occur. These results support the hypothesis that rat liver metabolism of HCE (reductive dehalogenation) occurs by CYP450. The report identifies PERC as a metabolite of HCE; however, the metabolite was not quantitatively measured.

Salmon et al. (1981) used Aroclor 1254-induced Sprague Dawley rats to quantify the dechlorination of HCE. In this case, dechlorination was measured by the release of radioactive Cl^- from the [^{36}Cl]-radiolabeled HCE substrate during incubation with liver microsomes from induced rats. The K_m and V_{max} were determined as 2.37 mM and 0.91 nmol/minute/mg protein, respectively. A control group of noninduced rats was not included.

Salmon et al. (1985) reported a follow-up study that used liver microsomes from noninduced rats (Wistar-derived Alderley Park strain) and a reconstituted CYP450 system from noninduced and phenobarbital-induced New Zealand White rabbits. Metabolic experiments of HCE using liver microsomes from noninduced rats yielded a K_m of 6.0 μM and a V_{max} of 3.55 nmol NADPH/minute/mg protein (2.41 nmol NADPH/minute/nmol CYP450). These results are not directly comparable to the previous study (Salmon et al., 1981) because of the use

of a different rat strain. A reconstituted CYP450 system from phenobarbital-induced New Zealand White rabbits yielded K_m and V_{max} values of 50 μM and 2.39 nmol NADPH/minute/nmol CYP450, respectively (Salmon et al., 1985). Microsomes from rabbits induced with β-naphthoflavone did not metabolize HCE. These results provide further evidence that the reductive dechlorination of HCE is catalyzed by phenobarbital-inducible CYP450 isoforms.

Yanagita et al. (1997) used recombinantly-expressed rat CYP450 1A2 in *Saccharomyces cerevisiae* to evaluate the in vitro metabolism of several chlorinated ethylenes and ethanes, including HCE. The metabolism of HCE by wild-type CYP450 1A2 under aerobic conditions resulted in the formation of PERC (3.7 nmol/2.5 nmol CYP450/hour), pentachloroethane (0.8 nmol/2.5 nmol CYP450/hour), and TCE (0.6 nmol/2.5 nmol CYP450/hour). CYP450 1A2 is a major hepatic CYP450 enzyme, but it is not a phenobarbital-inducible isoform; the major phenobarbital-inducible CYP450 enzymes are the 2A and 2B subfamilies. A follow-up study (Yanagita et al., 1998) that examined NADPH oxidation rates under anaerobic conditions found that CYP450 1A2 wild type had a V_{max} of 1.3 mol/mol CYP450/minute, a K_m of 0.25 mM, and an NADPH oxidation rate of 1.4 mol/mol CYP450/minute.

Table 3-6. Product formation rates and relative ratios of the products formed by CYP450 1A2 metabolism of HCE

CYP450 1A2	Product formation (nmol/nmol CYP450/minute)			Ratio of PERC: pentachloroethane + TCE
	PERC	Pentachloroethane	TCE	
Wild type	0.68	0.10	0.0034	6.6

Source: Yanagita et al. (1998).

Beurskens et al. (1991) used HCE as a reference compound to examine the metabolism of three hexachlorocyclohexane isomers. Liver microsomes from male Wistar rats that were induced with phenobarbital converted HCE to PERC and pentachloroethane at an initial dechlorination rate of 12.0 nmol/minute/nmol CYP450 under anaerobic conditions.

Van Dyke (1977) and Van Dyke and Wineman (1971) evaluated the dechlorination mechanisms of HCE and chlorinated olefins (alkenes) by using rat liver microsomes (a source of CYP450 enzymes). An initial study with HCE and other chlorinated ethanes found that the optimal configuration for dechlorination was a dichloromethyl group. HCE demonstrated a considerable amount of dechlorination (3.9%) in this in vitro study; however, the authors determined that HCE was unstable in aqueous solution and that this dechlorination was nonenzymatic based on the evidence of dechlorination in the absence of NADP.

Gargas and Andersen (1989) and Gargas et al. (1988) determined kinetic constants for HCE metabolism in the rat using exhalation rates and a physiologically based pharmacokinetic (PBPK) inhalation model described by Ramsey and Andersen (1984) for styrene. The V_{max}

(scaled to a 1.0 kg rat) was 1.97 ± 0.05 mg/hour, or 8.3 μmol/hour. The K_m was 0.80 mg/L, or 3.38 μM.

3.4. ELIMINATION

No studies are available that evaluated the elimination of HCE in humans. Animal studies indicated that the major routes of HCE elimination are either fecal or by expired air (Mitoma et al., 1985; Fowler, 1969; Jondorf et al., 1957). The sheep studies (Fowler, 1969) indicate that orally administered HCE is eliminated by the fecal route without absorption and metabolism while the rodent studies (Mitoma et al., 1985) provided evidence that HCE is absorbed and eliminated by exhalation. It is unknown why there is a discrepancy between the studies in sheep and rodents.

Rabbits fed [^{14}C]-radiolabeled HCE at 0.5 g/kg (Jondorf et al., 1957) eliminated 14–24% of the radioactivity in expired air during a 3-day period following exposure. Only 5% of the radiolabel was detected in urine. Fecal measurements were not conducted.

Fowler (1969) orally administered HCE to Scottish Blackface and Cheviot cross sheep. Two Cheviot cross sheep were administered a single dose of 0.5 g/kg HCE and were confined to metabolism cages; urine and feces were collected over a period of 4 days for HCE analysis. More than 80% of the total fecal excretion of HCE occurred in the first 24 hours, and only small amounts were detected in the urine. To assess bile concentrations of HCE, two Scottish Blackface sheep were fasted for 24 hours and anaesthetized with pentobarbitone sodium. The hepatic duct was cannulated to collect bile; HCE was injected at a dose of 0.5 g/kg (15% w/v in olive oil) into the rumen and lower duodenum. Bile was collected continuously, with 2 mL retained every 30 minutes for analysis. HCE was detected in bile of anaesthetized sheep at 15 minutes, compared with 27 minutes for blood and at maximum, HCE was 8- to 10-fold greater in bile.

Mitoma et al. (1985) evaluated excretion of HCE in Osborne-Mendel rats and B6C3F$_1$ mice following 4 weeks of administration of an MTD (500 mg/kg-day in rats, 1,000 mg/kg-day in mice). Excretion of radiolabel was monitored for 48 hours following administration of a tracer dose of [^{14}C]-HCE. The findings are presented in Table 3-4. Most of the radiolabel was detected in expired air, indicating this to be a major route of elimination. The authors did not investigate whether the exhaled material was parent compound or volatile metabolite, assuming that it was the parent compound. A low percentage of the exhaled radioactivity was in the form of CO_2, with rats exhaling slightly more than mice. The amount of radioactivity in the excreta, on the other hand, was lower in rats than in mice (Table 3-4). The excreta contained 6.3 and 16.2% of the radiolabel in rats and mice, respectively.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

No physiologically based toxicokinetic (PBTK) models for HCE have been developed specifically for mammalian species. Models for waterborne chloroethanes have been reported for rainbow trout and channel catfish; however, these are outside the scope of this toxicological review and are not described.

Gargas and Andersen (1989) and Gargas et al. (1988) determined kinetic constants for HCE metabolism in the rat using exhalation rates and a PBPK inhalation model described by Ramsey and Andersen (1984) for styrene. These reports by Gargas and Andersen (1989) and Gargas et al. (1988) do not describe a PBTK model for HCE, only kinetic constants for metabolism by inhalation. During these breath chamber experiments, fur deposition (fur loading) was observed to occur. At an exposure concentration of 53.3 ppm HCE at 6 hours, the chemical mass in body tissues was 7.29 mg and the chemical mass on fur was 0.6 mg (7.6% of total chemical mass).

4. HAZARD IDENTIFICATION

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

There are few published studies relating to the toxicology of HCE in humans. Case reports of pneumonitis (Allen et al., 1992) and pneumonitis with evidence of liver abnormalities (Loh et al., 2008, 2006) have been described in soldiers exposed to smoke bombs containing HCE and zinc oxide. However, the smoke produced by this incineration is primarily zinc oxychloride and zinc chloride and it is not likely that these effects are a result of HCE. Some aluminum production processes involve the use of HCE in tablet or powder form, resulting in exposures to fumes containing hexachlorobenzene, octachlorostyrene, dioxins, dibenzofurans and other organochlorinated compounds. A case report of a hepatocellular carcinoma (Seldén et al., 1989), and limited data concerning some clinical serologic measures (Seldén et al., 1999, 1997) in aluminum foundry workers involved in this process are available, but these data are not directly relevant to the question of health effects of HCE in other settings. No epidemiologic studies of the carcinogenicity of HCE were included in a 1985 review of cancer epidemiology with respect to halogenated alkanes and alkenes (Axelson, 1985). A study of Swedish workers involved in smoke bomb production has provided some information pertaining to exposure levels and symptoms and clinical parameters relating primarily to liver and pulmonary function (Seldén et al., 1994, 1993).

Two separate studies were conducted on a small population of Swedish workers occupationally exposed to HCE while producing military white smoke munitions. The first study reported on biological exposure monitoring (Seldén et al., 1993) and the second study described health effects resulting from HCE exposure (Seldén et al., 1994). The smoke formulation was approximately 60% HCE, 30% titanium dioxide, 8% aluminum powder, 2% cryolite, and a trace of zinc stearate. At the time this study was conducted in 1989, no HCE dust was found in the air sample filters, but the integrated results of personal and stationary charcoal tube samples revealed approximate HCE concentrations by location of 10–30 mg/m^3 (milling/mixing), 5-25 mg/m^3 (pressing), <5 mg/m^3 (assembly room), and nondetectable (storage room) (Seldén et al., 1993).

In the first study (Seldén et al., 1993), the exposed group consisted of 12 people (six men and six women) ranging in age from 23 to 57 (mean, 31.4 years; median, 30 years) (Seldén et al., 1993). The principal control group (n = 12) consisted of assembly line workers from the same company who were unexposed to chlorinated hydrocarbons, but had some exposure to glass fiber dust. They were matched to the exposure group by sex and age (± 5 years), except in the case of one exposed male subject where only a younger control could be found. This latter-exposed male subject was excluded from the analysis of health effects (Seldén et al., 1994). A second

control group of formerly HCE-exposed workers (3 males, 10 females; age range, 31–57 years; mean, 43.6 years) was used in the biological exposure monitoring study.

Blood samples were collected for analysis of HCE concentration. For the exposed group, samples were drawn 5 weeks into a temporary production break (the "baseline" period), and the second samples were drawn 5 months later, after production had been under way for 5 weeks (the "production" period). Analyses of blood plasma HCE indicated that values for both control groups (n = 25) were all below the limit of detection (<0.02 μg/L).

Exposed subjects were stratified into three subgroups (n = 4) of perceived exposure (low, medium, or high) based on information pertaining to work tasks, presence at work, and use of protective equipment. At baseline, the HCE concentrations in 10 of the samples from exposed workers were in the range of <0.02–0.06 μg/L, one sample was 0.15 μg/L, and one was 0.52 μg/L. The last sample was from an individual who had remained in an HCE-contaminated area during the baseline period. Plasma HCE levels in the production period increased by nearly 100-fold over that of the baseline samples (mean 7.30 ± 6.04 μg/L compared with 0.08 ± 0.14 μg/L in the production and baseline samples, respectively, $p < 0.01$). Although the magnitude of individual increases varied considerably, there was a significant ($p < 0.05$) linear trend for values in the low, medium, and high exposure subgroups (means of 3.99, 7.14, and 10.75 μg/L, respectively). These results demonstrate that a considerable increase in plasma HCE can occur after a relatively brief occupational exposure, even though workers used fairly sophisticated personal protective equipment.

As noted above, 11 of the subjects from the first study (Seldén et al., 1993) and their 11 age- and sex-matched controls were included in the second health effects study (Seldén et al., 1994). Data pertaining to 15 clinical symptoms (including headaches, sleep quality, palpations, difficulty concentrating, tension/restlessness, frequency of coughing, watery eyes/runny nose, itching/other skin problems, shortness of breath/chest discomfort, general health) were obtained from self-administered questionnaires for the exposed workers and the company controls. Similar data had been obtained in a previous study of 130 metal shop workers, and these workers were used as a second, "historical" comparison group in the analysis of the symptom data. Whole blood and serum samples from the 11 exposed and 11 matched company controls were analyzed for routine clinical parameters. Spot urine samples were analyzed for hemoglobin, protein, and glucose. Lung function was assessed by measuring vital capacity and 1-second forced expiratory volume (FEV_1).

The matched company controls reported more symptoms of ill health than exposed subjects, although the differences were not statistically significant. Although not statistically significant, the exposed group reported a higher prevalence of "dry skin/dry mucous membranes" (3/11 or 27%) than the matched controls (1/9, 9%) or historical controls (13/130, 10%), and a higher prevalence of "itching/other skin problems" (3/11, 27%) than the historical controls (16/130, 12%). The prevalence of "itching/other skin problems" in the matched controls

(3/11, 27%) was the same as that in the exposed group. These symptoms centered on the wrist and neck areas, and the authors suggest that this could reflect exposure to HCE through joints in the protective equipment, or possibly a "traumiterative effect of the equipment itself." Clinical examination revealed no dermatological or respiratory mucous membrane abnormalities in either group. The authors noted that a previous unpublished study of the plant workers (but with primarily different workers) had also found dermatologic complaints in up to 90% of the exposed workers.

All of the spot urine tests were normal, and there was no evidence of an effect of HCE exposure on pulmonary function as measured by vital capacity and FEV_1. Exposed subjects had significantly higher levels of serum creatinine, urate, and bilirubin than controls ($p < 0.05$), although the group means were still in the normal range. One exposed subject had a marginally elevated level of serum alanine aminotransferase (ALT) (70.5 U/L versus ≤41.1 U/L reference), while one control subject displayed increased levels of serum ALT and aspartate aminotransferase (AST) (67.6 and 186.4 U/L, respectively; 41.1 U/L reference for each). The control individual's values returned to normal after 8 months, while the exposed subject's serum ALT value worsened to 87.6 U/L 4 months later (Seldén et al., 1994). Available data pertaining to these liver function tests from 1982, when exposure levels at the worksite were higher than in the current study, did not show elevations in these liver enzymes in this individual at that time. Within the exposed group, there was no correlation between plasma HCE concentrations and the clinical chemistry parameters, although the authors do not discuss the power limitations of this exposure-response analysis (Seldén et al., 1993). In summary, these studies demonstrated HCE exposure in the smoke bomb production workers, but the health effects study is too small to reach definitive conclusions. The interpretation of small differences in clinical parameters, within the normal range, is uncertain. Based on the available data, the possible dermatologic/mucosal effects and hepatic effects are the areas in most need of additional research.

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

4.2.1. Oral

4.2.1.1. Subchronic Exposure

Two subchronic toxicity assays for HCE were reported (NTP, 1989; Gorzinski et al., 1985, 1980). The Gorzinski et al. (1985, 1980) study (16 weeks) reported histopathological evaluations that found kidney degeneration in males, kidney degeneration in females, and minimal hepatic effects. The NTP (1989) study (13 weeks) reported kidney effects in male rats such as degeneration and necrosis of renal tubular epithelium, hyaline droplet formation, and tubular regeneration and tubular casts. Female rats in this study exhibited a dose-response increase in the incidence of hepatocellular necrosis of the centrilobular area. The NTP (1989)

study suggested that male rats may be more susceptible to kidney effects whereas female rats may be more susceptible to liver effects.

Gorzinski et al. (1980) conducted a 16-week toxicity study in male and female F344 rats. Ten rats/sex/dose were exposed via the diet, formulated to deliver doses of 3, 30, or 100 mg HCE/kg-day (purity 99.4%). However, due to sublimation of HCE from the feed, the actual doses were reported as 1.3, 20, or 82 mg/kg-day and later based on feeding and diurnal eating patterns were determined to be 1, 15, or 62 mg/kg-day, respectively (Gorzinski et al., 1985). Gorzinski et al. (1980) is a Research and Development (R&D) Report by Dow Chemical and is not publicly available. The data for this study were published in the peer-reviewed literature by Gorzinski et al. (1985) and are presented in detail below.

Gorzinski et al. (1985) fed 1, 15, or 62 mg/kg-day HCE (purity 99.4%) to F344 rats (10 rats/sex/dose) for 16 weeks. As described in Section 3.2, HCE concentrations in male kidneys were proportionately increased with administered dose while the increases in females were not proportionate. At the high dose, male rats displayed statistically significant increases in absolute and relative kidney weights accompanied by macroscopically observed alterations. Male rats displayed slight hypertrophy and/or dilation of proximal convoluted tubules of the kidneys at incidences of 0/10, 1/10, 7/10, and 10/10 for the 0, 1, 15, and 62 mg/kg-day dose groups, respectively. The increased incidence of slight hypertrophy and/or dilation of proximal convoluted tubules was statistically significant in males at the 15 and 62 mg/kg-day doses. Male rats displayed atrophy and degeneration of renal tubules at incidences of 1/10, 2/10, 7/10, and 10/10 for the 0, 1, 15, and 62 mg/kg-day dose groups, respectively. The increased incidence of atrophy and degeneration of renal tubules was statistically significant in males at the 15 and 62 mg/kg-day doses. Female rats did not display hypertrophy and/or dilation of proximal convoluted tubules of the kidneys at any dose, but did exhibit atrophy and degeneration of proximal tubules: 1/10, 1/10, 2/10, and 6/10 at the 0, 1, 15, and 62 mg/kg-day doses, respectively. However, the increased incidence of atrophy and degeneration of proximal tubules was only statistically significant in females at the 62 mg/kg-day dose. Male rats of the 62 mg/kg-day group exhibited statistically significant increases in absolute and relative liver weights; histopathology revealed a slight swelling of the hepatocytes in this group. Although female rats exhibited a statistically significant increase in relative liver weight at the high dose, there was no evidence of hepatotoxicity in the histopathological examination. The data for liver and kidney weights are presented in Table 4-1 and the data for the kidney effects are presented in Table 4-2.

Table 4-1. Body, kidney and liver weights of rats exposed to HCE in the diet for 16 weeks

Sex	Dose level (mg/kg-day)	Fasted body weight (g)	Liver		Kidney	
			Absolute (g)	Relative (g/100 g body weight)	Absolute (g)	Relative (g/100 g body weight)
Male[a]	0	314.4 ± 12.4	8.32 ± 0.27	2.65 ± 0.06	2.28 ± 0.08	0.73 ± 0.04
	1	328.0 ± 7.2	8.46 ± 0.22	2.58 ± 0.07	2.31 ± 0.09	0.70 ± 0.02
	15	329.0 ± 24.4	8.69 ± 0.80	2.64 ± 0.09	2.40 ± 0.15	0.73 ± 0.01
	62	324.2 ± 10.0	8.98 ± 0.54[b]	2.77 ± 0.12[b]	2.51 ± 0.12[b]	0.77 ± 0.02[b]
Female[a]	0	176.7 ± 6.9	4.65 ± 0.26	2.63 ± 0.06	1.40 ± 0.08	0.79 ± 0.03
	1	174.0 ± 7.9	4.74 ± 0.22	2.73 ± 0.11	1.38 ± 0.05	0.79 ± 0.03
	15	176.7 ± 4.6	4.79 ± 0.21	2.69 ± 0.09	1.39 ± 0.06	0.79 ± 0.04
	62	170.8 ± 5.1	4.71 ± 0.23	2.76 ± 0.10[b]	1.39 ± 0.05	0.81 ± 0.02

[a]Data are presented as means ± SD of 10 rats/sex.
[b]Statistically significant from control using Dunnett's test (p = 0.05).

Source: Gorzinski et al. (1985).

Table 4-2. Histopathological results on kidney in rats exposed to HCE in the diet for 16 weeks[a]

Organ	Effect	Sex	Dose (mg/kg-day)			
			0	1	15	62
Kidney	Slight hypertrophy and/or dilation of proximal convoluted tubules	Male	0	1	7[c]	10[c]
		Female	0	0	0	0
	Atrophy and degeneration of renal tubules[b]	Male	1	2	7[c]	10[c]
		Female	1	1	2	6[c]

[a]Data are presented as number of positive observations for 10 rats/sex/dose.
[b]Graded as slight in 1 of 10 male control rats and very slight in 1 of 10 control female rats. Severity of nephropathy was not reported for HCE-exposed rats.
[c]EPA determined statistical significance from control using Fisher's Exact Test (p = 0.05).

Source: Gorzinski et al. (1985).

The authors concluded that the no-observed-effect level for both male and female rats was 1 mg/kg-day. EPA considered 1 mg/kg-day as the male no-observed-adverse-effect level (NOAEL) and 15 mg/kg-day as the lowest-observed-adverse-effect level (LOAEL), based on renal tubule toxicity in male rats. For female rats, EPA considered the NOAEL as 15 mg/kg-day and the LOAEL as 62 mg/kg-day, based on renal tubule toxicity.

NTP (1989) conducted a 13-week study of HCE oral toxicity in F344/N rats. Groups of 10 rats/sex/dose were administered 0, 47, 94, 188, 375, or 750 mg/kg (purity >99%) by corn oil gavage, 5 days/week for 13 weeks. The time-weighted average (TWA) doses were 0, 34, 67, 134, 268, and 536 mg/kg-day, respectively. In the 536 mg/kg-day group, 5/10 male rats (only the 5 males that died were examined microscopically) and 2/10 female rats died before the end of the study. Mean body weights of 536 mg/kg-day male and female rats were decreased 19 and

4%, respectively, compared with controls. Statistically significant increases in liver weights are noted at doses of ≥67 mg/kg-day (females) and ≥134 mg/kg-day (males), and in kidney weights at doses of ≥268 mg/kg-day (females) and ≥67 mg/kg-day (males). Organ weight to body weight ratios (mg/g) generally increased in a dose-related manner for both male and female rats exposed to HCE (Table 4-3).

Table 4-3. Organ weight to body weight ratios for rats exposed to HCE for 13 weeks

	HCE dose by gavage (mg/kg-day)					
	0	**34**	**67**	**134**	**268**	**536**
***Male*[a]**						
Number	10	10	10	10	9	5
Body weight	340 ± 7.6	349 ± 8.8	343 ± 5.9	348 ± 5.9	319 ± 4.0	262 ± 13.5
Liver	35.8 ± 0.61	37.3 ± 0.37	36.0 ± 0.71	39.1 ± 0.62[c]	42.5 ± 0.74[c]	46.3 ± 0.95[c]
Brain	6.0 ± 0.30	5.7 ± 0.17	5.7 ± 0.10	5.8 ± 0.23	6.3 ± 0.21	7.2 ± 0.31[c]
Heart	2.8 ± 0.04	2.8 ± 0.04	2.9 ± 0.07	3.2 ± 0.17[b]	3.3 ± 0.18[c]	3.2 ± 0.10[b]
Kidney	3.0 ± 0.05	3.8 ± 0.37	4.1 ± 0.27[b]	4.7 ± 0.44[c]	5.2 ± 0.35[c]	4.7 ± 0.28[c]
Lung	4.2 ± 0.21	4.6 ± 0.40	4.4 ± 0.48	3.9 ± 0.22	3.9 ± 0.15	4.9 ± 0.50
Right testis	4.2 ± 0.05	4.8 ± 0.38	4.3 ± 0.10	4.4 ± 0.17	4.7 ± 0.05	5.3 ± 0.21[c]
Thymus	0.8 ± 0.04	0.8 ± 0.06	0.6 ± 0.02	0.8 ± 0.10	0.7 ± 0.04	0.6 ± 0.06
***Female*[a]**						
Number	10	10	10	10	10	8
Body weight	206 ± 3.7	210 ± 3.9	208 ± 2.6	200 ± 2.9	203 ± 4.3	189 ± 3.8
Liver	32.2 ± 0.56	33.4 ± 0.63	34.3 ± 0.39[b]	36.3 ± 0.44[c]	42.0 ± 0.60[c]	52.4 ± 0.88[c]
Brain	8.7 ± 0.17	8.6 ± 0.14	8.6 ± 0.10	9.0 ± 0.14	9.0 ± 0.15	9.5 ± 0.17[c]
Heart	2.9 ± 0.04	3.0 ± 0.05	3.0 ± 0.03	3.0 ± 0.04	3.1 ± 0.07	3.4 ± 0.07[c]
Kidney	3.1 ± 0.04	3.2 ± 0.05	3.2 ± 0.07	3.2 ± 0.06	3.6 ± 0.05[c]	4.1 ± 0.10[c]
Lung	4.2 ± 0.09	4.1 ± 0.09	4.2 ± 0.10	4.1 ± 0.06	4.2 ± 0.08	4.5 ± 0.13
Thymus	1.1 ± 0.05	1.1 ± 0.05	1.1 ± 0.04	1.0 ± 0.06	1.1 ± 0.07	0.8 ± 0.05[c]

[a]Data are presented as mean ± SE in mg/g, except for body weight in g
[b]Statistically different from controls, $p<0.05$
[c]Statistically different from controls, $p<0.01$

Source: NTP (1989).

Kidney effects (characterized by hyaline droplet formation, tubular regeneration, and tubular casts), similar to the toxicity noted in the 16-day study also conducted by NTP (1989), were observed in 90% of 34 mg/kg-day males and in males from all other HCE dose groups (incidence data only reported for the 34 mg/kg-day dose group). NTP (1989) reported that the severity of these effects increased with dose (data not presented by NTP). These kidney effects were not observed in any of the treated females. At the 536 mg/kg-day dose, 5/10 males died. Kidneys from these five animals were examined microscopically and revealed papillary necrosis, degeneration, and necrosis of the renal tubular epithelium. Hepatocellular necrosis of the centrilobular area was observed in 2/5 males and 8/10 females at the 536 mg/kg dose, 1/10 males and 4/10 females at the 268 mg/kg-day dose, and 2/10 females at the 134 mg/kg-day dose. Additionally, males of the 536 mg/kg-day dose group exhibited hemorrhagic necrosis of the

urinary bladder. EPA considered the female rat NOAEL as 67 mg/kg-day and the LOAEL as 134 mg/kg-day, based on hepatocellular necrosis. A NOAEL could not be identified for male rats since kidney effects were observed in 90% or more of the male rats at all tested doses (compared to none of the controls). EPA considered the LOAEL for male rats as 34 mg/kg-day (lowest dose tested), based on kidney lesions.

4.2.1.2. Chronic Exposure and Carcinogenicity

The National Toxicology Program (NTP) and National Cancer Institute (NCI) conducted two chronic toxicity/carcinogenicity bioassays in rats and one in mice. Increased incidences of renal tubular hyperplasia, renal adenoma or carcinoma, adrenal medulla hyperplasia, pheochromocytomas, and malignant pheochromocytomas were noted in male F344/N rats; female rats did not develop HCE-related tumors (NTP, 1989). Osborne-Mendel rats of both sexes in the NCI (1978) study exhibited tumor types that have been previously identified as spontaneous lesions in this strain, and do not provide evidence of carcinogenicity. $B6C3F_1$ mice of both sexes exhibited hepatocellular carcinomas, although only male mice demonstrated a dose response with tumor incidence (NCI, 1978). Based on the body of evidence accumulated by these studies, NTP and NCI concluded that there was evidence of HCE carcinogenicity in male F344 rats and mice of both sexes, respectively, but there was no evidence of carcinogenicity in female F344 or male and female Osborne-Mendel rats (NTP 1989; NCI, 1978).

NTP (1989) conducted a chronic toxicity/carcinogenicity bioassay in F344/N rats. Groups of 50 male rats/dose were administered 0, 10, or 20 mg/kg-day (TWA doses of 0, 7, or 14 mg/kg-day, respectively, after adjusting for continuous exposure) of HCE (purity >99%) by corn oil gavage, 5 days/week for 103 weeks. Groups of 50 female rats/dose were administered 0, 80, or 160 mg HCE/kg by corn oil gavage, 5 days/week for 103 weeks (TWA doses of 0, 57, or 114 mg/kg-day, respectively, after adjusting for continuous exposure). These sex-specific doses were selected based on the results of the 13-week study conducted by NTP (1989) that demonstrated kidney lesions in male rats at the lower doses and liver lesions in female rats at the higher doses. All animals were necropsied.

Mean body weights of the 14 mg/kg-day male rats were 5–6% lower than controls after week 81. Mean body weights of the 114 mg/kg-day female rats were 5–9% lower between weeks 41 and 101. Nephropathy, characterized by tubular cell degeneration and regeneration, tubular dilatation and atrophy, glomerulosclerosis, interstitial fibrosis, and chronic inflammation, was observed in both treated and control rats. Incidences of male nephropathy were 48/50 in controls, 48/50 in the 7 mg/kg-day dose group, and 47/50 in the 14 mg/kg-day dose group. The mean severity scores for nephropathy in male rats increased with dose (2.34 ± 0.14, 2.62 ± 0.15, and 2.68 ± 0.16 in the 0, 7, and 14 mg/kg-day groups, respectively), with the 14 mg/kg-day group being statistically significantly higher than the control group. While the mean severity scores did not show more than a 15% increase over control in the high-dose group, an

examination of the various grades of severity revealed more moderate and marked nephrotoxicity in treated male rats compared with controls, which predominantly exhibited mild nephropathy (Table 4-4).

In light of these variations in severity, EPA considered the responses observed in both the control and treated male rats associated with more severe (moderate and marked severity) nephropathy to better distinguish the HCE-related effects. Incidences of male nephropathy (that were of moderate or marked severity) were 18/50, 24/50, and 30/50 in the control, 7, and 14 mg/kg-day dose groups, respectively.

Additional kidney effects were noted in the male rats (presented in Table 4-5). Linear mineralization of the renal papillae was increased in a dose-dependent manner: 15/50 (30%) and 32/50 (64%) in the 7 and 14 mg/kg-day dose groups, respectively, compared with 2/50 (4%) in controls. Hyperplasia of the pelvic transitional epithelium was increased in treated rats (14% in 7 and 14 mg/kg-day HCE dose groups) compared to 0% of control rats. Nonneoplastic lesions such as casts (4%), cytomegaly (4%), chronic inflammation (4%), and focal necrosis (2%) were observed in some of the male rats administered 14 mg/kg-day. An increased incidence of renal tubule pigmentation was noted in 4/50 (8%) of the 7 mg/kg-day dose group and 5/50 (10%) of the 14 mg/kg-day dose group, compared with 1/50 (2%) in the controls. Regeneration of the renal tubule was observed in three males administered 14 mg/kg-day HCE.

Incidences of female nephropathy were 22/50 for controls, 42/50 in the 57 mg/kg-day dose group, and 44/49 in the 114 mg/kg-day dose group. The severity scores for nephropathy in female rats were statistically significantly increased in both treated groups: 0.72 ± 0.13 (mean $\pm$ SE) in controls, 1.38 ± 0.11 in the 57 mg/kg-day group, and 1.69 ± 0.12 in the 114 mg/kg-day group. Examination of the various grades of severity shows mild and moderate nephropathy in treated females compared with controls which predominantly presented less than minimally severe nephropathy (Table 4-4).

Similar to the male rats, the incidence of nephropathy associated with the more severe (mild and moderate) responses was considered in the females rats. Therefore, incidences of female nephropathy (that were of mild or moderate severity) were 12/50, 25/50, and 32/50 in the control, 57, and 114 mg/kg-day dose groups, respectively.

Additional kidney effects in female rats included linear mineralization of the renal papillae, although the incidence was not dose-dependent: 14/50 (28%) in vehicle controls, 22/50 (44%) in the 57 mg/kg-day dose, and 13/50 (26%) in the 114 m/kg-day dose. Female rats also exhibited casts (4% at 114 mg/kg-day) and chronic inflammation (2% at both 57 and 114 mg/kg-day). Pigmentation of the renal tubule was present in 4, 4, and 6% of control, 57, and 114 mg/kg-day females, respectively. Renal tubule regeneration was observed in treated females (but not controls); 4% of the 57 mg/kg-day dose group and 2% of the 114 mg/kg-day dose group. Only male rats demonstrated an increase in hyperplasia of the pelvic transitional epithelium and a dose-dependent increase in incidences of mineralization along the renal papillae.

Table 4-4. Incidence and severity of nephropathy in male and female rats treated with HCE

	Dose (mg/kg-day)					
Severity	**0**	**7**	**14**	**0**	**57**	**114**
	Male			***Female***		
None (0)	2	2	3	28	8	5
Minimal (1)	4	3	4	10	17	12
Mild (2)	26	21	13	10	23	25
Moderate (3)	11	10	16	2	2	7
Marked (4)	7	14	14	0	0	0
Total Incidence (minimal to marked)	48	48	47	22	42[b]	45[b]
Total # of rats	50	50	50	50	50	49
Overall severity[c]	2.34 ± 0.14	2.62 ± 0.15	2.68 ± 0.16[a]	0.72 ± 0.13	1.38 ± 0.11[b]	1.69 ± 0.12[b]

[a]Authors reported as statistically significantly different from controls, $p < 0.05$.
[b]Authors reported as statistically significantly different from controls, $p < 0.01$.
[c]Mean ± SE.

Source: NTP (1989).

Table 4-5. Additional kidney effects in HCE-treated rats

	HCE Dose (mg/kg-day)					
	Males			***Females***		
	Vehicle control	**7**	**14**	**Vehicle control**	**57**	**114**
Renal tubule pigmentation	1/50 (2%)	4/50 (8%)	5/50 (10%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Linear mineralization of renal papillae	2/50 (4%)	15/50 (30%)[a]	32/50 (64%)[a]	14/50 (28%)	22/50 (44%)	13/50 (26%)
Hyperplasia of the pelvic transitional epithelium	0/50 (0%)	7/50 (14%)[a]	7/50 (14%)[a]	Not observed	Not observed	Not observed

[a]EPA determined statistical significance using Fisher's exact test, $p < 0.05$.

Source: NTP (1989).

EPA considered the male LOAEL as 7 mg/kg-day based on increased incidence of moderate or marked nephropathy (Table 4-4), hyperplasia of the pelvic transitional epithelium (Table 4-5), increased incidence of renal tubule pigmentation (Table 4-5), and linear mineralization of the renal papillae (Table 4-5). EPA considered 57 mg/kg-day the female LOAEL, based on dose-related increases in incidence and severity (minimal to marked) nephropathy. The male and female NOAELs could not be established as toxic effects were observed at the lowest doses tested.

Renal tubular hyperplasia was observed at an increased incidence in treated male rats: 4/50 (8%) in the 7 mg/kg-day dose and 11/50 (22%; statistically significantly higher than controls) in the 14 mg/kg-day dose, compared with 2/50 (4%) for control (Table 4-6). Only one female rat, administered 57 mg/kg-day, exhibited renal hyperplasia. Dose-related increases in the incidence of combined renal adenomas and carcinomas were observed in males rats administered HCE at doses of 7 (4%) and 14 mg/kg-day (14%, statistically significantly higher than controls) compared with controls (2%). No HCE-related tumors were observed in female rats. NTP concluded that these data provided evidence of carcinogenicity in male rats based on a comparison with the historical controls in the study laboratory (1/300; 0.3 ± 0.8%) and in NTP studies (10/1,943; 0.5 ± 0.9%).

Table 4-6. Renal tubular hyperplasia and tumor incidences in HCE-treated male rats

	Vehicle control	7 mg/kg-day HCE	14 mg/kg-day HCE
Hyperplasia	2/50 (4%)	4/50 (8%)	11/50 (22%)[a]
Adenoma	1/50 (2%)	2/50 (4%)	4/50 (8%)
Carcinoma	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adenoma or carcinoma	1/50 (2%)	2/50 (4%)	7/50 (14%)[a]

[a]Significantly different from vehicle controls, $p < 0.01$.

Source: NTP (1989).

This study demonstrates specificity for HCE-induced renal effects in male rats. The males of both dose groups were administered 8 times less HCE than the corresponding females. However, the male rats demonstrated more severe nephropathy than female rats. Male, but not female rats, also exhibited renal hyperplasia and tumors. NTP (1989) indicated that the renal hyperplasia and tumors observed in the HCE-treated male rats represented a morphologic continuum.

Effects in the adrenal gland were also noted in HCE-treated rats. Hyperplasia of the adrenal medulla was reported in 9 and 20% of male rats administered 7 and 14 mg/kg-day HCE, respectively, compared with 12% of controls. Female rats in the control (10%) and 114 mg/kg-day-treated (15%) groups exhibited hyperplasia of the adrenal medulla; this effect was not observed in the 57 mg/kg-day dose group.

Adrenal medullary lesions were observed in male rats, but not female rats (Table 4-7). Pheochromocytoma incidences were statistically significantly increased in the 7 mg/kg group (26/45, 58%). The increase of pheochromocytomas 14 mg/kg-day group (19/49, 39%) was not statistically significant compared with controls (14/50, 28%). There were no statistically significant differences in the incidences of malignant pheochromocytomas and complex pheochromocytomas (defined as pheochromocytomas containing nervous tissue in addition to the typical adrenal medullary cells) between controls and treated male rats. The combined

incidence of all three types of pheochromocytomas was statistically significantly increased in males treated with 7 mg/kg-day HCE (62%) but not in males treated with 14 mg/kg-day HCE (43%) when compared with vehicle controls (30%) and historical controls in the study laboratory (75/300; 25 ± 7%) and in NTP studies (543/1,937; 28 ± 11%). NTP concluded that the increased incidences of pheochromocytomas in male rats were possibly treatment-related.

Table 4-7. Adrenal medullary lesions in HCE-treated male rats

	Control	7 mg/kg-day	14 mg/kg-day
Focal hyperplasia	6/50 (12%)	4/45 (9%)	10/49 (20%)
Pheochromocytoma	14/50 (28%)	26/45 (58%)[a]	19/49 (39%)
Complex pheochromocytoma	0/50	0/45	2/49 (4%)
Malignant pheochromocytoma	1/50 (2%)	2/45 (4%)	1/49 (2%)
Combined pheochromocytoma	15/50 (30%)	28/45 (62%)[a]	21/49 (43%)

[a]Significantly different from vehicle controls, $p < 0.01$.

Source: NTP (1989).

The NCI (1978; Weisburger, 1977) conducted a chronic toxicity/carcinogenicity bioassay in Osborne-Mendel rats. HCE (purity >98%) at doses of 0, 250, or 500 mg/kg-day was administered by corn oil gavage to 50 rats/sex/dose for 5 days/week for 78 weeks. Following termination of exposure, animals were observed for 33–34 weeks for a total duration of 111-112 weeks. Twenty rats/sex were used for the untreated and vehicle controls. Starting in week 23, rats treated began a 5-week cyclic rotation that involved 1 week without exposure followed by dosing for 4 weeks. After adjustment from 5 days/week for 78 weeks, with the 5-week cyclic rotation for part of the time, to continuous exposure over the standard 2 years for a chronic bioassay, the TWA doses were 113 and 227 mg/kg-day.

Mortality was accelerated in the HCE-treated rats (NCI reported a statistically significant association between increased dose and mortality). The 113 and 227 mg/kg-day males exhibited survival rates of 24/50 (48%) and 19/50 (38%), respectively, compared with 14/20 (70%) in the untreated controls and 11/20 (55%) in vehicle controls (seven rats in the vehicle control group were sacrificed in week 60). Mortality in the treated groups occurred early in the bioassay. Approximately 20% of the high- and low-dose males died by weeks 15 and 45, respectively, compared with 90 weeks until 20% mortality for the controls. Survival rates for the female rats were 14/20 (70%) for both the untreated and vehicle controls, and 27/50 (54%) and 24/50 (48%) for the 113 and 227 mg/kg-day dose groups, respectively. Mortality also occurred early in the bioassay for the female rats. Approximately 20% of the high- and low-dose females died by weeks 25 and 30, respectively, compared with 110 weeks until 20% mortality for the controls.

Chronic inflammatory kidney lesions were observed in both control and treated rats: male rats exhibited incidences of 15/20 (75%) in untreated controls, 14/20 (70%) in vehicle controls, 32/49 (65%) in the 113 mg/kg-day dose group, and 25/50 (50%) in the 227 mg/kg-day dose group; female rats exhibited incidences of 8/20 (40%) in untreated controls, 4/20 (20%) in

vehicle controls, 18/50 (36%) in the 113 mg/kg-day dose group, and 20/49 (41%) in the 227 mg/kg-day dose group. Tubular nephropathy (characterized by degeneration, necrosis, and the presence of large hyperchromatic regenerative epithelial cells) was observed in 45 and 66% of males and 18 and 59% of females in the 113 and 227 mg/kg-day dose groups, respectively. These effects were not observed in the untreated or vehicle controls. EPA considered the LOAEL as 113 mg/kg-day (lowest dose tested), based on a dose-related increase in the incidence of nephropathy in both males and females. The NOAEL could not be identified.

Tumor types exhibited by male rats surviving at least 52 weeks included kidney tubular cell adenoma, pituitary chromophobe adenoma, thyroid follicular cell adenoma or carcinoma, and testicular interstitial cell tumors (Table 4-8). Due to the high mortality in the 227 mg/kg-day males, statistical analyses of male rat tumors were based only on those rats surviving at least 52 weeks. Increased incidences of kidney tubular cell adenoma (4/37) and pituitary chromophobe adenoma (4/32) were observed in the male rats of the 113 mg/kg-day dose group but not in the 227 mg/kg-day group. Male vehicle controls did not exhibit kidney tubular cell adenomas, although 11% (2/18) exhibited pituitary chromophobe adenomas. Thyroid follicular cell adenoma or carcinoma were observed in 11, 8, and 18% in vehicle control, 113, and 227 mg/kg-day males, respectively; high dose males also demonstrated the shortest time to first tumor of 60 weeks, compared with vehicle control (111 weeks) and low-dose males (92 weeks). Testicular interstitial cell tumors were not observed in vehicle control or 113 mg/kg-day males, but were observed in 10% of 227 mg/kg-day males.

Table 4-8. Tumor incidences[a] in male rats gavaged with HCE

Tumor type	Vehicle control	113 mg/kg-day	227 mg/kg-day
Kidney tubular cell adenoma	0/18 (0%)	4/37 (11%)	0/29 (0%)
Weeks to first tumor	–	86	–
Pituitary chromophobe adenoma	2/18 (11%)	4/32 (13%)	0/24 (0%)
Weeks to first tumor	105	104	–
Thyroid follicular cell adenoma or carcinoma	2/18 (11%)	3/36 (8%)	5/28 (18%)
Weeks to first tumor	111	92	60
Testis interstitial cell tumor	0/18 (0%)	0/36 (0%)	3/29 (10%)
Weeks to first tumor	–	–	109

[a]Due to early accelerated mortality the statistical analyses for the incidences of tumors are based on animals surviving at least 52 weeks

Source: NCI (1978).

Tumor types exhibited by female rats included kidney hamartoma (nonneoplastic overgrowth), pituitary chromophobe adenoma, thyroid follicular cell adenoma or carcinoma, mammary gland fibroadenoma, and ovary granulose cell tumors (Table 4-9). Females administered 227 mg/kg-day HCE had an incidence of 6% for kidney hamartoma, while none of these tumors were observed in the vehicle control or 113 mg/kg-day female rats. The increased

incidences of the remaining tumor types observed in female rats were not dose-dependent. Incidence of pituitary chromophobe adenomas, thyroid follicular cell adenoma or carcinomas, and mammary gland fibroadenomas was lower in HCE-treated animals than in controls. Ovary granulose cell tumors were increased in the low dose group, compared to controls, although none of the female rats in the high dose group exhibited this tumor. NCI (1978) noted that all of these tumor types had been encountered previously as spontaneous lesions in the Osborne-Mendel rat, and the authors reported no statistical differences in frequencies were observed between treated and control rats. NCI concluded that there was no evidence of carcinogenicity in this rat study.

Table 4-9. Tumor incidences in female rats gavaged with HCE

Tumor type	Vehicle control	113 mg/kg-day	227 mg/kg-day
Kidney hamartoma	0/20 (0%)	0/50 (0%)	3/49 (6%)
Weeks to first tumor	–	–	112
Pituitary chromophobe adenoma	7/20 (35%)	15/50 (30%)	6/46 (13%)
Weeks to first tumor	89	89	112
Thyroid follicular cell adenoma or carcinoma	2/20 (10%)	3/47 (6%)	3/47 (6%)
Weeks to first tumor	111	112	109
Mammary gland fibroadenoma	6/20 (30%)	13/50 (26%)	9/50 (18%)
Weeks to first tumor	106	57	94
Ovary granulosa cell tumor	1/20 (5%)	4/48 (8%)	0/49 (0%)
Weeks to first tumor	111	111	–

Source: NCI (1978).

NCI (1978; Weisburger, 1977) conducted a chronic oral study in 50 $B6C3F_1$ mice/sex/dose administered 0, 500, or 1,000 mg/kg-day HCE (purity >98%) via corn oil gavage for 5 days/week for 78 weeks. Following exposure termination, animals were observed for 12-13 weeks for a total duration of 90–91 weeks. Twenty mice/sex were included as untreated and vehicle controls. Starting in week 9, the doses were increased to 600 and 1,200 mg/kg-day; no explanation was provided for this change in dose. After adjustment from 5 days/week for 78 weeks to continuous exposure, the TWA doses were 360 and 722 mg/kg-day. Survival rates were unexpectedly low in males, particularly in the control and low-dose groups: 25 and 5% in the vehicle and untreated control groups and 14 and 58% in the 360 and 722 mg/kg-day dose group, respectively. NCI (1978) did not suggest a reason why more high-dose male mice survived compared with the low-dose and control males. Individual animal data were not available to make survival adjustments to the tumor incidence data discussed below. Survival rates in females were 80 and 85% in vehicle and untreated control groups and 80 and 68% in the 360 and 722 mg/kg-day dose groups, respectively. As a result of the low survival rates in the vehicle and untreated male control groups, NCI compared tumor incidences in the dosed males and females to the pooled vehicle control data derived from concurrently run bioassays for several other chemicals. Animals were all of the same strain, housed in the same room,

intubated with corn oil, tested concurrently for a least 1 year, and were examined by the same pathologists.

Chronic inflammation of the kidney was observed in control and treated male mice: 67, 80, 66, and 18% of untreated controls, pooled vehicle controls, low dose, and high dose, respectively. Female mice in the pooled vehicle control group (15%) and 722 mg/kg-day (2%), but not the untreated control and 360 mg/kg-day dose groups, exhibited chronic kidney inflammation. Tubular nephropathy (characterized by degeneration of convoluted tubule epithelium at the junction of the cortex and medulla, enlarged dark staining regenerative tubular epithelium, and infiltration of inflammatory cells, fibrosis and calcium deposition) was not observed in untreated or pooled vehicle controls of either sex, but was observed in mice treated with HCE: 49/50 and 47/49 in males and 50/50 and 45/49 in females in the 360 and 722 mg/kg-day dose groups, respectively. Information on the severity of these effects at the different dose levels was not presented. No other HCE-related nonneoplastic effects were observed. EPA considered 360 mg/kg-day as the LOAEL for this study based on tubular nephropathy. EPA considered that a NOAEL was not established.

Increases in the incidence of hepatocellular carcinomas were observed in male and female mice exposed to HCE (Table 4-10). Hepatocellular adenomas were not noted in the report. NCI (1978) reported statistically significant increases in the incidence of hepatocellular carcinomas in 30 and 63% of 360 and 722 mg/kg-day males, compared with 10 and 15% of pooled vehicle and matched vehicle controls, respectively. Female mice also demonstrated an increased tumor response, 40 and 31% of 360 and 722 mg/kg-day females compared with 3 and 10% of pooled vehicle and matched vehicle controls, respectively. Although the increases in HCE-treated females were not dose-dependent, a higher incidence of hepatocellular carcinomas was observed at the low dose (20/50) compared with the high dose (15/49). NCI concluded that HCE was carcinogenic in both sexes of $B6C3F_1$ mice (1978).

