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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)**

September 2011

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

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TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

α2u-g	α2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	United States Environmental Protection Agency
FDA	Food and Drug Administration
FEV1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood:gas partition coefficient
Hb/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

LC50	median lethal concentration
LD50	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MTD	maximum tolerated dose
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PERC	tetrachloroethene, tetrachloroethylene, perchloroethylene
POD	point of departure
POD[ADJ]	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
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
TWA	time-weighted average
UF	uncertainty factor
UFA	interspecies uncertainty factor
UFH	intraspecies uncertainty factor
UFS	subchronic-to-chronic uncertainty factor
UFD	database deficiencies uncertainty factor
U.S.	United States of America

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

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

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

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1 INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 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 ($\leq$ 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 μg/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, 1991c), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994b), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994a), *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, 2000c), *Supplementary Guidance*

for Conducting Health Risk Assessment of Chemical Mixtures (U.S. EPA, 2000b), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005a), *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 HCE was based on the chemical name, Chemical Abstracts Service Registry Number (CASRN), and multiple common synonyms. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. Primary, peer-reviewed-literature was reviewed through May 2011, and was included where that literature was determined to be critical to the assessment. The relevant literature included publications on HCE which were identified through Toxicology Literature Online (TOXLINE), PubMed, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Environmental Mutagens Information Center (EMIC) and Environmental Mutagen Information Center Backfile (EMICBACK) databases, the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate. No new publications were identified since the release of the external peer review draft Toxicological Review.

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). 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 U.S. Food and Drug Administration (FDA) withdrew approval for this use in 1971 (ATSDR, 1997c). HCE is primarily used by the military for smoke pots, smoke grenades, and pyrotechnic devices (ACGIH, 2001; ATSDR, 1997c; U.S. EPA, 1991b; IARC, 1979). HCE has also been used as a polymer additive, a moth repellant, a plasticizer for cellulose esters, and an insecticide solvent, and in metallurgy for refining aluminum alloys (ATSDR, 1997c; U.S. EPA, 1991a). HCE was also identified in the headspace of chlorine-bleach-containing household products (Odabasi, 2008). Certain physical and chemical properties are shown below in Table 2-1 (ACGIH, 2001; ATSDR, 1997c; Vogel and Nivard, 1993; Budavari et al., 1989; 1989; Weast et al., 1986; Spanggord et al., 1985; Verschueren, 1983; Mabey et al., 1982; Callahan et al., 1979).

Figure 2-1   Structure of HCE

Table 2-1   Physical properties of HCE

Property	Hexachloroethane
CASRN	67-72-1
Synonyms[a]	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
Specific Gravity	2.091 at 20°C
Water solubility[a]	50 mg/L at 22°C; 14 mg/L at 25°C
Log K_{ow}	3.82[b], 3.34[c], 4.14[d]
Log K_{oc}	4.3
Vapor pressure	0.5 mmHg at 20°C; 1.0 mmHg at 32.7°C
Henry's law constant	$2.8 \times 10\text{-}3$ (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] ChemIDplus Advanced Database (http://chem.sis.nlm.nih.gov/chemidplus/) and ACGIH (1991); [b]Howard (1989); [c]Callahan et al. (1979); [d]Hansch et al. (1995).

HCE is produced by the chlorination of tetrachloroethylene (PERC) in the presence of ferric chloride (ATSDR, 1997c; U.S. EPA, 1991b; Fishbein, 1979; IARC, 1979). HCE was produced in the United States (U.S.) for commercial distribution from 1921 to 1967, but is currently not commercially distributed (ATSDR, 1997c; IARC, 1979). In the 1970s, U.S. producers of HCE reported that HCE was not distributed, but was only used in-house or recycled (ATSDR, 1997c); U.S. distributors in the 1970s imported HCE from France, Spain, and the United Kingdom (ACGIH, 2001; ATSDR, 1997c). U.S. production plus imports of HCE totaled 10 million–50 million pounds in 1986, 1 million–10 million pounds in 1990, 10 million–50 million pounds in 1994, 500,000–1 million pounds in 1998, 10,000–500,000 pounds in 2002, and 1–10 million pounds in 2006 (NTP, 2011).

3 TOXICOKINETICS

3.1 Absorption

No studies have evaluated HCE absorption in humans by oral or inhalation exposure. HCE was identified in follicular fluid of women undergoing in vitro fertilization (IVF) during an analysis for environmental contaminants (Younglai et al., 2002). These data indicate the potential for HCE uptake, but not the source or route of exposure. The dermal absorption rate of HCE has been described as limited (ATSDR, 1997c); 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).

Oral exposure studies in animals have 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 that 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 in corn oil indicated that the amounts absorbed were 65–71 and 72–88%, respectively, based on the amount of radiolabel detected in expired air and total excreta (i.e., both urine and fecal excreta combined).

3.2 Distribution

There are limited data on the distribution of HCE in humans (Younglai et al., 2002). Animal studies (Gorzinski et al., 1985; Nolan and Karbowski, 1978; Fowler, 1969) have consistently demonstrated that HCE is distributed to fat ,kidney, liver, and 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 couples undergoing IVF. HCE was 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 serum obtained from females during oocyte retrieval for IVF. The study authors did not make any conclusions with regards to the level of HCE in follicular fluid and its effect on human fertility.

Fowler (1969) evaluated the tissue distribution of HCE, PERC, and pentachloroethane in sheep. Brain, fat, kidney, liver, muscle, blood, and bile were evaluated for HCE (see Table 3-1). To assess bile

concentrations of HCE, two of the HCE exposed Scottish Blackface sheep (Sheep 1 and Sheep 2) were fasted for 24 hours and anaesthetized with sodium pentobarbital (Table 3-1). The hepatic duct was cannulated to collect bile; HCE was injected at a dose of 500 mg/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. Anesthesia was maintained for 8.5 hours, after which time the sheep were sacrificed and tissues were taken within 10 minutes of death. HCE was widely distributed and the highest levels were found in fat of Sheep 1. Fat from different sites did not show significant variation in HCE concentration. Sheep 2 had only trace amounts of HCE in tissue. HCE was detected in bile of anaesthetized sheep at 15 minutes, compared with detection at 27 minutes for blood; at maximum, HCE was 8–10-fold greater in bile.

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

| | Concentration (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 delivered 100 mg/kg-day [later determined to be 62 mg/kg-day by Gorzinski et al. (1985)] for 57 days. After exposure, the rats were returned to an HCE-free control diet and sacrificed (groups of three or four rats) 0, 3, 6, 13, 22, and 31 days after starting the HCE-free diet. 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 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).

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

Days after cessation of HCE exposure	HCE tissue concentrations (n = 3 or 4) (mean ± SD µg/g tissue)			
	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 ± standard deviation (SD).
[b]One sample was lost and a mean ± SD could not be calculated.
[c]ND: less than 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 male and female rats after dietary exposure 3, 30, and 100 mg/kg-day of HCE for 110–111 days (16 weeks). The 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 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 increasing 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 hypothesized that 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[a] (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 (3)	0.260 ± 0.035 (2)	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 (3)	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
[a]n=4 for each tissue/sex/dose group, except where noted in parentheses.					