Table 4-10. Incidence of hepatocellular carcinomas in mice

	Pooled vehicle control[a]	Matched vehicle control	360 mg/kg-day	722 mg/kg-day
Males	6/60 (10%)	3/20 (15%)	15/50 (30%)[b]	31/49 (63%)[c]
Females	2/60 (3%)	2/20 (10%)	20/50 (40%)[c]	15/49 (31%)[c]

[a]As a result of the exceptionally low survival rates in the vehicle and untreated control groups, NCI used the pooled vehicle control data derived from concurrently run bioassays for several other chemicals. Animals were all of the same strain and housed in the same room. Incidences reported were not adjusted for survival.
[b]Statistically significant, $p = 0.008$.
[c]Statistically significant, $p < 0.001$.

Source: NCI (1978).

4.2.2. Inhalation

4.2.2.1. Subchronic Exposure

Median lethal concentration (LC_{50}) values for HCE have not been reported. Only one study is available in the peer-reviewed literature that evaluated the subchronic (Weeks et al., 1979) inhalation toxicity of HCE. Weeks et al. (1979) exposed Sprague-Dawley rats, beagle dogs, Hartley guinea pigs, and *Coturnix japonica* (Japanese quail) to HCE for 6 weeks. The effects observed in these species include neurotoxicity, reduced body weight gain, increased organ weights, and some evidence of respiratory tract irritation.

Weeks et al. (1979) exposed Sprague-Dawley rats (25/sex/concentration) to control air, 15, 48, or 260 ppm HCE (145, 465, and 2,517 mg/m^3, respectively; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observation was carried out for 12 weeks. An oxygen consumption test was also conducted. The authors reported that in the 2,517 mg/m^3 group, body weight gain of male, but not the nonpregnant female, rats was reduced beginning in the third week of exposure (although quantitative information was not reported). All rats in the 2,517 mg/m^3 group exhibited tremors, ruffled pelt, and red exudates around the eyes following the fourth week of exposure. The authors reported that in the male rats, relative kidney, spleen, and testes weights were significantly increased; in the female rats, only relative liver weights were significantly increased (although quantitative information was not reported). One male and one female rat died during the fourth week. During the observation period, treatment-related effects disappeared. No gross changes were evident at necropsy; however, after sacrifice, male and female rats of the 2,517 mg/m^{3}group had a higher incidence and severity of mycoplasma-related lesions in nasal turbinates, trachea, and lung compared with controls. The authors concluded these lesions were related to potentiation of an endemic mycoplasia infection rather than a direct effect of HCE exposure. However, no data were presented demonstrating the presence of mycoplasia in the lung. There were no histopathological differences observed between control and exposed rats sacrificed 12 weeks postexposure. No treatment-related effects were observed in the rats exposed to 145 and 465 mg/m^3 HCE.

In the oxygen consumption test, male rats (5/concentration) were tested prior to and following exposure to 145, 465, or 2,517 mg/m^3 HCE for 15 minutes, 3 days/week for the duration of the study (6 weeks). The 2,517 mg/m^3 rats exhibited significantly decreased mean rates of consumption both prior to (15%) and after (13%) HCE exposure. The authors suggested that this decrease in oxygen consumption, while nonspecific, is indicative of an alteration in basal metabolic rate. No histopathological effects were observed at this concentration. EPA considered 465 mg/m^3 the NOAEL and 2,517 mg/m^3 the LOAEL, based on reduced body weight gain, and increased organ weights.

Weeks et al. (1979) also exposed male Sprague-Dawley rats (15/concentration) exposed to 15, 48, or 260 ppm HCE (145, 465, or 2,517 mg/m^3) for 6 hours/day, 5 days/week for 6 weeks and examined them for behavioral changes related to learned and unlearned responses (described

in detail in Section 4.4.3.2). Similar to the other treated rats, body weight gain was reduced. Final mean body weight gain in male rats was reduced 2, 5, and 10% (statistically significant) in the 145, 465, and 2,517 mg/m^3 dose groups, respectively, compared with controls. Additionally, relative lung, liver, kidney, and testes weights were increased (quantitative information not reported) compared with controls.

Weeks et al. (1979) also exposed four male beagle dogs/concentration to control air, 15, 48, or 260 ppm HCE (145, 465, and 2,517 mg/m^3, respectively; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observation was carried out for 12 weeks. Blood samples were evaluated for blood chemistry parameters. In addition, the beagle dogs underwent pulmonary function tests prior to and following exposure. One dog died within 5 hours of exposure to 2,517 mg/m^3. The remaining animals in the 2,517 mg/m^3 group exhibited signs of neurotoxicity consisting of tremors, ataxia, hypersalivation, head bobbing, and facial fasciculations. No blood parameters were significantly affected and no exposure-related histopathological lesions were observed following necropsy on dogs sacrificed 12 weeks postexposture. Dogs evaluated for pulmonary functions while anesthetized did not display any significant effects. The HCE-exposed dogs did not display any treatment-related toxicity at 12 weeks postexposure. EPA considered 465 mg/m^3 the NOAEL and 2,517 mg/m^3 the LOAEL, based on neurotoxic effects.

Weeks et al. (1979) also exposed male Hartley guinea pigs (10/concentration) to control air, 15, 48, or 260 ppm HCE (145, 465, and 2,517 mg/m^3, respectively; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observation was carried out for 12 weeks. Guinea pigs were also evaluated for sensitization potential following inhalation exposure to HCE. Two guinea pigs died during each of the fourth and fifth weeks, resulting in four total deaths. Guinea pigs of the 2,517 mg/m^3 group displayed reductions in body weight beginning at the second week of exposure and significantly increased liver to body weight ratios (quantitative information was not reported). No treatment-related effects were observed in the other exposure groups. EPA considered the NOAEL as 465 mg/m^3 and the LOAEL as 2,517 mg/m^3, based on decreased body weight and significantly increased relative liver weight.

Weeks et al. (1979) also exposed male and female quail (*C. japonica*, 20/concentration) to control air, 15, 48, or 260 ppm HCE (145, 465, and 2,517 mg/m^3, respectively; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observation was carried out for 12 weeks. The only effects observed were in 2 of 10 quail in the 2,517 mg/m^3 group exhibited excess mucus in nasal turbinates after 6 weeks. The authors considered the excess mucus to be transient based on the lack of any inflammation or histopathological effects. Although the study authors considered the excess mucus to be a transient effect, EPA notes that the lack of inflammation and histopathological effects does not preclude the presence of more sensitive indicators of immune response (e.g., antibodies or other immune signaling chemicals) unable to be detected with methods available to the study authors. EPA considered 2,517 mg/m^3 (highest

exposure concentration) as the NOAEL, while the LOAEL could not be established from this study.

4.2.2.2. Chronic Exposure

No chronic exposure studies were identified.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral

Weeks et al. (1979) exposed 22 pregnant Sprague-Dawley rats/dose to 50, 100, or 500 mg/kg HCE (purity 99.8%) by gavage on gestation days (GDs) 6–16. Gavage controls received corn oil, and positive controls received 250 mg/kg aspirin. Dams orally administered 500 mg/kg HCE displayed tremors on GDs 15 and 16. Body weight gain of the 500 mg/kg dams was significantly lower than controls beginning on GD 8. Rats in the 500 mg/kg group exhibited an increased incidence of mucopurulent nasal exudates compared with controls. Approximately 70% of the orally exposed 500 mg/kg group had upper respiratory tract irritation; 20% had subclinical pneumonitis, compared with 10% in controls.

The aspirin-positive control group produced fetuses with lower body weights, and malformations such as hydrocephalus, spina bifida, and cranioschesis. None of the fetuses exhibited any significant skeletal or soft tissue anomalies, although fetuses from dams gavaged with 500 mg/kg HCE displayed significantly lower gestation indices, a lower number of viable fetuses/dam, and higher fetal resorption rates compared with controls (data not shown). This study concluded that HCE did not produce teratogenic effects at doses that were not maternally toxic.

Shimizu et al. (1992) evaluated the teratogenicity of HCE (purity not specified) in pregnant Wistar rats at doses of 0, 56, 167, or 500 mg/kg administered by gavage during GD 7-17 (20–21 rats/dose). The dams of the 500 mg/kg dose group exhibited significantly decreased weight gain after the second day of HCE treatment (8^{th} day of pregnancy); dams in the 167 mg/kg dose group displayed significantly decreased weight gain after the fourth day of treatment (10^{th} day of pregnancy) but not after the treatment ended on the 18^{th} day of pregnancy. Food intake was also significantly decreased in the 500 and 167 mg/kg dose groups after the second and third days, respectively, of HCE treatment; however, intake was normal when treatment ended. Dams in both the 167 and 500 mg/kg dose groups exhibited decreased motor activity (incidence and method of analysis not reported); dams in the 500 mg/kg dose group also exhibited piloerection and subcutaneous hemorrhage. These effects decreased or disappeared when HCE exposure ended. An autopsy performed on dams on GD 20, 3 days post-HCE exposure, revealed three rats with whitening of the liver in the 500 mg/kg dose group. The significance of this observation is unknown. No deaths occurred in any of the dose groups.

There were no significant differences between the HCE treatment and control groups with respect to the number of corpora lutea, number of implants, and number of live fetuses (Table 4-11). There was no significant difference in the incidence of dead or resorbed fetuses except for a significant increase during the late stage of pregnancy in the 500 mg/kg dose group (6.4% versus none in the control). Fetuses in the 500 mg/kg dose group also displayed significantly decreased body weight; 2.5 ± 0.57 (mean ± SD) and 2.3 ± 0.45 g in male and female fetuses, compared with 3.3 ± 0.20 and 3.1 ± 0.24 g in male and female controls, respectively.

Table 4-11. Summary of HCE effects on pregnant Wistar rats and their fetuses

	Dose (mg/kg)			
	0	**56**	**167**	**500**
Number of dams	20	20	20	21
% of dead or resorbed fetuses	8.7	9.2	7.0	14.7
Early stage	8.7	8.8	6.1	13.1
Late stage		0.4	0.9	6.4[a]
Body weight of live fetuses (g)[b]				
Male	3.3 ± 0.20	3.3 ± 0.17	3.2 ± 0.21	2.5 ± 0.57[a]
Female	3.1 ± 0.24	3.0 ± 0.20	2.9 ± 0.17	2.3 ± 0.45[a]

[a]Significantly different from control, $p < 0.01$.
[b]Values are mean ± SD.

Source: Shimizu et al. (1992).

Investigators examined the fetuses for external anomalies and found one case of acaudate in the 500 mg/kg dose group. Other anomalies included two fetuses with subcutaneous hemorrhage in the 167 and 500 mg/kg dose groups, and one case of hyposarca in the 500 mg/kg dose group. No skeletal malformations were observed in any group although a statistically significant increase in skeletal variations was observed in the 500 mg/kg (60.3%) group compared with controls (1.3%). Skeletal variations were significantly increased in the 500 mg/kg group (2 cases in the lumbar rib and 78 cases in the rudimentary lumbar rib) and nonsignificantly increased in the 167 mg/kg group (6 cases in the rudimentary lumbar rib) compared with controls (2 cases in the rudimentary lumbar rib) (Table 4-12). The degree of ossification (including number of sternebrae, number of proximal and middle phalanges, and number of sacral and caudal vertebrae) was significantly decreased in the 500 mg/kg dose group. No visceral malformations were observed and no significant differences in visceral anomalies were noted. The authors of this study concluded that there was no indication of teratological effects in rats for dose levels of HCE below 500 mg/kg. Shimizu et al. (1992) established a NOAEL of 56 mg/kg for dams and 167 mg/kg for fetuses. EPA considered the LOAEL for dams as 167 mg/kg-day, based on decreased motor activity and significantly decreased body weight.

EPA considered the LOAEL for fetuses as 500 mg/kg, based on significantly increased skeletal variations and significantly decreased ossification and fetal body weight.

Table 4-12. Summary of skeletal effects on fetuses from HCE-exposed rats

	Dose (mg/kg)			
	0	**56**	**167**	**500**
Number of fetuses examined	136	136	136	137
Percent of fetal variations	1.3	0	3.8	60.3[a]
Number of fetuses with variations				
Lumbar rib	0	0	0	2
Rudimentary lumbar rib	2	0	6	78
Ossification[b]				
Number of sternebrae	4.7 ± 0.07	4.5 ± 0.08	4.5 ± 0.08	3.4 ± 0.27[a]
Number of proximal and middle phalanges				
Fore limb	3.2 ± 0.05	3.1 ± 0.04	3.1 ± 0.04	2.9 ± 0.11[a]
Hind limb	4.0 ± 0.01	4.0 ± 0.01	4.0 ± 0.01	3.4 ± 0.23[a]
Number of sacral and caudal vertebrae	6.9 ± 0.06	6.9 ± 0.08	7.0 ± 0.04	5.7 ± 0.37[a]

[a]Significantly different from control, $p < 0.01$.
[b]As reported by Shimizu et al., the litter was used as the statistical unit for calculation of fetal values, thus these values represent the means ± SD of litter means within each group.

Source: Shimizu et al. (1992).

4.3.2. Inhalation

Weeks et al. (1979) exposed 22 pregnant Sprague-Dawley rats/concentration to control air, 15, 48, or 260 ppm HCE (145, 465, and 2,517 mg/m^3, respectively; purity 99.8%) by inhalation on GDs 6-16. Dams in the 2,517 mg/m^3 group displayed tremors during GDs 12–16. Body weight gain of the dams was significantly lower than controls beginning on GD 8 for the 2,517 mg/m^3 group, and beginning on GD 14 for the 465 mg/m^3 group. Rats in the 465 and 2,517 mg/m^3 groups exhibited an increased incidence of mucopurulent nasal exudates compared with controls. Inflammatory exudate was observed in the lumen of the nasal turbinates of 85% of the 465 mg/m^3 group and 100% of the 2,517 mg/m^3 group. The authors attributed the increased exudate to an endemic mycoplasia infection.

Fetuses of HCE-treated dams did not exhibit any significant skeletal or soft tissue anomalies. This study concluded that HCE did not produce teratogenic effects at concentrations that were not maternally toxic. EPA considered the NOAEL for the dams as 465 mg/m^3 and the LOAEL as 2,517 mg/m^3, based on neurological effects (tremors). EPA considered 2,517 mg/m^3 (highest concentration tested) as a fetal NOAEL, based on the lack of treatment-related effects, while a fetal LOAEL could not be established from this study.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute Exposure Studies

4.4.1.1. Oral

Several studies evaluated acute toxicity of HCE in animal species and reported lethal dose concentrations. Oral lethal doses ranged from 2,332 to 8,640 mg/kg in rats, >1,000 mg/kg in male rabbits, and 4,970 mg/kg in guinea pigs (Kinkead and Wolfe, 1992; Weeks et al., 1979). According to the Hodge and Sterner Scale, these lethal doses place HCE in low toxicity range (Hodge and Sterner, 1949). Reynolds (1972) administered a single dose of HCE (purity not specified) at 26 mmol/kg (6,155 mg/kg) by gavage in mineral oil to male rats and reported that liver function (assessed by microsomal protein concentration, antipyrine demethylase activity, NADP-neotetrazolium reductase activity, glucose 6-phosphatase activity, and conjugated diene concentration in microsomal lipids) was unaffected 2 hours after exposure. Kinkead and Wolfe (1992) determined the oral median lethal dose (LD_{50}) for HCE (purity not specified) in male and female Sprague-Dawley rats (5 rats/sex/dose) was 4,489 mg/kg (95% confidence limits [CLs], 2,332–8,640 mg/kg). A study in sheep that was conducted at high doses (500-1,000 mg/kg) found reduced hepatic function (Fowler, 1969).

Weeks et al. (1979) and Weeks and Thomasino (1978) determined acute oral toxicity values for Sprague-Dawley rats, New Zealand White rabbits, and Hartley guinea pigs by administering a single dose of HCE (99.8% purity) dissolved in corn oil (50% w/v) or methylcellulose (5% w/v) via stomach tube. Approximate lethal dosages (ALD) or LD_{50} values were calculated after a 14-day observation period (Table 4-13). All LD_{50} values were > 1,000 mg/kg.

Table 4-13. Summary of acute exposure data in rats, rabbits, and guinea pigs

			Lethal value		
Species	**Treatment**	**Diluent**	**mg/kg**	**95% CL**	**Slope**
Rabbit, male	Oral ALD	Methylcellulose	>1,000		
Rat, male	Intraperitoneal (i.p.) ALD	Corn oil	2,900		
Rat, male	Oral ALD	Corn oil	4,900		
Rat, female	Oral LD_{50}	Corn oil Methylcellulose	4,460 7,080	3,900–5,110 6,240–8,040	9.3 19.9
Rat, male	Oral LD_{50}	Corn oil Methylcellulose	5,160 7,690	4,250–6,270 6,380–9,250	6.1 8.5
Guinea pig, male	Oral LD_{50}	Corn oil	4,970	4,030–6,150	4.7
Rabbit, male	Dermal LD_{50}	Water paste	≥32,000		

Sources: Weeks et al. (1979); Weeks and Thomasino (1978).

Fowler (1969) orally administered a single dose of HCE (purity not specified) through a drenching bottle to Scottish Blackface and Cheviot cross sheep at three dose levels: 500 (six sheep), 750 (one sheep), and 1,000 mg/kg (one sheep). Hepatotoxicity was assessed by

measurement of plasma enzyme activities and bromsulphthalein dye clearance tests, which are widely-used indices of hepatic function in sheep. Plasma activities of glutamate dehydrogenase (GDH), sorbitol dehydrogenase (SDH), ornithine carbamoyl transferase (OCT), and AST were determined daily until they reached stable levels. Increases in these enzymes are indicative of hepatic damage. HCE exposure resulted in a 3-6-fold increase in GDH, with the exception of one sheep that exhibited a 55-fold increase. SDH was increased 3-6-fold, and OCT was increased 2-10-fold. GDH, SDH, and OCT levels peaked at 48 hours and returned to normal within 4–5 days. AST increased only slightly. Bromosulphophthalein dye clearance tests found a reduction in transfer from liver cells to bile at 72 hours after HCE exposure, indicating reduced hepatic function.

4.4.1.2. Inhalation

One study is available in the peer-reviewed literature that evaluated acute (Weeks and Thomasino, 1978) inhalation exposure to HCE. Weeks and Thomasino (1978) exposed six male rats/concentration (strain not specified, although one table in the report indicated strain as Sprague-Dawley) to 260 and 5,900 ppm HCE (2,500 or 57,000 mg/m^3) for 8 hours and to 1,000 ppm HCE (17,000 mg/m^3) for 6 hours. Postexposure observation was carried out for 14 days. Male rats exposed for 8 hours to 2,500 mg/m^3 HCE displayed no toxic signs during exposure or for 14 days thereafter. Body weight gain was slightly, but not statistically significantly, reduced over the 14-day exposure period. Male rats exposed for 8 hours to 57,000 mg/m^3 HCE displayed severe toxic signs including death. At 6 hours, one rat had a staggered gait. At 8 hours, 2/6 rats were dead. The surviving rats showed statistically significant reductions in mean body weight on exposure day 0 (7%), 1 (21%), 3 (19%), 7 (15%), and 14 (15%), compared with controls. Necropsy did not reveal any gross exposure-related lesions. Microscopy revealed that two of the four surviving rats had minimally to moderately severe subacute diffuse interstitial pneumonitis and vascular congestion. Additionally, a purulent exudate of the nasal turbinates was observed in one control and one treated rat. The authors concluded that this effect was not exposure-related but rather indicative of a low-grade endemic upper respiratory disease. The male rats exposed for 6 hours to 17,000 mg/m^3 showed slight reductions in body weight gain on postexposure days 1 (5%) and 3 (4%) and body weights similar to controls for the remaining 11 days of the postexposure period. Two of the six rats demonstrated a staggered gait. No exposure-related gross or histopathological changes were observed in tissues and organs.

4.4.2. Short-term Exposure Studies

Several studies evaluated short-term toxicity of HCE in animal species. A 12-day study in male New Zealand White rabbits found liver degeneration and necrosis, as well as tubular nephrosis in the kidney, indicating that both the liver and kidney are potential target tissues for

HCE-induced toxicity (Weeks et al., 1979). Short-term toxicity assays in rats (16 and 21 days) demonstrated kidney effects in males (NTP, 1996, 1989) but not females (NTP, 1989).

Weeks et al. (1979) conducted a 12-day study of HCE in male New Zealand White rabbits. Five rabbits/dose were administered a daily oral dose via a stomach tube of 100, 320, or 1,000 mg/kg HCE (purity 99.8%) suspended in 5% aqueous methylcellulose. Blood was drawn from the central ear artery of the rabbits on treatment days 1, 4, 8, and 12, and on day 4 following termination of dosing. Serum was analyzed for the following parameters: glutamic oxaloacetic transaminase (SGOT; also known as AST), glutamic pyruvic transaminase (SGPT; also known as ALT), blood urea nitrogen (BUN), alkaline phosphatase, bilirubin, total protein, potassium and sodium. On the 4th day following the termination of dosing, rabbits were necropsied and the following tissues were examined: eye, brain, lung, kidney, liver, spleen, heart, stomach, pancreas, large intestine, skeletal muscle, bone, urinary bladder, small intestine, and testes.

The 1,000 mg/kg dose group exhibited significantly reduced body weight (beginning on treatment day 7) and increased relative liver and kidney weights. The 320 mg/kg dose group exhibited significantly reduced body weight beginning on day 10. The 100 mg/kg dose group did not display any changes. The 320 and 1,000 mg/kg dose groups displayed liver degeneration and necrosis, including fatty degeneration, coagulation necrosis, hemorrhage, ballooning degeneration, eosinophilic changes, and hemosiderin-laden macrophages and giant cells. These effects were not observed in controls or rabbits of the 100 mg/kg dose group. Liver lesions increased in severity in a dose-related manner in which the effects were more severe in the 1,000 mg/kg group compared with the 320 mg/kg group. Tubular nephrosis of the convoluted tubules in the corticomedullary region of the kidney was also observed in the rabbits of the 320 and 1,000 mg/kg dose groups. These animals also exhibited tubular nephrocalcinosis of a minimal degree. The only blood chemistry parameters that were affected were significantly decreased potassium and glucose levels in the 320 and 1,000 mg/kg groups. EPA considered the NOAEL as 100 mg/kg and the LOAEL as 320 mg/kg, based on dose-related increases in severity of liver and kidney lesions.

The NTP (1989) conducted a 16-day study of oral HCE toxicity in F344/N rats. Groups of five rats/sex/dose were administered 0, 187, 375, 750, 1,500 or 3,000 mg HCE/kg (purity >99%) for 12 doses over 16 days by corn oil gavage. TWA doses were 0, 140, 281, 563, 1,125, and 2,250 mg/kg-day, respectively. Necropsy was performed on all rats; all organs and tissues were examined for grossly visible lesions and histopathology. All rats of the 1,125 and 2,250 mg/kg-day dose groups and 1/5 males and 2/5 females from the 563 mg/kg-day dose group died before the end of the study. Final mean body weights (statistical analyses were not reported) were decreased by 25% in males of the 563 mg/kg-day dose group; female body weights were decreased by 37% in the 563 mg/kg-day dose group. Microscopic observations of the kidneys revealed hyaline droplet formation in the cytoplasm of renal tubular epithelium in all

the treated males, and tubular cell regeneration and eosinophilic granular casts of cell debris in tubule lumina of male rats administered 140 and 281 mg/kg-day. EPA considered 140 mg/kg-day (lowest dose tested) a male rat LOAEL based on kidney tubule lesions, while a NOAEL could not be established for male rats. EPA considered the female rat LOAEL as 563 mg/kg-day, based on a dose-related decrease in body weight, and the female rat NOAEL as 281 mg/kg-day.

NTP (1996) conducted a 21-day study of oral HCE toxicity in male F344/N rats. Groups of five rats/dose were administered 0.62 or 1.24 mmol HCE/kg-day (146 or 293 mg/kg-day, respectively; purity 100%) by corn oil gavage. Necropsies were performed on all rats; the right kidney, liver, and right testis were weighed and underwent histopathological evaluation. Urine samples were collected during an overnight period that began 4 days before the end of the study. Urinalysis included measurements of volume, specific gravity, creatinine, glucose, total protein, AST, γ-glutamyl transferase (GGT), and *N*-acetyl-β-D-glucosaminidase (NAG). A Mallory-Heidenhain stain was used for kidney sections to evaluate protein droplets, particularly hyaline droplet formation. Cell proliferation analyses were performed on kidney sections and were scored by a labeling index indicating the percentage of proximal and distal tubule epithelial cells in S-phase.

Results from the measured endpoints/parameters are summarized in Table 4-14. Absolute and relative kidney weights were significantly increased in both dose groups; absolute and relative (significant at high dose) liver weights were increased in both dose groups. Rats of the 293 mg/kg-day group also exhibited significantly lower urinary creatinine and specific gravity, while glucose and urine volume were greater than controls. AST and NAG activities were significantly higher than in controls. Nephropathy, consisting of hyaline droplet accumulation, was observed in the male rats in addition to increased incidences of tubule regeneration (3/5 and 4/5 for 146 and 293 mg/kg-day, respectively) and granular casts (4/5 and 3/5 for 146 and 293 mg/kg-day, respectively). The mean proliferating cell nuclear antigen (PCNA) labeling index was significantly increased 5.7- and 9.2-fold, compared with controls, in the 146 and 293 mg/kg-day dose groups. EPA did not identify a NOAEL because effects (including increased absolute and relative kidney weight, increased AST and NAG activity, increased PCNA labeling index, and nephropathy) were observed at the low dose level. EPA considered 146 mg/kg-day a LOAEL based on statistically significant increases in kidney lesions and urinalysis parameters.

Table 4-14. Summary of toxicity data from male rats exposed to HCE for 21 days

	Vehicle control	146 mg/kg-day HCE	293 mg/kg-day HCE
Right kidney weight[a]			
Absolute (g)	1.009 ± 0.025	1.157 ± 0.011[b]	1.250 ± 0.022[b]
Relative (mg/g)	3.19 ± 0.04	3.77 ± 0.06[b]	4.07 ± 0.05[b]
Liver weight[a]			
Absolute (g)	11.041 ± 0.291	11.959 ± 0.178	13.479 ± 0.390
Relative (mg/g)	34.82 ± 0.60	39.01 ± 0.92	43.84 ± 0.64[b]
Right testis weight[a]			
Absolute (g)	1.412 ± 0.037	1.409 ± 0.023	1.430 ± 0.016
Relative (mg/g)	4.47 ± 0.09	4.60 ± 0.11	4.66 ± 0.05
Urinalysis			
Creatinine (mg/dL)	143.22 ± 18.12	79.56 ± 11.01	54.48 ± 3.06[b]
Glucose (μg/mg creatinine)	169 ± 3	344 ± 30	446 ± 23[b]
Protein (mU/mg creatinine)	1,322 ± 59	1,748 ± 257	2,980 ± 103
AST (mU/mg creatinine)	6 ± 1	40 ± 6[c]	66 ± 5[b]
GGT (mU/mg creatinine)	1,456 ± 47	1,547 ± 66	1,897 ± 73
NAG (mU/mg creatinine)	11 ± 0	23 ± 2[c]	36 ± 1[b]
Volume (mL/16 h)	4.2 ± 0.8	7.5 ± 9	10.6 ± 1.1[b]
Specific gravity (g/mL)	1.038 ± 0.005	1.024 ± 0.003	1.020 ± 0.001[b]
PCNA labeling index (mean ± SE)	0.13 ± 0.02	0.74 ± 0.19[c]	1.2 ± 0.2[c]

[a]Data are mean ± SE.
[b]Significantly different from control ($p \leq 0.01$).
[c]Significantly different from control ($p \leq 0.05$).

Source: NTP (1996).

4.4.3. Neurological

Neurological endpoints for HCE toxicity have been evaluated in several HCE toxicity studies. The studies listed below provide limited evidence that HCE produces neurological effects; however, it is unknown if the central nervous system (CNS) effects are due to the parent compound or the metabolites. Although there are few studies on the neurological effects associated with HCE exposure, the database is extensive for two of its proposed metabolites, PERC and TCE. Studies have shown that PERC and TCE readily cross the blood brain barrier resulting in CNS depressive effects. Sheep exposed to high doses of HCE (500-1,000 mg/kg) developed facial muscle tremors (Fowler, 1969; Southcott, 1951), and a staggering uncoordinated gait (Southcott, 1951). Sprague-Dawley rats evaluated for HCE-induced effects on avoidance latency (i.e., learned behavior) and spontaneous motor activity (i.e., unlearned behavior) exhibited slight, but not statistically significant, behavioral effects at 2,517 mg/m^3. Male and female rats exhibited tremors and ruffled pelt at 2,517 mg/m^3 as well (Weeks et al., 1979). Beagle dogs developed signs of neurotoxicity such as tremors, ataxia, hypersalivation, and head bobbing, following exposure to 2,517 mg/m^3 HCE. Dogs showed similar signs of

neurotoxicity intermittently throughout the HCE exposures, with signs disappearing overnight. During an observation period of 12 weeks following exposure, these symptoms were not observed (Weeks et al., 1979).

4.4.3.1. Oral Studies

Fowler (1969) orally administered a single dose of HCE (purity not specified) to Scottish Blackface and Cheviot cross sheep at three dose levels: 500 (10 sheep), 750 (1 sheep), and 1,000 mg/kg (1 sheep). Slight facial muscle tremors were noted in three sheep between 1 and 4 hours after dosages of 500-1,000 mg/kg HCE. The HCE dose level for the individual sheep exhibiting facial tremors was not specified by the authors. Fowler (1969) also examined two sheep administered 0.3 mL/kg PERC and two sheep administered 0.3 mL/kg pentachloroethane, two proposed major metabolites of HCE. The sheep exposed to PERC exhibited no effects following exposure, while the sheep exposed to pentachloroethane exhibited narcosis One pentachloroethane-exposed animal was recumbent within 30 minutes of exposure, exhibiting flaccid limbs, depression of normal reflexes, and labial tremors. The sheep regained normal posture 9 hours postexposure and appeared normal 72 hours postexposure. The second pentachloroethane-treated sheep became recumbent within 20 minutes of exposure and exhibited labial tremors. However, unlike the first sheep, this animal appeared normal 1.5 hours postexposure. EPA considered the LOAEL as 500 mg/kg (lowest dose tested), based on neurotoxic effects (tremors), while a NOAEL could not be established from this data.

Southcott (1951) treated 30 Merino Wethers sheep suffering from liver fluke infections with 15 g HCE-bentonite dispersible powder (13.5 g HCE, 445 mg/kg; 15 sheep) or 30 g HCE-bentonite (27 g HCE, 906 mg/kg; 15 sheep). The purity of the HCE was not specified. Two sheep died a day after treatment and nine others were unable to rise and stand. One of the severely affected sheep (i.e., unable to rise and stand) was from the 445 mg/kg HCE group and the other eight were from the 906 mg/kg group. Some severely affected animals (two from the 445 mg/kg group) could walk if placed on their feet, but displayed a staggering, uncoordinated gait and fell again. The lips, face, neck, and forelegs were afflicted by fine muscular tremors that were observed in most of the animals. EPA considered the LOAEL as 445 mg/kg (lowest dose tested), based on neurological effects consisting of tremors, staggering, uncoordinated gait, and inability to stand, while a NOAEL could not be established from this study.

As described in Section 4.3.1, Shimizu et al. (1992) reported decreased motor activity (incidence and method of analysis not reported) in pregnant Wistar rats (20–21 rats/dose) at doses of 167 and 500 mg/kg administered by gavage during GD 7–17. These effects decreased or disappeared when HCE exposure ended. Weeks et al. (1979) exposed 22 pregnant Sprague-Dawley rats/dose to 50, 100, or 500 mg/kg HCE by corn oil gavage on GDs 6–16. Dams orally administered 500 mg/kg HCE displayed tremors on GDs 15 and 16.

4.4.3.2. Inhalation Studies

Weeks et al. (1979) exposed male Sprague-Dawley rats (15/concentration) to air, 15, 48, or 260 ppm HCE (145, 465, or 2,517 mg/m^3, respectively; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Learned behavior endpoints evaluated using an avoidance latency task by measuring the time it took the rats to avoid foot shock by escaping into a safe compartment and unlearned behavior endpoints (spontaneous motor activity; evaluated by photobeam interruptions) were measured in the animals. The avoidance latency task was conducted prior to exposure, 1 day into exposure, after 3 weeks of exposure and after 6 weeks of exposure. Spontaneous motor activity was tested after 3 and 6 weeks of exposure.

Avoidance latency was slightly but not significantly increased in the 465 and 2,517 mg/m^3 groups at 6 weeks (median 3.9 and 3.3 seconds, respectively) compared with control (median 2.2 seconds). Spontaneous motor activity counts were slightly, but not significantly increased in the HCE-treated rats (mean ± SD): 231 ± 77 for 145 mg/m^3, 183 ± 109 for 465 mg/m^3, and 201 ± 102 for 2,517 mg/m^3, compared with control rats (163 ± 74). Weeks et al. (1979) concluded that the rats did not display obvious signs of behavioral toxicity. However, tremors and a ruffled pelt were noted in a separate experiment in male and female rats exposed to 2,517 mg/m^3 HCE during the fourth week of exposure. Tremors and lack of grooming are indicators of neurobehavioral effects (Kulig et al., 1996). The investigators sacrificed the rats 12 weeks after the last exposure and reported that all measurable changes (e.g., brain histopathology, body weights) were comparable to controls.

Weeks et al. (1979) also exposed 22 pregnant Sprague-Dawley rats/concentration and 4 beagle dogs/concentration to 145, 465, and 2,517 mg/m^3 HCE by inhalation. Rat dams in the 2,517 mg/m^3 group displayed tremors during GDs 12–16. Dogs in the 2,517 mg/m^3 exposure group developed tremors, ataxia, hypersalivation, and displayed severe head bobbing, facial muscular fasciculations, and held their eyelids closed during exposure. One dog experienced convulsions and died within 5 hours after initial exposure. The surviving dogs exhibited less severe symptoms during exposure, but recovered overnight after removal from exposure.

4.4.4. Immunological

Ten male Hartley guinea pigs/dose were exposed by inhalation to control air or three concentrations of HCE (purity 99.8%): 15, 48, or 260 ppm (145, 465, and 2,517 mg/m^3, respectively; Weeks et al., 1979). Exposures were conducted for 6 hours/day, 5 days/week for 3 weeks. Following exposure, animals were allowed to rest for 2 weeks. The guinea pigs were then challenged with a single intradermal injection of 0.1% HCE in saline. A sensitization response was not produced.

4.4.5. Dermatological

Yamakage and Ishikawa (1982) examined certain patients suffering from systemic scleroderma (SSD) for potential exposure to solvents. These patients also presented with localized scleroderma with bilateral distribution of multiple skin lesions reminiscent of those observed in several cases of occupational or agent-induced scleroderma. Of nine such patients, seven had had significant subchronic/chronic exposure (5–44 years), while an eighth had had a significant acute exposure (2 weeks). The solvents involved were reported as "variable and mostly unidentified." As an experimental follow-up, groups of ddY mice received intraperitoneal (i.p.) injections daily for 17 days with 1 of 10 experimental solvents, as well as with 0.9% saline to mitigate treatment lethality. For HCE, 17 mice were injected daily with 0.01 mL of HCE (purity not specified) and 0.1 mL of 0.9% saline. Along with naphtha ("Esso No. 5") and n-hexane, HCE was found by double-blind histological examination and electron microscopy to be a significant inducer of sclerodermatous changes in skin taken from the animals' backs, near the forelimbs. HCE treatment resulted in evident dermal sclerosis in five mice, slight fibrosis in another, and no change in nine; two mice died. PERC, a primary metabolite of HCE, was similarly tested in 10 mice. Injections of 0.005 mL (+ saline) resulted in evident dermal sclerosis in one mouse, slight fibrosis in two, no change in six, and death in one. Even though the experimental route of exposure used is generally irrelevant to humans, the skin lesions produced by HCE were "fundamentally similar" to those produced by control reference solvents that have been implicated in human occupational SSD. Thus, this study provides indirect evidence that suggests HCE may be capable of inducing SSD-type conditions in humans.

Weeks and Thomasino (1978) conducted two dermal studies in male New Zealand White rabbits. A single 24-hour application of 500 mg of technical dry HCE to intact and abraded skin of six rabbits did not result in primary irritation of intact or abraded skin when assessed at 24 hours, 72 hours, or 7 days after exposure. HCE was placed in Irritation Category IV (no irritation). In the second study, HCE was applied as a paste in 0.5 mL of distilled water. Intact skin displayed no edema and barely perceptible erythema at 24 hours. Abraded skin displayed barely perceptible erythema in one rabbit with moderate to slight erythema reactions. HCE was placed in Irritation Category III (mild or slight irritation).

4.4.6. Eye Irritation

Weeks and Thomasino (1978) applied a single, 24-hour dose of 100 mg dry HCE to one eye of six male New Zealand White rabbits. Moderate corneal damage, iritis, and conjunctivitis was observed in 5/6 rabbits 24, 48, and 72 hours after exposure. No effects were observed 7 days after exposure. HCE was placed in Irritation Category II for eye effects (corneal opacity reversible within 7 days or persisting for 7 days).

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

4.5.1. Genotoxicity

In vivo genotoxicity studies have not been performed in humans exposed to HCE. In vivo exposure to animals resulted in predominantly nonpositive results. Similarly, in vitro genotoxicity studies conducted in microorganisms, cultured mammalian cells, and insects (Table 4-15) were largely nonpositive both in the presence and absence of exogenous metabolic activation. Ashby and Tennant (1988) examined genotoxic carcinogenesis in a set of 222 chemicals tested in rodents by NCI/NTP; HCE did not induce mutagenicity in *Salmonella typhimurium* reverse mutation tester strains. NTP's technical report on the toxicity and carcinogenicity of HCE in F344/N rats concluded that HCE (purity >99%) was not significantly genotoxic, and that the increased incidence of tumors occurred through a mechanism other than one involving the induction of mutations (NTP, 1989). In an examination of putative "nongenotoxic" carcinogens on the basis of their reported mutagenicity per se (the ability to induce alterations in DNA structure or content, i.e., gene mutation, chromosomal aberrations [CAs], or aneuploidy), HCE was categorized as having insufficient data for evaluation (Jackson et al., 1993). Studies conducted by Lohman and Lohman (2000) considering DNA damage, recombination, gene mutation, sister chromatid exchange (SCE), micronuclei (MN), CA, aneuploidy, and cell transformation as endpoints indicate that the genetic activity profile for HCE is predominantly nonpositive. However, some positive findings have been reported in assays for gene conversion, somatic mutation/recombination, DNA adducts, and SCEs.

Table 4-15. Summary of genotoxicity studies of HCE

Test system	Genetic endpoint	Strain/cells	Results	Reference	Comments
In vitro tests					
Bacterial	Gene reversion/ *S. typhimurium*	TA98; TA100; TA1535; TA1537; TA1538	– (±S9)[a]	Simmon and Kauhanen (1978)	
		TA98; TA100; TA1535; TA1537; TA1538	– (±S9)[a]	Weeks et al. (1979)	
		TA98; TA100; TA1535; TA1537	– (±S9)[a]	Haworth et al. (1983)	Liquid preincubation protocol
		TA98; TA100; TA1535; TA1537	– (±S9)[a]	Milman et al. (1988)	
	Forward mutations	BA13	– (±S9)[a]	Roldán-Arjona et.al. (1991)	Liquid preincubation protocol
	SOS test	TA1535/pSK1002	– (±S9)[a]	Nakamura et al. (1987)	*umu* test; Liquid preincubation protocol
Mammalian	CAs	Chinese hamster ovary (CHO)	– (±S9)[a]	Galloway et al. (1987)	

Table 4-15. Summary of genotoxicity studies of HCE					
Test system	**Genetic endpoint**	**Strain/cells**	**Results**	**Reference**	**Comments**
	SCEs	CHO	– (–S9)[a], + (+S9)[a]	Galloway et al. (1987)	HCE precipitation at doses causing positive results
	MN	AHH-1	–	Doherty et al. (1996)	Human cell line
		MCL-5	–	Doherty et al. (1996)	Human cell line
		h2E1	–	Doherty et al. (1996)	Human cell line
	Cell transformation	BALB/c-3T3	–	Milman et al. (1988)	
	DNA adduct formation (nonhuman)	Wistar rats, calf thymus DNA	+ DNA binding in liver, kidney, lung, and stomach	Lattanzi et al. (1988)	DNA adducts not identified
		BALB/c mice, calf thymus DNA	+ DNA binding in liver, kidney, lung, and stomach	Lattanzi et al. (1988)	DNA adducts not identified
Fungi	Mitotic recombination	*S. cerevisiae* D3	– (±S9) [a]	Simmon and Kauhanen (1978)	
		S. cerevisiae D4	– (±S9) [a]	Weeks et al. (1979)	
		S. cerevisiae D7	– (±S9) [a]	Bronzetti et al. (1990, 1989)	
	Aneuploidy	*Aspergillus nidulans* P1 diploid	–	Crebelli et al. (1995,1992, 1988)	
In vivo tests					
Rat	Rat liver foci	Osborne-Mendel	– (initiation) +(promotion)	Milman et al. (1988)	Initiation or promotion protocols
	DNA adduct formation (Nonhuman)	Wistar rats	Weakly + DNA binding in liver	Lattanzi et al. (1988)	Adducts not identified
Mice	Micronucleus induction	CD-1 mice	–	Crebelli et al. (1999)	
	Replicative DNA synthesis	B6C3F1 mice	+	Yoshikawa (1996); Miyagawa et al. (1995)	Hepatic cell proliferation
		BALB/c mice	Moderately + DNA binding in liver	Lattanzi et al. (1988)	Adducts not identified
Human Lymphocytes		Isolated human lymphocytes	+ (±S9)	Tafazoli et al. (1998)	
	DNA strand breaks	Human lymphocyte cultures	–	Tafazoli et al. (1998)	Comet assay

Table 4-15. Summary of genotoxicity studies of HCE					
Test system	**Genetic endpoint**	**Strain/cells**	**Results**	**Reference**	**Comments**
Drosophila	Mitotic recombination	Drosophila	Weakly +	Vogel and Nivard (1993)	Eye mosaic assay

[a]S9 is a supernatant fraction from 9000 ×g centrifugal spin.

Using the standard Ames assay for reversion of *S. typhimurium* histidine tester strains (TA1535, TA1537, TA1538, TA98, and TA100), Simmon and Kauhanen (1978) found HCE to be nonmutagenic at concentrations of 5,000 or 10,000 μg HCE/plate (purity not specified), both in the absence and presence of an exogenous Aroclor 1254-stimulated rat liver supernatant fraction from 9,000 × g centrifugal spin (S9) metabolic activation system. HCE was reported to be slightly toxic at the 10,000 μg/plate concentration in the absence of the S9 mix. Weeks et al. (1979) also reported nonpositive results using the same tester strains, test protocol, solvent, and metabolic activation system over a concentration range of 0.1–500 μg HCE/plate (purity 99.8%). Further, as a part of NTP's mutagenicity screening program, HCE was dissolved in dimethylsulfoxide (DMSO) and tested in two independent trials in two separate laboratories over a collective concentration range of 1–10,000 μg/plate. HCE was nonpositive for induction of reverse mutation in *S. typhimurium* (tester strains TA1535, TA1537, TA98, and TA100), with and without S9 metabolic activation (NTP, 1989; Haworth et al., 1983). Finally, HCE (purity >97%) was reported to be nonpositive in several Ames tester strains, both with and without S9 from the Aroclor 1254-induced livers of both sexes of Osborne Mendel rats and B6C3F1 mice (Milman et al., 1988).

Using a different *S. typhimurium* indicator strain, BA13, in a liquid preincubation protocol of the Ara test, Roldán-Arjona et al. (1991) found HCE to be nonpositive. This bacterial assay examines the ability of an agent to induce forward mutations from L-arabinose sensitivity to resistance, and theoretically might be expected to detect a broader range of mutagens than reverse-mutation assays. HCE (purity 98%) was dissolved in DMSO and tested over a concentration range of 1.5–30.0 μmol/plate (355–7,102 μg/plate), both with and without rat liver S9 metabolic activation. Of the 16 chemicals tested in this study, HCE was the only one that did not demonstrate any toxicity, which the authors speculated was probably related to its low solubility in water. HCE (purity not specified) was nonpositive when assayed in the *umu* test using *S. typhimurium* tester strain TA1535/pSK1002 (Nakamura et al., 1987). This study also employed a liquid preincubation protocol, and was conducted both with and without rat liver S9 metabolic activation, up to a concentration of 42 μg/mL (solvent, water or DMSO, was not specified for individual test agents). Although the available data indicate that HCE is not mutagenic to Salmonella, Legator and Harper (1988) suggested that this may be related to inadequate reductive dechlorination (i.e., if HCE is activated by metabolic pathways not present in the in vitro system used).

HCE was assayed for its ability to induce mitotic recombination in tester strain D3 of the yeast *S. cerevisiae* (Simmon and Kauhanen, 1978). No significant activity over a concentration range of 0.1-5.0% HCE (1–50 mg/mL; purity not specified), with or without exogenous rat liver S9 metabolic activation was observed. In addition, nonpositive findings for HCE were reported by Weeks et al. (1979) using the *S. cerevisiae* D4 strain.

Bronzetti et al. (1989) evaluated HCE (purity not specified) for mitotic gene conversion at the trp locus and reverse point mutation at the ilv locus in the *S. cerevisiae* D7 tester strain. Two-hour liquid suspension exposures were conducted both on a logarithmic growth phase culture having high levels of CYP450 metabolizing enzymes, and on stationary growth phase cultures either with or without exogenous liver S9 mix. Exposures were from 5 to 12.5 mM (1.2–3.0 mg/mL) and were reportedly limited by solubility. HCE was inactive for both gene conversion and reverse mutation in stationary cultures with or without S9, and for reverse mutation in the logarithmic culture. However, statistically significant ($p \leq 0.05$–0.001) increases in revertant frequency of more than twofold over background were observed at every concentration (Bronzetti et al., 1989).

The ability of various halogenated hydrocarbons to induce aneuploidy in the P1 diploid strain of the mold *Aspergillus nidulans* has been reported (Crebelli et al., 1995, 1992, 1988). Liquid suspension exposures (3 hours) to concentrations of 0.0025–0.04% HCE (0.005-0.84 mg/mL; purity >98%) resulted in survival rates of 100-48%. Exposure to these concentrations did not induce mitotic malsegregation of chromosomes.

A number of studies have evaluated the effects of in vivo and in vitro HCE exposures on various cytogenetic endpoints in higher organisms (Crebelli et al., 1999; Tafazoli et al., 1998; Doherty et al., 1996; Vogel and Nivard, 1993; NTP, 1989; Galloway et al., 1987). Crebelli et al. (1999) utilized the mouse bone marrow micronucleus test to investigate the in vivo induction of micronucleated polychromatic erythrocytes (MNPCE) by 10 aliphatic halogenated hydrocarbons, including HCE. CD-1 mice (5/sex/concentration) were injected i.p. with HCE doses of 2,000 or 4,000 mg/kg (purity >98%), representing approximately 40 and 70-80% of the LD_{50}, respectively. Animals were sacrificed and bone marrow cells harvested at 24 and 48 hours post treatment. At least 5,000 polychromatic erythrocytes/animal were analyzed. HCE treatment induced clinical signs of general toxicity, but no significant increases in the frequency of MNPCE in any treated group.

Vogel and Nivard (1993) utilized a Drosophila eye mosaic assay to monitor genetic damage in somatic cells, predominantly interchromosomal mitotic recombination, caused by the exposure of larvae to various chemicals. In the case of HCE (3% ethanol solvent; purity not specified), adult flies of the C-1 cross were permitted to lay eggs for 3 days on food supplemented with 10 mM HCE. Examination for light spots in the normally colored eyes of the resulting flies revealed what the authors classified as a weak positive response for HCE—a reproducible increase of not more than a doubling of the spontaneous frequency at a dose

associated with toxicity. The authors suggested that the effect was unspecific and likely not related to genotoxicity.

HCE was evaluated for its ability to induce MN and DNA damage in isolated human lymphocytes from two donors (Tafazoli et al., 1998). Lymphocytes were exposed for 3 hours in the presence of exogenous metabolic activation (S9 mix), or for 48 hours in the absence of S9. Results using cells from one donor ("A") were reported for HCE (purity >99%) for exposures of 0.05–1.00 mM (0.012–0.24 mg/mL) in the presence of S9. Neither toxicity nor MN induction was evident. Cells from the other donor ("D") were exposed to higher HCE concentrations of 1-16 mM (a saturating concentration; 0.24–3.79 mg/mL), both with and without S9. Toxicity (measured as a significant decrease in the relative division index) was still not observed, but statistically positive results for percent cells with MN were recorded at HCE concentrations of 1 and 8 mM (0.24 and 1.89 mg/mL, respectively) in the absence of S9 (12 and 11%, respectively, versus a control value of 5.5%, $p < 0.05$), and at 1 mM (0.24 mg/mL) in the presence of S9 (19.8% versus a control value of 9%, $p < 0.01$). In the second part of the study, lymphocyte cultures exposed to test agents for 3 hours with and without S9 were assessed for DNA damage (breaks, alkali-labile sites) using the Comet assay. HCE did not affect the measured DNA damage parameters (tail length, fraction of total cellular DNA in the tail, and tail moment).

Doherty et al. (1996) examined in vitro induction of MN by HCE in three human cells lines with metabolic competence; lymphoblastoid AHH-1 (native CYP1A1 activity), MCL-5 (transfected with cDNAs encoding human CYP1A2, 2A6, 3A4, 2E1, and microsomal epoxide hydrolase), and h2E1 (with cDNA for human CYP2E1). Exponentially growing cultures were exposed for approximately one cell cycle (18 hours for AHH-1, 24 hours for MCL-5 and h2E1) to 0, 0.01, 0.05, or 0.1 mM HCE (purity not specified; 0, 0.002, 0.012, or 0.024 mg/mL, respectively), and then processed for scoring of kinetochore-positive and kinetochore-negative MN. No MN formation was observed in any of the three cell lines in response to HCE exposure. However, MN induction was enhanced by exposure to an HCE metabolite, PERC, in h2E1 and MCL-5 cells.

Induction of CAs and SCEs in cultured Chinese hamster ovary (CHO) cells exposed to HCE was investigated as part of an NTP screening program for genotoxicity (NTP, 1989; Galloway et al., 1987). Concentrations for analysis were selected based on observations of cell confluence and mitotic cell availability. HCE concentrations (purity >99%) ranged from 10 to 1,000 μg/mL (0.01–1.0 mg/mL). For both endpoints, linear regression was used to test for dose-response trends. For individual doses, induction of CA was considered significant if *p* values (adjusted by Dunnett's method to correct for multiple dose comparisons) relative to controls were ≤0.05, while increases of SCEs/chromosome ≥20% over control values were considered significant. For CA, duration of exposure was 8–10 hours in the absence of S9 metabolic activation and 2 hours in the presence of S9. For induction of SCEs, exposure was 26 hours without S9 and 2 hours with S9 (followed by 24-hour incubation without HCE). CAs were

not observed in response to HCE exposure without S9. In the presence of S9, the first study (0.15–0.50 mg/mL HCE) did not induce CAs; however, the second study (0.20–0.40 mg/mL HCE) was judged equivocal due to a positive response at the low dose (15.0% cells with CA, versus 5.0% for the DMSO control). HCE (0.010–0.33 mg/mL) did not induce SCE in the absence of S9; however, positive results were obtained in the presence of S9 (0.10–1.0 and 0.40-1.0 mg/mL HCE).

In vitro cell transformation studies were conducted to understand the effect of HCE in the process of chemical carcinogenesis. In the absence of exogenous metabolic activation, a 3-day exposure to concentrations of HCE (purity >97%) from 0.16 to 100.0 μg/mL (0.00016-0.100 mg/mL) failed to induce morphological cell transformation in BALB/c-3T3 cells, as measured by the incidence of Type III foci (characterized by the authors as an aggregation of multilayered, densely stained cells that are randomly oriented and exhibiting a criss-cross array at the edge of the focus) (Milman et al., 1988; Tu et al., 1985). Milman et al. (1988) also examined the capacity of HCE to initiate and promote tumors in a rat liver foci assay. To assess initiation potential, 24 hours after partial hepatectomy, 10 young adult male Osborne-Mendel rats received the MTD of HCE in corn oil by gavage. Six days later, the animals received a 0.05% dietary exposure to the tumor promoter phenobarbital for 7 weeks. Following sacrifice, livers were examined histopathologically for foci containing GGT, a putative preneoplastic indicator. To assess promotion potential, animals were initiated 24 hours after partial hepatectomy with an i.p. injection of 30 mg of the tumor initiator, diethylnitrosamine (DEN). Six days later, the animals received the MTD of HCE in corn oil by gavage, 5 days/week for 7 weeks. The animals were sacrificed and their livers examined for the presence of GGT-positive foci. In these assays, HCE failed to demonstrate any initiating activity, but did show significant ($p < 0.05$) promoting capability (4.38 ± 1.04 GGT^+ foci/cm^2, versus 1.77 ± 0.49 for the corn oil control).

Yoshikawa and colleagues reported on the activity of HCE and other putative nongenotoxic (i.e., Ames-nonpositive) mouse hepatocarcinogens in an in vivo–in vitro hepatocyte replicative DNA synthesis (RDS) assay (Yoshikawa, 1996; Miyagawa et al., 1995). Groups of 4–5 male $B6C3F_1$ mice were exposed to single gavage doses of 0, 1,000, or 2,000 mg/kg HCE (purity not specified). The hepatocytes were prepared at 24, 39, or 48 hours after exposure. The 1,000 mg/kg HCE-treated hepatocytes prepared 39 hours after exposure yielded a positive mean RDS response of 1.21 ± 0.46% (investigators noted that an RDS incidence rate of 0.4% for any dose group was considered a positive response for the chemical). The remaining HCE groups were nonpositive with mean responses of 0.15–0.35%, while the solvent control mean was 0.26 ± 0.17%.

4.5.2. In Vitro and Ex Vivo Studies Using Isolated Target Tissues/Organs or Cells

A study using a rat liver foci assay (Milman et al., 1988, Story et al., 1986) found that HCE was a tumor promoter rather than an initiator. In vitro and in vivo assays were conducted to assess the ability of HCE to bind to DNA, RNA, and protein in several mouse and rat tissues (Lattanzi et al., 1988). This study reported binding of radiolabeled carbon to DNA, RNA, and protein was observed following [^{14}C]HCE administration in both in vitro and in vivo assays in mice and rats (Lattanzi et al., 1988), suggesting that either HCE or its metabolites may bind to these macromolecules. The role of this binding in mediating HCE-induced toxicity was not further evaluated.

Story et al. (1986) and Milman et al. (1988) conducted a rat liver foci assay to assess the initiation and promotion potential of HCE, along with eight other chlorinated aliphatics. Male Osborne-Mendel rats (10 rats/group) were given partial hepatectomies and then administered the initiation protocol or the promotion protocol. In the initiation protocol, the rats were administered by gavage the MTD of 2.1 mmol/kg (497 mg/kg) HCE (purity 98%), followed 6 days later with 7 weeks of phenobarbital in the diet at 0.05%. Control rats were administered by gavage either corn oil (negative control) or 30 mg/kg DEN (positive control), followed by the phenobarbital treatment. In the promotion protocol, rats were dosed with 30 mg/kg DEN by i.p. injection, followed 6 days later with the MTD of 497 mg/kg HCE, 5 days/week for 7 weeks. Phenobarbital was administered (in the same manner as HCE) as a positive control. Control rats were either administered DEN or water, followed by corn oil for the promotion phase. Livers were removed and stained for GGT activity. Results from the initiation protocol were nonpositive, with only a small number of GGT^+ foci (1.0 foci/cm^2 at most). However, initiation with DEN followed by HCE or phenobarbital resulted in statistically significant increases in GGT^+ foci (Table 4-16). Absolute and relative liver weights were increased by HCE in the promotion protocol. These results indicate that HCE is not an initiator in the rat liver foci assay, but is capable of promotion.

Table 4-16. Number of enzyme-altered foci in rat liver of the promotion protocol

	Total number of foci/cm^2	
Promotion treatment	**+ DEN initiation**	**- DEN initiation**
HCE	4.4 ± 1.0[a]	0.1 ± 0.2
Phenobarbital	3.9 ± 1.0[a]	0.3 ± 0.2
Corn oil	1.7 ± 0.5	0.2 ± 0.2

[a]Statistically different from DEN + corn oil control group, $p < 0.05$

Sources: Milman et al. (1988); Story et al. (1986).

Lattanzi et al. (1988) conducted in vivo and in vitro assays to assess the binding of [^{14}C] HCE (specific activity 14.6 mCi/mmol, radiochemical purity 98%) to nucleic acids in various organs from mice and rats following metabolic activation. For the in vivo studies,

6 male Wistar rats and 12 male BALB/c mice were injected i.p. with 127 μCi/kg HCE (purity 98%). The animals were fasted and sacrificed 22 hours after injection. The organs (liver, kidney, lung, and stomach) were removed, pooled, and processed to obtain DNA, RNA, and proteins. The in vitro studies examined microsomal and cytosolic fractions from these same organs. The incubation mixture included 2.5 μCi [^{14}C] HCE, 1.5 mg calf thymus DNA or polyribonucleotide, 2 mg microsomal proteins (plus 2 mg NADPH), and/or 6 mg of cytosolic proteins (plus 9.2 mg GSH). Coenzymes were not utilized with the controls. Measures for binding to macromolecules were determined by the presence of radiolabeled carbon from [^{14}C] HCE in the DNA, RNA, and protein. The presence of radiolabeled carbon may indicate HCE binding directly to the macromolecules or incorporation of radiolabeled carbon from intermediate metabolites into these macromolecules.

In vivo binding data for HCE are presented in Table 4-17. Binding to macromolecules was interpreted by the presence of radiolabeled carbon; however, HCE-specific metabolites were not measured. In both rats and mice, binding values (in pmol HCE/mg) for RNA were consistently much greater than those for DNA or protein. Greater binding to RNA was observed in the kidneys of rats and mice (5-28 times greater) compared with the binding measured in the livers, lungs, and stomachs. DNA exhibited the lowest amount of HCE binding. Species differences were evident for all three macromolecule types (DNA, RNA, and protein) with the mouse exhibiting much higher levels (9 times greater) of covalent binding for DNA in the liver than the rat. The binding was 2 and 3 times greater for mice than rats with RNA and protein, respectively, from the liver. The binding to DNA was similar between species, but slightly greater in mice, for the kidney, lung, and stomach analyses. According to classifications reported by Lutz (1986, 1979), the covalent binding index values calculated on rat and mouse liver indicate weak (rat) to moderate (mice) oncogenic potency in HCE-treated rodents.

Table 4-17. In vivo covalent binding of [^{14}C]HCE to DNA, RNA, and proteins from rat and mouse organs

	Liver[a]		Kidney[a]		Lung[a]		Stomach[a]	
(pmol/mg)	**Rat**	**Mouse**	**Rat**	**Mouse**	**Rat**	**Mouse**	**Rat**	**Mouse**
DNA (CBI[b])	0.43 ± 0.05[c] (15.1)[b]	3.92 ± 0.20[d] (140)[b]	0.42	0.50	0.14	0.35	0.26	0.37
RNA	46.59 ± 7.23[c]	108.08 ± 21.57[d]	232.94	564.98	15.55	60.10	8.33	21.04
Protein	4.94 ± 1.14[c]	14.99 ± 0.83[d]	2.59	4.91	0.89	3.42	0.80	2.41

[a]Data are from pooled organs from 6 male Wistar rats or 12 male BALB/c mice, except for liver (see indices).
[b]Covalent binding index (CBI) calculated according to Lutz (1986, 1979) as cited in Lattanzi et al. (1988). Classification of CBI values for oncogenic potency: strong, in the thousands; moderate, in the hundreds; weak, in the tens; and below one for nongenotoxic oncogens.
[c]Mean ± SE of six individual values.
[d]Mean ± SE of four values, each obtained from three pooled livers.

Source: Lattanzi et al. (1988).

In vitro binding data for HCE are presented in Table 4-18. Liver microsomes from rats and mice catalyzed HCE binding to DNA at comparable levels. Kidney microsomes from rats and mice produced statistically significantly greater amounts of HCE binding to DNA when compared with controls. Kidney microsomes from mice had a threefold increase in HCE binding to DNA when compared to controls, while kidney microsomes from rats had a twofold increase in HCE binding to DNA when compared to controls. Microsomes from lung and stomach in both species did not display increased DNA binding activity over corresponding controls in the absence of coenzymes. Cytosolic fractions from all organs in mice and rats exhibited higher levels of HCE binding to DNA than microsomal fractions. Mouse liver cytosols produced much greater levels of HCE binding to DNA than rat liver cytosols. When both microsomal and cytosolic fractions were in the incubation mixture, HCE binding to DNA was decreased for liver and kidney. SKF 525-A, a nonspecific CYP450 inhibitor, caused a 50.5% decrease in HCE binding to DNA (data not included in report). The addition of GSH to the microsomal fractions also resulted in inhibition of HCE binding to DNA (data not included in report). When microsomal and cytosolic fractions were heat-inactivated, HCE binding to DNA was similar to control, providing further support that HCE binding to DNA is enzymatically catalyzed. This study provided evidence that HCE is metabolized by microsomal CYP450 enzymes and cytosolic glutathione transferases, and that DNA binding may be increased following HCE metabolism.

Table 4-18. In vitro binding of [^{14}C]HCE to calf thymus DNA mediated by microsomal and/or cytosolic phenobarbital-induced fractions of rat and mouse organs

	Microsomes + NADPH		Cytosol + GSH		Microsomes + cytosol (+ NADPH, + GSH)	
	Rat	**Mouse**	**Rat**	**Mouse**	**Rat**	**Mouse**
Liver						
Standard[a]	90.83 ± 5.31[b]	105.39 ± 7.80[b]	195.51 ± 21.44[c]	346.17 ± 18.91[b]	95.06 ± 6.29[c]	133.44 ± 2.42[a]
Controls[a]	55.19 ± 4.90	46.96 ± 4.19	92.96 ± 26.07	128.56 ± 8.92	52.85 ± 12.93	99.84 ± 8.06
Kidney						
Standard	395.84 ± 78.58[c]	78.86 ± 6.85[c]	246.85 ± 35.39[c]	251.42 ± 45.38[c]	247.99 ± 3.40[b]	ND
Controls	136.26 ± 9.04	39.12 ± 5.34	88.82 ± 30.91	81.91 ± 9.93	144.61 ± 12.86	ND
Lung						
Standard	125.60 ± 22.37	87.37 ± 7.90	126.65 ± 16.84[b]	168.52 ± 19.41[b]	234.26 ± 28.35[b]	ND
Controls	121.13 ± 16.54	86.10 ± 3.27	40.23 ± 7.34	60.44 ± 21.90	56.27 ± 5.32	ND
Stomach						
Standard	94.41 ± 14.38	47.67 ± 17.00	289.58 ± 31.19[b]	228.74 ± 20.42[b]	76.79 ± 5.34[b]	ND
Controls	93.20 ± 15.24	47.12 ± 11.20	130.51 ± 4.01	51.52 ± 6.20	44.77 ± 2.28	ND

[a]Data (total DNA binding in pmol/mg) are reported as mean ± SE of three values; ND, not determined. Controls were conducted in the absence of coenzymes.
[b]Statistically different from control $p < 0.01$
[c]Statistically different from control $p < 0.05$

Source: Lattanzi et al. (1988).

4.5.3. Structure Activity Relationships

Several studies were conducted with the objective of defining structure activity relationships (SARs) of halogenated hydrocarbons and toxicity. NTP (1996) defined a group of chlorinated ethanes that resulted in hyaline droplet nephropathy in male F344/N rats and a group of halogenated ethanes that resulted in renal toxicity in the absence of hyaline droplet nephropathy. In a series of studies, Crebelli et al. (1995, 1992, 1988) evaluated chlorinated and halogenated hydrocarbons for their ability to induce chromosome malsegregation, lethality, and mitotic growth arrest in the mold *A. nidulans*.

NTP (1996) conducted a 21-day oral toxicity study with halogenated ethanes in male F344/N rats. Chemicals under investigation were 1,1,1,2-tetrachloroethane, 1,1,2,2-tetrachloroethane, pentachloroethane, 1,1,2,2-tetrachloro-1,2-difluoroethane, 1,1,1-trichloro-2,2,2-trifluoroethane, 1,2-dichloro-1,1-difluoroethane, 1,1,1-trichloroethane, 1,1,1,2-tetrabromoethane, 1,1,2,2-tetrabromoethane, pentabromoethane, and HCE (purity >98%). Groups of five male rats/dose were administered 0.62 or 1.24 mmol/kg-day of the halogenated ethane (for HCE, 146 and 293 mg/kg-day, respectively). Increased kidney weights and evidence of renal toxicity were observed in many of the rats administered halogenated ethanes; however, this was not always coincident with hyaline droplet nephropathy. Hyaline droplet nephropathy (assessed by Mallory-Heidenhain staining, which allows for greater sensitivity in evaluating hyaline droplets within the tubules of the kidney) was only observed in rats administered pentachloroethane, 1,1,1,2-tetrachloroethane, and HCE. RDS, indicated by PCNA labeling index, was increased in male rats administered chemicals that induced hyaline droplet nephropathy (pentachloroethane, 1,1,1,2-tetrachloroethane, and HCE) as well as pentabromoethane and 1,1,2,2-tetrachloroethane, compared with control rats. The increase in cell proliferation in the kidneys (as measured by the PCNA labeling index) observed with some of the halogenated ethanes that did not induce hyaline droplet nephropathy suggests the contribution of another toxic mechanism. NTP (1996) concluded that the capacity to induce hyaline droplet nephropathy in male rats was restricted to ethanes with four or more halogens, and only the chlorinated (compared with the fluorinated and brominated) ethanes were active. This study also predicted that if hyaline droplet nephropathy is the determining factor in the induction of renal tubule cell neoplasia, then chemicals such as bromo- or chlorofluoroethanes would be nonpositive for kidney neoplasia in 2-year cancer bioassays of male rats.

Crebelli et al. (1988) evaluated three chloromethanes and eight chlorinated ethanes (including HCE) for the induction of chromosome malsegregation in *A. nidulans*. Although 8 of the 11 compounds tested provided positive results including the 3 chloromethanes and 5 out of 8 chlorinated ethanes, HCE was nonpositive for chromosome malsegregation induction. Analyses of relationships between biological and chemical variables indicate that the ability of a chemical to induce chromosome malsegregation was not related to any of the chemical descriptors examined, including molecular weight, melting point, boiling point, refractive index,

octanol/water partition coefficient, and the free energy of binding to biological receptors. Because of the similarity of the chemical descriptors between the positive chlorinated ethanes, aside from 1,1,1-trichloroethane which was nonpositive, the authors argue against a previous hypothesis that nonspecific interactions with hydrophobic cellular structures is the mechanism of aneuploidy induction (Onfelt, 1987).

Crebelli et al. (1992) evaluated the ability of 24 chlorinated aliphatic hydrocarbons to induce chromosome malsegregation, lethality, and mitotic growth arrest in the mold, *A. nidulans*. Data were combined with previous data on 11 related compounds (Crebelli et al., 1988) to generate a database for quantitative structure-activity relationship (QSAR) analysis. Physico-chemical descriptors and electronic parameters for each chemical were included in the analysis. Out of the 24 chemicals, 19 were nonpositive for the induction of chromosome malsegregation; 5 chemicals produced reproducible increases in the frequency of euploid whole chromosome segregants. HCE was nonpositive for the induction of chromosome malsegregation. QSAR analyses on these 35 chlorinated aliphatic hydrocarbons indicate that toxicity, such as the induction of lethality, is primarily related to steric factors (the spatial orientation of reactive centers within a molecule) and measures of the volume occupied by an atom or functional group (molar refractivity). Measures of molar refractivity are a function of temperature, index of refraction, and atmospheric pressure. Mitotic growth arrest was also primarily related to molar refractivity. However, aneugenic activity was related to both molar refractivity and electronic factors, such as the ease in accepting electrons (described by density and the energy of the lowest unoccupied molecular orbital).

These QSAR studies (Crebelli et al., 1992, 1988) were expanded to include 20 additional halogenated hydrocarbons (Crebelli et al., 1995). Chemicals in this study were also assayed for lipid peroxidation in rat liver microsomes, and the authors reported a partial coincidence was found between the ability of a chemical to initiate lipid peroxidation and to disturb chromosome segregation at mitosis. This updated study concluded that electronic and structural parameters that determine the ease of homolitic cleavage of the carbon-halogen bond play a primary role in the peroxidative properties of haloalkanes.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

Table 4-19 summarizes the oral toxicity studies that have been reported in laboratory animals. The primary noncancer effects observed in these studies include decreased body weight or body weight gain, increased absolute and relative kidney weights, increased absolute and relative liver weights, various effects associated with renal tubule toxicity in the kidney, and hepatocellular necrosis. Developmental studies in rats did not consistently demonstrate fetal effects, especially in those cases where maternal toxicity was absent.

Acute and short-term toxicity tests in animals reported liver necrosis and tubular nephrosis in male rabbits (Weeks et al., 1979), and evidence of kidney effects such as nephropathy with hyaline droplet formation and tubular cell regeneration in male rats (NTP, 1996, 1989). Female rats in short-term toxicity tests displayed only decreased body weights at the LOAEL of 563 mg/kg-day with a NOAEL of 281 mg/kg-day (NTP, 1989). Oral LD_{50} values in rats ranged from 4,460 to 7,690 mg/kg (Weeks et al., 1979).

Table 4-19. Oral toxicity studies for HCE

Species	Dose (mg/kg-day)/ Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
New Zealand White rabbits, male (5/dose)	0, 100, 320 or 1,000 by oral; 12 days	100	320	Increased liver and kidney weights; liver degeneration and necrosis; tubular nephrosis and nephrocalcinosis	Weeks et al. (1979)
F344/N rats (5/sex/dose)	0, 140, 281, 563, 1,125, or 2,250 by gavage; 16 days	Male: not established Female: 281	Male: 140 Female: 563	Male: kidney effects (hyaline droplets, tubular cell regeneration) Female: decreased body weight	NTP (1989)
F344/N rats, male (5/dose)	0, 146 or 293 by gavage; 21 days	Not established	146	Increased kidney weight, nephropathy (hyaline droplets, tubule regeneration, granular casts), effects on urinalysis parameters	NTP (1996)
F344/N rats (10/sex/dose)	0, 34, 67, 134, 268, or 536 by gavage; 13 weeks	Male: not established Female: 67	Male: 34 Female: 134	Male: kidney effects in all dose groups Female: hepatocellular necrosis	NTP (1989)
F344 rats (10/sex/dose)	0, 1, 15, or 62 by diet; 16 weeks	Male: 1 Female: 15	Male: 15 Female: 62	Male: kidney atrophy, proximal tubule degeneration Female: kidney atrophy, tubule degeneration	Gorzinski et al. (1985)
Osborne-Mendel rats (50/sex/dose)	0, 113, or 227 by gavage; 78 weeks	Not established	113	Tubular nephropathy in both sexes	NCI (1978), Weisburger (1977)
$B6C3F_1$ mice (50/sex/dose)	0, 360, or 722 by gavage; 91 weeks	Not established	360	Tubular nephropathy in both sexes	NCI (1978); Weisburger (1977)
F344/N rats (50/sex/dose)	Male: 0, 7, or 14 Female: 0, 57, or 114 by gavage; 103 weeks	Not established	Male: 1 Female: 57	Male: Tubular nephropathy, renal tubular hyperplasia Female: Tubular nephropathy	NTP (1989)
Pregnant Sprague-Dawley rats (22/dose)	0, 50, 100, or 500 by gavage on GDs 6–16	Maternal: 100	Maternal: 500	Maternal: body weight decreased, increased mucus in nasal turbinates, subclinical pneumonitis Fetal: no effects	Weeks et al. (1979)
Pregnant Wistar rats (21/dose)	0, 56, 167, or 500 by gavage on GDs 7–17	Maternal: 56 Fetal: 167	Maternal: 167 Fetal: 500	Maternal: decreased weight gain and motor activity Fetal: reduced body weight increased incidence of skeletal variations, decreased ossification	Shimizu et al. (1992)

4.6.1.1. Nephrotoxicity

Two short-term studies in F344 rats (NTP, 1996; NTP, 1989, 16-day study) reported nephrotoxic effects at all administered doses in male rats. The formation of hyaline droplets accompanied by cell regeneration and eosinophilic granular casts was observed in the renal tubules of male rats administered 140-563 mg/kg-day HCE (NTP, 1989). Female rats did not exhibit any renal toxicity. In a 21- day study by NTP (1996), male rats exhibited increased absolute and relative kidney weights, tubular regeneration and granular casts, and increased labeling index in kidneys at doses of 146 and 293 mg/kg-day HCE. Tubular nephrosis, and to a minimal degree, tubular nephrocalcinosis were observed in the kidney of male New Zealand White rabbits administered 320 and 1,000 mg/kg-day (but not 100 mg/kg-day) HCE (Weeks et al., 1979). Compared with rabbits, the rats were more sensitive to renal effects induced by HCE. A gender-specific response was demonstrated in the male rats (NTP, 1989). However, the use of only male rats (NTP, 1996) and male rabbits (Weeks et al., 1979) in the other two studies makes it difficult to evaluate if the renal effects observed were gender specific.

Subchronic exposure in male F344/N rats demonstrated kidney effects including hyaline droplet formation, tubular regeneration, and tubular casts in male rats administered HCE ranging from 34 to 536 mg/kg-day in the NTP (1989) 13-week study. Males in the 536 mg/kg-day dose group also exhibited renal papillary necrosis and degeneration and necrosis of renal tubule epithelium (NTP, 1989). Female rats did not display these kidney effects. These results suggest a sex-specific difference in HCE toxicity. Another study (Gorzinski et al., 1985) in F344 rats reported slight hypertrophy and dilation of the renal tubules in males and renal tubule atrophy and degeneration in male and female rats. Evidence of kidney effects in female rats consisted of very slight renal tubular atrophy and degeneration observed histopathologically at the highest dose tested. EPA considered the NOAEL and LOAEL for male rats as 1 and 15 mg/kg-day, respectively, while the corresponding values in the females were 15 and 62 mg/kg-day, indicating greater sensitivity of the males to the renal effects of HCE. These data and tissue distribution information (see Section 3.2 Distribution) show that the male kidney accumulated higher HCE concentrations than the female kidney, indicating that the kidney is the primary target organ following oral exposure to HCE and there are potential gender differences in the distribution and metabolism of HCE. Consequently, male rats are likely more sensitive to the nephrotoxicity of HCE than female rats. Additionally, Gorzinski et al. (1985) is the only study of either short-term or subchronic duration to report renal effects in female rats.

Chronic toxicity tests were conducted by the NTP on F344/N rats and by NCI on Osborne-Mendel rats and B6C3F_1 mice (NTP, 1989; NCI, 1978). NTP (1989) administered much lower doses of HCE (7 and 14 mg/kg-day in males; 57 and 114 mg/kg-day in females) to the F344 rats compared with the Osborne-Mendel rats (113 and 227 mg/kg-day) in the NCI (1978) study. In the NTP (1989) chronic study, nephropathy (characterized as tubular cell degeneration and regeneration, dilation and atrophy, glomerulosclerosis, interstitial fibrosis, and

chronic inflammation) was observed in both male and female rats. In the case of the male rats, the response was roughly equivalent across the control and treated groups with nephropathy in more than 94% of animals. The high incidence of nephropathy observed in control rats could result of a spontaneous syndrome known as chronic progressive nephropathy (CPN) that is associated with aged rats, especially F344 and Osborne-Mendel strains (see Section 4.7.3.2.1 for additional discussion). To examine the effects of chronic HCE exposure separate from CPN, the nephropathy incidence in terms of severity was considered. The severity was increased in the treated male rats compared with the controls. In considering severity, the increases in incidence of male nephropathy (that was of moderate or marked severity) were 18/50 (36%), 24/50 (48%), and 30/50 (60%) in the control, 7, and 14 mg/kg-day dose groups, respectively. In females, both the incidence (44% of controls and approximately 84% of treated) and severity of nephropathy were dose-related. When considering the severity, incidences of female nephropathy (that were of mild or moderate severity) were 12/50 (24%), 25/50 (50%), and 32/50 (64%) in the control, 57, and 114 mg/kg-day dose groups, respectively.

Dose-related increases (30 and 64% in 7 and 14 mg/kg-day, respectively) in linear mineralization of the renal papillae and treatment-related increases (14% in 7 and 14 mg/kg-day) in hyperplasia of pelvic transitional epithelium in the kidney were observed in the male rats. In females, an increased incidence of mineralization was only noted at the low dose (44% at 57 mg/kg-day compared with 28% in controls). The low dose for the females was 8 times greater than that for the males yet the signs of nephropathy were more severe in the males.

In the NCI (1978) study, Osborne-Mendel rats of both sexes displayed chronic inflammatory kidney lesions in both control and treated groups, although tubular nephropathy (characterized by degeneration, necrosis, and the presence of large hyperchromatic regenerative epithelial cells) was observed only in the HCE-exposed male and female rats. There were dose-related increases in incidences of nephropathy in males (45 and 66%, respectively) and females (15 and 59%, respectively) administered 113 and 227 mg/kg-day HCE. The chronic toxicity test in $B6C3F_1$ mice (NCI, 1978) is the only study conducted in this species. Male mice experienced low survival in the control and 360 mg/kg-day (low-dose) groups. Chronic kidney inflammation was observed in 67 and 80% of males in the vehicle and untreated control groups, respectively, as well as in 66 and 18% of the 360 and 722 mg/kg-day HCE males. The report did not provide an explanation for the large response in the control and low dose mice and the relatively small response in the high dose group. Female mice exhibited chronic kidney inflammation only in vehicle controls (15%) and the high dose group (2%). Tubular nephropathy was observed in both dose groups of both sexes at high incidences (92-100%), and was characterized by degeneration of convoluted tubule epithelium with some hyaline casts. Enlarged dark staining regenerative tubular epithelium was also observed, with the kidney exhibiting infiltration of inflammatory cells, fibrosis, and calcium deposition. The response in

the treated male and female mice compared with the absence of nephropathy in the controls suggests that the doses used in this study were too high.

The available information for HCE-induced nephropathy in rats, mice, male rabbits, and sheep indicates that the male rat is the most sensitive sex/species to the renal toxicity of HCE. Limited, if any, information is available for species other than the rat; however, the doses that elicited toxic responses in mice (NCI, 1978), male rabbits (Weeks et al., 1979), and sheep (Fowler, 1969) were at least 45-fold greater than the lowest dose (7 mg/kg-day; NTP, 1989) that induced a statistically significant response in rats.

4.6.1.2. Hepatotoxicity

Short-term studies in rats (NTP, 1996), male rabbits (Weeks et al., 1979), and sheep (Fowler, 1969) reported hepatotoxicity at doses approaching ≥300 mg/kg-day. Male F344 rats exhibited significantly increased relative liver weights at the highest dose of 293 mg/kg-day. AST and NAG serum activities were also significantly higher than in controls. These effects were not observed at 146 mg/kg-day HCE (NTP, 1996). Liver degeneration and necrosis, including fatty degeneration, coagulation necrosis, hemorrhage, ballooning degeneration, eosinophilic changes, and hemosiderin-laden macrophages and giant cells were observed in male New Zealand White rabbits administered 320 and 1,000 mg/kg-day HCE (but not 100 mg/kg-day); increasing in severity with increasing dose. Sheep given single oral doses of 500-1,000 mg/kg of HCE exhibited plasma levels of GDH, SDH, and OCT that were increased twofold or more than levels in controls; indicating reduced hepatic function.

Effects in the liver of animals treated with HCE were observed in male and female rats in two subchronic studies (NTP, 1989; Gorzinski et al., 1985). Liver weight increased in a dose-related fashion from the lowest dose (34 mg/kg-day) to the highest (536 mg/kg-day). Females were more sensitive than males; severity and statistical significance increased in females at doses lower than those eliciting toxicity in male rats. Hepatocellular necrosis was noted in females at doses ranging from 134 to 156 mg/kg-day and in males at the two highest doses, 268 and 536 mg/kg-day (NTP, 1989). Gorzinski et al. (1985) reported slight swelling of hepatocytes in control and treated males, although the two highest doses (15 and 62 mg/kg-day) exhibited dose-related increases in incidences of swelling. Other than a statistically significant increase (5%) in liver weight at 62 mg/kg-day HCE, the females were not affected. This is in contrast to the hepatocellular effects noted in female rats in the NTP study (NTP, 1989). However, the highest dose used by Gorzinski et al. (1985), 62 mg/kg-day, is below the 67 mg/kg-day NOAEL for females of the NTP (1989) study, indicating that sufficient doses may not have been reached in Gorzinski et al. (1985) to cause hepatotoxicity in female rats.

There were no liver effects observed in the animals administered HCE for chronic durations. The range of doses in the subchronic assay (0, 34, 67, 134, 268, and 536 mg/kg-day on F344 rats; NTP, 1989) encompassed the doses used in the chronic assays for female F344 rats

(57 and 114 mg/kg-day; NTP, 1989) and Osborne-Mendel rats (113 and 227 mg/kg-day; NCI, 1978). Hepatocellular necrosis was observed in female rats in the subchronic, but not the chronic study. The LOAEL for female F344/N rat hepatocellular necrosis, 134 mg/kg-day, in the subchronic study (NTP 1989) occurred at a dose that exceeded the highest dose of the chronic study (NTP, 1989), suggesting that a sufficiently high dose may have not been achieved to elicit hepatocellular necrosis despite the longer exposure period. The NCI (1978) study in Osborne-Mendel rats was conducted with doses above the LOAEL for hepatocellular necrosis in female F344/N rats (NTP, 1989), but hepatocellular effects were not observed. Osborne-Mendel rats may not be as sensitive to HCE-induced hepatotoxicity as F344/N rats. The only study in mice (NCI, 1978; chronic) did not report any hepatotoxic effects other than the development of hepatocellular tumors.

HCE-induced liver effects were only observed in animals in short-term and subchronic studies. Female rats exhibited a greater sensitivity to liver effects as evidenced by the effects observed at lower doses compared with males (NTP, 1989). The implications of the slight swelling of hepatocytes in the absence of other histopathological effects at 15 and 62 mg/kg-day in male rats (Gorzinski et al., 1985) are unknown. Rabbits (males) and sheep demonstrated hepatic effects at doses at least fourfold greater than the lowest dose (67 mg/kg-day) that induced a statistically significant response in female rats.

4.6.1.3. Developmental Toxicity

Two developmental studies in rats indicated that HCE-induced teratogenicity in the presence of maternal toxicity (Shimizu et al., 1992; Weeks et al., 1979). In the Shimizu et al. (1992) study, maternal rats gavaged with 167 and 500 mg/kg HCE displayed decreased motor activity. At the high dose, dams also exhibited piloerection and subcutaneous hemorrhage. Fetuses of the 500 mg/kg dose displayed decreased body weight, skeletal variations such as rudimentary lumbar ribs, and ossification effects, but no skeletal malformations were observed. The NOAEL for this study was 56 mg/kg for the dams and 167 mg/kg for the fetuses. In Weeks et al. (1979), maternal rats gavaged with 500 mg/kg HCE displayed pulmonary effects such as increased incidence of mucopurulent nasal exudates, upper respiratory tract irritation, and subclinical pneumonitis. The fetuses did not exhibit any skeletal or soft tissue anomalies. The maternal LOAEL and NOAEL were 500 and 100 mg/kg, respectively.

4.6.1.4. Metabolite Toxicity

The potential metabolites of HCE are PERC and pentachloroethane (Fowler, 1969), which are subsequently metabolized to TCE, TCA, and/or trichloroethanol (see Figure 3-1). Exposure to these potential metabolites results in effects on the liver, kidneys, and nervous system, similar to effects observed following HCE exposure. Potential HCE metabolites PERC (Cal EPA, 2001), TCE (NTP, 1990, 1988; NCI, 1976), and TCA (Mather et al., 1990; Bull et al.,

1990) are associated with liver effects following exposure. Kidney effects have been reported following exposure to putative HCE metabolites PERC (JISA, 1993; NTP, 1986; NCI, 1977), pentachloroethane (NTP, 1996, 1983), TCE (NTP, 1990, 1988; NCI, 1976), and TCA (Mather et al., 1990). Neurological effects are also reported following exposure to the putative HCE metabolites PERC (JISA, 1993; NTP, 1986; NCI, 1977), TCE, and pentachloroethane.

This qualitative comparison suggests that metabolites formed during HCE metabolism could contribute to the liver, kidney, and neurological effects observed in animals exposed to HCE. However, the available metabolism data for HCE do not allow for the conclusive identification of metabolites or provide quantitative information on how potential metabolites contribute to effects associated with HCE exposure.

4.6.2. Inhalation

Inhalation toxicity has only been evaluated in a single 6-week repeat exposure study in multiple species performed by Weeks et al. (1979). There is some uncertainty regarding the exposure to HCE vapor because HCE would remain a vapor only when surrounded by heated air. However, as soon as the hot HCE vapor was mixed with room temperature air, most (but not all) vapor in the airstream would condense into fine particles (a solid aerosol). The data from this study are summarized in Table 4-20. The study authors reported NOAELs and LOAELS for beagle dogs, guinea pigs, and rats of 48 ppm (465 mg/m^3) and 260 ppm (2,517 mg/m^3), respectively. Neurological effects, such as tremors and ataxia, were observed in beagle dogs and in pregnant and nonpregnant Sprague-Dawley rats. Rats and guinea pigs exhibited reduced body weight gain and increased relative liver weight. Male rats also displayed increased relative spleen and testes weights. Behavioral tests were conducted in male Sprague-Dawley rats at the same exposure concentrations, and no significant effects were observed. Overall, the information on the inhalation toxicity of HCE is limited.

Table 4-20. Inhalation toxicity studies with HCE

Species	Concentration (mg/m^3)/duration[a]	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Effect	Reference
Male beagle dogs (4/concentration)	0, 145, 465, or 2,517; 6 weeks	465	2,517	Tremors, ataxia, hypersalivation, head bobbing, facial muscular fasciculations	Weeks et al. (1979)
Male Hartley guinea pigs (10/concentration)	0, 145, 465, or 2,517; 6 weeks	465	2,517	Reduced body weight, increased relative liver weight	Weeks et al. (1979)
Sprague-Dawley rats (25/sex/concentration)	0, 145, 465, or 2,517; 6 weeks	465	2,517	Males: reduced body weight gain, increased relative kidney, spleen, and testes weights Females: increased relative liver weight	Weeks et al. (1979)
C. Japonica (Japanese quail) (20/concentration)	0, 145, 465, or 2,517; 6 weeks	2,517	Not established	No effects	Weeks et al. (1979)
Pregnant Sprague-Dawley rats (22/concentration)	0, 145, 465, or 2,517; GDs 6-16	Maternal: 465	Maternal: 2,517	Maternal: tremors, decreased body weight Fetal: no effects	Weeks et al. (1979)
Male Sprague-Dawley rats (15/concentration)	0, 145, 465, or 2,517; 6 weeks	465	2,517	Behavioral tests: avoidance latency and spontaneous motor activity	Weeks et al. (1979)

[a]145, 465, and 2,517 mg/m^3 correspond to concentrations reported by Weeks et al. (1979) as 15, 48, and 260 ppm, respectively.

4.6.3. Mode-of-Action Information

Reports on HCE-induced human health effects are limited and confounded by coexposure to multiple solvents or other toxicants (e.g., HCE-zinc oxide smoke). Studies that observed substantial HCE exposure in smoke bomb production workers were too small to provide definitive conclusions on health effects.

Animal studies suggest that HCE is primarily metabolized to PERC and pentachloroethane by CYP450 enzymes of the liver, with likely subsequent metabolism to TCE. Metabolites identified in the urine include TCA, trichloroethanol, oxalic acid, dichloroethanol, dichloroacetic acid, and monochloroacetic acid. However, only 5% of a radiolabeled compound was measured in the urine, indicating that all of the urinary metabolites account for a small percentage of the dose. It is unknown whether unchanged HCE or its metabolites are responsible for the liver and kidney toxicities observed in animal studies. Only one study attempted to assess the extent of HCE metabolism in rats and mice and estimated that 24–29% of administered HCE is metabolized (Mitoma et al., 1985). This study did not quantify actual metabolite concentrations, so these estimations are of questionable accuracy.

The mode of action for HCE-induced kidney toxicity is unknown. HCE-induced nephropathy has been observed in both sexes of rats and mice. Specifically, short-term assays in

male rats showed nephropathy characterized by hyaline droplet accumulation and increased incidences of tubule regeneration and granular casts (NTP, 1996, 1989). Cell proliferation of kidney sections using PCNA labeling analysis was also increased (NTP, 1996). Subchronic and chronic animal bioassays confirmed these renal effects (NTP, 1989; Gorzinski et al., 1985; NCI, 1978). Chronic inflammatory kidney lesions and tubular nephropathy were observed in rats, and tubular nephropathy was also observed in mice (NCI, 1978). The mode of action for nephropathy is unknown. Some data suggest an α_{2u}-globulin mode of action could contribute to hexachloroethane-induced nephropathy. However, there is insufficient evidence to conclude that the kidney effects observed following HCE exposure (NTP, 1989) are related to an α_{2u}-globulin mode of action for the following reasons: (1) the lack of α_{2u}-globulin immunohistochemical data for HCE-induced nephrotoxicity and carcinogenicity, (2) the hyaline droplet accumulation is caused by excessive protein load that may not be exclusively related to α_{2u}-globulin accumulation, and (3) the existence of renal toxicity in female rats and male and female mice indicates that the nephrotoxic effects are not limited to an α_{2u}-globulin-induced sequence of lesions. It is also possible that advanced chronic progressive nephropathy (CPN), an age-related renal disease of laboratory rodents that occurs spontaneously, may contribute to the observed nephrotoxicity following HCE exposure. However, changes in severity of the nephropathy were observed to be greater in male rats exposed to HCE compared to controls indicating that HCE exposure exacerbated effects in the kidney. Additionally, HCE-exposed male rats demonstrated dose-dependent increases in incidence of mineralization of the renal papillae and hyperplasia of pelvic transitional epithelium. Neither of these effects increased in a dose-related manner in the controls or the HCE-exposed female rats, suggesting that CPN is not solely responsible for the nephropathy observed by NTP (1989). Lattanzi et al. (1988) conducted in vivo binding studies concluding that HCE could bind to DNA, RNA, and protein in the kidney.

The liver has been demonstrated to be a target organ in several animal species. Sheep (Fowler, 1969) and male rabbits (Weeks et al., 1979) exhibited hepatotoxicity characterized by clinical chemistry parameters that indicated reduced hepatic function and showed histopathological findings including hepatocellular necrosis. Subchronic studies showed statistically significant decreases in relative and absolute liver weight (Gorzinski et al., 1985) and statistically significant increases in relative liver weight and hepatocellular necrosis (NTP, 1989) in female F344/N rats. Studies of TCA (a potential metabolite of HCE) indicate that free radical generation may play a role in mediating toxicity particularly in the liver. However, no data are available demonstrating generation of free radicals following exposure to HCE and it is unknown whether unchanged HCE or its metabolites are responsible for the liver and kidney toxicities observed in animal studies. Town and Leibman (1984) reported lipid peroxidation (as indicated by a statistically significant increase in the formation of malondialdehyde and conjugated dienes) following treatment with HCE (8 mM). The authors suggested the involvement of a free radical.

However, this mode of action has not been explored or further addressed in the literature for HCE.

The presence of radiolabeled carbon measured by in vivo binding studies suggested that HCE can bind to DNA, RNA, and protein (Lattanzi et al., 1988). Binding to macromolecules was interpreted by the presence of radiolabeled carbon; however, radiolabeled carbon may have been incorporated into these macromolecules from intermediary HCE metabolites. In the rat, higher levels of DNA, RNA, and protein binding were observed in the kidney and liver compared with the lung and stomach. The mouse demonstrated the highest levels of DNA and protein binding in the liver and RNA binding in the liver and kidney. Studies using CYP450 indicate that HCE must be metabolized to reactive intermediates prior to binding to macromolecules. Therefore, renal toxicity and hepatotoxicity may also involve HCE binding to DNA, RNA, or protein, resulting in cytotoxicity and contributing to the cytotoxic damage from radicals.

The neurological effects observed in beagle dogs (Weeks et al., 1979) and sheep (Fowler, 1969; Southcott, 1951) are commonly observed effects of chlorinated hydrocarbons. These effects have not been extensively studied for HCE, and data are inadequate to determine a mode of action.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), HCE is "likely to be carcinogenic to humans" based on data from oral cancer bioassays in F344/N rats and B6C3F1 mice (NCI, 1978; NTP, 1989). No human data are available to assess the carcinogenic potential of HCE. NTP (1989) reported dose-dependent increases (statistically significant at the high dose) in the combined incidence of adenoma or carcinoma and increases (statistically significant at the low dose) in the incidence of pheochromocytomas in male F344/N rats. Tumors were not observed in the female F344/N rats in the NTP (1989) study. In addition, NCI (1978) observed statistically significant increases in the incidence of hepatocellular carcinomas in male and female $B6C3F_1$ mice. The male rats demonstrated a statistically significantly increased tumor response for hepatocellular carcinomas that was dose-related. The female mice displayed a statistically significantly elevated incidence of hepatocellular carcinomas at both doses, although no dose-related increase in tumor response was evident. The Osborne-Mendel rats in the NCI (1978) study did not provide consistent evidence of carcinogenicity. HCE was shown to be a promoter, but not an initiator, in an Osborne-Mendel rat liver foci assay (Milman et al., 1988; Story et al., 1986). Binding of radiolabeled carbon to DNA, RNA, and protein following administration of [^{14}C]HCE was observed in both in vitro and in vivo assays in mice and rats (Lattanzi et al., 1988).

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of hexachloroethane via the oral route demonstrates that tumors occur in tissues remote from the site of absorption. Information on the carcinogenic effects of hexachloroethane via the inhalation and dermal routes in humans or animals is absent. Based on the observance of systemic tumors following oral 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, hexachloroethane is "likely to be carcinogenic to humans" by all routes of exposure.