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

3.3 Metabolism

Data from 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. In vivo metabolism data for HCE are limited to three studies: Mitoma et al. (1985) in rats and mice; Jondorf et al. (1957) in rabbits; and Fowler (1969) in sheep. Each of these studies suggest limited metabolism for HCE. A variety of intermediary metabolites have also been identified in exhaled air and urine (Fowler, 1969; Jondorf et al., 1957). In vitro studies using liver microsomes indicated that HCE metabolism involves phenobarbital-inducible cytochrome P450 (CYP450) enzymes (Salmon et al., 1985; Town and Leibman, 1984; Nastainczyk et al., 1982a; Nastainczyk et al., 1982b; Salmon et al., 1981); however, no specific enzymes have been identified. The CYP450 enzymes induced by phenobarbital include those from the 2A, 2B, 2C, and 3A subfamilies. One study (Yanagita et al., 1997) found 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., 1982a; Nastainczyk et al., 1982b; 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.

Figure 3-1 provides a plausible 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 metabolites identified in in vivo (Mitoma et al., 1985; Fowler, 1969; Jondorf et al., 1957) and vitro studies (Town and Leibman, 1984; Nastainczyk et al., 1982a) were used in the derivation of Figure 3-1. The HCE metabolism data was supplemented with data on the metabolism of the PERC (ATSDR, 1997a), trichloroethylene (ATSDR, 1997b), and 1,1,2,2-tetrachloroethane (ATSDR, 2008) intermediary metabolites.

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 Plausible metabolic pathway of HCE

Mitoma et al. (1985) examined HCE distribution in male Osborne-Mendel rats and male B6C3F$_1$ mice to evaluate metabolism in the 48 hours after administration of 125 or 500 mg/kg radiolabeled HCE to the rats and 250 or 1,000 mg/kg radiolabeled HCE to the mice. Doses were 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. Four animals per dose were orally administered unlabeled HCE 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. 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 ± 9.59
Total metabolism (CO_2 + excreta + carcass)	28.72	23.95

Source: Reprinted with permission of Informa Healthcare©; Mitoma et al. (1985).

Recovery of the radiolabel was >90% for both rats and mice. The authors calculated total metabolism as the sum of the radiolabel present in carbon dioxide, excreta, and the carcass. This calculation is not an accurate estimate of metabolism because metabolites were not quantified. Data on the extent of metabolism for the radiolabeled material are presented in Table 3-5. Both rats and mice metabolized 30% of the parent compound, based on the mass balance between dose and the estimated sum of metabolites. This finding is consistent with the 60–70% of the unmetabolized radiolabel present in expired air. However, this conclusion assumed that all of the exhaled radiolabel in expired air 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.

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: Reprinted with permission of Informa Healthcare©; 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 5% of the applied radioactivity in urine over 72

hours, indicating slow metabolism. This finding is consistent with the Mitoma et al. (1985) study in rats and mice, in which approximately 2–4% of the label was found in urine after 48 hours. During the 72 hours, 14–24% of the radioactivity was detected in expired air, a lower percentage than seen for rats at a comparable (Mitoma et al., 1985). The remainder of the radioactivity was present in tissues and the intestinal tract, although the authors unable to quantify 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 observed in expired air). Quantitative data on the volatile metabolites in exhaled air were not reported.

Fowler (1969) orally administered HCE via drenching bottle 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 blood 24 hours after exposure. Following administration of 500 mg/kg HCE, 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 confirmed the presence of the metabolites PERC and pentachloroethane in liver slices.

The in vivo data on HCE metabolism are supported by in vitro studies of hepatic metabolism using liver microsomes. Two studies by Nastainczyk et al. (1982a; 1982b) reported that HCE is metabolized by phenobarbital-inducible CYP450 enzymes, which catalyze reductive dechlorination using 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 $\pm$ SD) provided support for reductive dehalogenation of HCE mediated by CYP450. Carbon monoxide inhibited the NADPH oxidation rate, further indicating that CYP450 enzymes were involved in the reaction. The major HCE metabolite of HCE reduction was PERC. Nastainzcyk et al. (1982a) determined that the stoichiometry of the reaction was represented by the following equation:

$$NADPH + H^+ + \underset{(HCE)}{Cl_3C - CCl_3} \xrightarrow{yields} NADP^+ + \underset{(PERC)}{Cl_2C = CCl_2} + 2H^+ + 2Cl^-$$

Because CYP450 is a one electron donor, Nastainczyk et al. (1982a; 1982b) proposed that 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 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. (1982a) found that the products of HCE reductive dechlorination were 99.5% PERC and 0.5% pentachloroethane at

physiological pH values. At a more basic 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.

To provide additional support for HCE reduction being catalyzed by CYP450, Nastainczyk et al. (1982a; 1982b) inhibited CYP450 using carbon monoxide, metyrapone (CYP450 3A inhibitor), or α-naphthoflavone (CYP450 1A and CYP450 1B inhibitor) [see Omiecinski (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 ± 8 and $79 \pm 10\%$, respectively. α-Naphthoflavone (10^{-4} M) did not inhibit HCE metabolism as effectively as metyrapone; inhibiting PERC formation by $13 \pm 2\%$ and pentachloroethane formation by $26 \pm 4\%$. These data indicate that CYP450 3A inhibition partially attenuated HCE metabolism, whereas inhibition of CYP450 1A and CYP450 1B did not attenuate HCE as much as CYP450 3A inhibition. 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 enzymes not inhibited in this study (i.e., CYP450 2A and CYP450 2B subfamilies).

Town and Leibman (1984) prepared liver microsomes from phenobarbital-induced male Holtzman rats to study the rate of HCE metabolism to PERC. The formation of PERC was favored in a low oxygen (O_2) environment, with 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 was omitted from the experiment, the metabolism of HCE to PERC was inhibited ($\geq$ 91%). In addition, the use of carbon monoxide (a monooxygenase inhibitor) arrested HCE metabolism. Enzymes responsible for metabolism of HCE to PERC were located in the microsomes (not the cytosol) of phenobarbital-treated rat livers. Formation of malondialdehyde and conjugated dienes was 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 (NZW) 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 identified PERC as a metabolite of HCE; however, the metabolite was not quantitatively measured.

Salmon et al. (1981) used liver microsomes from Sprague-Dawley rats to quantify HCE dechlorination. Dechlorination was measured by the release of radioactive Cl^- from the [^{36}Cl]-radiolabeled HCE substrate during incubation with liver microsomes from Aroclor 1254-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 NZW 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 NZW 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 enzymes.

Yanagita et al. ([1997](#)) used recombinantly-expressed rat CYP450 1A2 in baker's yeast (*Saccharomyces cerevisiae*) to evaluate the in vitro metabolism of HCE. CYP450 1A2 is not a phenobarbital-inducible hepatic CYP450 enzyme. 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). 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. Product formation rates and relative ratios of the products formed by metabolism of HCE from the Yanagita et al. ([1998](#)) study are shown in Table 3-6.

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

Units expressed as ([nmol product content per nmol CYP450] per minute). Experiments were repeated three times. Experiment errors were within 15%. Tetrachloroethylene (PERC); Pentachloroethylene; Trichlorethylene (TCE).

Source: Reprinted with permission of Elsevier©; 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 induced with phenobarbital) converted HCE to PERC and pentachloroethane at an initial dechlorination rate of 12 nmol/minute·nmol CYP450 under anaerobic conditions.

Van Dyke ([1977](#)) and Van Dyke and Wineman ([1971](#)) evaluated the mechanisms of HCE dechlorination using rat liver microsomes. 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-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 available studies evaluated the HCE elimination in humans. Animal studies indicated that the major routes of HCE elimination are either by fecal matter or by expired air ([Mitoma et al., 1985](#); [Fowler, 1969](#); [Jondorf et al., 1957](#)). Sheep studies ([Fowler, 1969](#)) indicated that orally administered HCE is eliminated by the fecal route without absorption and metabolism, while rodent studies ([Mitoma et al., 1985](#)) provided evidence that HCE is absorbed and eliminated by exhalation. It is unknown why there is a difference in elimination between 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 a single 500 mg/kg dose of HCE to two Cheviot cross in metabolism cages and collected urine and feces over 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.