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

There are currently no data from human studies pertaining to the carcinogenicity of HCE. NTP (1989) conducted a chronic toxicity/carcinogenicity bioassay in F344/N rats. Groups of 50 male rats/dose were administered TWA doses of 7 and 14 mg/kg-day of HCE (purity >99%) by corn oil gavage, 5 days/week for 103 weeks. Groups of 50 female rats/dose were administered, by corn oil gavage, 5 days/week for 103 weeks, TWA doses of 57 and 114 mg/kg-day. Male rats exhibited a dose-related, statistically significant increase in the incidence of combined renal adenomas or carcinomas at the highest dose. Combined renal adenomas or carcinomas were observed in 2, 4, and 14% of controls, 7, and 14 mg/kg-day males, respectively. No HCE-related renal tumors were observed in female rats. The combined incidence of all three types of pheochromocytomas (benign, malignant, and complex pheochromocytomas) was statistically significantly increased in males treated with 7 mg/kg-day HCE (62%) and increased in males treated with 14 mg/kg-day (43%) when compared with vehicle controls (30%) and historical controls in the study laboratory (75/300; 25 ± 7%) and in NTP studies (543/1,937; 28 ± 11%). No HCE-related adrenal gland tumors were observed in female rats.

The NCI (1978; Weisburger, 1977) conducted a chronic toxicity/carcinogenicity bioassay in Osborne-Mendel rats. HCE (purity >98%) at doses of 0, 250, or 500 mg/kg-day was administered by corn oil gavage to 50 rats/sex/dose for 5 days/week for 78 weeks. Following termination of exposure, rats were observed for 33–34 weeks for a total duration of 111-112 weeks. Twenty rats/sex were used for the untreated and vehicle controls. Starting in week 23, rats in the exposure groups began a 5-week cyclic rotation that involved 1 week without exposure followed by dosing for 4 weeks. After adjustment from 5 days/week for 78 weeks, with the 5-week cyclic rotation for part of the time, to continuous exposure over the standard 2 years for a chronic bioassay, the TWA doses were 113 and 227 mg/kg-day. Mortality

was increased in the 113 and 227 mg/kg-day males with survival rates of 24/50 (48%) and 19/50 (38%), respectively, compared with 14/20 (70%) in the untreated controls. Survival rates for the female rats were 14/20 (70%) for both the untreated and vehicle controls, and 27/50 (54%) and 24/50 (48%) for the 113 and 227 mg/kg-day dose groups, respectively.

All of the tumor types observed had been encountered previously as spontaneous lesions in the Osborne-Mendel rat and no statistical differences in frequencies were observed between treated and control rats. NCI concluded that there was no evidence of carcinogenicity in this rat study. Notably, the doses used in the Osborne-Mendel rats of the NCI (1978) study were approximately 16 times greater than those doses administered to F344 male rats by NTP (1989).

A $B6C3F_1$ mouse study conducted by NCI (1978; Weisburger, 1977), HCE (purity >98%) was administered by corn oil gavage at TWA doses of 360 and 722 mg/kg-day for 5 days/week for 78 weeks, followed by 12–13 weeks of an observation period (total 91 weeks). Survival rates in males were 5/20 (25%), 1/20 (5%), 7/50 (14%), and 29/50 (58%) in the vehicle control, untreated control and the 360 and 722 mg/kg-day dose groups, respectively. Survival rates in females were 80, 85, 80, and 68% in vehicle control, untreated control, 360 and 722 mg/kg-day groups, respectively. Both male and female mice exhibited statistically significantly increased incidences of hepatocellular carcinomas. The treated males demonstrated an increased tumor response for hepatocellular carcinomas that was dose-related: 30 and 63% in the 360 and 722 mg/kg-day dose groups, respectively, compared with 10% in pooled vehicle controls and 15% in matched vehicle controls. Females demonstrated an increased tumor response that was not dose related in that a higher incidence of hepatocellular carcinomas occurred at the low dose (40%) compared with the high dose (31%); pooled vehicle and matched vehicle controls had incidences of 3 and 10%, respectively. NCI concluded that HCE was carcinogenic in both sexes of $B6C3F_1$ mice.

Evidence of HCE's promotion (following treatment with DEN), but not initiation, potential was observed in the liver of male Osborne-Mendel rats administered a single gavage dose of 497 mg/kg HCE (Milman et al., 1988; Story et al., 1986). Lattanzi et al. (1988) reported in vivo and in vitro binding of HCE to DNA, RNA, and protein in mice and rats. In both rats and mice administered single i.p. injections of 127 μCi/kg [^{14}C]HCE, in vivo covalent binding of HCE for RNA was consistently much greater than that for DNA or protein. DNA exhibited the lowest amount of HCE binding. Species differences were evident for all three macromolecule types (DNA, RNA, and protein), with the mouse exhibiting much higher levels (9 times greater) of covalent binding for DNA in the liver than the rat. The binding was 2 and 3 times greater for mice than rats with RNA and protein, respectively, from the liver. The binding was similar between species, but slightly greater in mice, for the kidney, lung, and stomach analyses. In vitro covalent binding to DNA was observed at comparable levels in liver microsomes from both rats and mice following exposure to HCE. Kidney microsomes from rats and mice produced statistically significantly greater amounts of DNA binding compared with controls, with greater

amounts of DNA binding from mice (threefold increase) compared with rats (twofold increase). Microsomes from the lungs and stomachs in both species did not display increased DNA binding activity over corresponding controls.

Carcinogenicity associated with potential HCE metabolites

Potential metabolites of HCE include PERC, pentachloroethane, and TCE. Epidemiologic studies have reported associations between exposures to PERC and TCE and increased risks of several cancers including cancer of the lymphoid system, esophagus, cervix, bladder, kidney, and lung. PERC (NTP, 1986; NCI, 1977), pentachloroethane (NTP, 1983), and TCE (NTP, 1990; 1988; NCI, 1976) have also been evaluated for carcinogenicity in several chronic bioassays. Specifically, hepatocellular carcinomas have been reported for exposure to PERC, pentachloroethane, and TCE. Renal tubule adenomas have also been observed with exposure to PERC and pentachloroethane, with equivocal evidence for TCE. An increased incidence of pheochromocytomas was reported in studies of pentachloroethane, but not in studies of PERC or TCE. In addition, data suggest that some tumors are not shared between HCE and these potential metabolites (e.g., there is evidence of PERC-associated mononuclear cell leukemia in rats, but no reports of an association with HCE exposure). Based on the available data, the relative roles of the parental compound (HCE) and its metabolites in the carcinogenicity associated with exposure to HCE are unknown. However, the carcinogenicity of these HCE metabolites provides support for describing HCE as a rodent carcinogen.

4.7.3. Mode-of-Action Information

Hepatocellular and renal adenomas and carcinomas and pheochromocytomas were observed in rats and mice following oral exposure to HCE (NTP, 1989; NCI, 1978). The mode(s) of carcinogenic action of HCE in the liver, kidney, and adrenal gland is unknown. There are mode-of-action data suggesting that the induction of kidney tumors in male rats and liver tumors in male and female mice may involve the accumulation of α_{2u}-globulin in the kidney and increased cytotoxicity, inflammation, and regenerative cell proliferation in the liver, respectively.

4.7.3.1. Kidney Tumors

Description of the Hypothesized Mode of Action

Hypothesized mode of action

The mode of action for the carcinogenic effects of HCE in the kidney is unknown. Specifically, the key events leading to development of kidney tumors in male rats exposed to HCE have not been fully characterized. Some of the experimental data suggest that development of kidney tumors in male rats following exposure to HCE may involve an α_{2u}-globulin-mediated mode of action. Generally, kidney tumors observed in cancer bioassays are assumed to be

relevant for assessment of human carcinogenic potential. However, a number of chemicals have been shown to induce accumulation of α_{2u}-globulin in hyaline droplets in male rat kidney. The α_{2u}-globulin accumulation in hyaline droplets initiates a sequence of events that leads to renal nephropathy and, eventually, to renal tubular tumor formation. The phenomenon is unique to the male rats since female rats and other laboratory mammals administered the same chemicals do not accumulate α_{2u}-globulin in the kidney and do not develop renal tubule tumors (U.S. EPA, 1991b).

The lack of α_{2u}-globulin immunohistochemical data for HCE-induced nephrotoxicity and carcinogenicity supports the conclusion that there is insufficient evidence to establish the role of α_{2u}-globulin in HCE-induced kidney tumors. Furthermore, reported renal toxicity in female rats and male and female mice exposed to HCE suggest a mode of action other than α_{2u}-globulin nephropathy. In the absence of minimum information demonstrating the involvement of α_{2u}-globulin processes, male rat renal toxicity/tumors is considered relevant for risk assessment purposes.

Identification of Key Events

The U.S. EPA (1991c) Risk Assessment Forum Technical Panel report provides specific guidance for evaluating chemical exposure-related male rat renal tubule tumors for the purpose of risk assessment, based on an examination of the potential involvement of α_{2u}-globulin accumulation.

The protein α_{2u}-globulin is a member of a large superfamily of low-molecular-weight proteins and was first characterized in male rat urine. It has been detected in various tissues and fluids of most mammals, including humans. However, the particular isoform of α_{2u}-globulin commonly detected in male rat urine is considered specific for the male rat; moreover, the urine and kidney concentrations detected in the mature male rat are several orders of magnitude greater than in any other age, sex, or species tested (U.S. EPA, 1991c).

The hypothesized mode of action ascribed to α_{2u}-globulin-associated nephropathy is defined by a progressive sequence of effects in the male rat kidney, often culminating in renal tumors. The involvement of hyaline droplet accumulation in the early stages of nephropathy associated with α_{2u}-globulin-binding chemicals is an important difference from the sequence of events observed with classical carcinogens. The pathological changes that precede the proliferative sequence for classical renal carcinogens also include early nephrotoxicity (e.g., cytotoxicity and cellular necrosis) but no apparent hyaline droplet accumulation. Furthermore, the nephrotoxicity that can ensue from hyaline droplet accumulation is novel because it is associated with excessive α_{2u}-globulin accumulation. This α_{2u}-globulin accumulation is proposed to result from reduced renal catabolism of the α_{2u}-globulin chemical complex and is thought to initiate a sequence of events leading to chronic proliferation of the renal tubule epithelium. The histopathological sequence of events in mature male rats consists of the following (see Table 4-

21 for a summary of this sequence specific for HCE):

- Excessive accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium
- Sustained regenerative tubule cell proliferation
- Development of intralumenal granular casts from sloughed cellular debris associated with tubule dilatation and papillary mineralization
- Foci of tubule hyperplasia in the convoluted proximal tubules
- Renal tubule tumors

Table 4-21. Nephrotoxic effects characteristic of α_{2u}-globulin nephropathy observed in male and female rats administered HCE

Study, dose, duration, and sex	NTP, 1989 7 or 14 mg/kg-d (M); 57 or 114 mg/kg-d (F) 103 wks		NCI, 1978 113 or 227 mg/kg-d 104 wks		Gorzinski et al., 1985 1, 15, or 62 mg/kg-d 16 wks		NTP, 1989 34, 67, 134, 268, or 536 mg/kg-d 13 wks		NTP, 1996 146 or 293 mg/kg-d 3 wks		NTP, 1989 140, 281, or 563 mg/kg-d 16 d	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Accumulation of hyaline droplets							X		X	NT	X	
Accumulation of α_{2u}globulin in hyaline droplets	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Necrosis/degeneration	X	X	X	X	X	X	X			NT		
Tubular regeneration	X	X	X	X			X		X	NT	X	
Granular casts/dilatation	X	X	X	X	X		X		X	NT	X	
Papillary mineralization	X									NT		
Tubular hyperplasia	X								X	NT		

X = presence of effect
NT = not tested

In addition to this histopathological sequence, EPA (1991c) provides more specific guidance for evaluating chemically induced male rat renal tubule tumors for the purpose of risk assessment. To determine the appropriateness of the data for use in risk assessment, chemicals inducing renal tubule tumors in the male rat are examined in terms of three categories:

- The α_{2u}-globulin sequence of events accounts for the renal tumors.
- Other potential carcinogenic processes account for the renal tumors.
- The α_{2u}-globulin-associated events occur in the presence of other potential carcinogenic processes, both of which result in renal tumors.

Therefore, it is important to determine whether the α_{2u}-globulin process is involved and, if so, to what extent α_{2u} -globulin-associated events, rather than other processes, account for the tumor increase.

Determination of these elements requires a substantial database of bioassay data not only from male rats but also from female rats and mice, and such toxicity studies must demonstrate whether or not α_{2u}-globulin processes are operative. In the absence of minimum information demonstrating the involvement of α_{2u}-globulin processes, it should be assumed that any male rat renal toxicity/tumors are relevant for risk assessment purposes.

As outlined in the U.S. EPA Risk Assessment Forum Technical Panel report (U.S. EPA, 1991c), the following information from adequately conducted studies of male rats is used for demonstrating that the α_{2u}-globulin process may be a factor in any observed renal effects—an affirmative response in each of the three categories is required. If data are lacking for any of the criteria in any one category, the available renal toxicity data should be analyzed in accordance with standard risk assessment principles. The three categories of information and criteria are as follows:

- *Increased number and size of hyaline droplets in the renal proximal tubule cells of treated male rats*. The abnormal accumulation of hyaline droplets in the P_2 segment helps differentiate α_{2u}-globulin inducers from chemicals that produce renal tubule tumors by other modes of action.
- *Accumulating protein in the hyaline droplets is* α_{2u}*-globulin*. Hyaline droplet accumulation is a nonspecific response to protein overload, and, thus, it is necessary to demonstrate that the protein in the droplet is, in fact, α_{2u}-globulin.
- *Additional aspects of the pathological sequence of lesions associated with* α_{2u}*-globulin nephropathy are present.* Typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia. If the response is mild, not all of these lesions may be observed. However, some elements consistent with the pathological sequence must be demonstrated to be present.

Experimental Support for the Hypothesized Mode of Action

Strength, consistency, and specificity of association

NTP (1989)—16-day study

In a short-term exposure study, NTP (1989) administered 140, 281, 563, 1,125, or 2,250 mg/kg-day HCE to F344/N rats via gavage for 16 days. All of the surviving HCE-exposed male rats exhibited hyaline droplets in the cytoplasm of the renal tubular epithelium. Additionally, male rats exposed to 140 and 281 mg/kg-day HCE demonstrated tubular cell regeneration and eosinophilic granular casts of cell debris in the tubule lumina at the corticomedullary junction. NTP (1989) did not report regeneration or granular casts in the surviving males of the 563 mg/kg-day dose group. NTP (1989) did not report the incidence or severity of the lesions observed in the treated males. None of the nephrotoxic effects were observed at any HCE dose in the female rats or in the controls.

NTP (1996)—21-day study

In a second short-term exposure study, NTP (1996) administered 146 or 293 mg/kg-day HCE by oral gavage to male F344/N rats for 21 days. Marked hyaline droplet accumulation was observed and categorized by severity in relation to controls. The hyaline droplet accumulation exhibited by HCE-exposed male rats was characterized as two severity grades above the control rats. A Mallory-Heidenhain stain allowed for greater sensitivity in evaluating hyaline droplets within the tubules of the kidney and further supported the presence of the hyaline droplets in the kidney tubules. Increased incidence of tubular regeneration (60 and 100% in the 146 and 293 mg/kg-day dose groups, respectively) was also observed in male rats following HCE exposure. The severity of the tubular lesions was considered mild at both doses. Eosinophilic granular casts, of minimal to mild severity, were identified in the outer medullary tubules in male rats exposed to HCE: 80 and 60% in the 146 and 293 mg/kg-day HCE, respectively. There was a dose-related and statistically significant increase in the PCNA labeling index in HCE-treated male rats. The percentage of replicating proximal and distal tubule epithelial cells was increased 5.7-fold over controls in the 146 mg/kg-day dose group and 9.2-fold over the controls in 293 mg/kg-day dose group. The nephrotoxic effects reported by NTP (1996) were not noted in the control animals. Female rats were not included in this study; therefore, gender specificity was of the nephrotoxic effect were not examined.

NTP (1989)—13-week study

In a subchronic exposure study, NTP (1989) administered 34, 67, 134, 268, or 536 mg/kg-day HCE via gavage to F344/N rats for 13 weeks. Kidney effects were reported in male rats from all dose groups exposed to HCE exhibited exposure-related kidney effects, although incidence data was only reported for the 34 mg/kg-day dose group. These kidney

effects were characterized by hyaline droplet formation in the renal tubular epithelium, eosinophilic granular casts of cell debris in the tubular lumina at the corticomedullary region (with associated tubular dilatation), and tubular cell regeneration. The severity of these lesions increased with HCE exposure dose, though the severity grades were not reported. Furthermore, as the HCE exposure dose increased, the animals developed additional lesions. Renal papillary necrosis and renal tubule epithelium degeneration and necrosis were observed in all 536 mg/kg-day males (only the 5 male rats that died before the end of the study were analyzed microscopically).

Urinalysis in male rats administered HCE showed fine and course granules, cellular casts, and epithelial cells, findings that were consistent with the histopathological changes observed in the male rats. Kidney weights of HCE-exposed males were increased 27, 37, 57, 73, and 57% in 34, 67, 134, 268, and 536 mg/kg-day males, respectively (increases were statistically significant, compared with control kidney weights except the low-dose group). Female kidney weight was increased following HCE exposure: 16 and 32% (statistically significant) in the 268 and 536 mg/kg-day dose groups, respectively. Treated females showed no other HCE-exposure related kidney effects.

Gorzinski et al. (1985)—16-week study

Gorzinski et al. (1985) observed dose-related levels of HCE in the kidneys of male F344 rats fed 1, 15, or 62 mg/kg-day HCE for 16 weeks. HCE was also detected in the kidneys of female rats, although at much lower levels and did not increase proportionally with dose. Renal tubular atrophy and degeneration was observed in male rats: 20, 70, and 100% in the 1, 15, and 62 mg/kg-day dose groups, respectively. These renal degenerative effects were also noted in 10% of the male controls, although the authors noted that these lesions were graded as slight. Slight hypertrophy and/or dilation of the proximal convoluted tubules were noted in 10, 70, and 100% of the HCE-exposed male rats in the 1, 15, and 62 mg/kg-day dose groups, respectively. Slight hypertrophy and dilation of the proximal convoluted tubules were not observed in the male control rats. Peritubular fibrosis was also noted in the high-dose group males. Renal tubular atrophy and degeneration were observed in 10, 20, and 60% of female rats in the 1, 15, and 62 mg/kg-day dose groups respectively. These lesions were seen in one female control rat (10%), although the authors characterized the severity grade of the lesions as very slight.

Male rat sensitivity was evident in the histopathological changes seen in the HCE exposed male rats compared with the female rats. Renal effects were either observed in more male rats than female rats (statistical analyses were not reported) or did not occur in females. Additionally, kidney concentrations of HCE were much higher in male rats compared with female rats. Gorzinski et al. (1985) noted that the differences in HCE concentrations measured in male rat and female rat kidneys may explain the differences observed in the kidney effects (i.e., male sensitivity to HCE exposure).

NCI (1978)—78-week study

NCI (1978) conducted a carcinogenicity bioassay in Osborne-Mendel rats administered 113 and 227 mg/kg-day HCE via gavage for 5 days/week for 78 weeks. Chronic inflammatory kidney lesions were observed in both control and HCE-exposed rats. Male rats exhibited chronic inflammation in the kidney, 75, 70, 65, and 50% of untreated control, vehicle control, 113, and 227 mg/kg-day dose groups, respectively. Similarly, female rats showed an incidence of inflammatory lesions in 40, 20, 36, and 41% in the untreated control, vehicle control, 113, and 227 mg/kg-day dose groups, respectively. The control and HCE-exposed male rats exhibited greater sensitivity to the chronic inflammation compared with the female rats. NCI (1978) noted that these lesions observed in the control and HCE-exposed animals of both sexes were characteristic of age-related renal lesions. Some renal lesions observed in older rats could be related to a spontaneous syndrome known as chronic progressive nephropathy (CPN). CPN is associated with aged rats, especially F344, Sprague-Dawley, and Osborne-Mendel strains. CPN is frequently more severe in males compared with females. Hard et al. (1993) reported the pathologic features attributed to CPN including:

- Thickening of tubular and glomerular basement membranes
- Basophilic segments of proximal convoluted tubules with sporadic mitoses indicative of tubule cell proliferation
- Tubular hyaline casts of proteinaceous material originating the more distal portion of the nephron, mainly in the medulla, and later plugging a considerable length of the tubule
- Focal interstitial aggregations of mononuclear inflammatory cells within areas of affected tubules
- Glomerular hyalinization and sclerosis
- Interstitial fibrosis and scarring
- Tubular atrophy involving segments of proximal tubule
- Occasional hyperplastic foci in affected tubules (chronically in advanced cases)
- Accumulation of protein droplets in sporadic proximal tubules (in some advanced cases)

Several of the CPN pathological effects are similar to and can obscure the lesions characteristic of α_{2u}-globulin-related hyaline droplet nephropathy (Hard et al., 1993). Additionally, renal effects of α_{2u}-globulin accumulation can exacerbate the effects associated with CPN (U.S. EPA, 1991c). However, Webb et al. (1989) suggested that exacerbated CPN was one component of the nephropathy resulting from exposure to chemicals that induce α_{2u}-globulin nephropathy. Male rat sensitivity has been noted with both CPN and α_{2u}-globulin nephropathy.

With the exception of atrophy of proximal tubule, tubular cell proliferation, and hyaline casts of proteinaceous material, the histopathological effects associated with CPN are distinctive from those of α_{2u}-globulin nephropathy. Additionally, the urinalysis and serum chemistry of CPN-rats show albuminuria, hypoalbuminemia, and hypocholesterolemia as well as increased serum creatinine and urea nitrogen levels, whereas these changes in α_{2u}-globulin nephropathy are minimal (Hard et al. 1993).

NCI (1978) reported tubular nephropathy in HCE-exposed rats, but not in untreated or vehicle controls. Increased incidence of nephropathy described as tubular degeneration and necrosis and the presence of large hyperchromatic regenerative epithelial cells was observed in 45 and 66% of male rats exposed to 113 and 227 mg/kg-day HCE, respectively. Female rats also exhibited tubular nephropathy following HCE exposure: in 18 and 59% in the 113 and 227 mg/kg-day dose groups, respectively. In addition to the tubular nephropathy, observed effects overlying these lesions included focal pyonephritis, tubular ectasia, cast formation, chronic interstitial nephritis and fibrosis, and focal glomerulosclerosis. Renal tubular cell adenomas were observed in four male rats (11% incidence rate) exposed to 113 mg/kg-day HCE. Similar renal tumors were not observed in males from the high-dose group, males from the vehicle control, males from the untreated control, or female rats. NCI (1978) concluded that there was no evidence of HCE-exposure related carcinogenicity in Osborne-Mendel rats based on the lack of statistical significance and dose-response in the tumor incidence rate. However, it is possible that the truncated duration of HCE-treatment (78 weeks, cyclical) and the significantly accelerated mortality in the male rats did not allow enough time for the renal tubule tumors to develop. According to Goodman et al. (1980), the incidences of spontaneous renal tubule tumors in control male and female Osborne-Mendel rats (as recorded in the NCI Carcinogenesis Testing Program) were 0.3 and 0%, respectively. The incidence of renal adenomas (11%, first observed at 86 weeks; 8 weeks after the treatment period ended) following administration of 113 mg/kg-day HCE exceeded both the concurrent (0%) and historical (0.3%) controls in males.

NTP (1989)—103-week study

NTP (1989) administered 7 or 14 mg/kg-day HCE in corn oil via gavage to male F344/N rats for 103 weeks. Kidney effects consisting of tubular cell degeneration and atrophy, tubular dilatation, tubular cell regeneration, glomerulosclerosis, interstitial fibrosis, and chronic inflammation were observed in ≥94% of the HCE-exposed male rats. The incidence of nephropathy in male control rats was 96%. The mean severity of the kidney effects in male rats increased following HCE exposure: 2.34 ± 0.14, 2.62 ± 0.15, and 2.68 ± 0.16 (statistically significant) in the control, 7 mg/kg-day, and 14 mg/kg-day dose groups, respectively. Kidney effect severity was considered mild for the controls and mild to moderate for the HCE-exposed male rats. While the mean severity scores do not show more than a 15% increase over control in

the high-dose group, more moderate and marked nephropathy was observed in HCE-exposed male rats compared with controls. The incidence of severe (moderate or marked) nephropathy in males was 18/50, 24/50, and 30/50 in the control, 7, and 14 mg/kg-day dose groups, respectively. Additionally, the male rats exhibited increased incidence in linear mineralization of the renal papillae: 4, 30, and 64% in the control, 7 mg/kg-day, and 14 mg/kg-day dose groups, respectively. Pelvic epithelium hyperplasia was also observed in 14% of male rats exposed to either 7 or 14 mg/kg-day HCE. These hyperplastic effects were not observed in either the controls or the treated females.

NTP (1989) administered 57 or 114 mg/kg-day HCE in corn oil via gavage to female F344/N rats for 103 weeks. The incidence of nephropathy in female rats following chronic HCE exposure was 44, 84, and 90% for the control, 57, and 114 mg/kg-day dose groups, respectively. The severity scores for nephrotoxicity in female rats were statistically significantly increased in both treated groups: 0.72 ± 0.13, 1.38 ± 0.11, and 1.69 ± 0.12 in the control, 57 mg/kg-day, and 114 m/kg-day dose groups, respectively. The average severity of nephropathy was considered minimal for the controls and minimal to mild for the HCE-exposed female rats. Examination of the various grades of nephropathy severity shows more mild and moderate nephrotoxicity in HCE-exposed females compared with controls. In females, the incidence of severe (mild or moderate) nephropathy was 12/50, 25/50, and 32/50 in the control, 57, and 114 mg/kg-day dose groups, respectively (statistical analysis was not reported). Female rats also showed an increase in linear mineralization at 57 (44%) and 114 mg/kg-day (26%) compared with relatively high response in the controls (28%). This increase in linear mineralization was not dose-related. The HCE-exposed male rats also exhibited renal tubular hyperplasia, renal tubule adenomas, and renal tubule carcinomas. The combined renal adenoma or carcinoma incidence was 2, 4, and 14% (3, 6, and 24% after adjusting for intercurrent mortality) in the control, 7, and 14 mg/kg-day dose groups, respectively. There were no HCE-related neoplasms observed in female rats treated with 57 or 114 mg/kg-day HCE. NTP (1989) noted that the hyperplasia and tumors of the renal tubules represented a morphologic continuum. The hyperplasia incidence was observed in 4, 8, and 22% of the control, 7 mg/kg-day, and 14 mg/kg-day dose groups, respectively. The incidence of renal tubule neoplasia in male rats also exceeded historical controls (0.5%). Female rats did not exhibit renal tubule hyperplasia.

A sex difference was noted in the observed nephropathy, as males were more sensitive to HCE-exposure related nephropathy than females. This sex-specificity is apparent for the nephrotoxicity and grades of nephropathy severity in both control and HCE-treated groups. Although administered only one-eighth of the dose given to the female rats; the male rats demonstrated a greater incidence of nephropathy that was more severe and included additional kidney effects (i.e., increases in incidence of mineralization of the renal papillae and hyperplasia of pelvic transitional epithelium) compared with the female rats.

With the exceptions of glomerulosclerosis, interstitial fibrosis, and chronic inflammation, the observed nephrotoxic effects in the male rats are characteristic of α_{2u}-globulin nephropathy. However, NTP (1989) did not report accumulation of hyaline droplets containing the α_{2u}-globulin protein in the proximal tubule. It is possible that hyaline droplets were present considering that the 16-day and 13-week rats examined by NTP (1989) exhibited hyaline droplets; however, the hyaline droplets were likely obscured by the prevalence of the other lesions. Evidence of these effects in almost all of the control males and in treated and control female rats also complicates the characterization of the mode of action. Considering that α_{2u}-globulin nephropathy is typically male rat-specific, the appearance of nephrotoxic effects in the female rats as well as the male and female controls, and the identification of other effects not specifically associated with α_{2u}-globulin (i.e., glomerulosclerosis and interstitial fibrosis) suggests that the effects are not the result of α_{2u}-globulin accumulation.

Considering the strain and age of the rats in the chronic (103 weeks) NTP study (1989), it is also possible that the rats were affected by CPN (i.e., increased incidence of nephrotoxicity in the control rats). However, changes in severity of the nephropathy that are greater in the HCE-exposed animals indicate some chemical-related effects. Additionally, HCE-exposed male rats demonstrated dose-dependent increases in incidence of mineralization of the renal papillae and hyperplasia of pelvic transitional epithelium. Neither of these effects increased in a dose-related manner in the controls or the HCE-exposed female rats. Therefore, the treatment-related effects in male and female rats serve as evidence that CPN is not solely responsible for the nephropathy observed by NTP (1989).

Limitations in the available studies

These studies describe the effects associated with HCE exposure using a general, nonspecific term: tubular nephropathy (Weeks et al., 1979; NCI, 1978). This general term does not provide information on the specific histopathological changes characterizing the nephropathy. Additionally, the reported incidences of effects were grouped and measured as nephropathy rather than individual effects. Effects described in this way are difficult to interpret with regards to α_{2u}-globulin nephropathy. One study (NTP, 1996) was limited in its usefulness because the only in male rats were exposed and the experimental design sought to draw conclusions about SARs involved in the induction of hyaline droplet nephropathy of 11 halogenated ethanes. The study focused predominantly on the kidneys and the purpose of the study was to compare chlorinated ethanes, not examine the mode of action of HCE. The divergence in doses used for male and females in the NTP (1989) chronic exposure experiment highlighted the male sensitivity to HCE-induced nephrotoxicity. However, this study design made it difficult to otherwise compare the sexes. Additionally, three of the six HCE exposure studies utilized only two dose groups, limiting the ability to characterize the dose response of HCE-exposure related nephropathy.

Summary of evidence for strength, specificity, and consistency

Generally, kidney tumors observed in cancer bioassays are assumed to be relevant for assessment of human carcinogenic potential. However, when the mode-of-action evidence demonstrates that kidney tumors in male rats result from an α_{2u}-globulin-related mode of action, the data are not suitable for use in risk assessment (U.S. EPA, 1991b). The criteria for demonstrating the α_{2u}-globulin-related mode of action for risk assessment purposes have been defined (U.S. EPA, 1991b). Three criteria must be met: (1) increase in hyaline droplets in the renal proximal tubule cells; (2) determination that the accumulating protein in the droplets is α_{2u}-globulin; and (3) presence of additional pathological lesions associated with α_{2u}-globulin. The key event in the histopathological sequence for the α_{2u}-globulin-related mode of action is excessive accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules. None of the HCE studies performed the necessary immunohistochemical assays to confirm the presence of α_{2u}-globulin protein within the hyaline droplets observed following administration of HCE (NTP, 1996, 1989). It is unknown if HCE is binding to α_{2u}-globulin or to other proteins during the formation of hyaline droplets. This represents an important data gap considering that the presence of this protein is essential to identifying the α_{2u}-globulin-related mode of action. Therefore, there is insufficient evidence to support an α_{2u}-globulin-related mode of action for renal tumors following HCE exposure.

In addition, the data on female rats and mice of both sexes from chronic exposure studies (NTP, 1989; NCI, 1978) do not support the α_{2u}-globulin mode of action for HCE-exposure related nephropathy. The appearance and type of nephrotoxicity noted in control and female rats suggest a mode of action other than α_{2u}-globulin. NCI (1978) reported dose-related nephropathy in female rats that was not apparent in the controls. The dose-responsive kidney effects observed in the female rats treated with HCE suggests that a mode of action other than α_{2u}-globulin nephropathy was occurring. Nephropathy was also reported in male and female mice chronically-administered HCE (NCI, 1978). The NCI (1978) reported the appearance of renal tubular effects in almost all ($\geq$92%) of the HCE-treated male and female mice following chronic HCE exposure, but the mice did not develop renal tubule tumors. The presence of kidney effects in HCE-exposed male and female mice, which generally do not accumulate the α_{2u}-globulin protein, suggests that a mode of action other than α_{2u}-globulin nephropathy.

Dose-response concordance

The key event in the histopathological sequence for the α_{2u}-globulin-related mode of action is excessive accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules. The accumulation of α_{2u}-globulin in hyaline droplets must occur at lower doses than subsequent α_{2u}-globulin-related effects. None of the HCE studies performed the necessary immunohistochemical assays to confirm the presence of α_{2u}-globulin protein within the hyaline

droplets observed following administration of HCE (NTP, 1996, 1989). Therefore, this key event cannot be demonstrated from the available data.

Most of the effects characterizing the histopathological sequence of events in epithelial cells of the proximal tubules leading to renal tumors (U.S. EPA, 1991c) increased in incidence with dose of HCE in the short-term and subchronic exposure studies. Dose-related increases in nephrotoxicity and renal carcinogenicity were noted in the two chronic HCE exposure studies. The short-term and subchronic exposure studies did not report evidence of carcinogenicity in rats administered HCE. In the NTP (1989) study, male rats administered 7 or 14 mg/kg-day HCE for 2 years exhibited a dose-related increased incidence of renal tubule adenomas and carcinomas. Typical histopathological effects associated with α_{2u}-globulin nephropathy (tubular cell degeneration and atrophy, tubular dilatation, and tubular cell regeneration) were noted in almost all of the treated and untreated animals. A dose-response relationship was difficult to detect considering the number of animals affected by nephrotoxicity. However, dose-related increases over controls for toxic kidney effects such as linear mineralization, severity of nephrotoxicity, and renal tubule hyperplasia were observed. NTP (1989) did not report interim data; therefore, examinations were performed at study termination. Consequently, the nephrotoxicity (that is generally attributed to leading up to the formation of renal tubular tumors associated with α_{2u}-globulin) is reportedly increased at doses similar to those that induce tumor formation.

Overall, dose-related kidney effects were noted for almost all of the male rats administered HCE at doses ranging from 1 to 563 mg/kg-day. Even at the lowest HCE dose administered in the studies, renal effects were observed in male rats. Animals treated with greater amounts of HCE exhibited dose-related increases in incidence and severity of effect when compared with those of the lower dose groups. It is difficult to establish dose-response concordance between the noncancer nephropathy and the renal tubule tumors reported by NTP (1989). Renal tubule tumors were observed at 7 mg/kg-day HCE, the lowest dose administered for a chronic duration, which also induced significant nephropathy in HCE-exposed animals. The other studies that administered doses within an order of magnitude of 7 mg/kg-day were the NTP (1989) study [34 or 67 mg/kg-day for 13 weeks] and the Gorzinski et al. (1985) study [1, 15, or 62 mg/kg-day for 16 weeks]. Although nephropathy was noted in the shorter duration studies (NTP, 1996, 1989; Gorzinski et al., 1985), there only evidence of carcinogenicity was from the chronic exposure studies (NTP, 1989; NCI, 1978).

Temporal relationship

The key event in the histopathological sequence for the α_{2u}-globulin-related mode of action is excessive accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules. The accumulation of α_{2u}-globulin in hyaline droplets must occur first in the sequela leading to α_{2u}-globulin-related nephrotoxicity and tumor formation. None of the HCE studies performed the necessary immunohistochemical assays to confirm the presence of α_{2u}-globulin

protein within the hyaline droplets observed following administration of HCE (NTP, 1996, 1989). Therefore, this key event and the important temporal relationship for the accumulation of α_{2u}-globulin cannot be demonstrated from the available data.

Many of the histopathological effects associated with α_{2u}-globulin-related nephropathy were observed in animals treated with HCE in studies that varied in exposure duration from 16 days to 2 years. The sequence of histopathological events characteristic of the α_{2u}-globulin-related mode of action was noted in the chronic exposure study NTP (1989) that reported renal tubule adenomas and carcinomas. All of the studies (NTP, 1996, 1989; Gorzinski et al., 1985; NCI, 1978) that administered HCE for shorter durations than the NTP (1989) study reported similar histopathological changes, although an increase in renal tubule tumors was not observed. It is unknown if the nephropathy observed by NTP (1989) leads to the reported renal tubule tumors because the animals were only examined at the end of the 103-week study period. A temporal relationship cannot be distinguished from reported data.

Biological plausibility and coherence

The sequence of events including accumulation of α_{2u}-globulin protein in the renal tubules of male rats initiating a sequence of nephrotoxic events leading to renal tubule tumor formation is plausible (U.S. EPA, 1991c). These effects are typically not observed in female rats or other species due to the absence or minimal presence of the α_{2u}-globulin protein in these animals (Hard et al., 1993). Concluding that HCE is acting through an α_{2u}-globulin-associated mode of carcinogenic action is precluded by evidence of nephrotoxic effects in female rats in two chronic studies (NTP, 1989; NCI, 1978) and in male and female mice in one chronic study (NCI, 1978). Nephropathy associated with α_{2u}-globulin is generally not observed in female rats or other species due to the absence or minimal presence of the α_{2u}-globulin protein in these animals (Hard et al., 1993).

Other Possible Modes of Action

There is insufficient evidence to support an α_{2u}-globulin-related mode of action for renal tumors following HCE exposure. It is possible that advanced CPN may play a role in the incidence of nephrotoxicity and kidney tumors in male rats following HCE exposure. CPN is an age-related renal disease of laboratory rodents that occurs spontaneously. The observed renal lesions in male rats following exposure to HCE are effects commonly associated with CPN. Nephropathy (described as tubular cell degeneration and regeneration, tubular dilatation and atrophy, glomerulosclerosis, interstitial fibrosis, and chronic inflammation) was also observed following chronic HCE exposure in female rats in the NTP (1989) chronic study, as well as male and female mice following chronic HCE exposure (NCI, 1978). However, changes in severity of the nephropathy were observed to be greater in male rats exposed to HCE compared to controls

indicating that HCE exposure exacerbated effects in the kidney. Additionally, HCE-exposed male rats demonstrated dose-dependent increases in incidence of mineralization of the renal papillae and hyperplasia of pelvic transitional epithelium. Neither of these effects increased in a dose-related manner in the controls or the HCE-exposed female rats. The treatment-related effects in male and female rats serve as evidence that CPN is not solely responsible for the nephropathy observed by NTP (1989).

Conclusions about the Hypothesized Mode of Action

Support for the Hypothesized Mode of Action in Animals

The mode of action for the carcinogenic effects of HCE in the kidney is unknown. There is insufficient evidence to establish the role of α_{2u}-globulin in HCE-exposure related nephropathy. Studies following short-term, subchronic, and chronic exposure of male rats have reported renal lesions consistent with α_{2u}-globulin nephropathy (NTP, 1996, 1989; Gorzinski et al., 1985; NCI, 1978). The formation of renal tubule adenomas and carcinomas (preceded by hyperplasia) following chronic HCE exposure (NTP, 1989) are also consistent with an α_{2u}-globulin-related mode of action. However, the key event in the histopathological sequence of events demonstrating a α_{2u}-globulin-related mode of action (excessive accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules) leading to development of kidney tumors in male rats exposed to HCE has not been fully characterized. None of the HCE studies performed the necessary immunohistochemical assays to confirm the presence of α_{2u}-globulin protein within the hyaline droplets observed following administration of HCE (NTP, 1996, 1989). It is unknown if HCE is binding to α_{2u}-globulin or to other proteins during the formation of hyaline droplets. This represents an important data gap considering that the presence of this protein is essential to identifying this hypothesized mode of action. In addition, data are available that demonstrate kidney effects in female rats and mice of both sexes from chronic exposure studies (NTP, 1989; NCI, 1978). The NCI (1978) study reported dose-related nephropathy in female rats that was not apparent in the controls. Nephropathy was also reported in male and female mice chronically-administered HCE (NCI, 1978). The presence of kidney effects in HCE-exposed male and female mice, which generally do not accumulate the α_{2u}-globulin protein, suggests that a mode of action other than α_{2u}-globulin nephropathy.

Role of metabolites

Studies of proposed HCE metabolites such as PERC, pentachloroethane, and TCE revealed similar noncancer and cancer effects. PERC exposure caused toxic nephropathy and kidney tumors (NCI, 1977); pentachloroethane exposure resulted in chronic inflammation of the kidney, (NTP, 1983); and TCE exposure produced chronic nephropathy (NCI, 1976), toxic nephrosis, kidney cytomegaly (NTP, 1990), renal tubular adenomas (NTP, 1990), tubular cell cytomegaly, and toxic nephropathy (NTP, 1988). These studies consistently demonstrate that the

kidney is the principal target tissue for noncancer effects following exposure to putative HCE metabolites. Based on the available data, it is not possible to define the relative roles of the parental compound (HCE) or its metabolites in the kidney effects associated with exposure to HCE. However, the kidney effects of these potential HCE metabolites further support the kidney as the principal target organ of HCE exposure.

Relevance of the Hypothesized Mode of Action to Humans

Generally, kidney tumors observed in cancer bioassays are assumed to be relevant for assessment of human carcinogenic potential. However, for male rat kidney tumors, when the mode-of-action evidence convincingly demonstrates that the response is secondary to α_{2u}-globulin accumulation, the tumor data are not used in the cancer assessment (U.S. EPA, 1991b). There is insufficient evidence to conclude that the renal adenomas and carcinomas observed in male rats administered HCE (NTP, 1989) are related to an α_{2u}-globulin mode of action for the following reasons: (1) the lack of α_{2u}-globulin immunohistochemical data for HCE-induced nephrotoxicity and carcinogenicity, (2) the hyaline droplet accumulation is caused by excessive protein load that may not be exclusively related to α_{2u}-globulin accumulation, and (3) the existence of renal toxicity in female rats and male and female mice indicates that the nephrotoxic effects are not limited to an α_{2u}-globulin-induced sequence of lesions. Therefore, the renal adenomas and carcinomas observed in male rats administered HCE (NTP, 1989) were considered relevant to humans.

4.7.3.2. Liver Tumors

Description of the Hypothesized Mode of Action

Hypothesized mode of action

Hepatocellular carcinomas were observed in male and female $B6C3F_1$ mice administered 360 or 722 mg/kg-day HCE, via gavage, in a chronic oral bioassay conducted by NCI (1978). Tumor incidences in males of both dose groups were statistically significantly elevated compared with control groups, and demonstrated a dose response. Both dose groups of female mice presented statistically significantly elevated incidences of hepatocellular carcinoma compared with control groups but a dose response was not observed. The investigators did not find nonneoplastic liver effects (such as organized thrombus, inflammation, fibrosis, necrosis, infarctions, amyloidosis, or hyperplasia) in either sex.

The mode of action for the carcinogenic effects of HCE in the liver is unknown. Metabolism studies of HCE indicate that the major enzymes involved are phenobarbital-inducible CYP450s. These are primarily localized in the liver. Although tissue-specific metabolism of HCE has not been studied extensively, the majority of HCE metabolism is presumed to occur in the liver. HCE is proposed to metabolize to PERC and pentachloroethane and is likely, subsequently metabolized to TCE. It is possible that the HCE-

induced hepatocellular carcinomas in mice occur as a result of the binding of HCE metabolites to liver macromolecules and the generation of free radicals during HCE metabolism, causing key events in the carcinogenic process such as cytotoxicity, inflammation and regenerative cell proliferation. However, these potential key events have not been systematically evaluated for HCE.

In a 13-week study, hepatocellular necrosis of the centrilobular area was observed in rats (NTP, 1989). It is unknown if this could be considered a key event in the carcinogenic process because rats in the available studies (NTP, 1989; NCI, 1978) have not displayed hepatocellular neoplastic endpoints. Although mice demonstrated hepatocellular carcinoma, nonneoplastic effects such as hepatocellular necrosis were not observed (NCI, 1978). HCE-induced hepatocellular carcinomas in mice varied in microscopic appearance (NCI, 1978). Some carcinomas were characterized by well-differentiated hepatic cells with uniform cord arrangement, while others had anaplastic liver cells with large hyperchromatic nuclei, often with inclusion bodies and vacuolated pale cytoplasm. Arrangement of neoplastic liver cells also varied from short stubby cords to nests of cells, and occasional pseudo-acinar formations. Neoplasms in control mice did not vary in appearance from those in HCE-treated mice.

In vivo binding of radiolabeled carbon to DNA, RNA and protein from liver, kidney, lung, and stomach following administration of [^{14}C]HCE was consistently greater in mice compared with rats (Lattanzi et al., 1988). Binding to macromolecules was interpreted by the presence of radiolabeled carbon; however, radiolabeled carbon may have been incorporated into these macromolecules from intermediary HCE metabolites. In vitro binding studies using calf thymus DNA demonstrated that mouse liver cytosol (induced by phenobarbital) mediated more extensive DNA binding than rat liver cytosol (Lattanzi et al., 1988). Comparisons of HCE metabolism rates indicated that mice metabolize HCE at twice the rate of rats (Mitoma et al., 1985).

Cellular damage leading to cytotoxicity, inflammation, and regenerative cell proliferation is a possible consequence of this binding in the liver. The binding studies provide a line of evidence as to why the liver is the major carcinogenic target in the mouse, but not the rat. Regenerative cell proliferation has been evaluated in the kidney, but not in the liver of HCE-treated rats (NTP, 1996). RDS in hepatocytes was evaluated in mice treated with HCE (Yoshikawa, 1996; Miyagawa et al., 1995). This study reported ambiguous results; the lower HCE dose caused a statistically significant increase in RDS, whereas the higher dose did not (Yoshikawa, 1996; Miyagawa et al., 1995). Rat liver foci experiments provide support for the hypothesis that HCE acts as a tumor promoter, not as a tumor initiator (Milman et al., 1988; Story et al., 1986).

The in vivo binding data suggest that HCE is sequestered in the liver of mice and rats and metabolic data suggest that mice metabolize HCE at a greater rate compared with rats. Considering the greater potential for metabolism in mice compared with rats and the proposed

increase in DNA binding following metabolism of HCE (Lattanzi et al., 1988), the increased incidence of hepatocellular carcinomas in mice, but not rats, may be related to DNA binding. However, the DNA binding measurements were based solely on the presence of radiolabeled carbon; specific HCE metabolites were not identified. Therefore, this process does not take into account the possibility of normal biological mechanisms in which the radiolabeled carbon can be incorporated into the macromolecules via anabolic processes. All together, while it is possible that metabolism and binding in mice are involved in the development of liver tumors, the role of DNA binding in the mode of action for HCE-induced hepatotoxicity and carcinogenesis is not known and, as such, the mode of action is not known.

4.7.3.3. Pheochromocytomas

Description of the Hypothesized Mode of Action

Hypothesized mode of action

No studies were identified to determine a mode of action for HCE-induced tumors of the adrenal gland. Therefore, the mode of action for pheochromocytomas observed following oral exposure to HCE is unknown.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

No studies were located that address the susceptibility of populations or life stages to HCE-induced toxicity or carcinogenicity in humans.

4.8.1. Possible Childhood Susceptibility

No studies were located that addressed possible childhood susceptibility to HCE-induced toxicity or carcinogenicity. Although it is unknown if HCE toxicity is mediated by parent compound or its metabolites, CYP450 enzymes of the 2A, 2B, and 3A subfamilies and CYP450 1A2 are involved in HCE metabolism. Many drugs reportedly exhibit a higher systemic clearance in children than in adults (Evans et al., 1989). Blanco et al. (2000) compared liver microsomal CYP450 activities of humans aged <10 years with those aged >10-60 years and concluded that factors other than maximal CYP450 catalytic activities, such as reductions in hepatic blood flow, hepatic size, and oxygen supply in the elderly, may be responsible for age-related changes in drug clearance. Studies of fetal and neonatal livers indicate that CYP450 expression is similar to adult levels by a few months of age (Lacroix et al., 1997; Vieira et al., 1996; Cazeneuve et al., 1994; Treluyer et al., 1991). However, Dorne (2004) reported in a review article that Phase I (including CYP450 activities) and Phase II enzymatic activities are 1.3–1.5-fold higher in children (aged 1–16 years) compared with adults. Therefore, the extent to which variable age-related expression of CYP450 contributes to childhood susceptibility is unknown. Research in developmental expression of CYP450 is ongoing; conclusions regarding the differential expression of CYP450 are premature. Considering the substantial portion of

HCE that remains as parent compound, the impact, if any, of age on CYP450 expression and HCE metabolism cannot be assessed.

4.8.2. Possible Gender Differences

Toxicity studies in rats indicate that male rats are more sensitive to HCE-induced nephrotoxicity than females (NTP, 1989; Gorzinski et al., 1985; Gorzinski et al., 1980; NCI, 1978). Evidence suggests that female rats are more sensitive to HCE-induced hepatotoxicity. The reasons for these sex-specific differences are unknown, but may be related to sex-specific differences in tissue concentrations following HCE administration (i.e., higher concentrations observed in male rat tissues when compared with female rats, see Table 3-3), sex hormone differences, and/or gender difference in CYP450 activities. No additional studies were located that addressed possible gender differences for HCE-induced toxicity or carcinogenicity.

4.8.3. Other

CYP450 enzymes are polymorphic in the human population. Polymorphisms result in CYP450 enzymes with variant catalytic activity for substrates such as HCE. This could potentially result in decreased HCE detoxification or increased HCE bioactivation. Detoxification enzymes such as the GST family are also polymorphic in the human population, with variant catalytic activities that could affect the detoxification of HCE. There are numerous epidemiology studies in the literature examining CYP450 and GST polymorphisms and increases in cancer risks; however, none of these are specific for HCE.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

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

Data on the health effects of oral HCE exposure in humans are not available. The oral exposure database for HCE includes a 103-week gavage study in F344 rats (NTP, 1989), a 78-week gavage study in Osborne-Mendel rats (NCI, 1978), a 91-week gavage study in $B6C3F_1$ mice (NCI, 1978), a 16-week feeding study in F344 rats (Gorzinski et al., 1985), and a 13-week gavage study in F344 rats (NTP, 1989). The short-term study data were not considered in the selection of the principal study for the derivation of the RfD because the database contains reliable dose-response data from studies of subchronic and chronic durations. However, short-term studies in rats (NTP, 1996, 1989) were used to support findings in the subchronic and chronic studies. The available oral exposure studies identified kidney or liver effects associated with exposure to HCE. Reported effects include tubular nephropathy (NTP, 1989; NCI, 1978), atrophy and degeneration of renal tubules (NTP, 1989; Gorzinski et al., 1985), slight hypertrophy and/or dilation of proximal convoluted renal tubules (Gorzinski et al., 1985), linear mineralization of renal tubules (NTP, 1989), hyperplasia of the renal pelvic transitional epithelium (NTP, 1989), and hepatocellular necrosis (NTP, 1989).

In the NTP (1989) chronic study, HCE was administered via gavage at doses of 7 and 14 mg/kg-day in male F344 rats and 57 and 114 mg/kg-day in female F344 rats for 103 weeks. Nephropathy (characterized by tubular cell degeneration and regeneration, tubular dilatation and atrophy, glomerulosclerosis, interstitial fibrosis, and chronic inflammation) was observed in HCE treated rats of both sexes. Nephropathy was also reported in control rats of both sexes. Although a high incidence of nephropathy was observed in control rats, the study authors reported that the incidence of more severe nephropathy increased in dosed rats relative to controls (NTP, 1989). EPA considered the increase in severity of nephropathy in male rats by analyzing the incidence of greater than mild nephropathy. EPA determined that the increased incidence of moderate or marked nephropathy in males was statistically significant at the 14 mg/kg-day dose (see Table 5-1). EPA considered the increased severity of nephropathy in female rats by analyzing the incidence of nephropathy that was greater than minimal nephropathy. EPA determined that the increased incidences of mild to marked nephropathy were statistically significant in females at the 57 and 114 mg/kg-day doses (see Table 5-1). Linear mineralization of the renal papillae and hyperplasia of the renal pelvic epithelium were increased in a dose-dependent, statistically significant manner in the treated male rats. EPA determined that the increased incidences of linear mineralization of the renal papillae and hyperplasia of the renal pelvic epithelium were statistically significant in males at the 7 and 14 mg/kg-day doses (see Table 5-1). Considering the increased severity of nephropathy following HCE exposure and

dose-dependent increases in the incidence of mineralization of the renal papillae and hyperplasia of renal pelvic transitional epithelium in male rats, EPA determined that the nephropathy observed in the NTP (1989) study was exacerbated by HCE exposure. The NTP (1989) chronic study did not identify NOAELs for male or female rats as kidney effects were observed at the lowest doses tested. EPA considered the male rat LOAEL as 7 mg/kg-day based on increased incidence in moderate or marked tubular nephropathy (characterized by degeneration, necrosis, and regenerative epithelial cells), hyperplasia of the pelvic transitional epithelium, and linear mineralization of the renal papillae in the NTP (1989) study. EPA considered the female rat LOAEL as 57 mg/kg-day, based on dose-related increases in incidence and severity of nephropathy in the NTP (1989) study.

In the NCI (1978) chronic rat study, HCE was administered via gavage to groups of 50 male and 50 female Osborne-Mendel rats for 5 days/week, cyclically for 66 of the 78 weeks, followed by an observation period of 33-34 weeks (total of 112 weeks). The TWA doses of HCE were 113 and 227 mg/kg-day. Tubular nephropathy was observed in all groups of treated animals, but was not observed in either untreated or vehicle controls. Statistically significant increases in incidence of tubular nephropathy were observed at 113 and 227 mg/kg-day HCE in both male and female rats (see Table 5-1). The NCI (1978) study did not identify a NOAEL for tubular nephropathy in rats. EPA considered the LOAEL as 113 mg/kg-day, based on a dose-related increase in incidence of nephropathy in both male and female rats.

In the NCI (1978) chronic mouse study, HCE was administered via corn oil gavage to groups of 50 male and 50 female $B6C3F_1$ mice for 5 days/ week for 78 weeks followed by an observation period of 12-13 weeks (total of 90 weeks). Starting in week 9, the HCE doses were increased, though no explanation for the increase was provided. The TWA doses of HCE were 360 and 722 mg/kg-day. Because of low survival rates in the vehicle and untreated male control groups, NCI (1978) compared tumor incidences in the dosed males and females to the pooled vehicle control data derived from concurrently run bioassays for several other chemicals. NCI (1978) reported chronic kidney inflammation (i.e., tubular nephropathy characterized by degeneration of the convoluted tubule epithelium at the junction of the cortex and medulla and hyaline casts) in male and female $B6C3F_1$ mice administered 360 and 721 mg/kg-day HCE. EPA considered the LOAEL for this study as 360 mg/kg-day based on tubular nephropathy, while a NOAEL could not established from these data.

In Gorzinski et al. (1985), HCE was administered (in feed) to groups of 10 male and 10 female F344 rats at doses of 0, 1, 15, or 62 mg/kg-day for a period of 16 weeks. Kidney effects consisted of slight hypertrophy and/or dilation of proximal convoluted renal tubules and atrophy and degeneration of renal tubules. Slight hypertrophy and/or dilation of the proximal convoluted renal tubules was not observed in the control rats of either sex or in HCE exposed female rats. EPA determined that increases in slight hypertrophy and/or dilation of the proximal convoluted renal tubules were statistically significant in male rats treated with 15 or

62 mg/kg-day HCE (see Table 5-1). Atrophy and degeneration of renal tubules was observed in both male and female rats. EPA determined that increases in incidences of atrophy and degeneration of renal tubules were statistically significant in male rats treated with 15 or 62 mg/kg-day HCE and in female rats fed 62 mg/kg-day HCE (see Table 5-1). EPA considered the male rat LOAEL as 15 mg/kg-day and the male rat NOAEL as 1 mg/kg-day, based on increased incidence of the renal tubule effects. EPA considered the female rat LOAEL as 62 mg/kg-day and the female rat NOAEL as 15 mg/kg-day, based on increased incidence of renal tubule effects.

In the NTP (1989) subchronic study, HCE was administered via gavage to groups of 10 male and 10 female F344 rats at TWA doses of 0, 34, 67, 134, 268, and 536 mg/kg-day for 13 weeks. Kidney effects (i.e. hyaline droplet formation, renal tubular regeneration, and renal tubular casts) were observed in male rats from all HCE exposure groups, though incidence data was only provided for the 34 mg/kg-day dose group. NTP (1989) reported that the severity of kidney effects in male rats increased with dose, but no data on severity were presented. No kidney effects were reported in female F344 rats exposed to HCE. Liver effects were observed in male and female rats at higher doses of HCE and EPA determined that statistically significant increases in hepatocellular necrosis were observed in female rats exposed to 268 or 536 mg/kg-day HCE (see Table 5-1).

The short-term studies in rats were not used for subsequent dose-response assessment because of short study durations (12-day study [Weeks et al., 1979], 16-day study [NTP, 1989], 21-day study [NTP, 1996], and 1-day study [Fowler, 1969]) and the availability of reliable dose-response data from studies of subchronic and chronic durations. The effects and the incidence of kidney and liver effects from the studies considered for selection as the principal study, serving as the basis for the derivation of the RfD, are summarized in Table 5-1. As incidence data on kidney effects reported in the 13-week subchronic study (NTP, 1989) were limited to males in the 34 mg/kg-day dose group, these data are not presented in Table 5-1.

Table 5-1. Incidences of non-cancerous kidney and liver effects in rats following oral exposure to HCE

Study	Duration (route)	Strain/sex/species	Endpoint	Dose (mg/kg-day)	Incidence
Kidney Effects					
NCI (1978)	78 weeks (gavage)	Osborne-Mendel Male Rat	Tubular Nephropathy	0	0/20 (0%)
				113	22/49[a] (45%)
				227	33/50[a] (66%)
		Osborne-Mendel Female Rat	Tubular Nephropathy	0	0/20 (0%)
				113	9/50[a] (18%)
				227	29/49[a] (59%)
NTP (1989)	103 weeks (gavage)	F344 Male Rat	Moderate to Marked Tubular Nephropathy	0	18/50 (36%)
				7	24/50 (48%)
				14	30/50[a] (60%)
		F344 Female Rat	Mild to Marked Tubular Nephropathy	0	12/50 (24%)
				57	25/50[a] (50%)
				114	32/49[a] (65%)
NTP (1989)	103 weeks (gavage)	F344 Male Rat	Linear Mineralization	0	2/50 (4%)
				7	15/50 [a] (30%)
				14	32/50 [a] (64%)
NTP (1989)	103 weeks (gavage)	F344 Male Rat	Hyperplasia of the Renal Pelvic Transitional	0	0/50 (0%)
				7	7/50[a] (14%)
				14	7/50[a] (14%)
Gorzinski et al. (1985)	16 weeks (dietary)	F344 Male Rat	Slight Hypertrophy and/or Dilation of Proximal Convoluted Renal Tubules	0	0/10 (0%)
				1	1/10 (10%)
				15	7/10[a] (70%)
				62	10/10[a] (100%)
Gorzinski et al. (1985)	16 weeks (dietary)	F344 Male Rat	Atrophy and Degeneration of Renal Tubules	0	1/10 (0%)
				1	2/10 (20%)
				15	7/10[a] (70%)
				62	10/10[a] (100%)
		F344 Female Rat	Atrophy and Degeneration of Renal Tubules	0	1/10 (0%)
				1	1/10 (10%)
				15	2/10 (20%)
				62	6/10[a] (60%)
Liver Effects					
NTP (1989)	13 weeks (gavage)	F344 Male Rat	Hepatocellular Necrosis	0	0/10 (0%)
				33.5	0/10 (0%)
				67.1	0/10 (0%)
				134.3	0/10 (0%)
				267.8	1/10 (10%)
				535.7	2/5 (40%)
		F344 Female Rat	Hepatocellular Necrosis	0	0/10 (0%)
				33.5	0/10 (0%)
				67.1	0/10 (0%)
				134.3	2/10 (20%)
				267.8	4/10[a] (40%)
				535.7	8/10[a] (80%)

[a]EPA determined statistical significance using Fisher's Exact Test ($p < 0.05$)

These chronic and subchronic studies in rats and mice indicate that the kidney and liver are both target organs of HCE oral toxicity in rodents. Given the number of effects reported in

the kidney and the greater sensitivity of these effects in available studies, the kidney is the primary target of oral HCE exposure toxicity in rodents. HCE exposure resulted in a number of kidney effects: atrophy and degeneration of renal tubules in male and female F344 rats (Gorzinski et al., 1985), slight hypertrophy and/or dilation of proximal convoluted renal tubules in male F344 rats (Gorzinski et al., 1985), linear mineralization in male F344 rats (NTP, 1989), tubular nephropathy in male and female F344 rats (NTP, 1989), hyperplasia of the renal pelvic transitional epithelium in male F344 rats (NTP, 1989), and tubular nephropathy in male and female Osborne-Mendel rats (NCI, 1978). Further consideration was given to these endpoints as potential critical effects for the determination of the point of departure (POD) for derivation of the oral RfD.

Although the doses associated with hepatic effects were more than 10-fold higher than doses associated with kidney effects, data from the NTP (1989) study on incidence of hepatocellular necrosis from the female rats were subject to BMD modeling for comparison purposes. The data on the male rat liver effects from the NTP (1989) study were not considered because the incidence of hepatocellular necrosis was not significantly elevated above controls at any HCE dose. The kidney effects reported in the 13-week subchronic study (NTP, 1989) were not further considered because the lack of the incidence data for the control groups made it uncertain if the 34 mg/kg-day HCE dose represented a LOAEL. In addition, the HCE doses administered were more than four-fold higher than those doses associated with kidney effects in other subchronic (Gorzinski et al., 1985) and chronic (NTP, 1989) studies. The chronic study in $B6C3F_1$ mice (NCI, 1978) was not considered for selection as the principal study because HCE doses that induced kidney effects were more than 7-fold higher than doses associated with kidney effects in rats following subchronic (Gorzinski et al., 1985) or chronic (NTP, 1989; NCI, 1978) exposure. The ability of the chronic NTP (1989) study to inform the effects observed at the lowest dose tested in the Gorzinski et al. (1985) study is limited because the lowest dose tested in the chronic exposure study represented a LOAEL. Table 5-2 summarizes the BMD modeling results of the available data and the benchmark response (BMR) levels and the potential PODs are identified for each effect.

5.1.2. Methods of Analysis—Including Models

For this assessment, BMD approach (U.S. EPA, 2001) was employed to identify the potential POD for each of the endpoints described above. A BMR of 10% extra risk was considered appropriate for derivation under the assumption that it represents a minimally biologically significant response level (U.S. EPA, 2000b). All of the dichotomous dose-response models available in the EPA benchmark dose software (BMDS), version 2.0, were fit to the incidence data for kidney effects in male and female rats reported by NTP (1989), NCI (1978), and Gorzinski et al. (1985), as well as the incidence data for hepatocellular necrosis in female rats reported by NTP (1989). Details of the BMD dose-response modeling reported in Table 5-2

are presented in Appendix B (Table B-1). In addition, the BMD and BMDL modeling outcomes for a BMR of 5 and 1% are presented in Appendix B (Table B-2) for comparison with the 10% BMR.

From the BMD modeling analysis results presented in Table B-1, candidate PODs were selected. Table 5-2 summarizes the BMD modeling results of the available data and the benchmark response (BMR) levels and the potential PODs are identified for each effect.

Table 5-2. Summary of the BMD modeling results for the kidney

Study	Endpoint	Sex/species (group size)	Duration (route)	"Best-Fit" Model	Goodness of fit *p*-value	AIC	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gorzinski et al. (1985)	Slight hypertrophy and/or dilation of proximal convoluted renal tubules	Male Rats (n = 10)	16 weeks (dietary)	Gamma[a]	0.99	20.88	1.22	0.710
Gorzinski et al. (1985)	Atrophy and degeneration of renal tubules	Male Rats (n = 10)	16 weeks (dietary)	Gamma[b]	0.70	34.94	1.34	0.728
		Female Rats (n = 10)		Multistage 1°[c]	0.93	40.61	8.54	4.49
NCI (1978)	Tubular Nephropathy	Male Rats (n ≈ 50)	78 weeks (gavage)	Gamma[d]	0.93	133.68	21.22	16.99
		Female Rats (n ≈ 50)		Multistage 2°	0.94	116.09	80.63	41.89
NTP (1989)	Increased Severity of Tubular Nephropathy	Male Rats (n ≈ 50)	103 weeks (gavage)	Quantal-linear[e]	0.87	205.90	3.20	1.88
		Female Rats (n ≈ 50)		Gamma[f]	0.86	191.90	15.17	10.72
NTP (1989)	Linear Mineralization	Male Rats (n ≈ 50)	103 weeks (gavage)	Probit	0.51	147.66	3.98	3.22
NTP (1989)	Hyperplasia of the pelvic transitional epithelium	Male Rats (n ≈ 50)	103 weeks (gavage)	LogLogistic	0.48	84.42	7.05	4.48

[a]Gamma, Quantal-linear, and Weibull models had identical AIC, goodness of fit p-values, as well as BMD_{10} and $BMDL_{10}$ values.
[b]Gamma, Multistage 1°, and Quantal-linear models had identical AIC, goodness of fit p-values, as well as BMD_{10} and $BMDL_{10}$ values.
[c]Multistage 1° and Quantal-linear models had identical AIC, goodness of fit p-values, as well as BMD_{10} and $BMDL_{10}$ values.
[d]Gamma, Multistage 1°, and Weibull models had identical AIC, goodness of fit p-values, as well as BMD_{10} and $BMDL_{10}$ values.
[e]Quantal-linear and Multistage 1° models had identical AIC, goodness of fit p-values, as well as BMD_{10} and $BMDL_{10}$ values.
[f]Gamma, Quantal-linear, and Weibull models had identical AIC, goodness of fit p-values, as well as BMD_{10} and $BMDL_{10}$ values.

AIC = Akaike's Information Criteria (i.e., a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint).

The range of potential PODs (approximately 60-0.6 mg/kg-day) is about 100-fold. Kidney effects (i.e., tubular nephropathy, linear mineralization of the renal tubules, hyperplasia of the pelvic transitional epithelium, atrophy and degeneration of renal tubules, and slight

hypertrophy and/or dilation of the proximal convoluted renal tubules) observed in male rats resulted in lower candidate PODs than comparable effects in female rats.

The most sensitive effect observed in male rats exposed to HCE is slight hypertrophy and/or dilation of proximal convoluted renal tubules (Gorzinski et al., 1985). However, the potential POD for slight hypertrophy and/or dilation of proximal convoluted renal tubules (i.e., 0.710 mg/kg-day) is nearly identical to the candidate POD for atrophy and degeneration of renal tubules (i.e., 0.728 mg/kg-day). As tubular nephropathy in the chronic studies (NTP, 1989; NCI, 1978) was characterized as atrophy and degeneration of renal tubules, this endpoint has been consistently observed following HCE exposure in several studies. Therefore, atrophy and degeneration of renal tubules was selected as the candidate POD for this subchronic exposure study. As shown in Appendix B, the gamma, multistage 1°, logistic, probit, Weibull models in BMDS (version 2.0) provided adequate fits to the incidence data for atrophy and degeneration of renal tubules in male rats from the Gorzinski et al. (1989) 16-week study (Table B-1), as assessed by a chi-square goodness-of-fit p-values, as well as BMD_{10} and $BMDL_{10}$ values were identical for the gamma, multistage 1°, and quantal-linear model; therefore, the model with the lowest $BMDL_{10}$ was selected. The gamma, multistage 1°, and quantal-linear model had identical $BMDL_{10}$ values. Therefore, the BMD_{10} associated with a 10% extra risk for nephropathy in male rats of 1.34 mg/kg-day, and the lower 95% confidence limit on this BMD_{10} ($BMDL_{10}$) of 0.728 mg/kg-day was selected as the potential POD for these data.

The tubular nephropathy in male rats observed in the chronic exposure study (NTP, 1989) resulted in higher PODs than the atrophy and degeneration of renal tubules in male rats observed following 16 weeks of HCE exposure (Gorzinski et al., 1985). The ability of the chronic NTP (1989) study to inform the effects observed at the lowest dose tested in the Gorzinski et al. (1985) study is limited because the lowest dose tested in the chronic exposure study represented a LOAEL. Therefore, the Gorzinski et al. (1985) study was selected as the principal study and atrophy and degeneration of renal tubules in male rats was selected as the critical effect. The $BMDL_{10}$ of 0.728 mg/kg-day was selected as the POD and serves as the basis for the derivation of the oral RfD for HCE. This endpoint is supported by additional kidney effects associated with oral exposure to HCE and supports the weight of evidence for HCE-associated nephrotoxicity.

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

The derivation of the RfD for atrophy and degeneration of renal tubules in male F344 rats from the Gorzinski et al. (1985) 16-week toxicity study was calculated from the $BMDL_{10}$ of 0.728 mg/kg-day. The composite UF of 3,000 was comprised of the following:

- An interspecies UF (UF_A) of 10 was applied to account for the variability in extrapolating from rats to humans. Although the toxicokinetics have been minimally evaluated in animals, the toxicokinetics of HCE have not been fully characterized in either rats or humans.

- An intraspecies UF (UF_H) of 10 was applied to adjust for potentially sensitive human subpopulations in the absence of information on the variability of response to HCE in the human population. Current information is unavailable to assess human-to-human variability in HCE toxicokinetics and toxicodynamics.

- The study selected as the principal study was a 16 week study by Gorzinski et al (1985), a study duration that is minimally past the standard subchronic (90 day) study and falls well short of a standard lifetime study. Kidney effects were observed in male rats in the Gorzinski et al. (1985) subchronic study at doses below the range of exposure tested in the available chronic exposure studies. In addition, the ability of the available chronic studies to inform the effects observed at the low dose is limited because the lowest dose tested in the NTP (1989) chronic exposure study represented a LOAEL. Therefore, there are no data to exclude the possibility that chronic exposure could increase the severity of the observed kidney effects or could result in similar effects at lower doses. For these reasons, subchronic-to-chronic UF (UF_S) of 10 was used to account for the extrapolation from subchronic-to-chronic exposure duration.

- An UF for a LOAEL to a NOAEL extrapolation was not used because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% increase in the incidence of renal tubule atrophy and degeneration was selected under an assumption that it represents a minimal biologically significant change.

- A UF of 3 was applied to account for deficiencies in the HCE toxicity database, including the lack of a multigenerational reproductive study. The database includes studies in laboratory animals, including chronic and subchronic dietary exposure studies and two oral developmental toxicity studies. One of the available oral developmental toxicity studies demonstrated that HCE exposure decreased gestational indices and fetal viability, and increased resorptions with maternal toxicity at 500 mg/kg-day (Weeks et al., 1979). The second oral developmental toxicity study showed maternal toxicity at both the mid- and high doses (167 and 500 mg/kg-day) with decreased fetal body weight and increased late stage resorptions and skeletal variations at the high dose (Shimizu et al., 1992). The toxic effects observed in the developmental toxicity studies were observed at doses higher than those observed to induce renal toxicity in the subchronic and chronic toxicity studies. Therefore, in consideration of the entire database for HCE, a database UF of 3 was applied to account for the lack of a two-generational reproductive study.

Given the UFs established above, the RfD for HCE was calculated employing the following equation:

$$\begin{aligned} \text{RfD} &= \text{POD} \div \text{UF} \\ &= 0.728 \text{ mg/kg-day} \div 3{,}000 \\ &= 2 \times 10^{-4} \text{ mg/kg-day} \end{aligned}$$

5.1.4. RfD Comparison Information

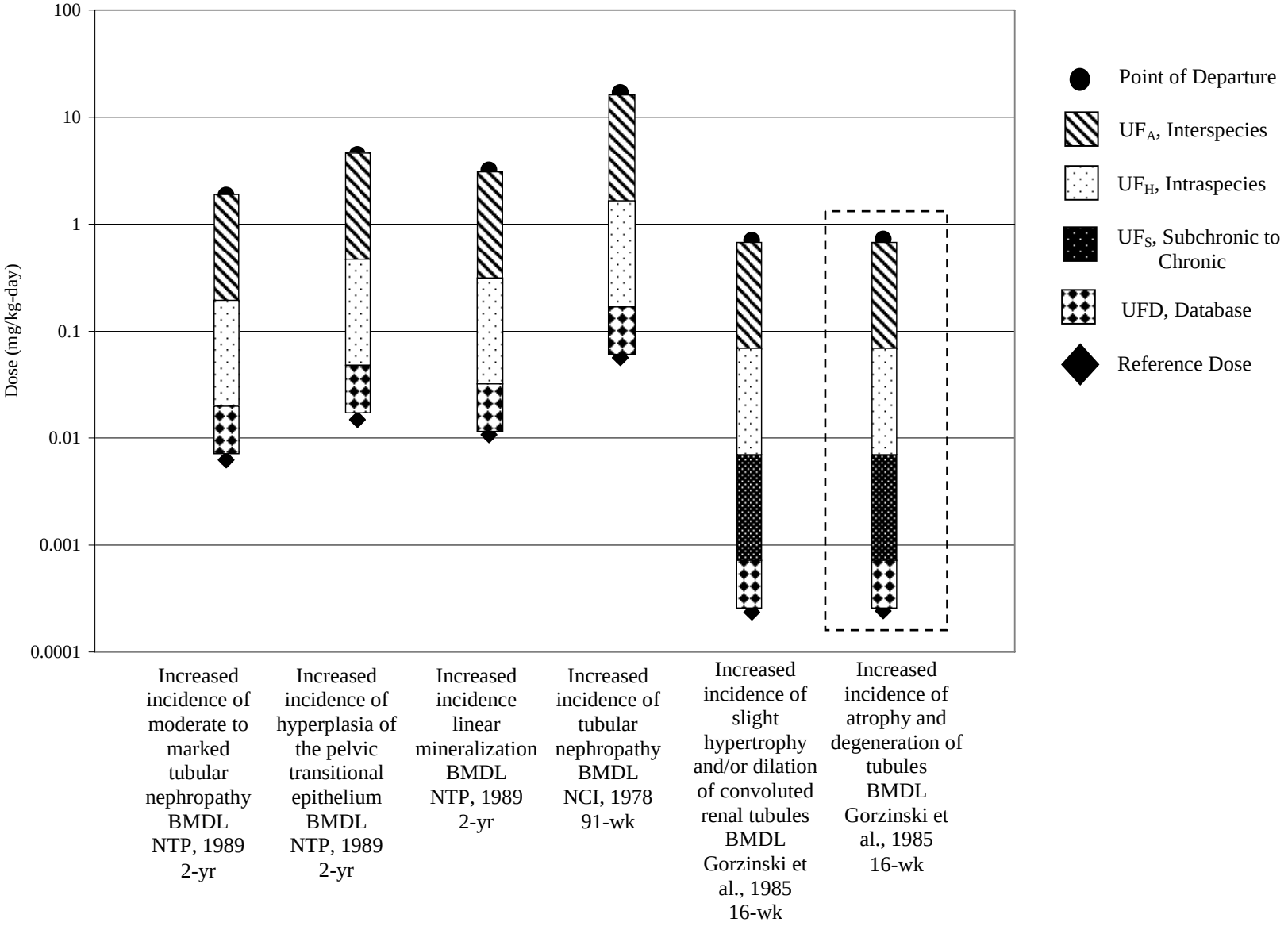
The predominant noncancer effect of acute, short-term, subchronic, and chronic oral exposure to HCE is renal toxicity. Table 5-3 presents the PODs for nephrotoxicity in male rats with applied uncertainty factors (UFs) and potential reference values. Figure 5-1 provides a graphical display of dose-response information from three studies that reported kidney toxicity in male rats following chronic and subchronic oral exposure to HCE, focusing on potential PODs that could be considered in deriving the oral RfD. As discussed in Sections 5.1.1 and 5.1.2, among those studies that demonstrated kidney toxicity, atrophy and degeneration of renal tubules in male F344 rats from the Gorzinski et al. (1985) study provided the POD for deriving the RfD (see dotted box in Figure 5-1). Potential reference values that might be derived from other studies are also presented. Only endpoints observed in male rats are presented because the database for HCE consistently showed that male rats exhibited greater sensitivity to HCE toxicity compared to females.

The nephropathy observed by NCI (1978) was similar to that reported by NTP (1989); however, the animals in the NTP study were exposed to and exhibited effects at a lower range of doses of HCE than those in the NCI study (Table 5-1). NTP (1989) described tubular nephropathy characterized by degeneration, necrosis, and regenerative epithelial cells in rats. Gorzinski et al. (1985) described similar renal effects characterized by atrophy and degeneration of renal tubules and slight hypertrophy and/or dilation of proximal convoluted tubules. Linear mineralization of the renal tubules, hyperplasia of the pelvic transitional epithelium, slight hypertrophy and/or dilation of the proximal convoluted tubules, increased severity of tubular nephropathy, and atrophy and degeneration or renal tubules were all reported in male rats exposed to HCE (NTP, 1989; Gorzinski et al., 1985). Additionally, nephropathy was observed in both male and female rats, whereas linear mineralization was only observed in male rats. Kidney effects were observed in male rats in the Gorzinski et al. (1985) study at doses below the range of exposure tested in the NTP (1989) study. In addition, the ability of the chronic studies to inform the effects observed at the low dose in the Gorzinski et al. (1985) study is limited because the lowest dose tested in the NTP (1989) chronic exposure study represented a LOAEL. The potential POD associated with atrophy and degeneration of renal tubules from the Gorzinski et al. (1985) study was lower than the POD based on increased severity of tubular nephropathy from NTP (1989). Therefore the POD based on atrophy and degeneration of renal tubules from the Gorzinski et al. (1985) study was selected to serve as the basis for the derivation of the RfD.

Table 5-3. Potential points of departure (PODs) for nephrotoxicity in male rats with applied uncertainty factors (UFs) and potential reference values

Potential PODs (mg/kg-day)		Total UF	UF_A	UF_H	UF_S	UF_D	Potential Reference Values (mg/kg-day)	Reference
Tubular nephropathy BMDL 2-year	16.99	300	10	10	1	3	0.0566	NCI (1978)
Hyperplasia of pelvic transitional epithelium BMDL 2-year	4.48	300	10	10	1	3	0.0149	NTP (1989)
Linear mineralization BMDL 2-year	3.22	300	10	10	1	3	0.0107	
Moderate to Marked Tubular Nephropathy BMDL 2-year	1.88	300	10	10	1	3	0.0075	
Slight hypertrophy and/or dilation of proximal convoluted renal tubules BMDL 16 week	0.710	3000	10	10	10	3	0.0002	Gorzinski et al. (1985)
Atrophy and degeneration of renal tubules BMDL 16 week	0.728	3000	10	10	10	3	0.0002	

Figure 5-1. Array of potential points of departure with applied uncertainty factors and potential reference values for nephrotoxic effects of studies in Table 5-3.



5.1.5. Previous RfD Assessment

In the previous RfD assessment for HCE, completed in 1987, the Gorzinski et al. (1985) study was employed in deriving the RfD using the NOAEL/LOAEL approach. In this study, the identified LOAEL for atrophy and degeneration of renal tubules was 15 mg/kg-day, with a corresponding NOAEL of 1 mg/kg-day. A composite UF of 1,000 was employed to account for the following three limitations or uncertainties: (1) interspecies extrapolation ($UF_A = 10$), (2) intraspecies variation ($UF_H = 10$), and (3) subchronic-to-chronic extrapolation ($UF_S = 10$). An RfD of 1×10^{-3} mg/kg-day was derived.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

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

The short-term inhalation toxicity studies on HCE is limited. Human studies demonstrated HCE exposure in smoke bomb production workers, but the sample sizes are too small to reach definitive conclusions regarding health effects and the exposure was likely a mixture of HCE and zinc oxide. There are no chronic studies available, and only a single subchronic inhalation study (in four species) which included a developmental toxicity experiment is available. Weeks et al. (1979) exposed Sprague-Dawley rats, male beagle dogs, male Hartley guinea pigs, and Japanese quail to HCE air concentrations of 145, 465, or 2,517 mg/m^3 for 6 hours/day, 5 days/week, for 6 weeks. Postexposure observations were carried out for 12 weeks.

As discussed in Section 4.4.3.2, toxic effects observed in treated rats, dogs, and guinea pigs (the quail did not show signs of toxicity) were at the highest exposure level, 2,517 mg/m^3, except for dams in of the 465 mg/m^3 exposure group in the developmental study; which exhibited significantly decreased body weight gain and an increased incidence (85%) of mucopurulent nasal exudate. This inflammatory exudate was observed in 100% of the dams treated with 2,517 mg/m^3. Similar to the dams, male and female rats exposed to 2,517 mg/m^3 HCE for 6 weeks exhibited mucopurulent exudate in the nasal turbinates. Excess mucus in the nasal turbinates was also observed in 2/10 quail in the 2,517 mg/m^3 concentration group. Effects of this nature were not observed in the 465 or 145 mg/m^3 rats and quail or in the treated guinea pigs and dogs.

Weeks et al. (1979) concluded that the excess mucus in two of the 2,517 mg/m^3 quails was a transient effect of the HCE exposure because there was no evidence of inflammatory cells or tissue damage. The authors attributed the increased incidence of respiratory lesions in rats to an endemic mycoplasia infection as evidenced by the histopathological observation of an increased incidence and severity of mycoplasia-related lesions in the nasal turbinates (mucopurulent exudate), trachea (lymphoid hyperplasia in the lamina propria), and lung (pneumonitis) of 2,517 mg/m^3 male and female rats. Similar lesions characteristic of respiratory

mycoplasmosis in rodents were detected in an oral developmental study in rats that paralleled the inhalation developmental study described above (both conducted by Weeks et al., 1979). Irritation of the upper respiratory tract was observed in approximately 70% of the pregnant rats (20% diagnosed with subclinical pneumonitis) orally exposed to 500 mg/kg HCE, compared with 10% of controls showing irritation and pneumonitis.

The presence of the infection in the rats in both the oral and inhalation studies and in the controls of the oral study suggests that respiratory tract effects are a potentiation of the underlying mycoplasia infection rather than a direct result of HCE exposure. Additionally, the reduced weight gain in the rats is likely related to the condition of the infected animals, considering mycoplasma-infected rodents generally gain less weight or lose weight compared with noninfected rodents (Xu et al., 2006; Sandstedt et al., 1997). Reduced weight gain was also observed in the 2,517 mg/m^3 guinea pigs, but mycoplasma infection was not reported (Weeks et al., 1979). Like rats and mice, guinea pigs can carry mycoplasma organism; however, they are not clinically affected (Fox et al., 1984; Holmes, 1984). However, no data was presented demonstrating the presence of mycoplasma in the lungs. Therefore, the respiratory tract effects cannot be excluded from consideration as a potential critical effect.

As discussed in Section 4.4.3, neurobehavioral effects were consistently observed in the rats and dogs exposed to 2,517 mg/m^3. The male and female rats in the 6-week study exhibited tremors and ruffled pelt. The pregnant rats developed tremors on GDs 12-16. Similarly, the dams exposed to 500 mg/kg HCE in the concurrent oral developmental study by Weeks et al. (1979) experienced tremors on GDs 15 and 16 of the 11-day exposure period. The HCE-exposed dogs showed tremors, ataxia, and hypersalivation, severe head bobbing, facial muscular fasciculations, and closed eyelids. These effects were noted in the dogs throughout the study, although they disappeared overnight during nonexposure time periods.

Supporting data for the study was reported in an acute study by Weeks and Thomasino (1978), in which a single 8-hour inhalation exposure to 2,500 or 57,000 mg/m^3 HCE and a single 6-hour exposure to 17,000 mg/m^3 HCE in male Sprague-Dawley rats resulted in neurological and lung effects. The male rats exposed to 57,000 mg/m^3 HCE had reduced body weight gain compared with controls over the 14 days postexposure. By 6 hours of exposure, one rat had a staggered gait. Necropsy did not reveal any gross exposure-related lesions, although microscopy revealed that two of these rats had subacute diffuse interstitial pneumonitis of minimal to moderate severity and vascular congestion associated with these lung effects. Following 6 hours of exposure to 17,000 mg/m^3, the six rats in this group showed reduced weight gain compared with controls and two of these rats exhibited a staggered gait. No exposure-related gross or histopathological changes were observed in tissues and organs. These effects were not noticeable 14 days postexposure.

The short-term inhalation study by Weeks et al. (1979), as the only repeated exposure study available, was selected as the principal study for the derivation of the RfC. This study

used three concentrations and incorporated a variety of endpoints (toxicological, teratological, neurological, pulmonary) across a range of species. The primary limitation of Weeks et al. (1979) is the minimal amount of quantitative information provided characterizing the reported effects. Several experiments only utilized one sex, and additional exposure concentration(s) between the mid- and high-concentration would have allowed for better characterization of the exposure-response curve. However, this study identified neurotoxicity, statistically significant decreases in body weight gain, and upper and lower respiratory tract irritation. The responses were generally observed following exposure to the highest concentration, and not in the two lower concentrations. Considering the uncertainty surrounding the body weight changes and respiratory tract irritation in the presence of the mycoplasma infection, these effects were not considered as the critical effect for the derivation of the RfC. Therefore, neurological effects following inhalation exposure to HCE were selected as the critical effect.

Table 5-4. Noncancerous effects observed in animals exposed to HCE via inhalation

Species	Dose/Duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Effect
Sprague-Dawley rats (25/sex/dose)	0, 145, 465, or 2,517 mg/m^3; 6 weeks	465 mg/m^3	2,517 mg/m^3	Males: neurotoxic effects (tremors and ruffled pelt), reduced body weight gain, increased relative, spleen, and testes weights Females: neurotoxic effects (tremors and ruffled pelt), increased relative liver weight
Male beagle dogs (4/dose)Male beagle dogs (4/dose)		465 mg/m^3	2,517 mg/m^3	Tremors, ataxia, hypersalivation, head bobbing, facial muscular fasciculations
Male Hartley guinea pigs (10/dose)Male Hartley guinea pigs (10/dose)		465 mg/m^3	2,517 mg/m^3	Reduced body weight, increased relative liver weight
Coturnix Japonica quail (20/dose)Coturnix Japonica quail (20/dose)		2517 mg/m^3	Not established	No effects observed
Pregnant Sprague-Dawley rats (22/dose)	0, 145, 465, or 2,517 mg/m^3 on GDs 6-16	Maternal: 465 mg/m^3	Maternal: 2,517 mg/m^3	Maternal: tremors Fetal: no effects

Source: Lattanzi et al. (1988).

5.2.2. Methods of Analysis—Including Models

The Weeks et al. (1979) study included three exposure groups (145, 465, and 2,517 mg/m^3) plus a control. Neurological effects were observed in male and female Sprague-Dawley rats, male beagle dogs, and pregnant Sprague-Dawley rats only at the highest dose tested. Incidence data were not reported. Application of BMD modeling was precluded because 100% of the high dose animals displayed neurological effects. Therefore, a NOAEL served as the POD. The NOAEL of 465 mg/m^3, identified in Weeks et al. (1979), was selected as the POD for the derivation of the RfC based on effects in male and female rats and male dogs exposed to HCE for 6 weeks and pregnant rats exposed on GDs 6-16. Although the NOAELs are the same, the male and female rats exposed to HCE for 6 weeks were selected as the study animals upon which to base the POD, as the duration of exposure for the dams in the teratology study was only 11 days and only 4 male dogs were exposed to HCE in the 6-week study.

The NOAEL is based on intermittent HCE inhalation exposures in male and female rats for 6 hours/day, 5 days/week. Thus, prior to deriving the RfC, this POD was adjusted for

continuous exposure (24 hours/day, 7 days/week). The duration-adjusted POD ($POD_{[ADJ]}$) is derived using the following equation (U.S. EPA, 1994b):

$$\begin{aligned} POD_{[ADJ]} &= (POD) \times (\text{hours of exposure/24 hours}) \times (\text{days of exposure/7 days}) \\ &= (465\ mg/m^3) \times (6/24\ \text{hours}) \times (5/7\ \text{days}) \\ &= 83.0\ mg/m^3. \end{aligned}$$

The *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (hereafter referred to as the RfC Methodology) recommends converting the $POD_{[ADJ]}$ to a human equivalent concentration (HEC) (U.S. EPA, 1994b). The RfC Methodology separates gases into three categories based on their water solubility and reactivity with tissues in the respiratory tract. Determining whether HCE is a Category 2 or 3 gas is difficult because data regarding the inhalation effects are very limited. HCE is slightly water soluble and although HCE has been observed in blood following oral exposures to HCE, it is unknown whether HCE accumulates in blood following inhalation exposure. Given this limited information, HCE is likely a Category 2 gas because it is slightly water soluble and causes effects distal to the site of inhalation exposure (i.e., systemic effects). For Category 2 gases, HEC values are calculated using methods for Category 1 gases for portal-of-entry effects and Category 3 methods for systemic effects (U.S. EPA, 1994b). In view of the fact that neurotoxicity is a systemic effect, the methods for Category 3 gases were used to derive the HEC.

The RfC Methodology (U.S. EPA, 1994b) suggests that HECs be estimated by applying to the duration-adjusted exposure level ($POD_{[ADJ]}$), a dosimetric adjustment factor (DAF) that is specific for the breathing characteristic of the species to be compared. The DAF for a Category 3 gas is based on the regional gas dose ratio (RGDR), where the RGDR is the ratio of the animal blood:gas partition coefficient $(H_{b/g})_A$ and the human blood:gas partition coefficient $(H_{b/g})_H$.

$$POD_{[HEC]} = POD_{[ADJ]} \times (H_{b/g})_A/(H_{b/g})_H$$

However, the human and animal blood partition coefficients for HCE are not known. In accordance with the RfC Methodology (U.S. EPA, 1994) when the partition coefficients are unknown a ratio of 1 is used. This results in a $NOAEL_{[HEC]}$ of 83.0 mg/m^3.

$$\begin{aligned} POD_{[HEC]} &= POD_{[ADJ]} \times (H_{b/g})_A/(H_{b/g})_H \\ &= 83.0\ mg/m^3 \times 1 \\ &= 83.0\ mg/m^3 \end{aligned}$$

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

The $NOAEL_{[HEC]}$ value of 83 mg/m^3 for evidence of neurotoxicity in Sprague-Dawley rats was used as the POD to derive the RfC for HCE. A composite UF of 3,000 was applied as follows:

- For animal-to-human interspecies differences (UF_A), an UF of 3 was applied to account for the uncertainty in extrapolating from laboratory animals to humans. This value is adopted by convention, where an adjustment from an animal-specific $NOAEL_{ADJ}$ to a $NOAEL_{HEC}$ has been incorporated. Application of an UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component associated with HCE is mostly addressed by the determination of an HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method and an UF of 3 is retained to account for uncertainty regarding the toxicodynamic differences between rats and humans.

- An intraspecies UF (UF_H) of 10 was applied to account for potentially sensitive human subpopulations in the absence of information on the variability of response to HCE in the human population. Information is currently unavailable to assess human-to-human variability in HCE toxicokinetics and toxicodynamics.

- A subchronic-to-chronic UF (UF_S) of 10 was applied to account for the use of the POD selected following a short-term duration of exposure to HCE to estimate a chronic exposure RfC.

- An UF for a LOAEL to a NOAEL extrapolation was not applied because this assessment utilized a NOAEL as the POD.

- A 10-fold UF was used to account for deficiencies in the toxicity database on inhalation exposure to HCE. There are no available human occupational or epidemiological studies of inhalation exposure to HCE. There are no chronic toxicity or multigeneration reproductive toxicity animal studies available for inhalation exposure to HCE. The toxicity data on inhalation exposure to HCE is very limited and largely restricted to one subchronic (6-week) inhalation study (Weeks et al., 1979) in rats, male dogs, male rabbits, and quail. The same investigators performed a developmental study and an acute study in rats. Maternal toxicity was observed at both doses. Fetuses of HCE-treated dams did not exhibit any significant skeletal or soft tissue anomalies. The toxic effects observed in the dams in the developmental study were similar to those observed in the rats exposed for 6 weeks, although additional effects were observed in the rats exposed

for a longer duration. The absence of teratogenic effects does not abrogate concern given the paucity of the inhalation database for HCE. In addition, the database lacks studies of neurotoxicity and developmental neurotoxicity, endpoints of concern based on the available inhalation data. Therefore, in consideration of the inhalation database for HCE, a database UF of 10 was applied.

Given the UFs established above, the RfC for HCE was calculated employing the following equation:

$$\begin{aligned} \text{RfC} &= \text{NOAEL}_{[\text{HEC}]} \div \text{UF} \\ &= 83\ \text{mg/m}^3 \div 3{,}000 \\ &= 0.028\ \text{mg/m}^3 \text{ or } 3 \times 10^{-2}\ \text{mg/m}^3. \end{aligned}$$

5.2.4. RfC Comparison Information

The predominant noncancer effect of short-term inhalation exposure to HCE is neurotoxicity. The other effects noted by Weeks et al. (1979) at the same dose level were decreases in body weight and increases in organ (liver or kidney) weights in male guinea pigs, male and female rats, and pregnant rats. As discussed in Sections 5.2.1 and 5.2.2, the neurotoxicity reported in the available inhalation study (Weeks et al., 1979) was selected for the RfC derivation because of the potential impact of the mycoplasma infection on the other endpoints. Based on the lack of alternative endpoints to be considered for the basis of the RfC, a graphical display of dose-response information from the short-term inhalation study was not provided. For the reasons discussed above and in Section 5.2.1, neurotoxic effects in male and female rats, pregnant rats, and male dogs reported by Weeks et al. (1979) are considered the most sensitive effects and were selected to serve as the basis for the derivation of the RfC for HCE.

5.2.5. Previous RfC Assessment

An RfC for HCE was not previously developed by the U.S. EPA. In the 1987 IRIS Summary, Weeks et al. (1979) was briefly summarized in the Additional Studies/Comments section for the oral RfD. The IRIS Summary (1987) stated that Weeks et al. (1979) administered HCE to rats by inhalation at 145, 465, or 2520 mg/mg^3, 6 hours/day during gestation. At the two highest doses, maternal toxicity was observed but there was no evidence of fetoxicity or teratogenicity. No additional discussion was presented in the IRIS Summary (1987) describing why this study was not used to develop an RfC.

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

The following discussion identifies uncertainties associated with the quantification of the RfD and RfC for HCE. Following EPA practices and guidance (U.S. EPA, 1994b, 1993), the UF approach was applied to the chosen PODs to derive an RfD and RfC (see sections 5.1.3 and 5.2.3). Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal study to human exposure, a diverse human population of varying susceptibilities, and database deficiencies.

The oral database includes short-term, subchronic, and chronic studies in rats and a chronic study in mice, and developmental studies in rats. Toxicity associated with oral exposure to HCE is predominantly reported as kidney toxicity, specifically, renal tubule nephropathy. The inhalation database includes a short-term study in rats, pregnant rats, male dogs, male guinea pigs, and quail. Toxicity associated with inhalation exposure to HCE in this study is mainly neurotoxicity. Critical data gaps have been identified in Chapter 4 and uncertainties associated with data deficiencies are more fully discussed below.

The RfD was derived from a $BMDL_{10}$ of 0.728 mg/kg-day, which was based on the observation of atrophy and degeneration of renal tubules in male F344 rats from the Gorzinski et al. (1985) 16-week toxicity study. The dose-response relationships for oral exposure to HCE and nephropathy in other studies of rats are also available for deriving an RfD, but are associated with higher NOAELs/LOAELs that are less sensitive than would be protected by the selected critical effect and corresponding POD. After consideration of all potential PODs, the RfD of 2×10^{-4} mg/kg-day was based on the observation of atrophy and degeneration of renal tubules in male F344 rats from the Gorzinski et al. (1985) 16-week toxicity study.