Mitoma et al. ([1985](#)) evaluated excretion of radiolabeled HCE in Osborne-Mendel rats and B6C3F$_1$ mice following 4 weeks of administration of an MTD (see Section 3.3). In both rats and mice, most of the radiolabel was detected in expired air, indicating this to be a major route of elimination. The authors assumed the radiolabel in expired air was the parent compound and did not investigate whether the exhaled air contained volatile HCE metabolites. Less than 2.5% he exhaled radioactivity was found in CO_2, with rats exhaling slightly more than mice. On the other hand, the amount of radioactivity in the excreta was lower in rats than in mice (Table 3-4).

3.5 Physiologically Based Pharmacokinetic Models

No physiologically based pharmacokinetic (PBPK) models for HCE have been developed specifically for mammalian species.

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 originally developed for styrene in rats ([Ramsey and Andersen, 1984](#)). The Gargas and Andersen ([1989](#)) and Gargas et al. ([1988](#)) reports do not describe a PBPK model for HCE, only kinetic constants for HCE metabolism by inhalation.

4 HAZARD IDENTIFICATION

4.1 Studies in Humans—Epidemiology, Case Reports, Clinical Controls

There are few studies related to HCE toxicology in humans. No epidemiology studies of HCE carcinogenicity were identified. Case reports of pneumonitis alone (Allen et al., 1992) and pneumonitis with evidence of liver abnormalities (Loh et al., 2008; Loh et al., 2006) have been described in soldiers exposed to smoke bombs containing HCE and zinc oxide. However, smoke bomb incineration produces a mixture of chemicals consisting primarily of zinc oxychloride and zinc chloride, and the reported health effects are consistent with zinc chloride exposure (NRC, 1997). Some aluminum production processes involve HCE, resulting in exposures to fumes containing hexachlorobenzene, octachlorostyrene, dioxins, dibenzofurans, and other organochlorinated compounds. A study of aluminum foundry workers provided a hepatocellular carcinoma case report (Seldén et al., 1989) and data concerning some clinical serologic measures (Seldén et al., 1997; Seldén et al., 1989), but these data are of limited use to inform health effects of HCE exposure in other settings. Studies of Swedish workers involved in smoke bomb production provided some information on exposure levels, as well as symptoms and clinical parameters relating primarily to liver and pulmonary function (Seldén et al., 1994; Seldén et al., 1993).

Two studies were conducted on Swedish workers occupationally exposed to HCE while producing military white smoke munitions. The smoke formulation was approximately 60% HCE, 30% titanium dioxide, 8% aluminum powder, 2% cryolite, and a trace of zinc stearate. 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 area), 5-25 mg/m^3 (pressing area), <5 mg/m^3 (assembly room), and nondetectable (storage room) (Seldén et al., 1993).The first study reported biological exposure monitoring (Seldén et al., 1993) and the second study described health effects resulting from HCE exposure (Seldén et al., 1994).

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. The exposed male subject without an age-matched control 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, blood samples were drawn at a time period 5 weeks into a temporary production break (the "baseline" period), and the second samples were drawn 5 months later, after production had been underway for 5 weeks

(the "production" period). Analyses of blood plasma HCE indicated that all values for both control groups (n = 25) were 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, 1 sample was 0.15 μg/L, and 1 sample was 0.52 μg/L. The last sample was from an individual who had remained in an HCE-contaminated area during the baseline period. During the production period, plasma HCE levels increased nearly 100-fold over the baseline-period plasma HCE levels (mean of 7.30 ± 6.04 μg/L in the production samples compared with 0.08 ± 0.14 μg/L in the baseline samples, $p < 0.01$). Although the magnitude of individual increases varied, 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 indicate that plasma HCE can increase after occupational exposure, even though workers used 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, and 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 controls reported more symptoms 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 in the exposed group. Skin symptoms centered on the wrist and neck areas which the authors suggested could reflect HCE exposure through joints in the protective equipment or a "traumiterative effect of the equipment itself." Clinical examination revealed no dermatological or respiratory mucous membrane abnormalities in either group.

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 an elevated level of serum alanine aminotransferase (ALT) (70.5 U/L versus ≤ 41.1 U/L reference), while one control subject had 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 increased to 87.6 U/L 4 months later (Seldén et al., 1994). Available data pertaining to liver function tests in this individual from 1982, when exposure levels at the worksite were higher than in the current study, did not show elevations in these liver enzymes at that time. Within the exposed group, there was no correlation between plasma HCE concentrations and clinical chemistry parameters, although the authors did 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 sample size from the health effects study was too small to reach definitive conclusions. The possible dermatologic/mucosal effects and hepatic effects 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; Gorzinski et al., 1980). The Gorzinski et al. (1985; 1980) study (16 weeks) reported kidney degeneration in male and female rats, as well as hepatic effects. The NTP (1989) study (13 weeks) reported degeneration and necrosis of renal tubular epithelium, hyaline droplet formation, and tubular regeneration and tubular casts in male rats. Female rats 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. Rats were exposed via the diet to 3, 30, or 100 mg HCE/kg-day; however, due to sublimation of HCE from the feed and diurnal eating patterns, actual doses 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 Report by Dow Chemical. The data from Gorzinski et al., (1980) were published in the peer-reviewed literature by Gorzinski et al. (1985).

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 and gross pathological alterations (Table 4-1). Male rats of the 62 mg/kg-day group exhibited statistically significant increases in absolute and relative liver weights (Table 4-1); histopathology revealed a slight swelling of the hepatocytes in the 15 and 62 mg/kg-day dose groups. Female rats

exhibited a statistically significant increase in relative liver weight at the high dose (Table 4-1), although there was no evidence of hepatotoxicity in the histopathological examination.

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 (Table 4-2). 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 (Table 4-2). 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, 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). The increased incidence of atrophy and degeneration of proximal tubules was statistically significant in females at the 62 mg/kg-day dose (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 Absolute (g)	Liver Relative (g/100 g body weight)	Kidney Absolute (g)	Kidney 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 in each treatment group (10 rats/sex/dose).
[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

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

[a]Data are presented as number of positive observations for 10 rats/sex/dose.
[b]EPA determined statistical significance from control using Fisher's Exact Test (p = 0.05).
[c]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.
[d]With a degree of peritubular fibrosis in high dose males.

Source: Gorzinski et al. (1985).

The authors concluded the no-observed-effect level for both male and female rats was 1 mg/kg-day. For male rats, EPA considered 1 mg/kg-day as no-observed-adverse-effect level (NOAEL) and 15 mg/kg-day as the lowest-observed-adverse-effect level (LOAEL), based on renal tubule toxicity. 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 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. At the highest dose, 5/10 male rats and 2/10 female rats died before the end of the study. Mean body weight in the 536 mg/kg-day exposure group decreased 19% in male rats and 4% in female rats when compared with controls. Statistically significant increases in liver weights were 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[d]	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[b]	42.5 ± 0.74[b]	46.3 ± 0.95[b]
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[b]
Heart	2.8 ± 0.04	2.8 ± 0.04	2.9 ± 0.07	3.2 ± 0.17[c]	3.3 ± 0.18[b]	3.2 ± 0.10[c]
Kidney	3.0 ± 0.05	3.8 ± 0.37	4.1 ± 0.27[c]	4.7 ± 0.44[b]	5.2 ± 0.35[b]	4.7 ± 0.28[b]
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[b]
Thymus	0.8 ± 0.04	0.8 ± 0.06 (9)	0.6 ± 0.02	0.8 ± 0.10 (8)	0.7 ± 0.04	0.6 ± 0.06 (3)
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[c]	36.3 ± 0.44[b]	42.0 ± 0.60[b]	52.4 ± 0.88[b]
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 (10)[b]
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[b]
Kidney	3.1 ± 0.04	3.2 ± 0.05	3.2 ± 0.07	3.2 ± 0.06	3.6 ± 0.05[b]	4.1 ± 0.10[b]
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 (9)	1.0 ± 0.06	1.1 ± 0.07	0.8 ± 0.05[b]

[a]Data are presented as mean ± SE in mg/g, except for body weight in grams.
[b]Statistically different from controls, $p < 0.01$
[c]Statistically different from controls, $p < 0.05$
[d]Number animals, except where (noted).

Source: NTP (1989).

Kidney effects (characterized by hyaline droplet formation, tubular regeneration, and tubular casts) were observed in 90% of 34 mg/kg-day males and in males from all other HCE dose groups. The authors reported incidence data only 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 exposed females. Kidneys from the 5 male rats that died following exposure to 536 mg/kg-day HCE underwent histopathological examination, which 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% 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). In the NCI (1978) study, Osborne-Mendel rats of both sexes exhibited tumor types previously identified as spontaneous lesions in this strain, while B6C3F$_1$ mice of both sexes exhibited hepatocellular carcinomas, although only male mice demonstrated a dose response with tumor incidence (NCI, 1978). NTP and NCI concluded there was evidence of HCE carcinogenicity in male F344/N rats and mice of both sexes, respectively, but there was no evidence of carcinogenicity in female F344/N rats or Osborne-Mendel rats (NTP, 1989; NCI, 1978).

NTP (1989) conducted a chronic toxicity/carcinogenicity bioassay in F344/N rats (Table 4-4). 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 based on the NTP (1989) subchronic study (Section 4.2.1.1) 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 exposed 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 significantly higher than controls. Although mean severity scores did not show more than a 15% increase over control in the high-dose group, examination of the severity of nephropathy revealed more moderate and marked nephropathy in exposed male rats compared with predominantly mild nephropathy in controls (Table 4-4).

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 significantly increased in both exposure 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 severity of nephropathy showed more mild and moderate nephropathy in exposed females compared with predominantly less than minimally severe nephropathy in controls. Females did not exhibit marked nephropathy in the control or exposed groups (Table 4-4).

Table 4-4   Incidence and severity of nephropathy in male and female rats exposed to 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]	44[b]
Total number 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, $p < 0.01$.
cMean $\pm$ SE.

Source: NTP ([1989](#)).

To identify HCE-related kidney nephropathy, EPA compared the incidences of more severe (moderate and marked severity) nephropathy between the control and exposed male rats. Incidences of moderate or marked nephropathy in males were 18/50, 24/50, and 30/50 in the control, 7, and 14 mg/kg-day dose groups, respectively. Similar to the male rats, the incidences of more severe (mild and moderate) nephropathy were considered in female rats. Incidences of mild or moderate nephropathy in females were 12/50, 25/50, and 32/50 in the control, 57, and 114 mg/kg-day dose groups, respectively.

Additional kidney effects were noted in male rats (Table 4-5). Linear mineralization of the renal papillae 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 increased in exposed male rats (14% in both 7 and 14 mg/kg-day HCE dose groups) compared to 0% of controls. Nonneoplastic lesions such as casts (4%), cytomegaly (4%), chronic inflammation (4%), and focal necrosis (2%) were observed in 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.

Additional kidney effects noted 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 mg/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 exposed 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 4-5   Additional kidney effects in HCE-exposed 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 7 mg/kg-day as the male LOAEL, 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 moderate) of nephropathy. Male and female NOAELs could not be established because renal effects were observed at the lowest doses tested.

Incidence of renal tubular hyperplasia increased in exposed male rats: 4/50 (8%) in the 7 mg/kg-day dose and 11/50 (22%; 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%, 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; $0.3 \pm 0.8\%$) and in NTP studies (10/1,943; $0.5 \pm 0.9\%$).

Table 4-6   Renal tubular hyperplasia and tumor incidences in HCE-exposed 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. Although males were exposed to less HCE than the corresponding females, exposed male rats demonstrated more severe nephropathy than the exposed female rats. NTP (1989) also observed more severe nephropathy in control male rats (i.e., mild nephropathy) than in control females (i.e., minimal nephropathy). Male rats, but not

female rats, also exhibited renal hyperplasia and tumors. NTP (1989) indicated that the renal hyperplasia and tumors observed in the HCE-exposed male rats represented a morphologic continuum.

Effects in the adrenal gland were also noted in HCE-exposed 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 (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-day group (26/45, 58%). The increase of pheochromocytomas in the 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 (i.e., pheochromocytomas containing nervous tissue in addition to adrenal medullary cells) between controls and exposed male rats. The combined incidence of all three types of pheochromocytomas was significantly increased in males from the 7 mg/kg-day dose group (62%), but not in males from the 14 mg/kg-day dose group (43%), when compared with vehicle controls (30%), historical controls in the study laboratory (75/300; $25 \pm 7\%$), and historical controls in NTP studies (543/1,937; $28 \pm 11\%$). NTP concluded that the increased incidences of pheochromocytomas in male rats were possibly treatment-related.

Table 4-7   Adrenal medullary lesions in HCE-exposed 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).

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 un exposed and vehicle controls. Starting in week 23, exposed rats began a 5-week cyclic rotation that involved 1 week without exposure followed by dosing for 4 weeks. After adjustment to continuous exposure, the TWA doses were 113 and 227 mg/kg-day.

Mortality was accelerated in the HCE-exposed rats and the authors 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

unexposed controls and 11/20 (55%) in vehicle controls (seven rats in the vehicle control group were sacrificed in week 60). Mortality in the exposed groups occurred early in the bioassay. Approximately 20% of the high- and low-dose males died by week 15 and week 45, respectively, compared with 90 weeks to reach 20% mortality for the controls. Survival rates for the female rats were 14/20 (70%) for both the unexposed 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 week 25 and week 30, respectively, compared with 110 weeks to reach 20% mortality for the controls.

Chronic inflammatory kidney lesions were observed in both control and exposed rats: male rats exhibited incidences of 15/20 (75%) in unexposed 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 unexposed 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 unexposed 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 established because renal effects were observed at the lowest dose tested.

Tumor types exhibited by male rats 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 in male rats gavaged with HCE

Tumor type	Vehicle control[a]	113 mg/kg-day[a]	227 mg/kg-day[a]
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. Rat strain : Osborne-Mendel.

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 granulosa cell tumors (Table 4-9). Kidney hamartomas were observed in 6% of the females administered 227 mg/kg-day HCE, while no kidney hamartomas 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. Incidences of pituitary chromophobe adenomas, thyroid follicular cell adenoma or carcinomas, and mammary gland fibroadenomas were lower in HCE-exposed animals than in controls. In the low-dose group, ovary granulosa cell tumors were increased compared to controls, although none of the females in the high-dose group exhibited ovary granulose tumors. NCI (1978) noted that all these tumor types had been encountered previously as spontaneous lesions in the Osborne-Mendel rat, and the authors observed no statistical differences in frequencies between exposed 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	–

Rat strain: Osborne-Mendel.