The derived RfD was quantified using a $BMDL_{10}$ for the POD. The selection of the BMD model for the quantitation of the RfD does not lead to significant uncertainty in estimating the POD since benchmark effect levels were within the range of experimental data. However, the selected models do not represent all possible models one might fit, and other models could be selected to yield different results, both higher and lower than those included in this assessment. Uncertainty exists in the selection of the BMR level utilized in the BMD modeling of the critical effect (atrophy and degeneration of renal tubules in male F344 rats) to determine the POD. In the absence of clear information to determine the level of change in atrophy and degeneration of renal tubules in male F344 rats related to a biologically significant change, a benchmark response (BMR) of 10% was selected for the modeling of the increased incidence was selected to represent a minimally biologically significant change.

The RfC was derived from $NOAEL_{[HEC]}$ value of 83 mg/m^3 for evidence of neurotoxicity in Sprague-Dawley rats from a short-term (6 week) inhalation study by Weeks et al. (1979). A POD based on a NOAEL or LOAEL is, in part, a reflection of the particular exposure concentration or dose at which a study was conducted. It lacks characterization of the dose-response curve and for this reason is less informative than a POD obtained from benchmark dose-response modeling. The short-term inhalation study in rats (Weeks et al., 1979) was

selected as the principal study and neurotoxicity was identified as the critical effect. A NOAEL of 465 mg/m^3 was selected to serve as the POD and the basis for derivation of the RfC.

Extrapolating from animals to humans adds further uncertainty. The effect and its magnitude at the concentration in rats are extrapolated to human response. Pharmacokinetic models are useful for examining species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not available for oral exposure to HCE. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans, so the 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfD. For the chronic RfC, a factor of 3 was adopted by convention where an adjustment from an animal specific $NOAEL_{ADJ}$ to a $NOAEL_{HEC}$ has been incorporated. Application of a full UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method and an UF of 3 is retained to account for this component.

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation needs consideration in extrapolating dose from a subset or smaller sized population, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population. In the absence of HCE-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation in the derivation of both the RfD and RfC. Human variation may be larger or smaller; however, HCE-specific data to examine the potential magnitude of over- or under-estimation are unavailable.

Uncertainties associated with data gaps in the HCE database have been identified. Data more fully characterizing potential multigenerational reproductive effects associated with both oral and inhalation HCE exposure are lacking. The oral database includes studies in laboratory animals, including chronic and subchronic dietary exposure studies and two oral developmental toxicity studies. The developmental studies show effects at doses higher than those observed to induce renal toxicity in the subchronic and chronic toxicity studies. Therefore, in consideration of the entire oral database for HCE, a database UF of 3 was considered appropriate to account for the lack of a two-generational reproductive study. There are no available human occupational or epidemiological studies of inhalation exposure to HCE. There are no standard chronic toxicity or multigeneration reproductive toxicity animal studies available for inhalation exposure to HCE. The toxicity data on inhalation exposure to HCE is very limited and largely restricted to one subchronic (6-week) inhalation study (Weeks et al., 1979) in rats, male dogs, male rabbits, and quail. The same investigators performed a developmental study and an acute study in rats. The developmental study in rats did not provide any evidence of teratogenic effects. However, this

data does not abrogate concern given the paucity of the inhalation database for HCE. In addition, the inhalation database lacks studies of developmental neurotoxicity, endpoints of concern based on the available inhalation data (critical effect for the RfC). Therefore, in consideration of the inhalation database for HCE, a database UF of 10 is was applied.

5.4. CANCER ASSESSMENT

There are no available studies on cancer in humans associated with exposure to HCE. NTP (1989) provided evidence of renal adenomas and carcinomas and pheochromocytomas and malignant pheochromocytomas in male F344/N rats in a 2-year cancer bioassay. NCI (1978) provided evidence of hepatocellular carcinomas in male and female $B6C3F_1$ mice in a 91-week cancer bioassay. Additionally, HCE was shown to be a promoter, but not an initiator, in an Osborne-Mendel rat liver foci assay (Milman et al., 1988; Story et al., 1986). Binding of radiolabeled carbon to DNA, RNA, and protein was observed following [^{14}C]HCE administration in both in vitro and in vivo assays in mice and rats (Lattanzi et al., 1988).

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), HCE is "likely to be carcinogenic to humans" based on dose-dependent, statistically significant increases in the incidence of renal adenoma or carcinoma combined in male F344/N rats, statistically significant increases in the incidence of pheochromocytomas/malignant pheochromocytomas combined in male F344/N rats (NTP, 1989), and statistically significant increases in the incidence of hepatocellular carcinomas in male and female $B6C3F_1$ mice (NCI, 1978).

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

Two animal studies were selected for BMD analysis and subsequent quantitative cancer assessment. In the first study, NTP (1989) reported statistically significantly elevated incidences of renal adenomas and carcinomas combined and pheochromocytomas, malignant pheochromocytomas, and complex pheochromocytomas combined in male F344 rats administered HCE via gavage for 2 years. Female rats in this study did not exhibit any HCE-related tumors. In the second study, NCI (1978) reported statistically significantly elevated incidences of hepatocellular carcinomas in both sexes of $B6C3F_1$ mice administered HCE via gavage for 78 weeks. However, male mice in this study demonstrated a dose-response relationship, while female mice did not.

5.4.2. Dose-response Data

The NTP (1989) administered, via gavage, TWA doses of 7 and 14 mg/kg-day HCE to male and female F344/N rats for 103 weeks. No HCE-related tumors were observed in female rats. Renal adenomas and carcinomas combined were observed in 2, 4, and 14% (statistically significant) of male rats administered 0 (controls), 7, and 14 mg/kg-day HCE, respectively. Male rats also exhibited increased incidences of pheochromocytomas and malignant

pheochromocytomas combined; 28, 58 (statistically significant), and 39% in the controls, 7 and 14 mg/kg-day dose groups, respectively (NTP, 1989). The NCI (1978) gavage study administered TWA doses of 0, 360, and 722 mg/kg-day HCE to male and female $B6C3F_1$ mice for 91-weeks. Statistically significant increases in the incidence of hepatocellular carcinomas were observed in 15, 30, and 63% of males and 10, 40, and 31% of females in the control, 360, and 722 mg/kg-day dose groups, respectively

Both NTP (1989) and NCI (1978) are well-designed studies, conducted in both sexes of two species with 50 animals/sex/dose. Each study utilized two dose groups of HCE and an untreated control group, with examination of a wide range of toxicological endpoints in both sexes of the rodents. Tumor incidences were elevated over controls at two sites in rats (NTP, 1989) and at one site in mice (NCI, 1978). Some limitations associated with the NCI (1978) study in mice include changes to the dosing regimen 9 weeks into the study, cyclical dosing periods, and decreased in all study groups for the male mice. Individual animal data were unavailable to perform time-to-tumor modeling or adjust the tumor incidences for survival before BMD modeling. The cancer incidence data are summarized in Table 5-5.

Table 5-5. Summary of incidence data in rodents orally exposed to HCE for use in cancer dose-response assessment

Study	Sex/strain/species	Endpoint	HCE dose (mg/kg-day)	Incidence
NTP (1989)	Male F344 rats	Kidney adenoma or carcinoma	0	1/50 (2%)
			7.1	2/50 (4%)
			14.3	7/50 (14%)[a]
NTP (1989)	Male F344 rats	Pheochromocytomas/ malignant pheochromocytomas	0	14/50 (28%)
			7.1	26/45 (58%)[a]
			14.3	19/49 (39%)
NCI (1978)	Male $B6C3F_1$ mice	Hepatocellular carcinoma	0	3/20 (15%)
			360	15/50 (30%)[a]
			722	31/49 (63%)[a]
NCI (1978)	Female $B6C3F_1$ mice	Hepatocellular carcinoma	0	2/20 (10%)
			360	20/50 (40%)[a]
			722	15/49 (31%)[a]

[a]Denotes statistical significance

5.4.3. Dose Adjustments and Extrapolation Methods

The HCE doses administered to laboratory animals were scaled to human equivalent doses (HEDs) according to EPA guidance (U.S. EPA, 2005a, 1992). More specifically, animal doses were converted to HEDs by assuming that doses in animals and humans are toxicologically equivalent when scaled by body weight raised to the ¾ power, as follows:

$$\frac{Dose(mg/day)_{[animal]}}{BW^{3/4}_{[animal]}} = \frac{Dose(mg/day)_{[human]}}{BW^{3/4}_{[human]}}$$

The body weights for the laboratory animals used in the scaled human dose conversions are the mean body weights reported in the studies for each dose group. The following formula was used for the conversion of oral animal doses to oral HEDs:

$$\text{Scaled human dose (HED)} = \text{animal dose} \times (\text{animal body weight}/\text{human body weight})^{1/4}$$

Therefore, the HCE doses of 7 and 14 mg/kg-day employed by NTP (1989) in rats were converted to HEDs, as follows:

$$\text{Scaled human dose (HED)} = 7 \text{ mg/kg-day} \times (0.483 \text{ kg}/70 \text{ kg})^{1/4}$$
$$= 2.05 \text{ mg/kg-day}$$

$$\text{Scaled human dose (HED)} = 14 \text{ mg/kg-day} \times (0.471 \text{ kg}/70 \text{ kg})^{1/4}$$
$$= 4.10 \text{ mg/kg-day}$$

Similarly, the HCE doses of 360 and 722 mg/kg-day employed by NCI (1978) in mice were converted to HEDs, as follows:

$$\text{Scaled human dose (HED)} = 360 \text{ mg/kg-day} \times (0.033 \text{ kg}/70 \text{ kg})^{1/4}$$
$$= 53.05 \text{ mg/kg-day}$$

$$\text{Scaled human dose (HED)} = 722 \text{ mg/kg-day} \times (0.030 \text{ kg}/70 \text{ kg})^{1/4}$$
$$= 103.88 \text{ mg/kg-day}$$

These scaled human doses are used in the dose-response modeling described below.

The multistage model was the primary model considered for fitting the dose-response data and is given by:

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where:

$P(d)$ = lifetime risk (probability) of cancer at dose d

q_i = parameters estimated in fitting the model, i = 1, …, k

And extra risk is defined as $(P(d) - P(0))/(1-P(0))$.

The multistage model in BMDS (version 2.0) (U.S. EPA, 2008) was used to fit the multistage dose-response model to the incidence data summarized in Table 5-5 using the calculated HEDs in order to derive an oral slope factor for HCE. The BMR selected was the default value of 10% extra risk recommended for dichotomous models (U.S. EPA, 2000b). No data were excluded from the BMD multistage modeling.

As stated above, the multistage model was fit to the incidences of renal adenomas or carcinomas combined in male rats and hepatocellular carcinomas in male and female mice. In all cases, the 2° multistage model provided the best fit. The multistage model was also fit to the incidence of pheochromocytomas or malignant pheochromocytomas in male rats. The model exhibited a significant lack of fit for the pheochromocytomas (according to the χ^2 statistic with $p < 0.01$). Thus, this dataset was not useful for dose-response assessment because the tumor incidence is not a monotonic increasing function of dose, as demonstrated by the Cochran-Armitage Trend Test. Therefore, the BMD modeling results for the kidney and liver tumors in rats and mice, respectively, are summarized in Table 5-6, with more detailed results contained in Appendix B.

Table 5-6. Summary of BMD modeling results for oral cancer assessment of HCE

Study	Sex/strain/species	Endpoint	"Best-fit" Model	*p*-value	AIC	BMR	BMD_{10}	$BMDL_{10}$ or POD	Oralr Slope Factor (mg/kg-day)$^{-1}$
NTP (1989)	Male F344 rats	Renal adenomas/carcinomas combined	2° Multistage	0.75	71.19	0.1	3.73	2.44	0.040984
NCI (1978)	Male $B6C3F_1$ mice	Hepatocellular carcinomas	2° Multistage	0.83	146.47	0.1	37.03	14.44	0.006925
NCI (1978)	Female $B6C3F_1$ mice	Hepatocellular carcinomas	2° Multistage	0.03	149.77	0.1	286.24	136.88	0.000730

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the mode of action of the carcinogen and the shape of the cancer dose-response curve. The linear approach is used as a default option if the mode-of-action of carcinogenicity is not understood (U.S. EPA, 2005a). In the case of HCE, the mode of carcinogenic action of HCE in the kidneys and livers of rats and mice is unknown. There is some data in experimental animals evaluating α_{2u}-globulin accumulation and toxicity in the kidney. As described in Section 4.7.3.1., two principal factors contribute to the conclusion that the available data do not support an α_{2u}-globulin mode of action for the development of renal tumors: (1) the lack of information identifying the α_{2u}-globulin protein in HCE-treated rats, and (2) evidence of nephropathy in female rats as well as male and female mice (because the α_{2u}-

globulin-related mode of action is specific for male rats). Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with HCE exposure.

5.4.4. Oral Slope Factor and Inhalation Unit Risk

The oral slope factor was derived from the $BMDL_{10}$ (the lower bound on the exposure associated with a 10% extra cancer risk) by dividing the BMR by the $BMDL_{10}$ and represents an upper bound on cancer risk associated with a continuous lifetime exposure to HCE. In accordance with the U.S. EPA (2005a) guidelines, an oral slope factor for renal tumors in male rats of 0.04 $(mg/kg\text{-}day)^{-1}$ was calculated by dividing the human equivalent $BMDL_{10}$ of 2.44 mg/kg-day into 0.1 (10%) (Appendix B). An oral slope factor for hepatocellular tumors in male mice of 0.007 $(mg/kg\text{-}day)^{-1}$ was calculated by dividing the human equivalent $BMDL_{10}$ of 14.44 mg/kg-day into 0.1 (10%) (Appendix B). An oral slope factor for hepatocellular tumors in female mice of 0.0007 $(mg/kg\text{-}day)^{-1}$ was calculated by dividing the human equivalent $BMDL_{10}$ of 136.88 mg/kg-day into 0.1 (10%) (Appendix B).The oral slope factors were derived by linear extrapolation to the origin from the POD and represent upper-bound estimates. The rats exhibited greater sensitivity to HCE-induced carcinogenicity than the mice. Thus, the risk estimate associated with the male rats that developed renal adenomas or carcinomas was selected as the oral slope factor of 0.04 $(mg/kg\text{-}day)^{-1}$ for HCE. The slope of the linear extrapolation from the central estimate (i.e., BMD) is 0.1/37.03 mg/kg-day or 3×10^{-3} $(mg/kg\text{-}day)^{-1}$.

In the absence of any suitable data on the carcinogenicity of HCE via the inhalation route, an inhalation unit risk has not been derived in this evaluation.

5.4.5. Uncertainties in Cancer Risk Values

Extrapolation of data from animals to estimate potential cancer risks to human populations from exposure to HCE yields uncertainty. Several types of uncertainty may be considered quantitatively, whereas others can only be addressed qualitatively. Thus, an overall integrated quantitative uncertainty analysis cannot be developed. Major sources of uncertainty in the cancer assessment for HCE are summarized in Section 5.4.5.1 and in Table 5-7.

Table 5-7. Summary of uncertainties in the HCE cancer risk assessment

Consideration/ approach	Impact on oral slope factor[a]	Decision	Justification
Human relevance of male mouse tumor data	Human risk could ↓ or ↑, depending on relative sensitivity; if rodent tumors proved not to be relevant to humans, oral cancer risk estimate would not apply, (i.e., human risk would ↓)	Kidney and adrenal gland tumors in male rats and liver tumors in male and female mice are relevant to human exposure	There are no mode of action data to guide extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across species. HCE is a multi-site carcinogen although direct site concordance is generally not assumed (U.S. EPA, 2005a); consistent with this view, some human tumor types are not found in rodents.
Bioassay	Alternatives could ↑ or ↓ oral slope factor by an unknown extent	NTP study	Alternative bioassays in rats were unavailable. NCI (1978) bioassay in mice was available, although mice were less sensitive than rats to HCE carcinogenicity and were not utilized in estimating carcinogenic risk to humans.
Species/gender choice	Human risk could ↑ or ↓, depending on relative sensitivity	Incidence of renal adenoma/carcinoma in male rats	It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. Increased tumor incidence in mice resulted in lower risk estimate than rats. No increase of kidney tumors in female rats.
Dose metric	Alternatives could ↑ or ↓ oral slope factor by an unknown extent	Used administered exposure	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not identified.
Low-dose extrapolation procedure	Departure from EPA's *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ oral slope factor an unknown extent	Multistage model to determine POD, linear low-dose extrapolation from POD (default approach)	Available mode of action data do not inform selection of dose-response model; linear approach employed in absence of support for an alternative approach.
Cross-species scaling	Alternatives could ↓ or ↑ the oral slope factor (e.g., 3.5-fold ↓ [scaling by body weight] or ↑ 2-fold [scaling by $BW^{2/3}$])	$BW^{3/4}$ (default approach)	There are no data to support alternatives. Because the dose metric was not an AUC, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks.
Statistical uncertainty at POD	↓ oral slope factor 1.5-fold if BMD used as the POD rather than lower bound on POD	BMDL (preferred approach for calculating reasonable upper bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% confidence interval on administered exposure.
Human population variability in metabolism and response/sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity, including whether children are more sensitive.

[a]↑ = increase; ↓ = decrease

5.4.5.1. Sources of Uncertainty

Relevance to humans

The modes of action for the kidney (adenomas/carcinomas) and adrenal gland tumors (pheochromocytomas) in male rats and liver tumors (hepatocellular carcinomas) in male and female mice are unknown. There is some data in experimental animals evaluating α_{2u}-globulin accumulation and toxicity in the kidney. As described in Section 4.7.3, two principal factors contribute to the conclusion that the available data do not support an α_{2u}-globulin mode of action for the development of renal tumors. First, the presence of kidney effects in HCE-exposed male and female mice, which generally do not accumulate the α_{2u}-globulin protein, suggests that a mode of action other than α_{2u}-globulin nephropathy. Second, none of the HCE studies performed the necessary immunohistochemical assays to confirm the presence of α_{2u}-globulin protein within the hyaline droplets observed following administration of HCE (NTP, 1996, 1989). This represents a data gap, as the presence of α_{2u}-globulin is necessary to support an α_{2u}-globulin mode of action.

The relevance of the mode of action of liver tumor induction to humans was considered in Section 4.7.2. There is no evidence in humans for hepatic cancer associated with HCE exposure. The experimental animal literature, however, shows that oral exposure to HCE induces liver tumors in male and female mice. It is possible that the HCE-induced hepatocellular carcinomas in mice occur as a result of the binding of HCE metabolites to liver macromolecules and the generation of free radicals during HCE metabolism, causing key events in the carcinogenic process such as cytotoxicity, inflammation and regenerative cell proliferation. Limited information exists to distinguish the similarities and differences between experimental animals and humans in terms of HCE metabolism or toxicity. However, these potential key events have not been systematically evaluated for HCE.

In humans, pheochromocytomas are rare catecholamine-producing neuroendocrine tumors that are usually benign, but may also present as or develop into a malignancy (Eisenhofer et al., 2004; Lehnert et al., 2004; Edstrom Elder et al., 2003; Goldstein et al., 1999). Hereditary factors in humans have been identified as important in the development of pheochromocytomas (Eisenhofer et al., 2004). Therefore, in the absence of information indicating otherwise, the kidney and adrenal gland tumors in male rats and liver tumors in male and female mice are considered relevant to humans.

Bioassay selection

The study by NTP (1989) was used for the development of an oral slope factor. This study was conducted in both sexes of F344/N rats and used 50 male and 50 female rats per dose group. Test animals were allocated among two dose levels of HCE and an untreated control group. Animals were observed twice daily and examined weekly (for 14 weeks) then monthly

for body weight and monthly for feed consumption. Animals were necropsied and all organs and tissues were examined grossly and microscopically for histopathological lesions for a comprehensive set of toxicological endpoints in both sexes.

Choice of species/gender

The oral slope factor for HCE was quantified using the tumor incidence data for male rats, which were found to be more sensitive than male or female mice to the carcinogenicity of HCE. The oral slope factor calculated from male rats was higher than the slope factors calculated from male and female mice. As there is no information to inform which species or gender of animals would be most applicable to humans, the most sensitive group was selected for the basis of the oral slope factor. Though the mode of action for observed kidney tumors in rodents is unknown, the evidence suggesting the kidney as a target organ of HCE toxicity in both species lends strength to the concern for human carcinogenic potential.

Dose metric

HCE is likely metabolized to PERC and pentachloroethane; however, it is unknown whether a metabolite or some combination of parent compound and metabolites is responsible for the observed toxicity and carcinogenicity of HCE. If the actual carcinogenic moiety(ies) is(are) proportional to administered exposure, then use of administered exposure as the dose metric provides an unbiased estimate of carcinogenicity. On the other hand, if this is not the most relevant dose metric, then the impact on the human equivalent slope factor is unknown; the low-dose cancer risk value may be higher or lower than that estimated, by an unknown amount.

Choice of low-dose extrapolation approach

The mode of action is a key consideration in clarifying how risks should be estimated for low-dose exposure. A linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with HCE exposure, in the absence of information to inform the dose-response at low doses. The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the mode of action for HCE were known is of interest, but data on the mode of action of HCE are limited and the mode of action is not known.

Etiologically different tumor types were not combined across sites prior to modeling, in order to allow for the possibility that different tumor types can have different dose-response relationships because of varying time courses or other underlying mechanisms or factors. The human equivalent oral slope factors estimated from the tumor sites with statistically significant increases ranged from 0.007 to 0.04 per mg/kg-day, a range less than one order of magnitude, with greater risk coming from the male rat kidney data.

Choice of model

All risk assessments involve uncertainty, as study data are extrapolated to make inferences about potential effects in humans from environmental exposure. The largest sources of uncertainty in the HCE cancer risk estimates are interspecies extrapolation and low-dose extrapolation. There are no human data from which to estimate human cancer risk; therefore, the risk estimate must rely on data from studies of rodents exposed to levels greater than would occur from environmental exposures.

Without human cancer data or better mechanistic data, the relevance of the rodent cancer results to humans is uncertain. The occurrence of increased incidences of kidney and adrenal gland tumors in male rats, and liver tumors in male and female mice exposed to HCE from the oral route of exposure suggests that HCE is potentially carcinogenic to humans as well. However, the lack of concordance in tumor sites between the two rodent species makes it more difficult to quantitatively estimate human cancer risk.

Regarding low-dose extrapolation, in the absence of mechanistic data for biologically based low-dose modeling or mechanistic evidence supporting a nonlinear approach (see the discussion at the beginning of Section 5.4.3), a linear low-dose extrapolation was carried out from the $BMDL_{10}$. It is expected that this approach provides an upper bound on low-dose cancer risk for humans. The true low-dose risks cannot be known without additional data.

With respect to uncertainties in the dose-response modeling, the two-step approach of modeling only in the observable range (U.S. EPA, 2005a) and extrapolating from a POD in the observable range is designed in part to minimize model dependence. Furthermore, the multistage model used provided an adequate fit to all the datasets. The ratios of the BMD_{10} values to the $BMDL_{10}$ values give some indication of the uncertainties in the dose-response modeling. These ratios did not exceed a value of 2.6, indicating that the estimated risk is not influenced by any unusual variability relative to other assessments.

Cross-species scaling

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

Human population variability

The extent of inter-individual variability or sensitivity to the potential carcinogenicity of HCE is unknown. There are no data exploring whether there is differential sensitivity to HCE carcinogenicity across life stages. In addition, neither the extent of interindividual variability in HCE metabolism nor human variability in response to HCE has been characterized. Factors that could contribute to a range of human response to HCE include variations in CYP450 levels

because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of HCE or antioxidant protection systems. This lack of understanding about potential susceptibility differences across exposed human populations thus represents a source of uncertainty. Humans are expected to be more heterogenous than laboratory animals, and this variability is likely to be influenced by ongoing or background exposures, diseases, and biological processes.

5.4.6. Previous Cancer Assessment

The previous HCE cancer assessment by U.S. EPA reported in IRIS was last revised in 1994. The quantitative cancer assessment was based on the incidence of hepatocellular carcinomas in male mice in the NCI (1978) study. The current risk value is derived from the incidence of renal adenomas or carcinomas in male rats (NTP, 1989), resulting in an approximately 2.8-fold higher than the oral slope factor derived in the previous assessment using BMD modeling based the hepatocellular carcinomas in male mice in the NCI (1978) study.

In addition, the scaled human doses were calculated using a slightly different formula than is current practice:

$$\text{Scaled human dose} = \text{animal dose} \times (\text{animal weight}/\text{human body weight})^{1/3} \times (546/637)$$

The difference in the animal-to-human dose scaling procedure is due to the fact that current practice bases dose equivalence on the ¾ power of body weight instead of the previous ⅔ power of body weight.

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

6.1. HUMAN HAZARD POTENTIAL

HCE is a halogenated hydrocarbon consisting of six chlorines attached to an ethane backbone. HCE was produced in the U.S. from 1921 to 1967, but is currently not commercially distributed. HCE is primarily used in the military for smoke pots, smoke grenades, and pyrotechnic devices. In the past, HCE was used as antihelminthic for the treatment of sheep flukes but is no longer used for this purpose since the Food and Drug Administration withdrew approval for this use in 1971. HCE has also been used as a polymer additive, moth repellant, plasticizer for cellulose esters, insecticide solvent, and in metallurgy for refining aluminum alloys.

There is limited information on the toxicity of HCE in humans. Current understanding of HCE toxicology is based on the limited database of animal studies. After absorption by oral exposure, HCE is primarily distributed to fat tissue. Toxicokinetic studies in animals indicated that HCE is also localized and metabolized in the liver and kidney. Kidney concentrations of HCE were higher in male rats than female rats (Gorzinski et al., 1985; Nolan and Karbowski, 1978). Studies of HCE metabolism indicated that the major CYP450 enzymes involved are phenobarbital-inducible, which include the 2A, 2B, and 3A subfamilies (Salmon et al., 1985, 1981; Town and Leibman, 1984; Nastainczyk et al., 1982, 1981). HCE is putatively metabolized via a pentachloroethyl free radical to PERC and pentachloroethane. Pentachloroethane is then metabolized to TCE. TCE and PERC are further metabolized by hepatic oxidation to several urinary metabolites including TCA, trichloroethanol, oxalic acid, dichloroethanol, dichloroacetic acid, and monochloroacetic acid (Mitoma et al., 1985; Nastainczyk et al., 1982, 1981; Bonse and Henschler, 1976; Fowler, 1969; Jondorf et al., 1957). Metabolism is minimal based on the few studies that provided quantitative data on metabolites. However, several of these metabolites have demonstrated liver and kidney toxicities similar to HCE.

The kidney has consistently been shown as the target for toxicity in acute, subchronic, and chronic toxicity bioassays in animals (NTP, 1996, 1989; Gorzinski et al., 1985; NCI, 1978). Noncancer effects include kidney degeneration (tubular nephropathy, necrosis of renal tubular epithelium, hyaline droplet formation, tubular regeneration and tubular casts) and hepatocellular necrosis. Hepatotoxicity was noted in animals exposed to HCE, although endpoints of this nature have not been evaluated in laboratory animals as fully as the renal effects. Hepatocellular necrosis was reported in female rats (NTP, 1989), but was not evaluated in a chronic exposure study of mice (NCI, 1978). The mouse study (NCI, 1978) focused on tumorigenic endpoints and not noncancer effects.

There is no information available describing the metabolism of HCE following exposure via inhalation. The inhalation database for HCE contains one acute (Weeks and Thomasino,

1978) and one short-term (Weeks et al., 1979) study. Neurological effects, such as tremors and ataxia, were observed in male beagle dogs, male and female rats, and pregnant rats. Other effects included reduced body weight gain and increased relative liver weight in rats and guinea pigs exposed to HCE via inhalation. Male rats also displayed increased relative spleen and testes weights.

Cancer effects observed in animal studies include hepatocellular carcinomas in mice and renal adenomas or carcinomas and pheochromocytomas in rats. Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), HCE is "likely to be carcinogenic to humans" because HCE induced kidney and adrenal gland tumors in male rats and liver tumors in male and female mice. Studies evaluating the carcinogenicity in humans exposed to HCE are unavailable. The carcinogenicity incidence data in male rats (NTP, 1989) were used to develop quantitative cancer risk assessments for HCE. The consistency of the kidney and liver as target organs in different species for HCE distribution and metabolism, and both noncancer and cancer endpoints, provides support for the evaluation of these endpoints as relevant to humans.

6.2. DOSE RESPONSE

6.2.1. Oral Noncancer

Subchronic and chronic bioassays in rats and mice have identified the following endpoints after exposure to HCE: tubular nephropathy, atrophy and degeneration of renal tubules, and hepatocellular necrosis. In female rats, tubular nephropathy, atrophy and degeneration of the renal tubules, and hepatocellular necrosis were observed in a statistically significant dose-response manner (NTP, 1989; Gorzinski et al., 1985; NCI, 1978). Tubular nephropathy, severity of nephropathy, and atrophy and degeneration of the renal tubules in male rats demonstrated a statistically significant dose response. Although mice were evaluated in a chronic exposure study (NCI, 1978), noncancer effects were not reported because this study was focused on tumorigenic endpoints.

The most sensitive endpoint identified for effects of HCE by oral exposure relate to kidney toxicity in the 16-week feeding study by Gorzinski et al. (1985) in male rats. Gorzinski et al. (1985) was selected as the principal study and atrophy and degeneration of renal tubules in male rats were chosen as the critical effect for the derivation of the oral RfD. This study included both sexes of F344 rats, 10 animals/sex/dose, and three dose groups plus controls (0, 1, 15, and 62 mg/kg-day). Dose-response analyses of the noncancer endpoint, atrophy and degeneration of renal tubules (Gorzinski et al., 1985), using EPA's BMDS, resulted in a POD of 0.728 mg/kg-day. A composite UF of 3,000 was applied to the POD to derive an oral RfD of 2×10^{-4} mg/kg-day.

Confidence in the principal study, Gorzinski et al. (1985) is high. The 16-week study is a well-conducted study that used three dose groups plus a control. NTP (1989) also conducted 16-day, 13-week, and 103-week studies that supported the results observed in the 16-week study.

Application of BMD modeling provided a POD upon which to base the derivation of the RfD. The critical effect on which the RfD is based is well-supported by other oral short-term, subchronic, and chronic studies. Confidence in the database is low to medium because the database includes acute, short-term, subchronic, and chronic toxicity studies and developmental toxicity studies in rats and chronic carcinogenicity bioassays in rats and mice. The database lacks a multigenerational reproductive study and studies in other species. Overall confidence in the RfD is low to medium.

6.2.2. Inhalation Noncancer

The inhalation toxicity database is limited to a single 6-week repeat-exposure study by Weeks et al. (1979). This study reported a NOAEL of 465 mg/m^3 and a LOAEL of 2,517 mg/m^3 in several species including Sprague-Dawley rats, male beagle dogs, and male Hartley guinea pigs. The effects described in this report include neurotoxicity, reduced body weight gain, and increased relative liver, spleen and testes weights. Based on neurological effects in Sprague-Dawley rats, the NOAEL of 465 mg/m^3 was selected to serve as the POD. Adjustments for continuous exposure and for the HEC, resulted in the $POD_{[HEC]}$ of 83 mg/m^3. An UF of 3,000 was applied to derive an inhalation RfC of 3×10^{-2} mg/m^3. Confidence in this toxicity value is low because of the short duration of the underlying study and the lack of support from other inhalation studies.

Confidence in the principal study, Weeks et al. (1979), is low. The 6-week study was conducted in several species (including male dogs, male and female rats, male guinea pigs, and quail). The study used three exposure groups (145, 465, and 2,517 mg/m^3) plus a control. The study is limited by the relatively short exposure duration (6 weeks) and minimal reporting of effects, especially quantitative changes. Application of BMD modeling was precluded based on a 100% response in animals for the neurological effects and the lack of quantitative information. Therefore, a NOAEL served as the POD. The critical effect on which the RfD is based is supported by the oral short-term study conducted by the same investigators and two subchronic studies. Confidence in the database is low because the database includes one acute and one short-term toxicity study in multiple species and one developmental toxicity study in rats. The database lacks studies by another laboratory and a multigenerational reproductive study. Overall confidence in the RfC is low.

6.2.3. Cancer

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), HCE is "likely to be carcinogenic to humans" by all routes of exposure. This descriptor is based on evidence of carcinogenicity from animal studies. HCE induced statistically significant increases in the incidence of kidney and adrenal gland tumors in male rats and liver tumors in male and female mice. The NTP (1989) rat study was selected for dose-response assessment based on

statistically significant increased incidences of renal adenomas and carcinomas and adrenal pheochromocytomas and malignant pheochromocytomas in male rats. This study was used for development of an oral slope factor. This was a well-designed study, conducted in both sexes of F344 rats with 50 rats/sex/dose; typical of carcinogenicity bioassays. Test animals were allocated among two dose levels (7 and 14 mg/kg-day) and an untreated control group. Animals were observed twice daily and examined weekly (for 14 weeks) then monthly for body weight and monthly for feed consumption. Animals were necropsied and all organs and tissues were examined grossly and microscopically for histopathological lesions for a comprehensive set of toxicological endpoints in both sexes. Renal adenomas and carcinomas and pheochromocytomas and malignant pheochromocytomas observed in male rats (NTP, 1989) were not seen in female rats or other species orally-exposed to HCE. Hepatocellular carcinomas were observed in male and female mice, but not in the rats. The male $B6C3F_1$ mice tumor incidence data (NCI, 1978) demonstrated evidence of carcinogenicity and a low-dose quantitative risk estimate was derived. The cancer risk associated with mice exposed to HCE was less sensitive than that of rats. Thus, the oral slope factor derived for HCE is based on the increased incidence of kidney tumors in male rats.

A linear approach was applied in the dose-response assessment for HCE, in which the mode of action is unknown, consistent with U.S. EPA's (2005a) *Guidelines for Carcinogen Risk Assessment*. The guidelines recommend the use of a linear extrapolation as a default approach when the available data are insufficient to establish a mode of action for a tumor site. As discussed in Section 4.7, the mechanism leading to the formation of the kidney and adrenal tumors in rats and the liver tumors in mice following oral exposure to HCE is unknown. The database for HCE lacks information on the mode of action and the shape of the curve in the region below the POD; therefore, a linear extrapolation was performed in determining the oral slope factor in the derivation of a quantitative estimate of cancer risk for ingested HCE.

Increased incidence of renal adenomas and carcinomas in a 2-year rat bioassay (NTP, 1989) served as the basis for the oral cancer dose-response analysis. A multistage model using linear extrapolation from the POD was performed to derive an oral slope factor of $4 \times 10^{-2}(\text{mg/kg-day})^{-1}$ for HCE. Extrapolation of the experimental data to estimate potential cancer risk in human populations introduces uncertainty in the risk estimation for HCE. Uncertainty can be considered quantitatively; however, some uncertainty can only be addressed qualitatively. For this reason, an overall integrated quantitative uncertainty analysis cannot be developed. However, EPA's development of the cancer quantitative assessment for HCE included consideration of potential areas of uncertainty. The following summarizes these considerations.

A biologically-based model was not supported by the available data; therefore, a multistage model was the preferred model. The multistage model can accommodate a wide variety of dose-response shapes and provides consistency with previous quantitative dose-

response assessments for cancer. Linear low-dose extrapolation from a POD determined by an empirical fit of tumor data has been judged to lead to plausible upper bound risk estimates at low doses for several reasons. However, it is unknown how well this model or the linear low-dose extrapolation predicts low dose risks for HCE. An adjustment for cross-species scaling ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between rats and humans based on the assumption that equal risks result from equivalent constant lifetime exposures.

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

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

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APPENDIX B: Benchmark Dose Modeling Output

Table B-1. Dose-response modeling results using BMDS (Version 2.0) based on non-cancerous kidney and liver effects in rats following oral exposure to HCE

Study	Endpoint	Sex/species	Fitted model[a]	*p*-Value	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Kidney effects							
NCI (1978)	Tubular nephropathy	Male rat	**Gamma**	**0.93**	**133.68**	**21.22**	**16.99**
			Multistage 1°	**0.93**	**133.66**	**21.25**	**17.01**
			Weibull	**0.93**	**133.68**	**21.22**	**16.99**
		Female rat	Gamma	1.00	117.47	87.24	50.63
			Multistage 2°	**0.94**	**116.09**	**80.63**	**41.89**
			Logistic	0.42	118.61	95.19	73.25
			Probit	0.53	118.14	91.25	69.20
			Weibull	1.00	117.47	84.22	48.62
NTP (1989)	Moderate to marked Tubular nephropathy	Male rat	Logistic	0.99	205.88	3.84	2.62
			Multistage 1°	**0.87**	**205.90**	**3.20**	**1.88**
			Probit	0.99	205.88	3.81	2.60
			Quantal-linear	**0.87**	**205.90**	**3.20**	**1.88**
	Mild to marked Tubular nephropathy	Female rat	**Gamma**	**0.86**	**191.90**	**15.17**	**10.72**
			Logistic	0.46	192.42	23.06	18.33
			Multistage 1°	0.78	192.96	15.91	11.14
			Probit	0.47	192.40	22.55	18.04
			Quantal-linear	**0.86**	**191.90**	**15.17**	**10.72**
			Weibull	**0.86**	**191.90**	**15.17**	**10.72**
NTP (1989)	Linear mineralization	Male rat	Logistic	0.36	148.11	4.30	3.45
			Multistage 1°	0.20	148.90	1.75	1.40
			Probit	**0.51**	**147.66**	**3.98**	**3.22**
NTP (1989)	Hyperplasia of the pelvic transitional epithelium	Male rat	Gamma	0.42	84.64	7.33	4.87
			Logistic	0.03	90.96	11.41	8.77
			LogLogistic	**0.48**	**84.42**	**7.05**	**4.48**
			LogProbit	0.07	87.89	8.38	6.51
			Multistage 2°	0.42	84.64	7.33	4.87
			Probit	0.03	90.53	10.86	8.26
			Weibull	0.42	84.64	7.33	4.87
			Quantal-linear	0.42	84.64	7.33	4.87
Gorzinski et al. (1985)	Atrophy and degeneration of renal tubules	Male rat	**Gamma**	**0.70**	**34.94**	**1.34**	**0.728**
			Multistage 1°	**0.93**	**32.94**	**1.34**	**0.728**
			Logistic	0.89	32.97	3.30	1.98
			Probit	0.89	32.95	3.08	1.95
			Quantal-linear	**0.93**	**32.94**	**1.34**	**0.728**
			Weibull	0.69	34.92	1.72	0.729
		Female rat	Gamma	0.99	42.47	13.80	4.56
			Multistage 1°	**0.93**	**40.61**	**8.54**	**4.49**
			Logistic	0.98	40.51	17.40	11.07
			Probit	0.99	40.49	16.10	10.51
			Quantal-linear	**0.93**	**40.61**	**8.54**	**4.49**
			Weibull	0.98	42.47	13.71	4.56
Gorzinski et al. (1985)	Slight hypertrophy and/or dilation of proximal convoluted tubules	Male rat	**Gamma**	**0.99**	**20.88**	**1.22**	**0.710**
			Logistic	0.66	23.91	4.85	2.71
			LogLogistic	0.68	23.89	1.23	0.308
			LogProbit	0.54	24.26	2.11	1.01
			Multistage 2°	0.94	22.84	1.33	0.713
			Probit	0.67	23.85	4.28	2.54
			Weibull	**0.99**	**20.88**	**1.22**	**0.710**
			Quantal-linear	**0.99**	**20.88**	**1.22**	**0.710**
Liver effects							
NTP (1989)	Hepatocellular necrosis	Female rat	**Gamma**	**0.93**	**38.62**	**118.04**	**60.18**
			Multistage 1°	0.68	40.56	53.82	35.19
			Logistic	0.55	41.58	156.22	107.49
			Probit	0.61	40.95	148.49	102.71
			Weibull	0.91	38.91	114.68	56.75

[a]For all models, a BMR of 0.1 was employed in deriving the estimates of the benchmark dose (BMD_{10}) and its 95% lower confidence limit ($BMDL_{10}$). Modeling output is provided for models that represent the POD for each of the kidney endpoints and these models are highlighted in bold font.

Based on the incidence of tubular nephropathy in male rats (NCI, 1978), the logistic and probit models exhibited significant lack-of-fit ($p < 0.1$), while the gamma, multistage (1°) and Weibull models had p-values > 0.1. All three of these models that showed adequate fit yielded the same AIC values, as well as nearly equivalent BMD_{10} and $BMDL_{10}$ estimates of 21.22 and 16.99 mg/kg-day, respectively. Therefore, the potential POD selected for this dataset is 16.99 mg/kg-day.

Based on the incidence of tubular nephropathy in female rats (NCI, 1978), only the 1° multistage model exhibited significant lack-of-fit. Of the models that did not show significant lack-of-fit (i.e., gamma, multistage 2°, logistic, probit and Weibull models), the $BMDL_{10}$ estimates were within a factor of three of each other suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. Therefore, the multistage 2° model $BMDL_{10}$ of 41.89 mg/kg-day was selected as the potential POD for this dataset.

In fitting the available dichotomous dose-response models to the incidence of moderate to marked tubular nephropathy in male rats (NTP, 1989), the gamma and Weibull models exhibited significant lack-of-fit ($p < 0.1$). The models that did not show significant lack-of-fit (i.e., logistic, multistage 1°, quantal-linear, and probit) yielded $BMDL_{10}$ estimates that were within a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. The AIC values were identical; therefore, the model with the lowest $BMDL_{10}$ was selected. The multistage 1° and quantal-linear model had identical $BMDL_{10}$ values, therefore the $BMDL_{10}$ of 1.88 mg/kg-day was selected as the potential POD for this dataset.

Based on the incidence of mild to marked tubular nephropathy in female rats (NTP, 1989), none of the models exhibited significant lack-of-fit. These models (i.e., gamma, logistic, multistage 1°, probit, quantal-linear, and Weibull models) yielded $BMDL_{10}$ estimates that were within a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. The gamma, quantal-linear, and Weibull models had identical AIC values; therefore, the model with the lowest $BMDL_{10}$ was selected. The $BMDL_{10}$ values for these models were identical, therefore the $BMDL_{10}$ of 10.72 mg/kg-day was selected as the potential POD for this dataset.

In fitting the available dichotomous dose-response models to the incidence of linear mineralization in male rats (NTP, 1989), the gamma and the Weibull models exhibited significant lack-of-fit ($p < 0.1$). Of the models that did not show significant lack-of-fit (i.e., logistic, multistage 1°, and probit), the resulting $BMDL_{10}$ estimates were within a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. Therefore the probit model $BMDL_{10}$ of 3.2 mg/kg-day was selected as the potential POD for this dataset.

In fitting the available dichotomous dose-response models to the incidence of hyperplasia of the pelvic transitional epithelium in male rats (NTP, 1989), the logistic, logprobit and probit models exhibited significant lack-of-fit ($p < 0.1$). Of the models that did not show significant lack-of-fit (i.e., gamma, loglogistic, multistage 2°, Weibull, and quantal-linear), the resulting $BMDL_{10}$ estimates were within a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. Therefore the loglogistic model $BMDL_{10}$ of 4.48 mg/kg-day was selected as the potential POD for this dataset.

In fitting the available dichotomous dose-response models to the incidence of atrophy and degeneration of renal tubules in male and female rats (Gorzinski et al., 1985), none of the models exhibited a significant lack-of-fit in either sex. For male rats, these models (i.e., gamma, multistage 1°, logistic, probit, quantal-linear, and Weibull) yielded $BMDL_{10}$ estimates that were within a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. The AIC values for the gamma, multistage 1°, and quantal-linear were identical; therefore, the model with the lowest $BMDL_{10}$ was selected. All of the $BMDL_{10}$s were identical for these models; therefore, the $BMDL_{10}$ of 0.728 mg/kg-day was selected as the potential POD for this dataset.

For female rats, these models (i.e., gamma, multistage 1°, logistic, probit, quantal-linear, and Weibull) yielded $BMDL_{10}$ estimates that were within a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. The AIC values for the multistage 1° and quantal-linear models were identical; therefore, the model with the lowest $BMDL_{10}$ was selected. The multistage 1° $BMDL_{10}$ of 4.49 mg/kg-day was selected as the potential POD for this dataset.

In fitting the available dichotomous dose-response models to the incidence of slight hypertrophy and/or dilation of proximal convoluted tubules in male rats (Gorzinski et al., 1985), none of the models exhibited a significant lack-of-fit. For male rats, these models (i.e., gamma, logistic, loglogistic, logprobit, multistage 2°, probit, Weibull, and quantal-linear) yielded $BMDL_{10}$ estimates that were within a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. The AIC values for the gamma, Weibull, and quantal-linear models were identical; therefore, the model with the lowest $BMDL_{10}$ was selected. All of the $BMDL_{10}$s were identical for these models, therefore the $BMDL_{10}$ of 0.710 mg/kg-day was selected as the potential POD for this dataset.

Based on the incidence of hepatocellular necrosis in female rats (NTP, 1989), none of the dichotomous dose-response models exhibited a significant lack-of-fit. All of these models (i.e., gamma, multistage 1°, logistic, probit, and Weibull) yielded $BMDL_{10}$ estimates that were within

a factor of three of each other, suggesting no appreciable model dependence. As the $BMDL_{10}$ values did not show large variation, the model with the lowest AIC value was selected. Therefore, the gamma model $BMDL_{10}$ of 60.18 mg/kg-day was selected as the potential POD for this dataset.

For comparison purposes, BMD modeling for the above endpoints was also conducted using BMRs of 5 and 1%. The modeling results are included in Table B-2.