Source: NCI (1978).

NCI (1978; Weisburger, 1977) also conducted a chronic 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 termination of exposure, animals were observed for 12–13 weeks for a total duration of

90–91 weeks. Twenty mice/sex were included as unexposed 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 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 un exposed 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 unexposed 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 unexposed male control groups, NCI compared tumor incidences in the dosed males and females to vehicle control data pooled from bioassays for hexachloroethane, trichloroethane, and 1,1,2-trichloroethane. NCI reported that animals were all of the same strain, housed in the same room, intubated with corn oil, tested concurrently for at least 1 year, and examined by the same pathologists.

Chronic inflammation of the kidney was observed in control and exposed male mice: 67, 80, 66, and 18% of unexposed 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 unexposed 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 unexposed or pooled vehicle controls of either sex, but was observed in mice exposed 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 and no renal tumors were observed in either sex. EPA considered 360 mg/kg-day as the LOAEL for this study based on tubular nephropathy. A NOAEL was not established because renal effects were observed at the lowest dose tested.

The incidence of hepatocellular carcinomas increased 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 incidence of hepatocellular carcinomas, 40 and 31% of 360 and 722 mg/kg-day females compared with 3 and 10% of pooled vehicle and matched vehicle controls, respectively. The increase in hepatocellular carcinoma in HCE-exposed females was not dose-dependent, with a higher incidence of 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 (NCI, 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 unexposed 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 (B6C3F$_1$) 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

Only one study in the peer-reviewed literature 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 included neurotoxicity, reduced body weight gain, increased organ weights, and evidence of respiratory tract irritation.

Weeks et al. (1979) exposed male, female, and pregnant female Sprague-Dawley rats (21-25/sex/concentration) to control air, 145, 465, and 2,517 mg/m^3 HCE (purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observation was carried out for 12 weeks. An O$_2$ consumption test was also conducted in male rats. The authors reported that male rats in the 2,517 mg/m^3 group had reduced body weight gain of male rats beginning in the third week of exposure, although quantitative information was not reported. The authors reported that nonpregnant female rats, in the 2,571 mg/m^3 did not reduced have body weight gain. 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. Although quantitative information was not reported, the authors reported that the high dose male rats had significantly increased relative kidney, spleen, and testes weights; in the high dose female rats, only relative liver weights were significantly increased. One male and one female rat exposed to 2,517 mg/m^3 HCE died during the fourth week, but the authors did not report a cause of death. During the 12 week postexposure observation period, exposure related effects disappeared. No gross changes were evident at necropsy after the postexposure observation period; however, male and nonpregnant female rats from the 2,517 mg/m^3 group (sacrificed immediately after the 6 week inhalation exposure) had a higher incidence and severity of mycoplasma-related lesions in nasal turbinates, trachea, and lung compared with controls. The authors concluded that these lesions were related to potentiation of an endemic mycoplasma infection rather than a direct effect of HCE exposure. However, no data were presented demonstrating the presence of mycoplasma 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 O$_2$ 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 prior to (15%) and after (13%) exposure to HCE. 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 examined male Sprague-Dawley rats (15/concentration) to 145, 465, or 2,517 mg/m^3 HCE for 6 hours/day, 5 days/week for 6 weeks and examined them for behavioral changes (see Section 4.4.3.2). 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 compared with controls, although quantitative information not reported.

Weeks et al. (1979) also exposed four male Beagle dogs/concentration to control air, 145, 465, and 2,517 mg/m^3 HCE (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 and the 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 postexposure. 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, 145, 465, and 2,517 mg/m^3 HCE (purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observation was carried out for 12 weeks. Guinea pigs were evaluated for sensitization potential following inhalation exposure to HCE. Two guinea pigs died during the fourth week and two guinea pigs died in the fifth week. 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, although 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,* 10/sex/concentration) to control air, 145, 465, and 2,517 mg/m^3 HCE (purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observation was carried out for 12 weeks. The only observed effect after 6 weeks was excess mucus in nasal turbinates in 2/10 quail in the 2,517 mg/m^3 group. 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 as the NOAEL, while the LOAEL could not be established from this study because no effects were observed at highest dose tested.

4.2.2.2 **Chronic Exposure and Carcinogenicity**

No inhalation 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, significantly reduced body weight gain beginning on GD8, and an increased incidence of mucopurulent nasal exudates. Approximately 70% of the orally exposed 500 mg/kg group had upper respiratory tract irritation; 20% had subclinical pneumonitis, compared with 10% in controls.

None of the fetuses exhibited significant skeletal or soft tissue anomalies. Fetuses from the 500mg/kg HCE exposure group displayed significantly lower gestation indices, lower numbers of viable fetuses/dam, and higher fetal resorption rates compared with controls (data not reported in the publication). It was unclear if the authors used a litter-based design for statistical analyses of fetal gestational indices, and the authors did not provide incidence data on the fetal gestational indices. EPA considered the maternal NOAEL and LOAEL as 100 and 500 mg/kg, respectively, based on neurological effects (tremors) and body weight decreases. EPA considered the developmental NOAEL and LOAEL to be the same as the maternal values, based on decreased viability and increased resorption rates.

Shimizu et al. (1992) evaluated HCE teratogenicity by administering of 0, 56, 167, or 500 mg/kg HCE (purity not specified) to pregnant Wistar rats (20–21 rats/dose) via gavage during GDs 7-17. Dams in the 500 mg/kg dose group exhibited significantly decreased weight gain after the second day of HCE treatment (8th day of pregnancy); dams in the 167 mg/kg dose group displayed significantly decreased weight gain after the fourth day of treatment (10th day of pregnancy), but not after the exposure ended. 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 exposure ended. Dams in both the 167 and 500 mg/kg dose groups exhibited decreased motor activity, although incidence and method of analysis was 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 GD20, 3 days post-HCE exposure, revealed three dams 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 exposure and control groups with respect to the numbers of corpora lutea, implants, or 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 (Table 4-11). Male and female fetuses in the 500 mg/kg dose group also displayed significantly decreased body weight compared to controls (Table 4-11). The authors stated that the litter was used as the statistical unit for calculations of the fetal values.

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	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 $\pm$ SD.

Source: Shimizu et al. (1992).

The investigators (Shimizu et al., 1992) examined the fetuses for external anomalies. One fetus in the 500 mg/kg dose group had no tail. Other external 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 group compared with controls (Table 4-12). Skeletal variations were increased in the 500 mg/kg group and the 167 mg/kg group, with the increase in the 500 mg/kg group statistically significant compared with controls (Table 4-12). The degree of ossification (including numbers of sternebrae, proximal and middle phalanges, and 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 concluded that there was no indication of teratological effects in rats for dose levels of HCE below 500 mg/kg. The authors stated that the litter was used as the statistical unit for calculations of the fetal values. Shimizu et al. (1992) established a NOAEL of 56 mg/kg for dams and 167 mg/kg for fetuses. EPA considered 167 mg/kg-day the LOAEL for dams, based on decreased motor activity and significantly decreased body weight. EPA considered 500 mg/kg LOAEL for fetuses, based on significantly increased skeletal variations, significantly decreased ossification, and significantly decreased 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	127
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. (1992), the litter was used as the statistical unit for calculation of fetal values; thus, these values represent the means $\pm$ 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, 145, 465, and 2,517 mg/m^3 HCE (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 GD8 for the 2,517 mg/m^3 group, and beginning on GD14 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 mycoplasma infection.

Fetuses of HCE-exposed dams did not exhibit any significant skeletal or soft tissue anomalies. It was unclear from the publication if a litter-based design was used for the statistical analysis of the fetal gestational indices, and incidence data for the fetal gestational indices were not provided. EPA considered 465 mg/m^3 the NOAEL for the dams and 2,517 mg/m^3 the LOAEL for the dams, based on neurological effects (tremors). EPA considered 2,517 mg/m^3 as a developmental NOAEL, while a developmental LOAEL could not be established because no effects were observed at the highest dose tested.

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 4,460 to 7,690 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 6,155 mg/kg HCE (purity not specified) by gavage in mineral oil to male rats and reported that liver function was unaffected 2 hours after exposure. Kinkead and Wolfe (1992) determined that 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 limit [CL]: 2,332–8,640 mg/kg).

Weeks et al. (1979) and Weeks and Thomasino (1978) determined acute oral toxicity values for Sprague-Dawley rats, NZW 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 gavage. 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

Species	Treatment	Diluent	Lethal value		
			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	4,460	3,900–5,110	9.3
		Methylcellulose	7,080	6,240–8,040	19.9
Rat, male	Oral LD_{50}	Corn oil	5,160	4,250–6,270	6.1
		Methylcellulose	7,690	6,380–9,250	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. 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. Increases in these enzymes are indicative of hepatic damage. In addition, bromsulphthalein 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**

Median lethal concentration (LC_{50}) values for HCE have not been reported. One study has evaluated single acute inhalation exposures to HCE (Weeks and Thomasino, 1978). Six male rats/concentration (strain not specified, although one table in the report indicated strain as Sprague-Dawley) were exposed to 2,500 or 57,000 mg/m^3 HCE for 8 hours and to 17,000 mg/m^3 HCE 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 effects either during exposure or for 14 days thereafter. Body weight gain was reduced, but not statistically significantly, over the 14-day observation period. Male rats exposed for 8 hours to 57,000 mg/m^3 HCE displayed effects, including death. At 6 hours, one rat had a staggered gait and by 8 hours, 2 rats were dead. The surviving rats showed statistically significant reductions in mean body weight on postexposure days 0 (7%), 1 (21%), 3 (19%), 7 (15%), and 14 (15%), compared with controls. Necropsy did not reveal any gross exposure-related lesions. 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 exposed rat. The authors concluded that this effect was not exposure-related, but rather was indicative of a low-grade endemic upper respiratory disease. The male rats exposed for 6 hours to 17,000 mg/m^3 showed reductions in body weight gain on postexposure day 1 (5%) and day 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**

Three animal studies have evaluated short-term toxicity of HCE. A 12-day study in male NZW 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) administered 100, 320, or 1,000 mg/kg-day HCE (purity 99.8%) via a stomach tube to male NZW rabbits (5/dose) for 12 days. 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. Rabbits were necropsied on the fourth day after termination of dosing, 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 exposure 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 effects. 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, with more severe effects 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 100 mg/kg the NOAEL and 320 mg/kg the LOAEL, 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 died before the end of the study, while 1 male and 2 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. Histopathology revealed hyaline droplet formation in the cytoplasm of renal tubular epithelium in the kidneys of all exposed 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 the male rat LOAEL based on kidney tubule lesions, while a NOAEL for male rats could not be established because effects were observed at the lowest dose. EPA considered 563 mg/kg-day the female rat LOAEL, based on a dose-related decrease in body weight, and 281 mg/kg-day the female rat NOAEL.

NTP (1996) administered 146 or 293 mg/kg-day HCE (purity 100%) via corn oil gavage to male F344/N rats (5/dose) for 21-days. All rats were necropsied; 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 to evaluate protein droplets, particularly hyaline droplet formation, in kidney sections. 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 liver weights were increased in both dose groups (significant at high dose). Rats of the 293 mg/kg-day group also exhibited significantly lower urinary creatinine and specific gravity, while glucose and urine volumes were greater than controls. AST and NAG activities were significantly higher than in controls in both dose groups (Table 4-14). Nephropathy (i.e., hyaline droplet accumulation) was observed in the male rats, as were 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 in both dose groups compared with controls (Table 4-14). EPA considered 146 mg/kg-day a LOAEL based on statistically significant increases in kidney lesions and urinalysis parameters. A NOAEL could not be established because effects were observed at the lowest dose tested (Table 4-14).

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)	$11.041 \pm 0.291(4)$	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	56.48 ± 3.06^b
Glucose (μg/mg creatinine)	169 ± 3	344 ± 30	446 ± 23^b
Protein (mU/mg creatinine)	$1,322 \pm 59$	$1,748 \pm 257$	$2,980 \pm 103$
AST (mU/mg creatinine)	6 ± 1	40 ± 6^c	66 ± 5^b
GGT (mU/mg creatinine)	$1,456 \pm 47$	$1,547 \pm 66$	$1,897 \pm 73$
NAG (mU/mg creatinine)	11 ± 0	23 ± 2^c	36 ± 1^b
Volume (mL/16 h)	4.2 ± 0.8	7.5 ± 0.9	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 $\pm$ SE)	0.13 ± 0.02	0.74 ± 0.19^c	1.2 ± 0.2^c

[a]Data are mean $\pm$ SE. (For all groups, n=5, unless otherwise noted).
[b]Significantly different from control ($p \leq 0.01$, Dunnet's test)
[c]Significantly different from control ($p \leq 0.05$, Dunnet's test)

Source: NTP (1996).

4.4.3   Neurological

Several studies have provided evidence that oral and inhalation HCE exposure produced central nervous system (CNS) effects; however, it is unknown if the neurological effects were due to the parent compound or the metabolites. Sheep exposed to HCE developed facial muscle tremors (Fowler, 1969; Southcott, 1951) and a staggering uncoordinated gait (Southcott, 1951). Sprague-Dawley rats did not show statistically significant effects of HCE exposure on avoidance latency or spontaneous motor activity. However, male rats, female rats, and pregnant rat dams exhibited tremors and/or ruffled pelt following HCE exposure (Weeks et al., 1979). Beagle dogs developed signs of neurotoxicity following HCE exposure. (Weeks et al., 1979).

4.4.3.1   Oral Studies

Fowler (1969) orally administered acute doses of HCE to sheep (see Section 4.4.1.1) and reported slight facial muscle tremors 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 in the study. EPA considered the 500 mg/kg LOAEL, based on neurotoxic effects (tremors), while a NOAEL could not be established from these data because effects were observed at the lowest dose tested.

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. One day after treatment, two sheep died 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.

Shimizu et al. ([1992](#)) reported decreased motor activity (incidence and method of analysis not reported) in pregnant Wistar rats following gestational exposure to 167 or 500 mg/kg HCE(see Section 4.3.1). These effects decreased or disappeared when HCE exposure ended. Similarly, Weeks et al. ([1979](#)) reported that pregnant Sprague-Dawley rats gestationally exposed to 500 mg/kg HCE displayed tremors on GDs 15 and 16 (see Section 4.3.1).