Table B-2. Dose-response modeling results using BMDS (Version 2.0) for BMRs of 1, 5, and 10% based on non-cancerous kidney and liver effects in rats following oral exposure to HCE

Study	Endpoint	Sex/ species	Fitted model[a]	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	BMD_{05} (mg/kg-day)	$BMDL_{05}$ (mg/kg-day)	BMD_{01} (mg/kg-day)	$BMDL_{01}$ (mg/kg-day)
Kidney effects									
NCI (1978)	Tubular nephropathy	Male rat	Gamma	21.22	16.99	10.33	8.27	2.02	1.62
			Multistage 1°	21.25	17.01	10.35	8.28	2.03	1.62
			Weibull	21.22	16.99	10.33	8.27	2.02	1.62
		Female rat	Multistage 2°	80.63	41.89	56.26	21.18	24.90	4.28
NTP (1989)	Moderate to marked tubular nephropathy	Male rat	Multistage 1°	3.20	1.88	1.56	0.91	0.30	0.18
			Quantal-linear	3.20	1.88	1.56	0.91	0.30	0.18
	Mild to marked tubular nephropathy	Female rat	Gamma	15.17	10.72	7.39	5.22	1.45	1.02
			Quantal-linear	15.17	10.72	7.39	5.22	1.45	1.02
			Weibull	15.17	10.72	7.39	5.22	1.45	1.02
NTP (1989)	Linear mineralization	Male rat	Probit	3.98	3.22	2.36	1.80	0.58	0.40
NTP (1989)	Hyperplasia of the pelvic transitional epithelium	Male rat	LogLogistic	7.05	4.48	3.34	2.12	0.64	0.41
Gorzinski et al. (1985)	Atrophy and degeneration of renal tubules	Male rat	Gamma	1.34	0.73	0.66	0.35	0.13	0.07
			Multistage 1°	1.34	0.73	0.65	0.35	0.13	0.07
			Quantal-linear	1.34	0.73	0.65	0.35	0.13	0.07
		Female rat	Multistage 1°	8.54	4.49	4.16	2.19	0.82	0.43
			Quantal-linear	8.54	4.49	4.16	2.19	0.82	0.43

Study	Endpoint	Sex/ species	Fitted model[a]	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	BMD_{05} (mg/kg-day)	$BMDL_{05}$ (mg/kg-day)	BMD_{01} (mg/kg-day)	$BMDL_{01}$ (mg/kg-day)
Gorzinski et al. (1985)	Slight hypertrophy and/or dilation of proximal convoluted tubules	Male rat	Gamma	1.22	0.71	0.60	0.35	0.12	0.07
			Weibull	1.22	0.71	0.60	0.35	0.12	0.07
			Quantal-linear	1.22	0.71	0.60	0.35	0.12	0.07
Liver effects									
NTP (1989)	Hepatocellular necrosis	Female rat	Gamma	118.03	60.18	84.66	33.34	41.75	8.60

NCI (1978) Tubular Nephropathy in Male Rats
Gamma Model

```
 ================================================================
        Gamma Model. (Version: 2.13;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpCDF.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpCDF.plt
                                            Thu Apr 09 14:55:06 2009
 ================================================================

 BMDS Model Run NCI 1978 Tubular Nephropathy Male Rat - Gamma Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy
   Power parameter is restricted as power >=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



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0238095
                          Slope =   0.00474439
                          Power =      1.01848


           Asymptotic Correlation Matrix of Parameter Estimates

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

                  Slope

     Slope            1



                         Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0               NA
          Slope       0.00496352      0.000693669          0.00360396
0.00632309
          Power                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	-65.7706	3			
Fitted model	-65.8419	1	0.142715	2	0.9311
Reduced model	-82.1514	1	32.7616	2	<.0001

AIC: 133.684

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20	0.000
113.0000	0.4293	21.035	22.050	49	0.293
227.0000	0.6759	33.795	33.000	50	-0.240

Chi^2 = 0.14 d.f. = 2 P-value = 0.9308

Benchmark Dose Computation

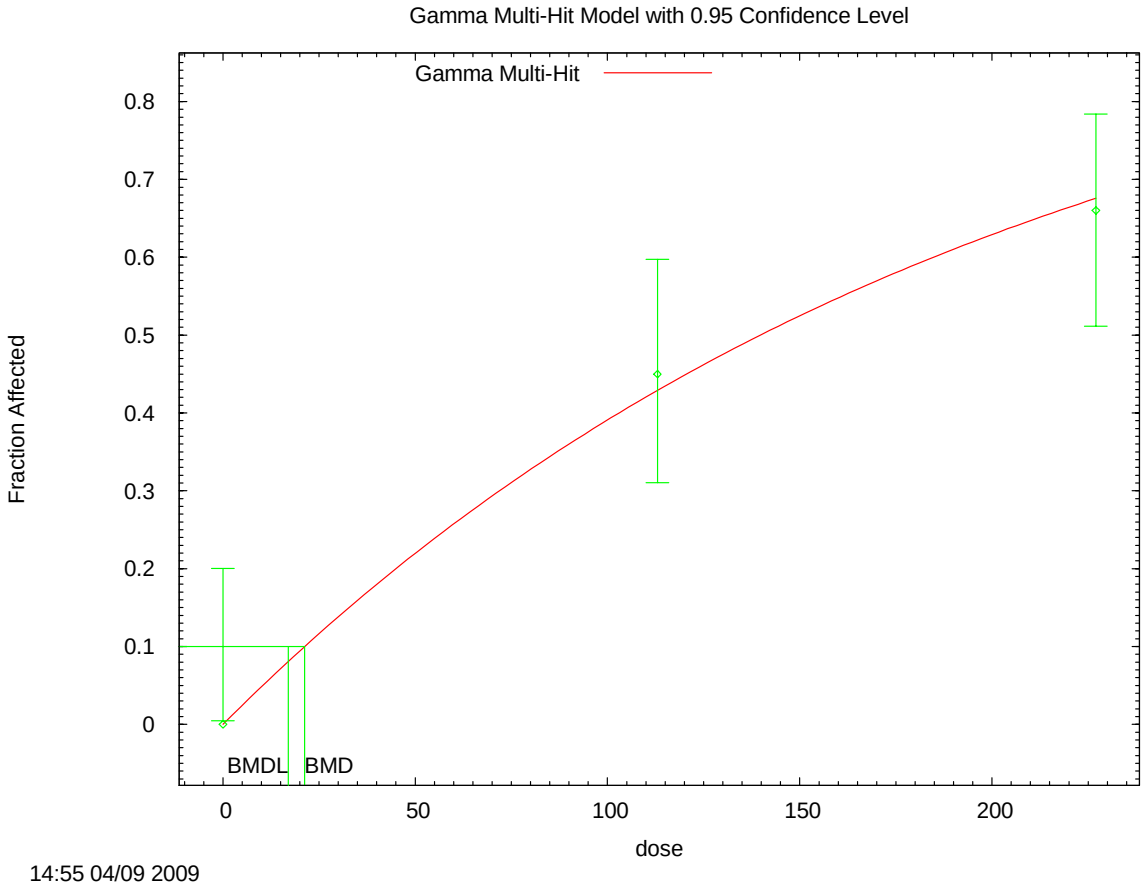
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 21.227

BMDL = 16.9904


Gamma Multi-Hit Model with 0.95 Confidence Level
Gamma Multi-Hit
Fraction Affected
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
50
100
150
200
dose
14:55 04/09 2009

Multistage 1°

```
================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\UNSAVED1.(d)
         Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                Thu Sep 14 09:09:29 2006
 ================================================================

 BMDS Model Run NCI 1978 Tubular Nephropathy Male Rat - Multistage 1 degree Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy

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


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



                  Default Initial Parameter Values
                     Background =    0.0201528
                        Beta(1) =   0.00475168


           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 )

                Beta(1)

   Beta(1)            1



                         Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0            *                *                   *
        Beta(1)       0.00495719            *                *                   *

* - Indicates that this value is not calculated.
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-65.7706	3			
Fitted model	-65.8277	1	0.114158	2	0.9445
Reduced model	-82.1514	1	32.7616	2	<.0001

AIC: 133.655

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20	0.000
113.0000	0.4289	21.015	22.050	49	0.299
227.0000	0.6754	33.772	33.000	50	-0.233

Chi^2 = 0.14 d.f. = 2 P-value = 0.9307

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

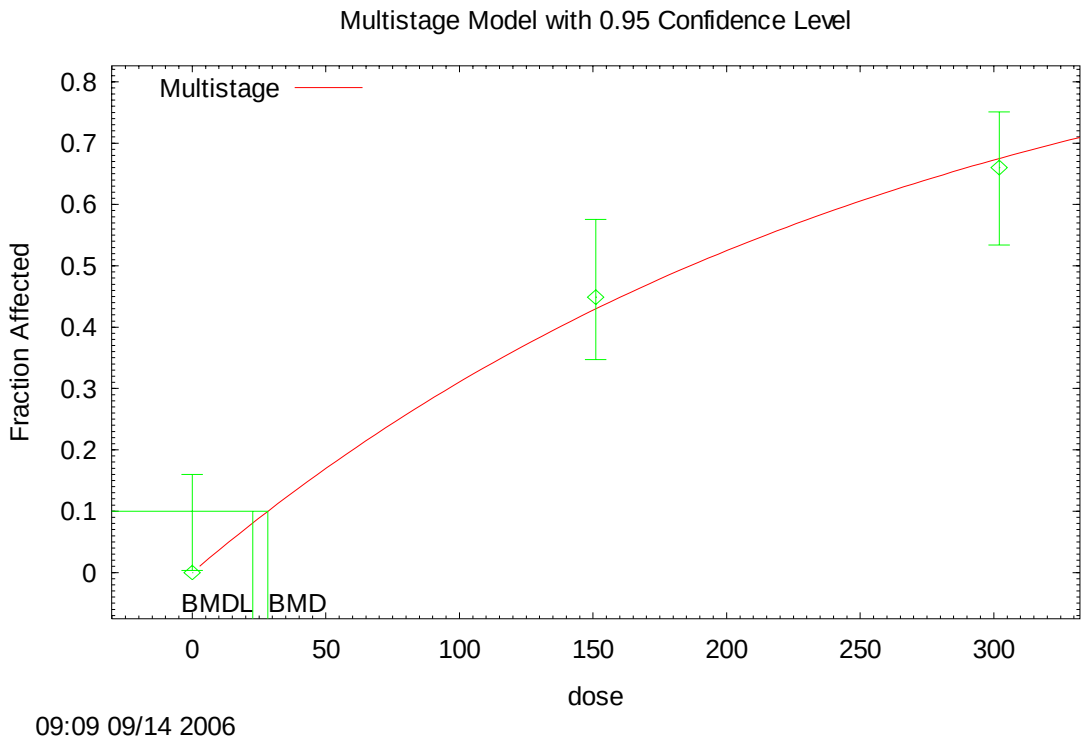
Confidence level = 0.95

BMD = 21.2541

BMDL = 17.0107

BMDU = 26.9612

Taken together, (17.0107, 26.9612) is a 90 % two-sided confidence interval for the BMD



Weibull

```
 ================================================================
         Weibull Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
         Input Data File: C:\BMDS\UNSAVED1.(d)
         Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                Thu Sep 14 09:13:24 2006
 ================================================================

 BMDS Model Run NCI 1978 Tubular Nephropathy Male Rat - Weibull Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy
   Power parameter is restricted as power >=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



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0238095
                          Slope =   0.00453277
                          Power =      1.00295


           Asymptotic Correlation Matrix of Parameter Estimates

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

                  Slope

     Slope            1



                          Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0               NA
          Slope       0.00496352      0.000693669          0.00360396
0.00632309
          Power                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	-65.7706	3			
Fitted model	-65.8419	1	0.142715	2	0.9311
Reduced model	-82.1514	1	32.7616	2	<.0001

AIC: 133.684

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20	0.000
113.0000	0.4293	21.035	22.050	49	0.293
227.0000	0.6759	33.795	33.000	50	-0.240

Chi^2 = 0.14 d.f. = 2 P-value = 0.9308

Benchmark Dose Computation

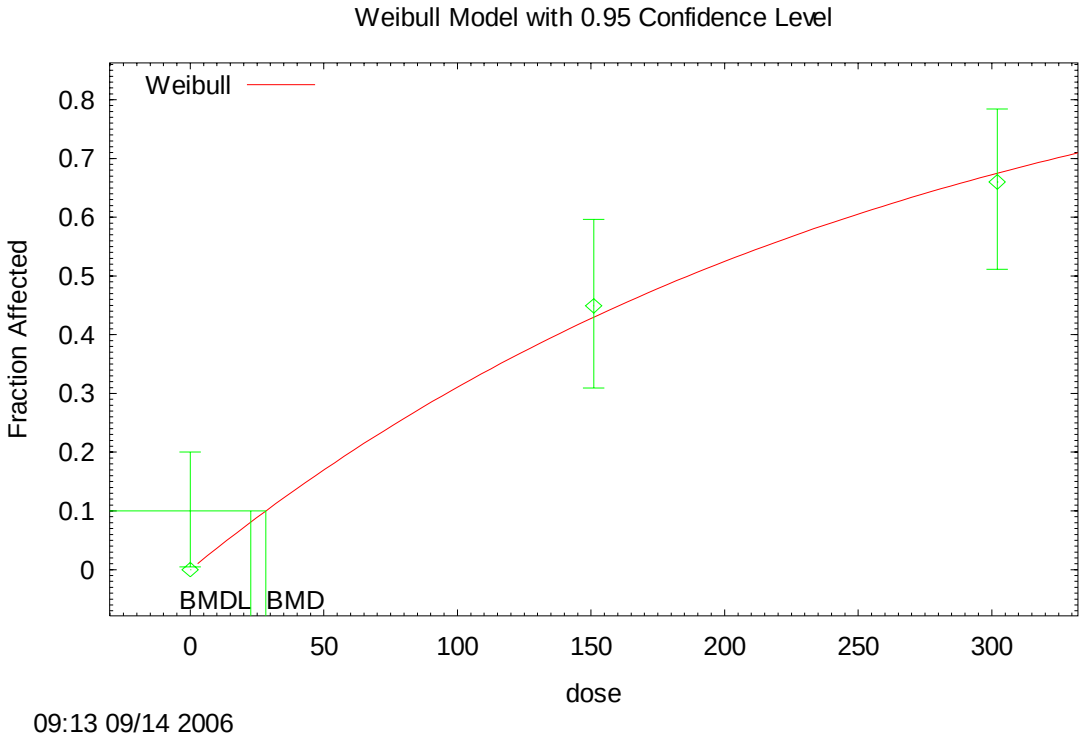
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 21.227

BMDL = 16.9904


Weibull Model with 0.95 Confidence Level
Weibull
Fraction Affected
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
50
100
150
200
250
300
dose
09:13 09/14 2006

NCI (1978) Tubular Nephropathy in Female Rats
Multistage 2°

```
================================================================
        Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
        Input Data File: C:\BMDS\UNSAVED1.(d)
        Gnuplot Plotting File:  C:\BMDS\UNSAVED1.plt
                                                Thu Apr 09 16:18:29 2009
 ================================================================

 BMDS Model Run - NCI 1978 Tubular Nephropathy Female Rat - Multistage 2 degree Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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 = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy

 Total number of observations = 3
 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
                        Beta(1) =           0
                        Beta(2) = 1.74381e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -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(2)

   Beta(2)           1



                           Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0           *               *                 *
        Beta(1)                0           *               *                 *
        Beta(2)     1.62048e-005           *               *                 *

* - Indicates that this value is not calculated.
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-56.7357	3			
Fitted model	-57.0429	1	0.614339	2	0.7355
Reduced model	-74.4688	1	35.466	2	<.0001

AIC: 116.086

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20	0.000
113.0000	0.1869	9.346	9.000	50	-0.125
227.0000	0.5661	27.741	28.910	49	0.337

Chi^2 = 0.13 d.f. = 2 P-value = 0.9374

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

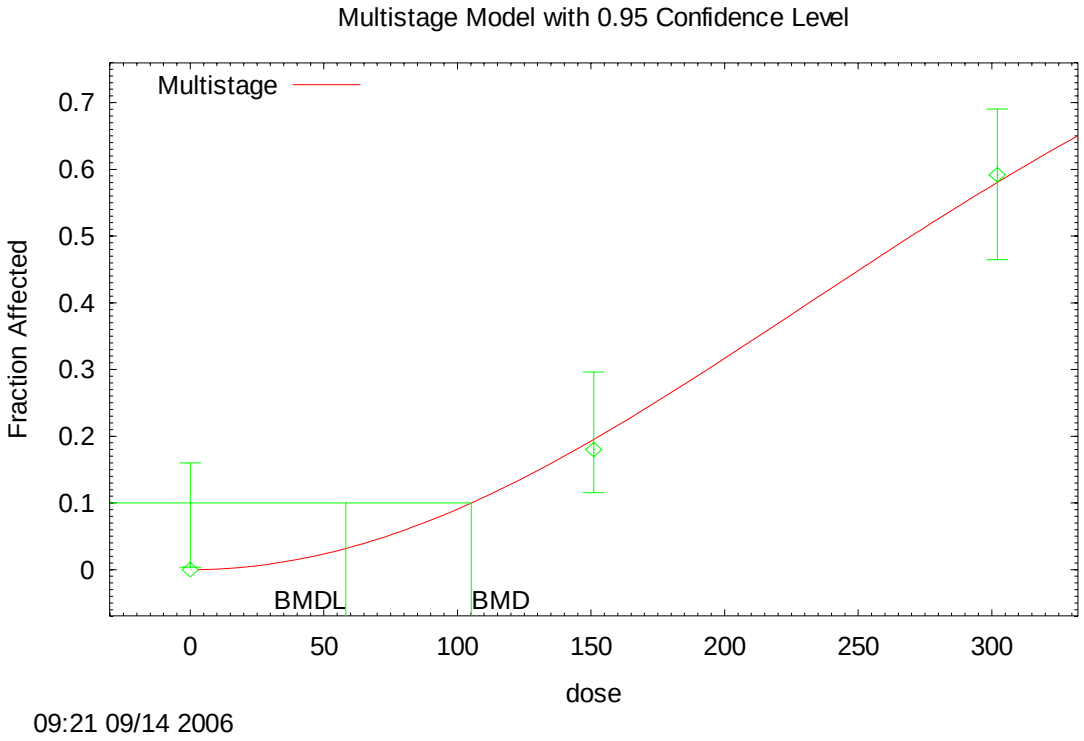
Confidence level = 0.95

BMD = 80.6338

BMDL = 41.8864

BMDU = 93.2552

Taken together, (41.8864, 93.2552) is a 90 % two-sided confidence interval for the BMD


Multistage Model with 0.95 Confidence Level
Multistage
Fraction Affected
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
50
100
150
200
250
300
dose
09:21 09/14 2006

NTP (1989) Male Rat Nephropathy
Multistage 1° Model

```
 ================================================================
        Multistage Model. (Version: 3.0;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp9D5.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp9D5.plt
                                            Wed Apr 08 11:27:18 2009
 ================================================================

 BMDS Model Run NTP 1989 Tubular Nephropathy Male Rat - Multistage 1 degree
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy

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


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



                  Default Initial Parameter Values
                     Background =     0.354136
                        Beta(1) =    0.0335717


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.72

   Beta(1)        -0.72            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.356651            *                *                 *
        Beta(1)        0.0329547            *                *                 *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table
```

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-100.939	3			
Fitted model	-100.952	2	0.0258029	1	0.8724
Reduced model	-103.852	1	5.82641	2	0.0543

AIC: 205.903

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3567	17.833	18.000	50	0.049
7.0000	0.4892	24.459	24.000	50	-0.130
14.0000	0.5944	29.721	30.000	50	0.080

Chi^2 = 0.03 d.f. = 1 P-value = 0.8724

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

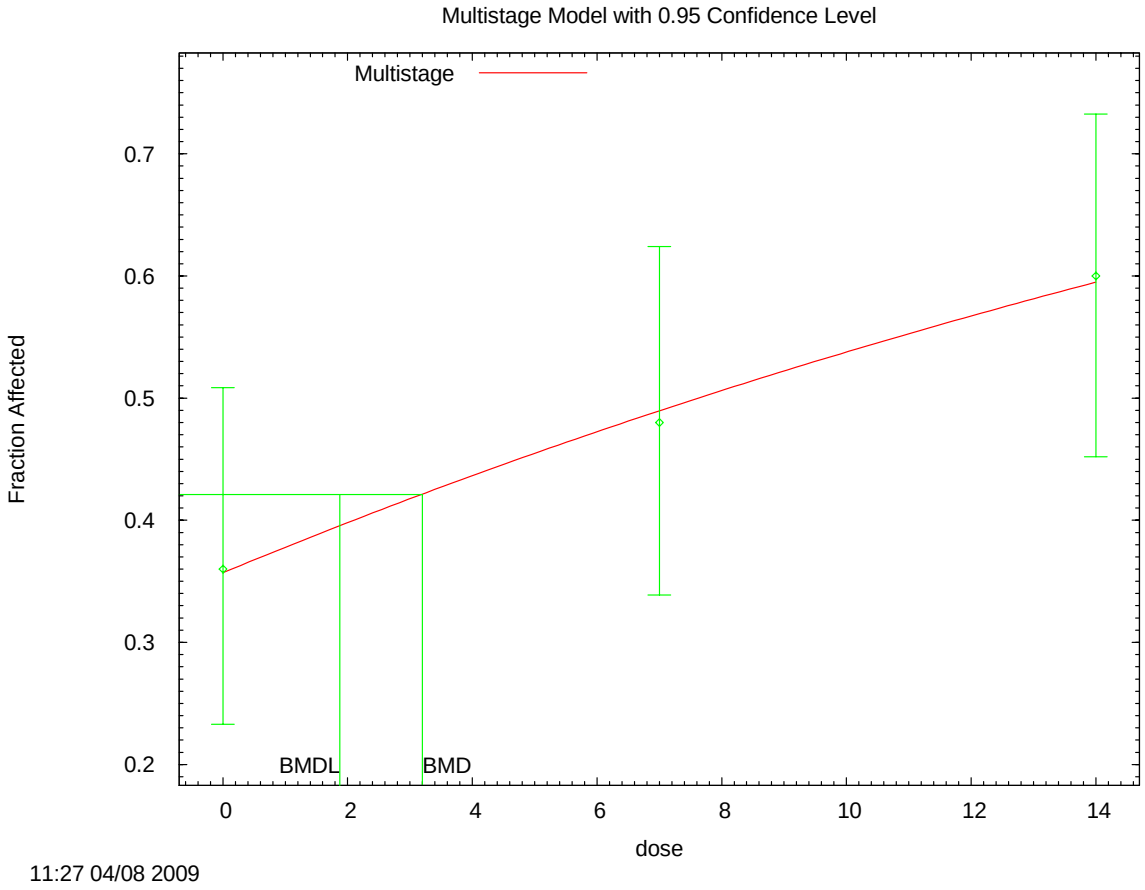
Confidence level = 0.95

BMD = 3.19713

BMDL = 1.8769

BMDU = 10.0721

Taken together, (1.8769 , 10.0721) is a 90 % two-sided confidence interval for the BMD


Multistage Model with 0.95 Confidence Level
Multistage
Fraction Affected
0.7
0.6
0.5
0.4
0.3
0.2
BMDL
BMD
0
2
4
6
8
10
12
14
dose
11:27 04/08 2009

Quantal-linear Model

```
 ================================================================
        Quantal Linear Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpA17.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpA17.plt
                                             Wed Apr 08 13:34:31 2009
 ================================================================

 BMDS Model Run NTP 1989 Tubular Nephropathy Male Rat - Quantal-linear Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy

   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 =     0.362745
                          Slope =    0.0329154
                          Power =            1   Specified


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background        Slope

Background            1        -0.66

     Slope        -0.66            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.356651        0.0642145            0.230793
0.482509
          Slope        0.0329547        0.0137884             0.00593
0.0599794



                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -100.939         3
```

Fitted model	-100.952	2	0.0258029	1	0.8724
Reduced model	-103.852	1	5.82641	2	0.0543

AIC: 205.903

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3567	17.833	18.000	50	0.049
7.0000	0.4892	24.459	24.000	50	-0.130
14.0000	0.5944	29.721	30.000	50	0.080

Chi^2 = 0.03 d.f. = 1 P-value = 0.8724

Benchmark Dose Computation

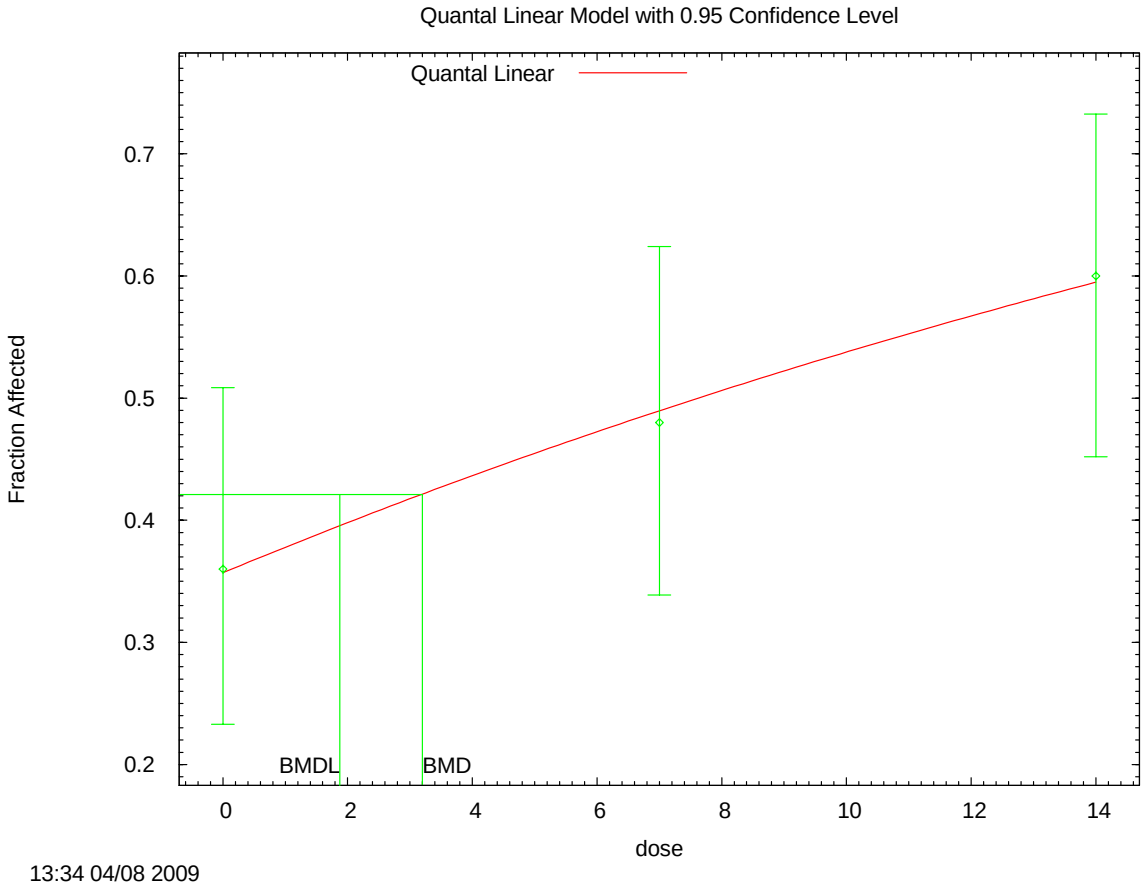
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.19713

BMDL = 1.8769


Quantal Linear Model with 0.95 Confidence Level
Quantal Linear
Fraction Affected
0.7
0.6
0.5
0.4
0.3
0.2
BMDL
BMD
0
2
4
6
8
10
12
14
dose
13:34 04/08 2009

NTP (1989) Female Rat Nephropathy

Gamma Model

```
 ================================================================
        Gamma Model. (Version: 2.13;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpD9.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpD9.plt
                                              Fri Apr 10 10:19:37 2009
 ================================================================

 BMDS Model Run NTP 1989 Tubular Nephropathy Female Rat - Gamma Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy
   Power parameter is restricted as power >=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



                  Default Initial (and Specified) Parameter Values
                     Background =     0.245098
                          Slope =    0.0111213
                          Power =          1.3


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background        Slope

Background            1        -0.55

     Slope        -0.55            1



                                  Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.242452        0.0592711            0.126283
0.358621
          Slope       0.00694477        0.0016862          0.00363988
0.0102497
          Power                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	-93.9362	3			
Fitted model	-93.9519	2	0.0312372	1	0.8597
Reduced model	-102.85	1	17.8276	2	0.0001345

AIC: 191.904

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2425	12.123	12.000	50	-0.040
57.0000	0.4901	24.504	25.000	50	0.140
114.0000	0.6568	32.182	31.850	49	-0.100

Chi^2 = 0.03 d.f. = 1 P-value = 0.8596

Benchmark Dose Computation

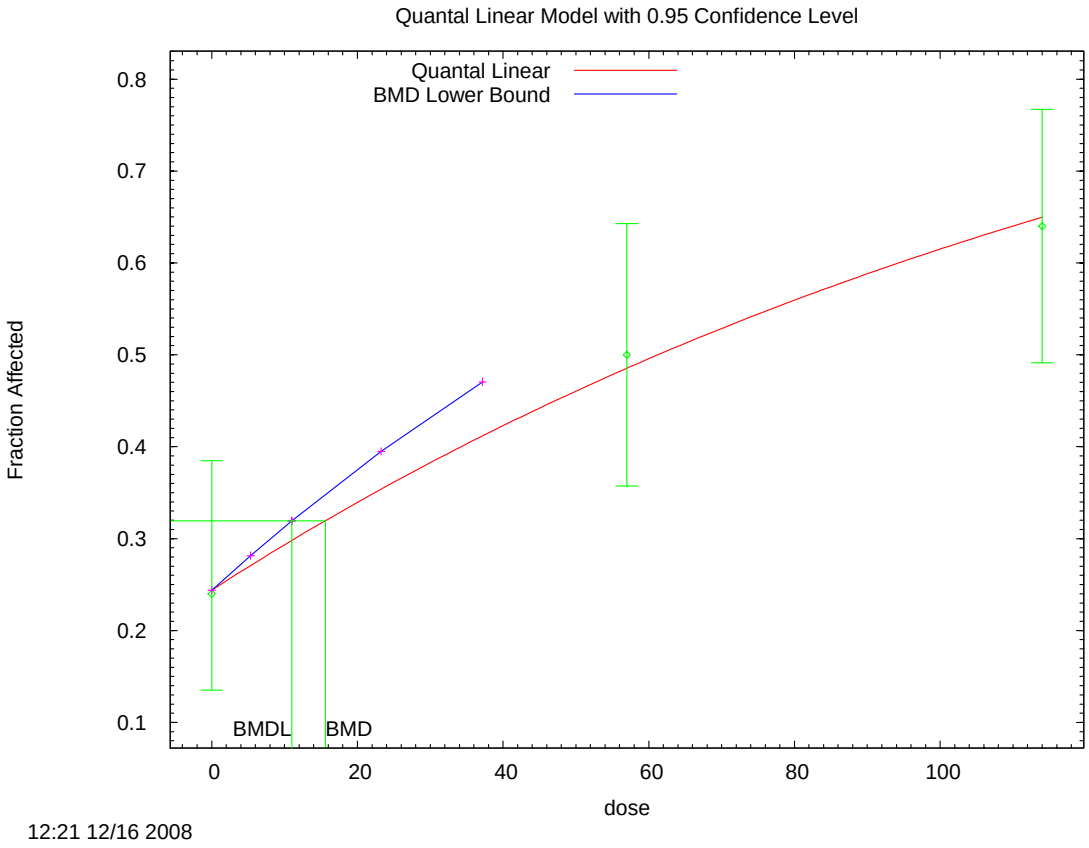
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 15.1712

BMDL = 10.7248


Quantal Linear Model with 0.95 Confidence Level
Quantal Linear
BMD Lower Bound
Fraction Affected
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
BMDL
BMD
0
20
40
60
80
100
dose
12:21 12/16 2008

Quantal-linear Model

```
 ================================================================
        Quantal Linear Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpE4.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpE4.plt
                                            Fri Apr 10 10:36:29 2009
 ================================================================

 BMDS Model Run NTP 1989 Tubular Nephropathy Female Rat - Quantal-linear Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy

   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 =     0.245098
                          Slope =   0.00666772
                          Power =            1   Specified


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background        Slope

Background            1        -0.55

     Slope        -0.55            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.242451        0.0592711            0.126282
0.358621
          Slope       0.00694478        0.0016862          0.00363989
0.0102497



                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -93.9362         3
```

Fitted model	-93.9519	2	0.0312372	1	0.8597
Reduced model	-102.85	1	17.8276	2	0.0001345

AIC: 191.904

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2425	12.123	12.000	50	-0.040
57.0000	0.4901	24.504	25.000	50	0.140
114.0000	0.6568	32.182	31.850	49	-0.100

Chi^2 = 0.03 d.f. = 1 P-value = 0.8596

Benchmark Dose Computation

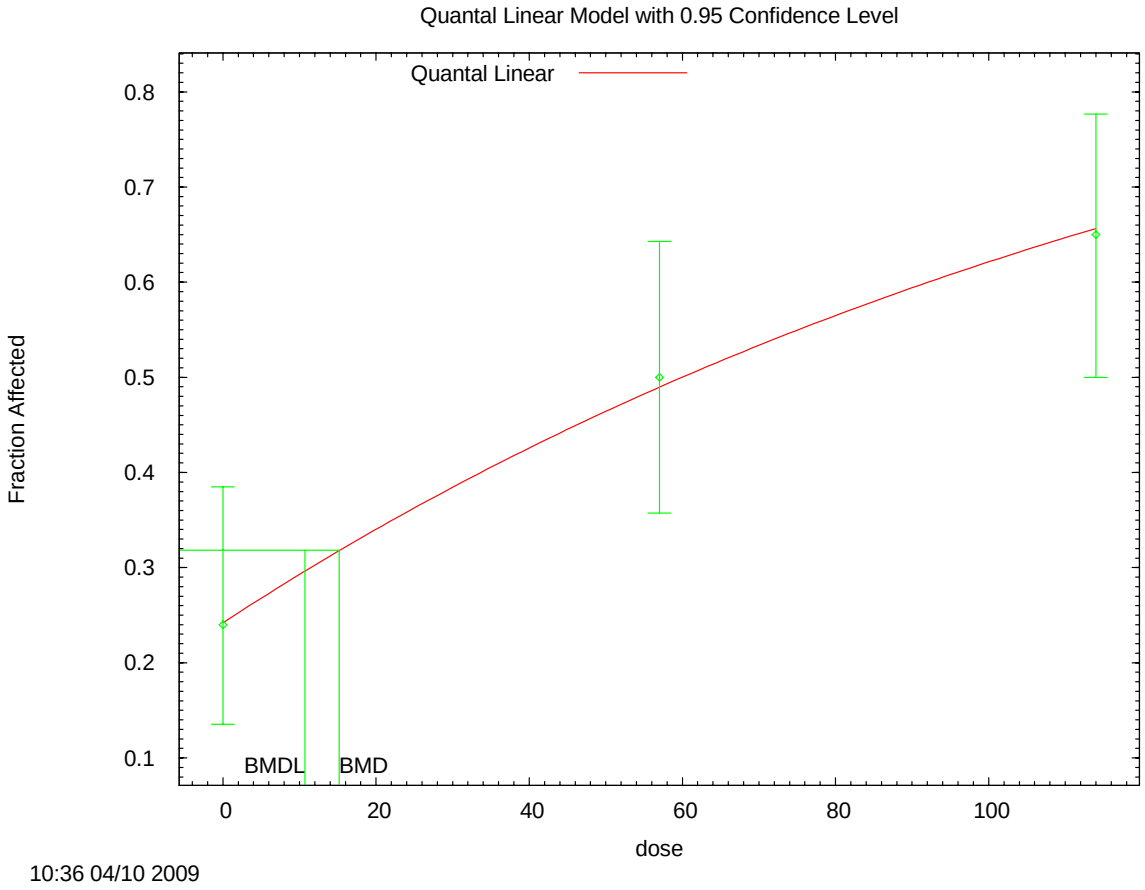
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 15.1712

BMDL = 10.7248


Quantal Linear Model with 0.95 Confidence Level
Quantal Linear
Fraction Affected
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
BMDL
BMD
0
20
40
60
80
100
dose
10:36 04/10 2009

Weibull Model

```
 ================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpE3.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpE3.plt
                                            Fri Apr 10 10:34:27 2009
 ================================================================

 BMDS Model Run NTP 1989 Tubular Nephropathy Female Rat - Weibull Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = PercentPositiveModerateMarkedTubularNephropathy
   Independent variable = ularNephropathy
   Power parameter is restricted as power >=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



                  Default Initial (and Specified) Parameter Values
                     Background =     0.245098
                          Slope =   0.00666772
                          Power =            1


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background        Slope

Background            1        -0.55

     Slope        -0.55            1



                                 Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.242451        0.0592711            0.126282
0.358621
          Slope       0.00694478        0.0016862          0.00363989
0.0102497
          Power                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	-93.9362	3			
Fitted model	-93.9519	2	0.0312372	1	0.8597
Reduced model	-102.85	1	17.8276	2	0.0001345

AIC: 191.904

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2425	12.123	12.000	50	-0.040
57.0000	0.4901	24.504	25.000	50	0.140
114.0000	0.6568	32.182	31.850	49	-0.100

Chi^2 = 0.03 d.f. = 1 P-value = 0.8596

Benchmark Dose Computation

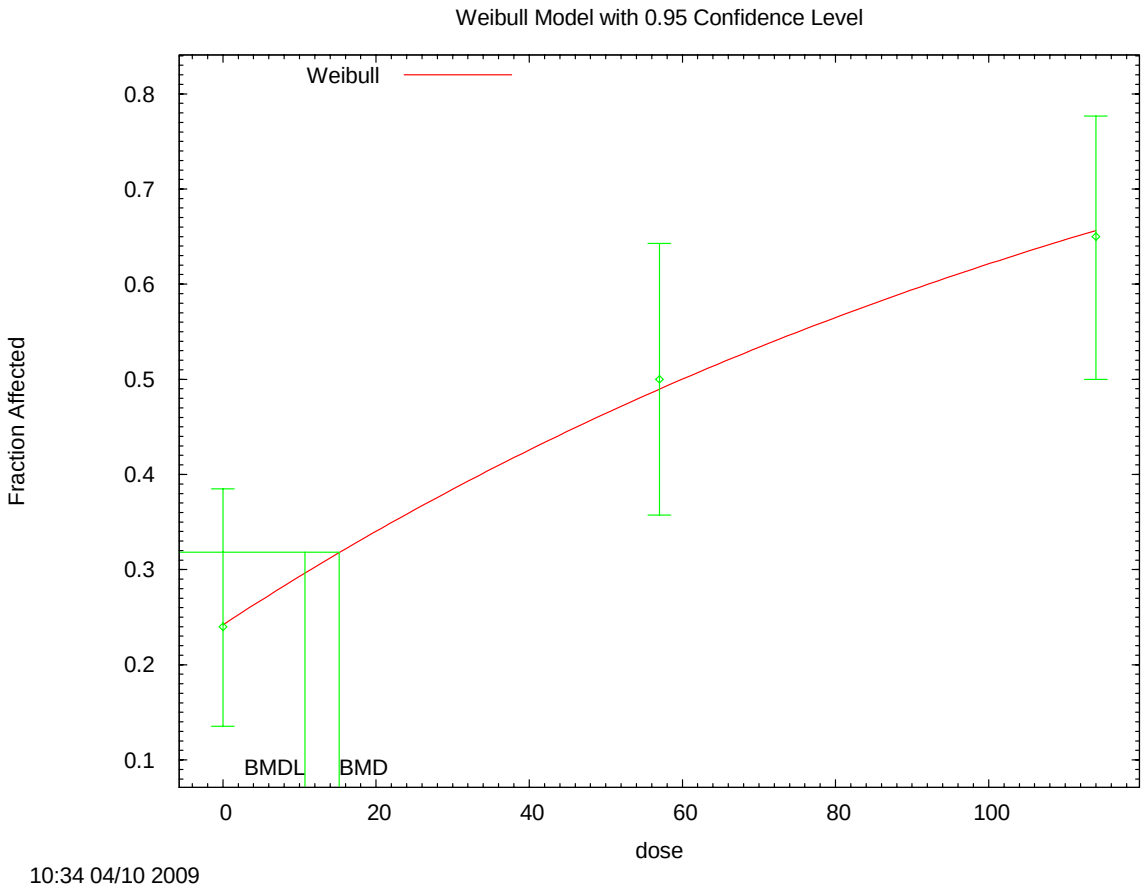
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 15.1712

BMDL = 10.7248


Weibull Model with 0.95 Confidence Level
Weibull
Fraction Affected
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
BMDL
BMD
0
20
40
60
80
100
dose
10:34 04/10 2009

NTP (1989) Linear Mineralization in Male Rats

Probit Model

```
 ================================================================
        Probit Model. (Version: 3.1;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpA33.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpA33.plt
                                          Wed Apr 08 14:24:02 2009
 ================================================================

 BMDS Model Run NTP 1989 Linear Mineralization Male Rat - Probit Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = PercentPositiveLinearMineralization
   Independent variable = ion
   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 =     -1.67551
                          slope =     0.149038


           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept        slope

 intercept            1        -0.87

     slope        -0.87            1


                                  Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
      intercept         -1.62793         0.244257            -2.10666            -
1.14919
          slope         0.144885        0.0238239           0.0981906
0.191579
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-71.6113	3			
Fitted model	-71.8283	2	0.433989	1	0.51
Reduced model	-94.7689	1	46.3152	2	<.0001

AIC: 147.657

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0518	2.589	2.000	50	-0.376
7.0000	0.2697	13.485	15.000	50	0.483
14.0000	0.6556	32.780	32.000	50	-0.232

Chi^2 = 0.43 d.f. = 1 P-value = 0.5129

Benchmark Dose Computation

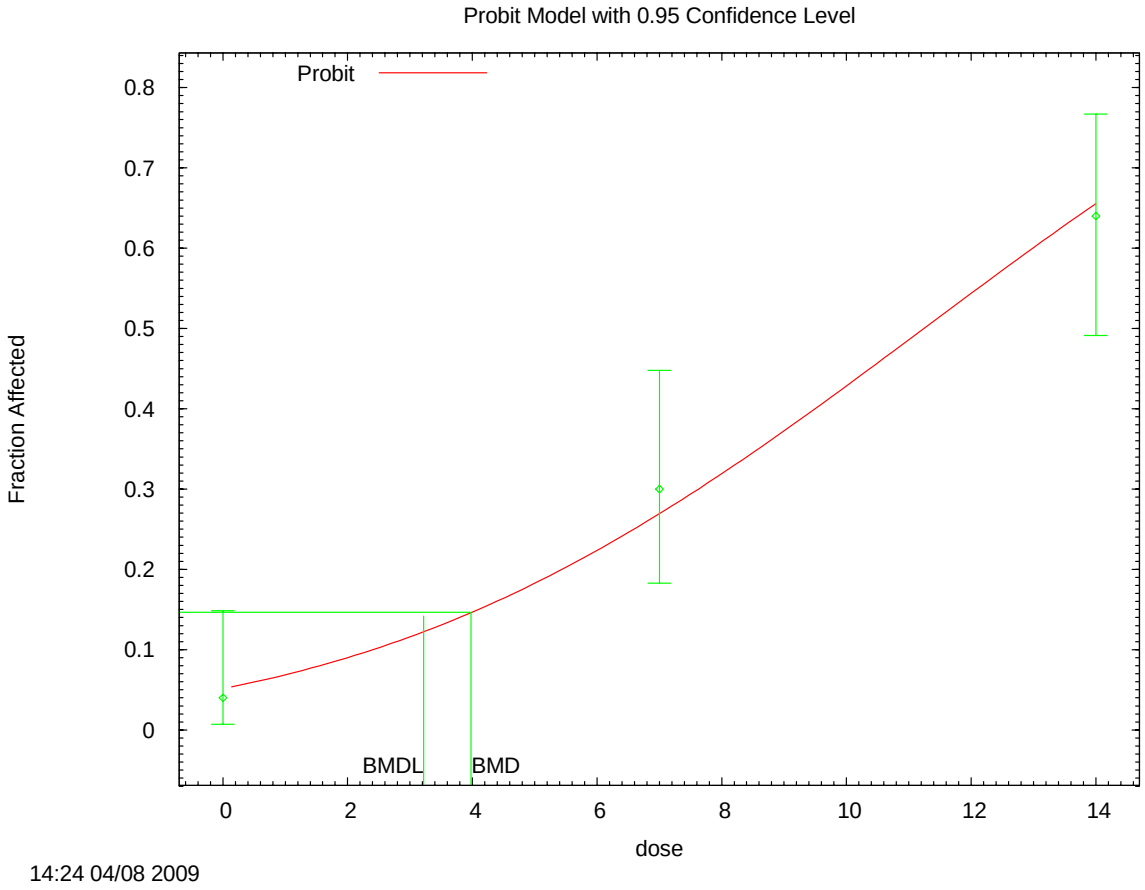
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.98089

BMDL = 3.21773


Probit Model with 0.95 Confidence Level
Probit
Fraction Affected
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
2
4
6
8
10
12
14
dose
14:24 04/08 2009

NTP (1989) Male Rat Hyperplasia of Pelvic Transitional Epithelium
LogLogistic Model

```
 ================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp4D5.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp4D5.plt
                                            Wed Aug 12 14:26:53 2009
 ================================================================

 BMDS Model Run – NTP 989 – Male Rat – Hyperplasia – LogLogistic Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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 =      -3.7612
                         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         -4.15077            *                *                  *
          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	-40.4963	3			
Fitted model	-41.2103	1	1.42796	2	0.4897
Reduced model	-46.5274	1	12.0622	2	0.002403

AIC: 84.4207

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
7.0000	0.0993	4.966	7.000	50	0.962
14.0000	0.1807	9.034	7.000	50	-0.748

Chi^2 = 1.48 d.f. = 2 P-value = 0.4761

Benchmark Dose Computation

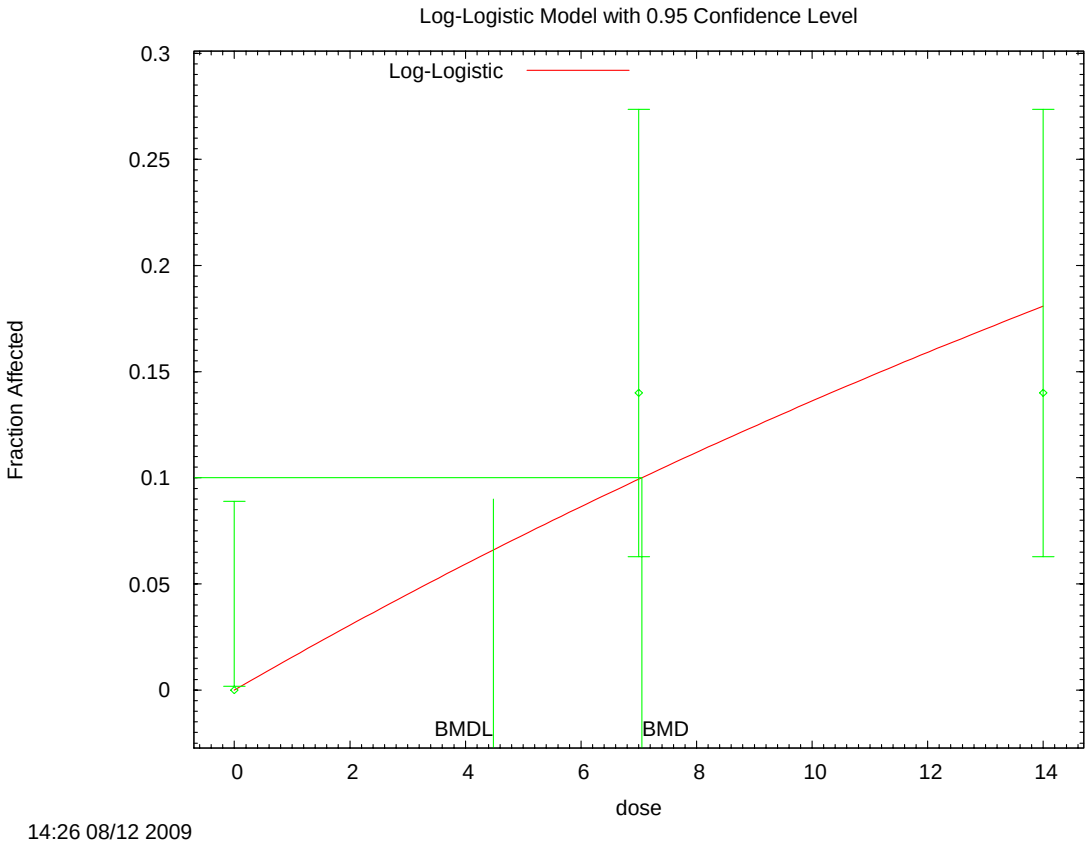
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.05365

BMDL = 4.48322


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
0.3
0.25
0.2
0.15
0.1
0.05
0
BMDL
BMD
0
2
4
6
8
10
12
14
dose
14:26 08/12 2009