4.4.3.2 Inhalation Studies

Weeks et al. ([1979](#)) examined neurological effects in male Sprague-Dawley rats (15/concentration) exposed to air, 145, 465, or 2,517 mg/m^3 HCE (purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Learned behavior was evaluated using an avoidance latency task by measuring the time it took the rats to avoid foot shock by escaping into a safe compartment. Unlearned behavior (i.e., spontaneous motor activity) was evaluated by photobeam interruptions. 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 and spontaneous motor activity counts were increased in the 465 and 2,517 mg/m^3 groups at 6 weeks compared with control, but the differences were not statistically significant. Weeks et al. ([1979](#)) concluded that the rats did not display signs of behavioral toxicity. However, Weeks et al. ([1979](#)) noted tremors and a ruffled pelt in male and female rats exposed to 2,517 mg/m^3 HCE during the fourth week of exposure in a separate experiment (see Section 4.2.2.1). In addition, Weeks et al. ([1979](#)) reported in a developmental toxicity experiment (see Section that pregnant Sprague-Dawley rat dams exposed to 2,517 mg/m^3 group displayed tremors during GDs 12–16 (see Section 4.3.2). Tremors are indicators of neurobehavioral effects and lack of grooming could be interpreted as an indicator of behavioral toxicity ([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](#)) reported that Beagle dogs exposed to 2,517 mg/m^3 HCE developed tremors, ataxia, hypersalivation, and displayed severe head bobbing, facial muscular fasciculations, and held their eyelids closed during exposure (see Section 4.2.2.1). 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

In a subchronic study, Weeks et al. (1979) exposed male Hartley guinea pigs to HCE via inhalation (see Section 4.2.2.1). Two weeks after termination of exposure, guinea pigs were 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 human patients suffering from systemic scleroderma (SSD) and localized scleroderma with bilateral distribution of multiple skin lesions for potential solvent exposure. Of nine such patients, seven had significant subchronic or chronic exposure (5–44 years) to solvents, while an eighth had a significant acute exposure (2 weeks) to solvents. The solvents involved were reported as "variable and mostly unidentified." As an experimental follow-up, Yamakage and Ishikawa (1982) administered daily intraperitoneal (i.p.) injections with 0.01 mL of HCE (purity not specified), as well as with 0.9% saline to mitigate exposure lethality, for 17 days in ddY mice (17 mice total). 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 one mouse, and no change in nine mice; two mice died. Even though this experimental route of exposure 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 provided indirect evidence that suggests that HCE may be capable of inducing SSD-type conditions in humans.

Weeks and Thomasino (1978) conducted two dermal studies in male NZW rabbits. A single 24-hour application of 500 mg of dry technical-grade 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, 500 mg 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 technical grade HCE to one eye of each of six male NZW 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 of HCE have not been performed in humans. In vivo HCE exposure in animals resulted in predominantly negative results. Similarly, in vitro HCE genotoxicity studies conducted in microorganisms, cultured mammalian cells, and insects (Table 4-15) were largely negative both in the presence and absence of exogenous metabolic activation. HCE did not induce mutagenicity in *Salmonella typhimurium* reverse mutation tester strains (Ashby and Tennant, 1988). The NTP toxicology and carcinogenesis studies concluded that HCE (purity >99%) was not significantly genotoxic in F344/N rats, and that increased tumors incidence occurred through a mechanism other than induction of mutations (NTP, 1989). In an examination of available mutagenicity and genotoxicity data (i.e., the ability to induce alterations in deoxyribonucleic acid [DNA] structure or content, gene mutation, chromosomal aberrations [CAs], or aneuploidy) from short-term tests with putative "nongenotoxic" carcinogens, HCE was categorized as having insufficient mutagenicity 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 indicated that the genetic activity profile for HCE is predominantly negative. 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)	Simmon and Kauhanen (1978)	
		TA98, TA100, TA1535, TA1537, TA1538	– (±S9)	Weeks et al. (1979)	
		TA98, TA100, TA1535, TA1537	– (±S9)	Haworth et al. (1983)	Liquid preincubation protocol
		TA98, TA100, TA1535, TA1537	– (±S9)	Milman et al. (1988)	
	Forward mutations	BA13	– (±S9)	Roldán-Arjona et al. (1991)	Liquid preincubation protocol
	SOS test	TA1535/pSK1002	– (±S9)	Nakamura et al. (1987)	*umu* test; Liquid preincubation protocol
Mammalian	CAs	Chinese hamster ovary (CHO)	– (±S9)	Galloway et al. (1987)	
	SCEs	CHO	– (–S9), + (+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)	Simmon and Kauhanen (1978)	
		S. cerevisiae D4	– (±S9)	Weeks et al. (1979)	
		S. cerevisiae D7	– (±S9)	Bronzetti et al. (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)	

				Yoshikawa (1996); Miyagawa et al. (1995)	Hepatic cell proliferation
	Replicative DNA synthesis (RDS)	B6C3F1 mice	+		
		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
Drosophila	Mitotic recombination	Drosophila	Weakly +	Vogel and Nivard (1993)	Eye mosaic assay

Using the standard Ames assay for reversion of *S. typhimurium* histidine tester strains (TA1535, TA1537, TA1538, TA98, and TA100), Simmon and Kauhanen (1978) found 5,000 or 10,000 μg HCE/plate (purity not specified) to be nonmutagenic, both in the absence and presence of an exogenous Aroclor 1254-stimulated rat liver 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 that a concentration range of 0.1–500 μg HCE/plate (purity 99.8%) yielded negative results using the same tester strains, test protocol, solvent, and metabolic activation system. Further, as a part of NTP's mutagenicity screening program, a concentration range of 1–10,000 μg/plate HCE was dissolved in dimethylsulfoxide (DMSO) and tested in two independent trials in two separate laboratories. HCE was negative 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 negative in several Ames tester strains, both with and without S9 metabolic activation (Milman et al., 1988).

Using a different *S. typhimurium* indicator strain, BA13, that could theoretically detect a broader range of mutagens than reverse-mutation assay, Roldán-Arjona et al. (1991) found HCE to be negative. 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. HCE did not demonstrate any toxicity, which the authors speculated was probably related to its low solubility in water. HCE (purity not specified) was negative when assayed in the *umu* test using *S. typhimurium* tester strain TA1535/pSK1002 (Nakamura et al., 1987). This study was also conducted both with and without rat liver S9 metabolic activation up to a concentration of 42 μg/mL (the solvent was not specified). Although the available data indicated 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 1–50 mg/mL HCE (purity not specified) was observed, either with or without exogenous rat liver S9 metabolic activation. In addition, negative findings for HCE were reported by Weeks et al. (1979) using the *S. cerevisiae* D4 strain.

Bronzetti et al. ([1989](#)) evaluated HCE for mitotic gene conversion at the trp locus and reverse point mutation at the ilv locus in the *S. cerevisiae* D7 tester strain. Exposures were from 1.2–3.0 mg/mL HCE (purity not specified) 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](#)).

HCE concentrations from 0.005–0.84 mg/mL HCE (purity >98%) to induce aneuploidy in the P1 diploid strain of the mold *Aspergillus nidulans* has been evaluated ([Crebelli et al., 1995](#); [Crebelli et al., 1992](#); [Crebelli et al., 1988](#)). Liquid suspension exposures (3 hours) resulted in survival rates of 100–48%. Exposure to these concentrations did not induce mitotic malsegregation of chromosomes.

Studies have evaluated the effects of in vivo and in vitro HCE exposures on cytogenetic endpoints in animals ([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 (MNPCEs) by HCE. CD-1 mice (5/sex/concentration) were injected i.p. with 2,000 or 4,000 mg/kg HCE (purity >98%), representing approximately 40 and 70–80% of the LD_{50}, respectively. Animals were sacrificed and bone marrow cells were harvested at 24 and 48 hours postexposure. HCE exposure resulted in clinical signs of general toxicity, but no significant increases in the frequency of MNPCEs.