Gorzinski (1985) Atrophy and Degeneration of renal tubules in Male Rats

Gamma Model

```
================================================================
        Gamma Model. (Version: 2.13;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpF14.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpF14.plt
                                            Thu Oct 08 08:59:00 2009
 ================================================================

 ndDegenRenalTubulesDataNoSeverityMaleRat.dax
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = Effect
   Independent variable = DOSE
   Power parameter is restricted as power >=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 (and Specified) Parameter Values
                     Background =     0.136364
                          Slope =    0.0871864
                          Power =          1.3


           Asymptotic Correlation Matrix of Parameter Estimates

             Background        Slope        Power

Background            1         0.52         0.64

     Slope         0.52            1         0.93

     Power         0.64         0.93            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.110626         0.107207          -0.0994949
0.320747
          Slope        0.0787607        0.0846932          -0.0872348
0.244756
          Power          1.00164          1.07041            -1.09632
3.0996
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-14.3635	4			
Fitted model	-14.4712	3	0.215359	1	0.6426
Reduced model	-27.7259	1	26.7248	3	<.0001

AIC: 34.9424

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1106	1.106	1.000	10	-0.107
1.0000	0.1777	1.777	2.000	10	0.185
15.0000	0.7265	7.265	7.000	10	-0.188
62.0000	0.9932	9.932	10.000	10	0.261

Chi^2 = 0.15 d.f. = 1 P-value = 0.6994

Benchmark Dose Computation

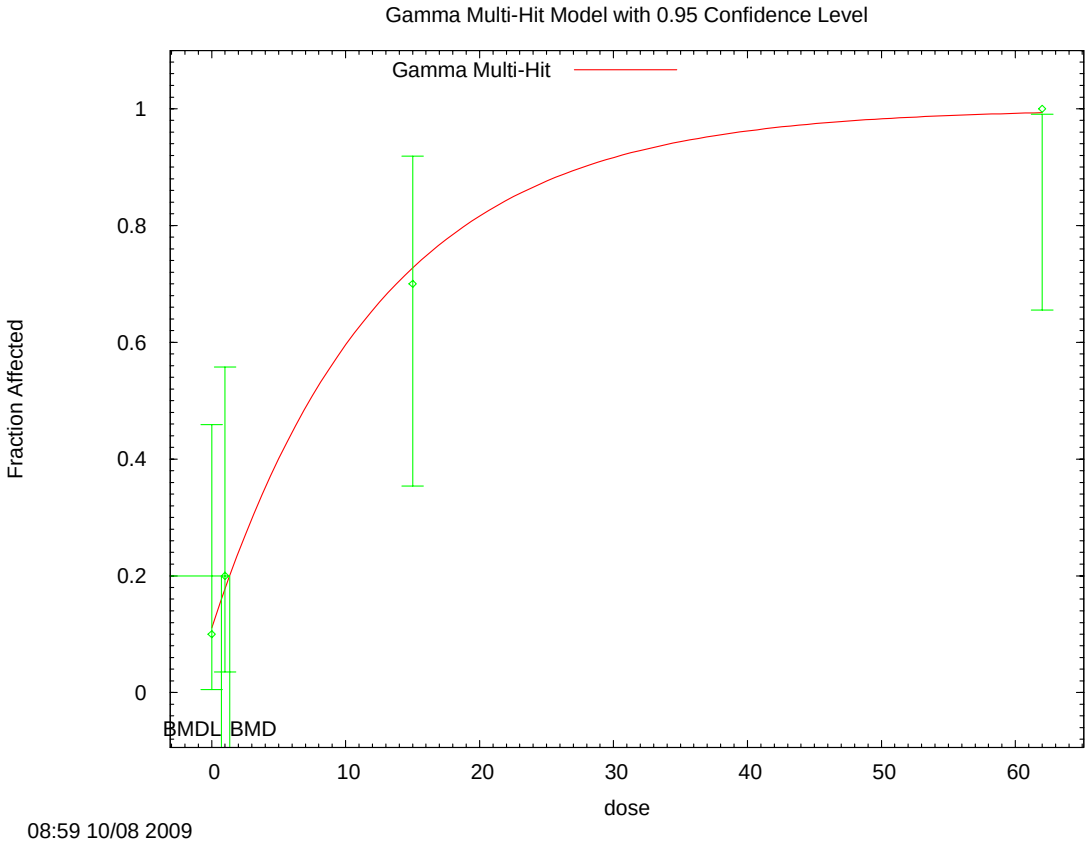
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.34399

BMDL = 0.727509



Multistage 1 degree Model

```
 ================================================================
        Multistage Model. (Version: 3.0;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpF17.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpF17.plt
                                            Thu Oct 08 09:00:57 2009
 ================================================================

 ndDegenRenalTubulesDataNoSeverityMaleRat.dax
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   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 = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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



                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) = 1.66732e+018


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1         -0.4

   Beta(1)         -0.4            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background          0.11052            *                *                  *
        Beta(1)        0.0786399            *                *                  *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

Full model	-14.3635	4			
Fitted model	-14.4712	2	0.215361	2	0.8979
Reduced model	-27.7259	1	26.7248	3	<.0001

AIC: 32.9424

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1105	1.105	1.000	10	-0.106
1.0000	0.1778	1.778	2.000	10	0.184
15.0000	0.7266	7.266	7.000	10	-0.189
62.0000	0.9932	9.932	10.000	10	0.261

Chi^2 = 0.15 d.f. = 2 P-value = 0.9283

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

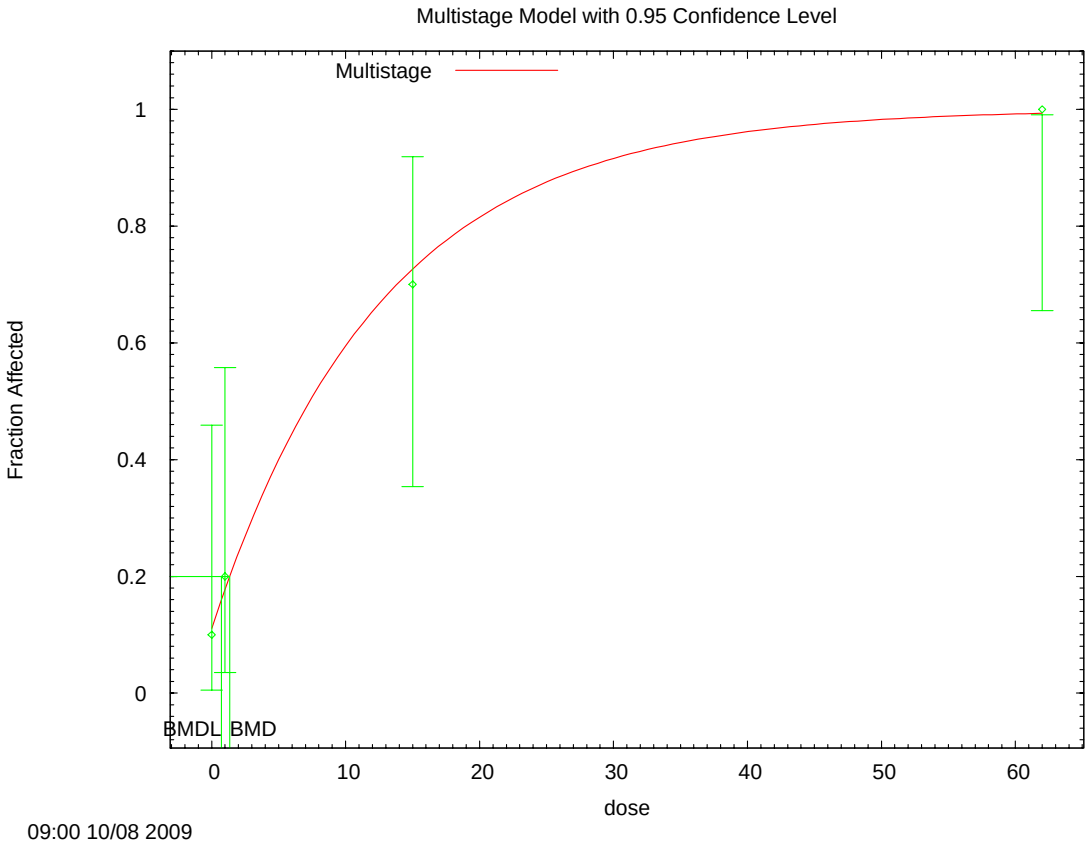
Confidence level = 0.95

BMD = 1.33978

BMDL = 0.727509

BMDU = 2.66189

Taken together, (0.727509, 2.66189) is a 90 % two-sided confidence interval for the BMD


Multistage Model with 0.95 Confidence Level
Multistage
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL BMD
0
10
20
30
40
50
60
dose
09:00 10/08 2009

Quantal-linear Model

```
 ================================================================
        Quantal Linear Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpF18.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpF18.plt
                                          Thu Oct 08 09:02:11 2009
 ================================================================

 ndDegenRenalTubulesDataNoSeverityMaleRat.dax
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = Effect
   Independent variable = DOSE

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



                  Default Initial (and Specified) Parameter Values
                     Background =     0.136364
                          Slope =     0.047491
                          Power =            1   Specified


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background        Slope

Background            1        -0.29

     Slope        -0.29            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background          0.11052        0.0819804          -0.0501583
0.271199
          Slope        0.0786399        0.0310542           0.0177749
0.139505



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -14.3635         4
   Fitted model        -14.4712         2      0.215361      2          0.8979
```

Reduced model	-27.7259	1	26.7248	3	<.0001

AIC: 32.9424

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1105	1.105	1.000	10	-0.106
1.0000	0.1778	1.778	2.000	10	0.184
15.0000	0.7266	7.266	7.000	10	-0.189
62.0000	0.9932	9.932	10.000	10	0.261

Chi^2 = 0.15 d.f. = 2 P-value = 0.9283

Benchmark Dose Computation

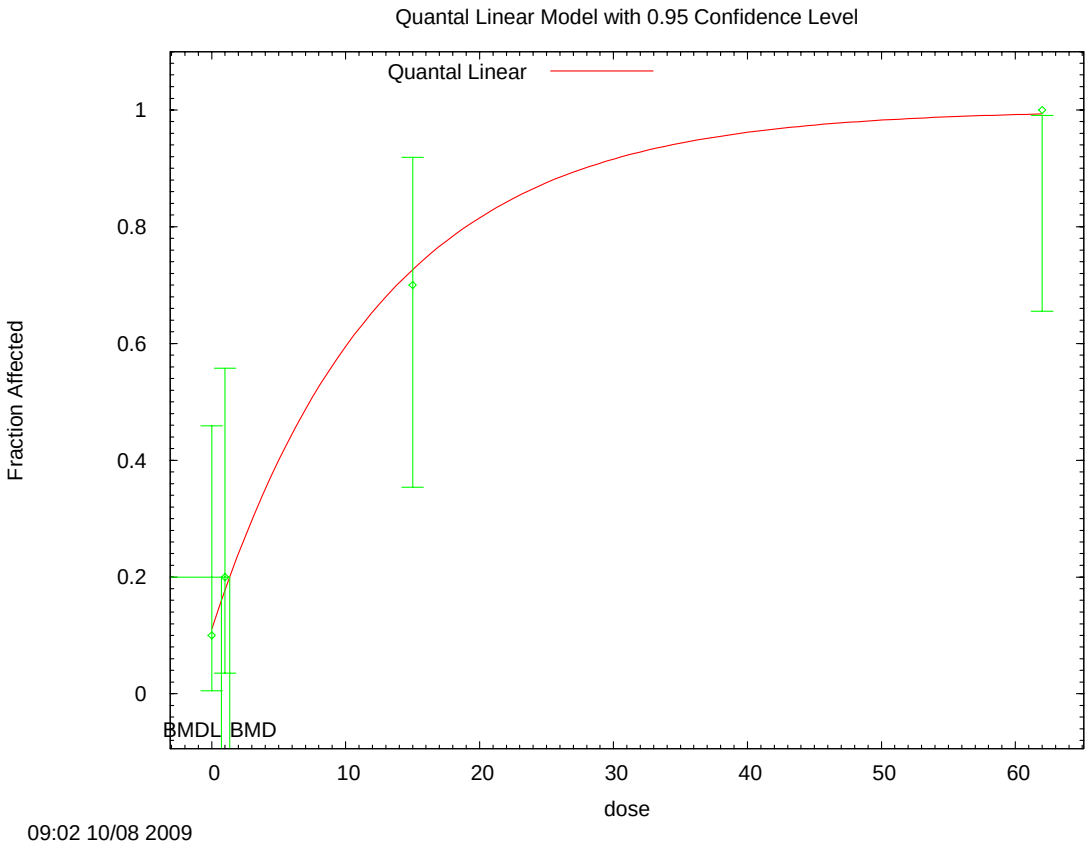
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.33978

BMDL = 0.727509



Gorzinski (1985) Atrophy and Degeneration of renal tubules in Female Rats

Multistage 1 degree Model

```
================================================================
        Multistage Model. (Version: 3.0;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpF51.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpF51.plt
                                            Thu Oct 08 09:34:37 2009
 ================================================================

 ndDegenRenalTubulesDataNoSeverityFemaleRat.dax
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   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 = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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



                  Default Initial Parameter Values
                     Background =    0.0749781
                        Beta(1) =    0.0133129


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.51

   Beta(1)        -0.51            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0885222            *                *                 *
        Beta(1)        0.0123308            *                *                 *

* - Indicates that this value is not calculated.
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.2358	4			
Fitted model	-18.3071	2	0.142532	2	0.9312
Reduced model	-22.4934	1	8.51521	3	0.03648

AIC: 40.6141

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0885	0.885	1.000	10	0.128
1.0000	0.0997	0.997	1.000	10	0.003
15.0000	0.2424	2.424	2.000	10	-0.313
62.0000	0.5757	5.757	6.000	10	0.156

Chi^2 = 0.14 d.f. = 2 P-value = 0.9330

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

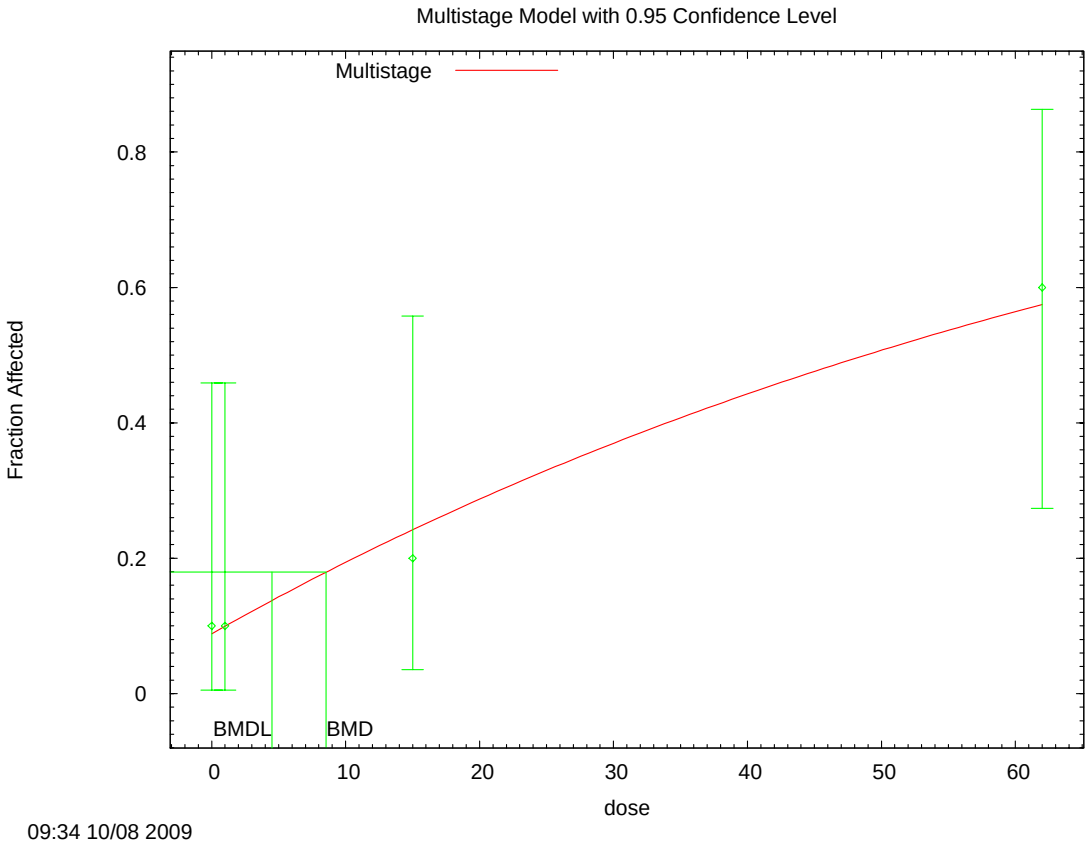
Confidence level = 0.95

BMD = 8.54451

BMDL = 4.49217

BMDU = 23.0819

Taken together, (4.49217, 23.0819) is a 90 % two-sided confidence interval for the BMD


Multistage Model with 0.95 Confidence Level
Multistage
Fraction Affected
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
10
20
30
40
50
60
dose
09:34 10/08 2009

Quantal-linear Model

```
================================================================
        Quantal Linear Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpF52.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpF52.plt
                                          Thu Oct 08 09:35:41 2009
 ================================================================

 ndDegenRenalTubulesDataNoSeverityFemaleRat.dax
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = Effect
   Independent variable = DOSE

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



                  Default Initial (and Specified) Parameter Values
                     Background =     0.136364
                          Slope =    0.0120518
                          Power =            1   Specified


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background        Slope

Background            1        -0.33

     Slope        -0.33            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0885253        0.0622082          -0.0334005
0.210451
          Slope        0.0123305       0.00562158          0.00131237
0.0233486



                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -18.2358         4
   Fitted model        -18.3071         2      0.142532      2          0.9312
```

```
Reduced model        -22.4934         1       8.51521      3         0.03648

          AIC:         40.6141
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0885	0.885	1.000	10	0.128
1.0000	0.0997	0.997	1.000	10	0.003
15.0000	0.2424	2.424	2.000	10	-0.313
62.0000	0.5756	5.756	6.000	10	0.156

Chi^2 = 0.14 d.f. = 2 P-value = 0.9330

Benchmark Dose Computation

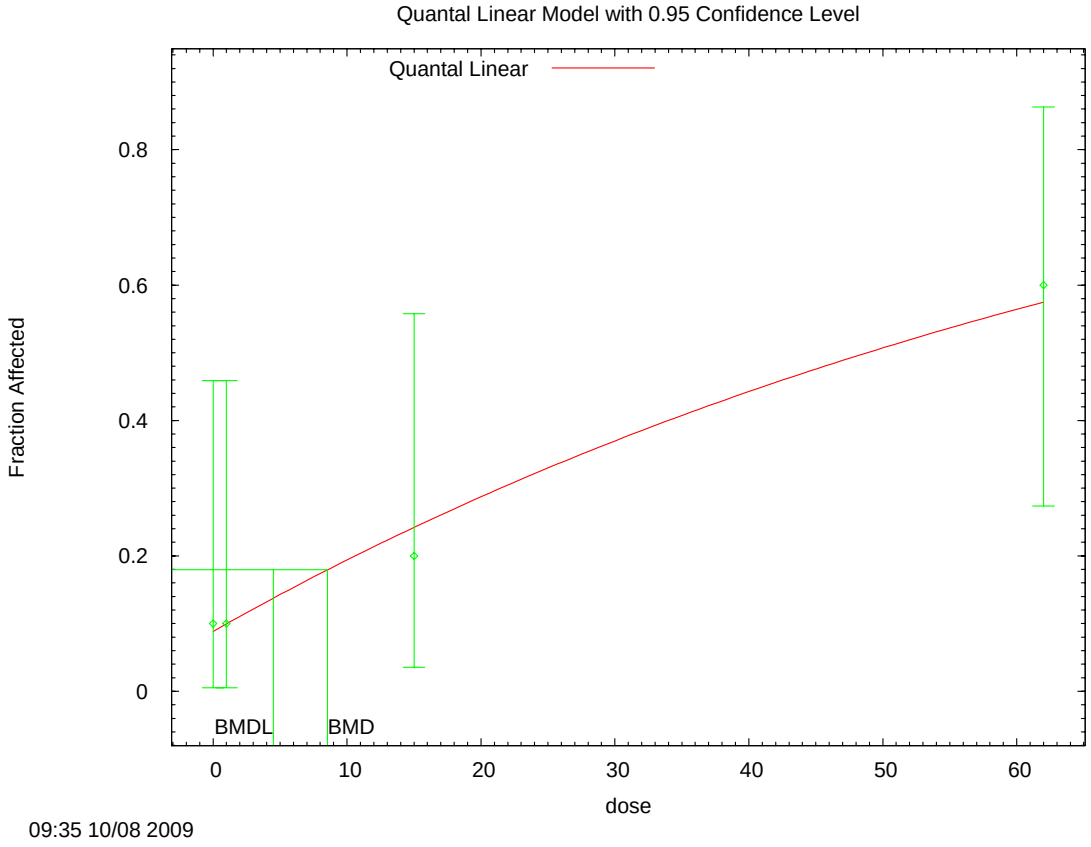
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 8.54473

BMDL = 4.49217



Gorzinski et al. (1985) Male Rat Hypertrophy and/or Dilation of Proximal Tubules
Gamma Model

```
 ================================================================
        Gamma Model. (Version: 2.13;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp4D6.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp4D6.plt
                                          Wed Aug 12 14:31:38 2009
 ================================================================

BMDS Model Run - Gorzinski et al (1985) - Male Rat – Hypertrophy/Dilation of Proximal
Tubules - Gamma Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = Effect
   Independent variable = DOSE
   Power parameter is restricted as power >=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 (and Specified) Parameter Values
                     Background =    0.0454545
                          Slope =    0.0907614
                          Power =          1.3


           Asymptotic Correlation Matrix of Parameter Estimates

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

                  Slope

     Slope            1



                                  Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
          Slope        0.0860249         0.029523           0.0281609
0.143889
          Power                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	-9.35947	4			
Fitted model	-9.44226	1	0.165576	3	0.9829
Reduced model	-27.5256	1	36.3322	3	<.0001

AIC: 20.8845

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
1.0000	0.0824	0.824	1.000	10	0.202
15.0000	0.7248	7.248	7.000	10	-0.176
62.0000	0.9952	9.952	10.000	10	0.220

Chi^2 = 0.12 d.f. = 3 P-value = 0.9893

Benchmark Dose Computation

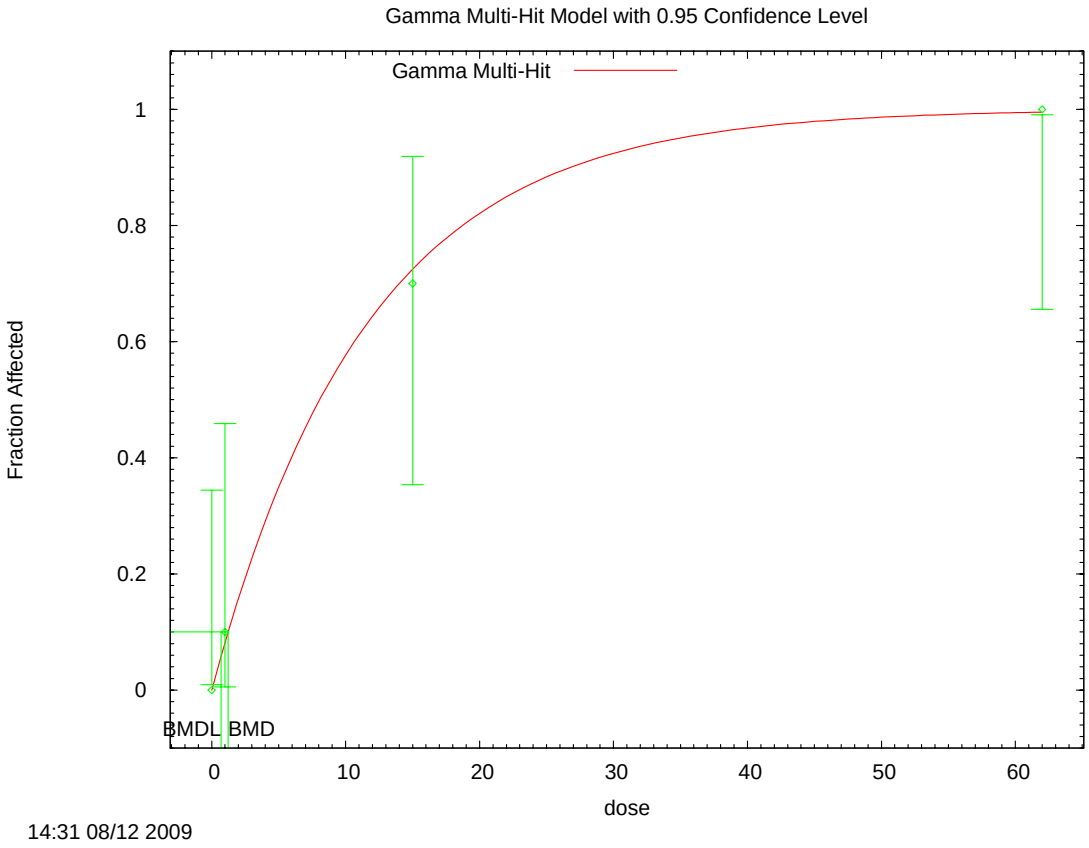
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.22477

BMDL = 0.710032


Gamma Multi-Hit Model with 0.95 Confidence Level
Gamma Multi-Hit
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL BMD
0
10
20
30
40
50
60
dose
14:31 08/12 2009

Weibull Model

```
================================================================
         Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp4D9.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp4D9.plt
                                          Wed Aug 12 14:35:51 2009
 ================================================================

 BMDS Model Run - Gorzinski et al (1985) - Male rats - Hypertrophy/Dilation of
Proximal Tubules - Weibull Model using Weibull Model (Version: 2.12;  Date:
05/16/2008)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = Effect
   Independent variable = DOSE
   Power parameter is restricted as power >=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 (and Specified) Parameter Values
                     Background =    0.0454545
                          Slope =    0.0491052
                          Power =            1


           Asymptotic Correlation Matrix of Parameter Estimates

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

                  Slope

     Slope            1



                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
          Slope         0.086025        0.0295231           0.0281608
0.143889
          Power                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	-9.35947	4			
Fitted model	-9.44226	1	0.165576	3	0.9829
Reduced model	-27.5256	1	36.3322	3	<.0001

AIC: 20.8845

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
1.0000	0.0824	0.824	1.000	10	0.202
15.0000	0.7248	7.248	7.000	10	-0.176
62.0000	0.9952	9.952	10.000	10	0.220

Chi^2 = 0.12 d.f. = 3 P-value = 0.9893

Benchmark Dose Computation

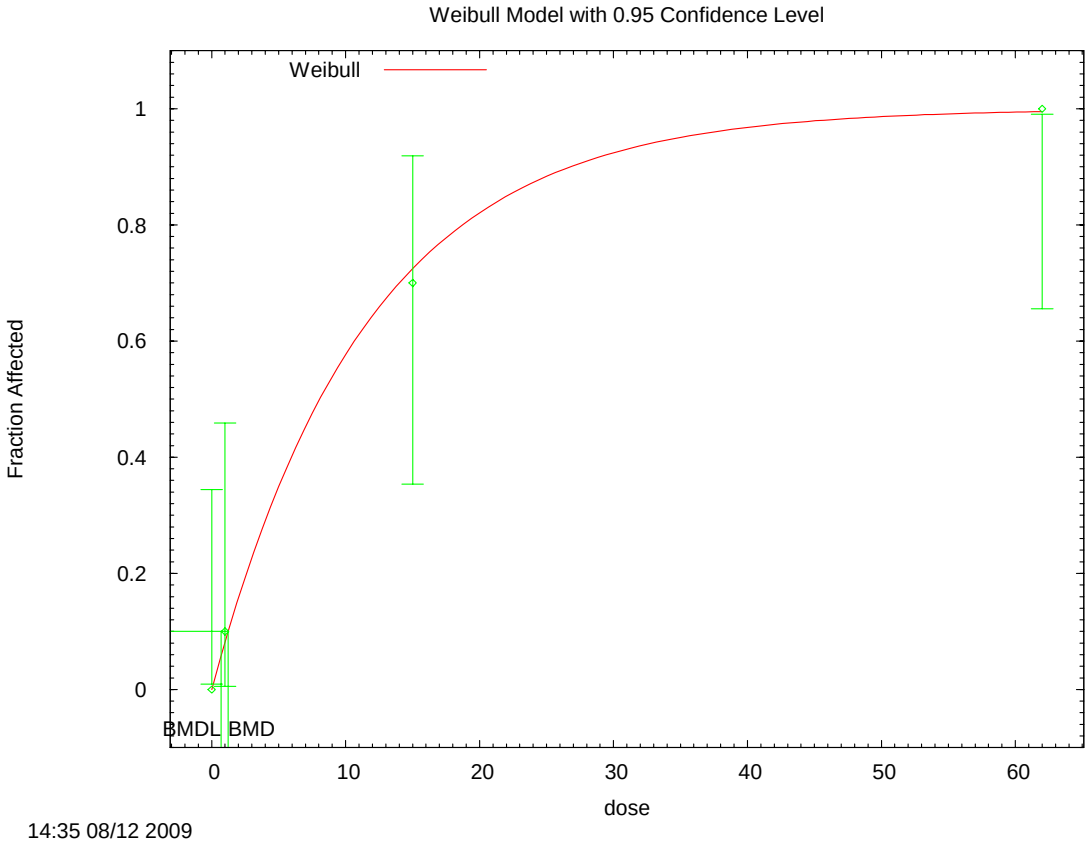
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.22477

BMDL = 0.710032



Quantal-linear Model

```
 ================================================================
        antal-linear Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp4DA.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp4DA.plt
                                              Wed Aug 12 14:37:26 2009
 ================================================================

 BMDS Model Run - Gorzinski et al (1985) - Male rats - Hypertrophy/Dilation Proximal
Tubules - Quantal-linear Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = Effect
   Independent variable = DOSE
   Power parameter is set to 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 (and Specified) Parameter Values
                     Background =    0.0454545
                          Slope =    0.0491052
                          Power =            1   Specified


           Asymptotic Correlation Matrix of Parameter Estimates

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

                  Slope

     Slope            1



                                 Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
          Slope        0.0860249         0.029523           0.0281608
0.143889

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	-9.35947	4			
Fitted model	-9.44226	1	0.165576	3	0.9829
Reduced model	-27.5256	1	36.3322	3	<.0001

AIC: 20.8845

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
1.0000	0.0824	0.824	1.000	10	0.202
15.0000	0.7248	7.248	7.000	10	-0.176
62.0000	0.9952	9.952	10.000	10	0.220

Chi^2 = 0.12 d.f. = 3 P-value = 0.9893

Benchmark Dose Computation

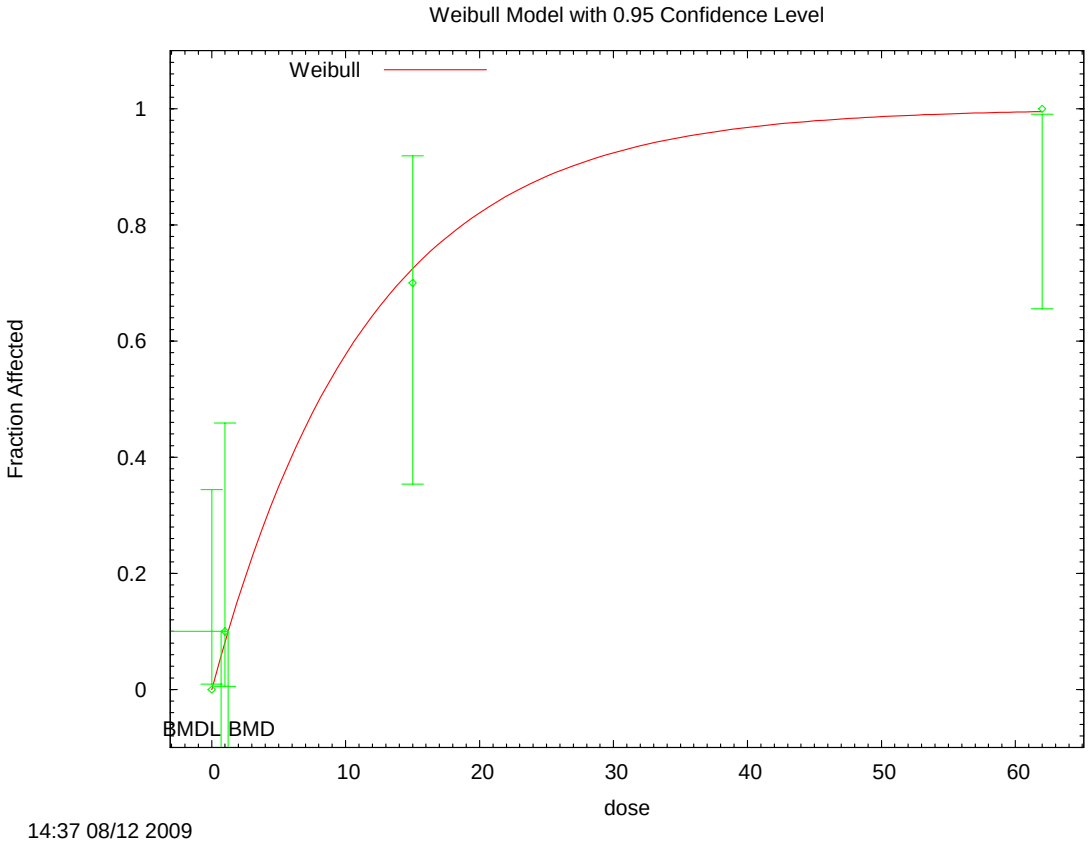
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.22477

BMDL = 0.710032



NTP (1989) Female Rat Hepatocellular Necrosis
Gamma Model

```
 ================================================================
        Gamma Model. (Version: 2.13;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpB62.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmpB62.plt
                                              Thu Apr 09 09:14:08 2009
 ================================================================

 BMDS Model Run NTP 1989 Hepatocellular Necrosis Female Rat - Gamma Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = PercentPositiveHepatocellularNecrosis
   Independent variable = rosis
   Power parameter is restricted as power >=1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial (and Specified) Parameter Values
                     Background =    0.0454545
                          Slope =   0.00743289
                          Power =      2.82109


           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 )

                  Slope        Power

     Slope            1         0.95

     Power         0.95            1



                                 Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
          Slope       0.00723384       0.00398244        -0.000571608
0.0150393
          Power          2.58447          1.14213            0.345944
4.823

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	-16.7382	6			
Fitted model	-17.3091	2	1.14186	4	0.8876
Reduced model	-32.5964	1	31.7164	5	<.0001

AIC: 38.6182

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
33.5000	0.0059	0.059	0.000	10	-0.244
67.1000	0.0300	0.300	0.000	10	-0.556
134.3000	0.1289	1.289	2.000	10	0.671
267.8000	0.4095	4.095	4.000	10	-0.061
535.7000	0.8159	8.159	8.000	10	-0.130

Chi^2 = 0.84 d.f. = 4 P-value = 0.9331

Benchmark Dose Computation

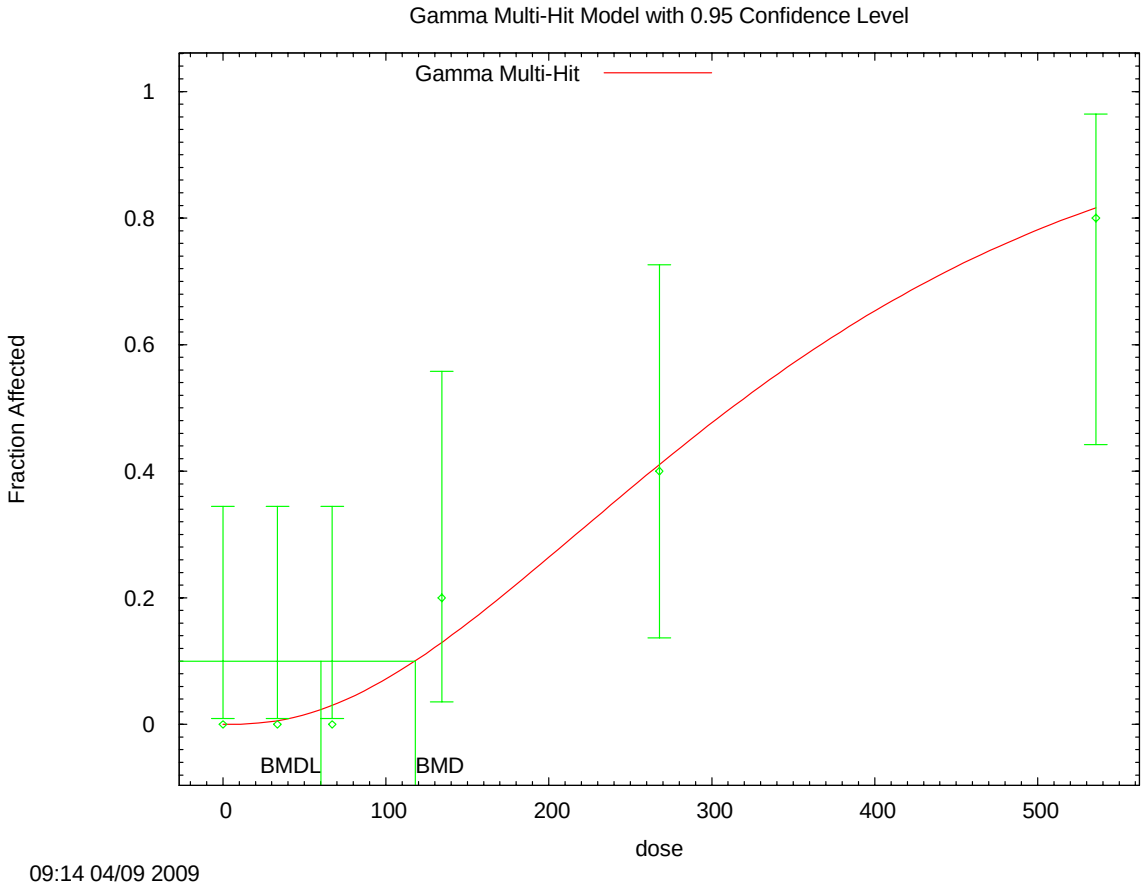
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 118.037

BMDL = 60.1812


Gamma Multi-Hit Model with 0.95 Confidence Level
Gamma Multi-Hit
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
100
200
300
400
500
dose
09:14 04/09 2009

NTP (1989) BMD Modeling of Adenoma/Carcinoma in Male Rats

Multistage 2°Model

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp6E8.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp6E8.plt
                                            Mon Apr 13 14:38:06 2009
 =================================================================

 BMDS Model Run NTP 1989 Kidney Adenoma-Carcinoma Male Rat - Multistage Cancer 2
degree Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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 = PercentAdenomaCarcinoma
   Independent variable = DOSE

 Total number of observations = 3
 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: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****



                  Default Initial Parameter Values
                     Background =     0.014541
                        Beta(1) =            0
                        Beta(2) =   0.00799069


           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        -0.67

   Beta(2)        -0.67            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0177261	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.00751246	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-33.5473	3			
Fitted model	-33.6008	2	0.106829	1	0.7438
Reduced model	-36.7395	1	6.38433	2	0.04108

AIC: 71.2015

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
i: 1					
0.0000	0.0177	0.887	1	50	0.129
i: 2					
2.0400	0.0481	2.407	2	50	-0.178
i: 3					
4.0900	0.1343	6.717	7	50	0.049

Chi-square = 0.10 DF= 1 P-value = 0.7510

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

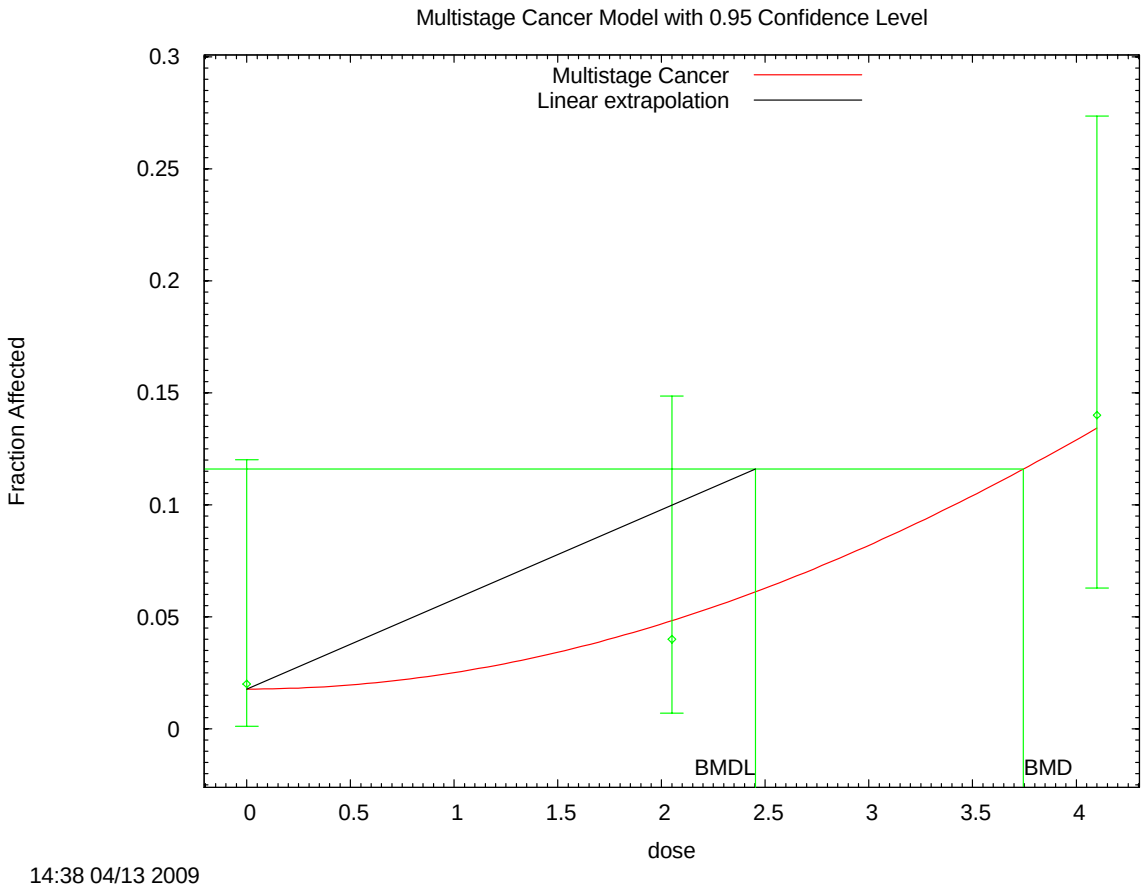
BMD = 3.74496

BMDL = 2.45283

BMDU = 9.24921

Taken together, (2.45283, 9.24921) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0407692


Multistage Cancer Model with 0.95 Confidence Level
Multistage Cancer
Linear extrapolation
Fraction Affected
0.3
0.25
0.2
0.15
0.1
0.05
0
BMDL
BMD
0
0.5
1
1.5
2
2.5
3
3.5
4
dose
14:38 04/13 2009

NCI (1978) BMD Modeling of Hepatocellular Carcinoma in Male Mice

Multistage 2°

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp7B8.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp7B8.plt
                                            Tue Apr 14 08:30:03 2009
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 BMDS Model Run NCI 1978 Hepatocellular Carcinoma Male Mice - Multistage Cancer 2
degree Model
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   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 = PercentHepatocellularCarcinoma
   Independent variable = DOSE

 Total number of observations = 3
 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: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence    ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****



                  Default Initial Parameter Values
                     Background =     0.141096
                        Beta(1) =            0
                        Beta(2) = 7.77012e-005


           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        -0.73

   Beta(2)        -0.73            1


                                  Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.146344	*	*	*
Beta(1)	0	*	*	*
Beta(2)	7.26074e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-71.2862	3			
Fitted model	-71.7199	2	0.867331	1	0.3517
Reduced model	-80.5752	1	18.5779	2	<.0001

AIC: 147.44

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1463	2.927	3.000	20	0.046
53.0500	0.3041	15.206	15.000	50	-0.063
103.8800	0.6101	29.892	30.870	49	0.286

Chi^2 = 0.09 d.f. = 1 P-value = 0.7666

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

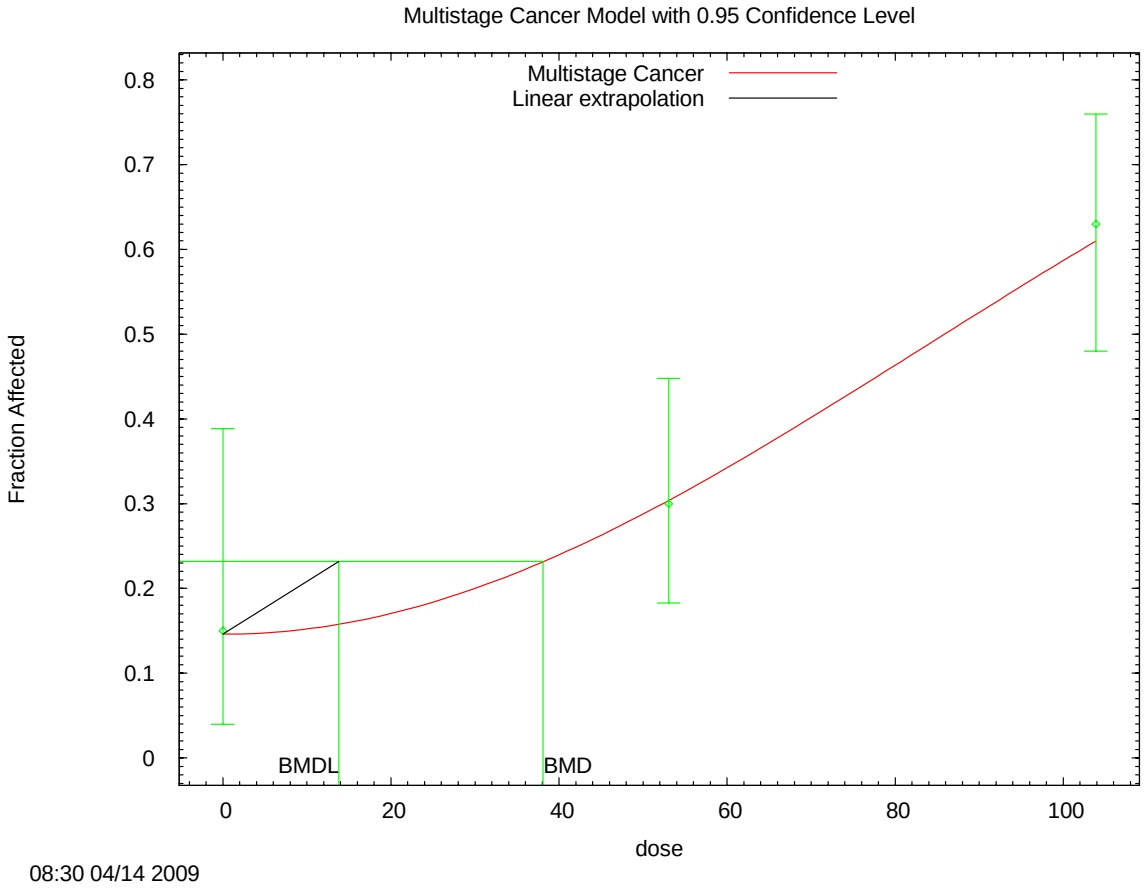
BMD = 38.0933

BMDL = 13.8018

BMDU = 49.5091

Taken together, (13.8018, 49.5091) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00724545


Multistage Cancer Model with 0.95 Confidence Level
Multistage Cancer
Linear extrapolation
Fraction Affected
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
20
40
60
80
100
dose
08:30 04/14 2009