Vogel and Nivard ([1993](#)) utilized a Drosophila eye mosaic assay to monitor genetic damage caused by the exposure of larvae to HCE. Drosophila larvae were exposed to food supplemented with 10 mM HCE (3% ethanol solvent; purity not specified) for 3 days. Based on their results, the authors classified HCE as a weak positive —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 not likely 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. Lymphocytes from one donor were exposed to 0.012–0.24 mg/mL HCE (purity >99%)in the presence of S9. Neither toxicity nor MN induction was evident. Cells from the other donor were exposed to 0.24–3.79 mg/mL HCE, both with and without S9. Although toxicity was not reported, statistically significant increases in the percent of cells with MN were observed at 0.24 and 1.89 mg/mL HCE concentrations in the absence of S9 (12 and 11%, respectively, versus a control value of 5.5%, $p < 0.05$). In presence of S9, statistically significant increased in MN were observed at 0.24 mg/mL (19.8% versus a control value of 9%, $p < 0.01$). In the second part of the study, lymphocyte cultures exposed to HCE for 3 hours with and without S9 were assessed for DNA damage 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, 0.002, 0.012, or 0.024 mg/mL HCE (purity not specified), then scored for kinetochore-positive and -negative MN. No MN formation was observed in any of the three cell lines in response to HCE exposure.

HCE was investigated for induction of CAs and SCEs in cultured Chinese hamster ovary (CHO) cells as part of an NTP screening program for genotoxicity (NTP, 1989; Galloway et al., 1987). HCE concentrations, selected on the basis of cell confluence and mitotic cell availability, ranged from 0.01–1.0 mg/mL HCE (purity >99%). For CAs and SCEs, linear regression was used to test for dose-response trends. Induction of CA was considered significant if p values relative to controls were ≤ 0.05, while increases of SCEs/chromosome $\geq 20\%$ over controls were considered significant. For CAs, the durations of exposure were 8–10 hours in the absence of S9 metabolic activation and 2 hours in the presence of S9. For induction of SCEs, exposure durations were 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, 0.15–0.50 mg/mL HCE did not induce CAs; however, 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). Exposure to 0.010–0.33 mg/mL HCE did not induce SCE in the absence of S9; however, positive results for 0.10–1.0 and 0.40–1.0 mg/mL HCE were obtained in the presence of S9.

In vitro cell transformation studies have examined the effect of HCE on the process of chemical carcinogenesis. In the absence of exogenous metabolic activation, a 3-day exposure to 0.00016–0.100 mg/mL HCE (purity >97%) did not induce morphological cell transformation in BALB/c-3T3 cells (Milman et al., 1988; Story et al., 1986; Tu et al., 1985). In a rat liver foci assay, Story et al. (1986) and Milman et al. (1988) examined the capacity of HCE to initiate and promote tumors. To assess initiation potential, 10 young adult male Osborne-Mendel rats received 500 mg/kg HCE (the MTD) by corn oil gavage 24 hours after partial hepatectomy. 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 i.p. injected with 30 mg of the tumor initiator, diethylnitrosamine (DEN) 24 hours after partial hepatectomy. Six days later, the animals received 500 mg/kg of HCE (the MTD) in corn oil by gavage, 5 days/week for 7 weeks. Animals were sacrificed and livers were examined for the presence of GGT-positive foci. In these assays, HCE failed to demonstrate any initiating activity, but did show statistically significant promoting capability (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: Promotion protocol

Promotion treatment	Total number of foci/cm^{2,a}	
	+ DEN initiation	- DEN initiation
HCE	4.38 ± 1.07^{b}(7)	0.1 ± 0.15(8)
Phenobarbital	3.89 ± 0.98^{b}(10)	0.3 ± 0.19(9)
Corn oil	1.77 ± 0.49^{c}(10)	0.2 ± 0.15(10)

[a]Means $\pm$ SE (with the number of animals in parentheses)
[b]Statistically different from DEN + corn oil control group, $p < 0.05$
[c]Statistically different from corn-oil-only (−DEN) control group, $p < 0.05$

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

Yoshikawa and colleagues reported on the effect of HCE exposure 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 administered single gavage doses of 0, 1,000, or 2,000 mg/kg HCE (purity not specified). Hepatocytes were prepared 24, 39, or 48 hours after exposure. Hepatocytes prepared 39 hours after exposure to 1,000 mg/kg HCE yielded a positive mean RDS response of $1.21 \pm 0.46\%$ (the investigators noted that an RDS incidence rate of 0.4% was considered a positive response). The remaining HCE exposure groups were negative with mean responses of 0.15–0.35%, while the solvent control mean was $0.26 \pm 0.17\%$.

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

In vitro and in vivo assays have assessed the ability of HCE to bind DNA, ribonucleic acid (RNA), and protein in several mouse and rat tissues (Lattanzi et al., 1988). In vitro and in vivo assays in mice and rats have demonstrated binding of radiolabeled carbon to DNA, RNA, and protein following [^{14}C]-HCE administration (Lattanzi et al., 1988), suggesting that either HCE or its metabolites bind these macromolecules. The role of macromolecule binding in mediating HCE toxicity was not further evaluated.

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 i.p. injected with 127 μCi/kg HCE (purity 98%; ~1 mg /kg HCE). The animals were fasted and sacrificed 22 hours after injection. 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. Measures for binding to macromolecules were determined by the presence of radiolabeled carbon from [^{14}C]-HCE in the DNA, RNA, and protein; however, HCE-specific metabolites were not measured. Therefore, 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. In both rats and mice, binding values (in pmol HCE/mg) for RNA were consistently greater than binding values for DNA or protein. Greater RNA binding 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 higher levels (9 times greater) of covalent binding for DNA in the liver than the rat. Binding to liver RNA and liver protein was 2 and 3 times greater in mice than rats, respectively. DNA binding was similar between species, but slightly greater in mice, for the kidney, lung, and stomach. According to Lutz (1986, 1979), the covalent binding index (CBI) values indicate weak (rat liver) to moderate (mice liver) oncogenic potency in HCE-exposed rodents.

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

(pmol/mg)	Liver[a]		Kidney[a]		Lung[a]		Stomach[a]	
	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]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 oncogenes.
[c]Mean ± SE of six individual values.
[d]Mean ± SE of four values, each obtained from three pooled livers.

Source: Reprinted with permission of Taylor & Francis©; Lattanzi et al. (1988).

In vitro binding data for HCE are presented in Table 4-18. Coenzymes were not utilized in the controls. Liver microsomes from rats and mice catalyzed HCE binding to DNA at comparable levels. Kidney microsomes from rats and mice produced significantly greater amounts of HCE binding to DNA. 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 compared to controls. Microsomes from lung and stomach in both species did not display increased DNA binding activity over corresponding controls. Cytosolic fractions from all organs in mice and rats exhibited higher levels of HCE binding to DNA than microsomal fractions, except for rat lung cystol. 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 compared to cytosolic fractions alone for liver. SKF 525-A, a nonspecific CYP450 inhibitor, caused a 50.5% decrease in HCE binding to DNA (data not included in report). Lattanzi et al. (1988) stated that addition of GSH to the microsomal fractions also resulted in inhibition of HCE binding to DNA. When microsomal and cytosolic fractions were heat-inactivated, HCE binding to DNA was similar to control. This study provided evidence that HCE is metabolized by microsomal CYP450 enzymes and cytosolic GSH 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: Reprinted with permission of Taylor & Francis©; Lattanzi et al. (1988).

4.5.3   Structure Activity Relationships

Several studies were conducted with the objective of defining structure activity relationships (SARs) between 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 (see Section 4.4.2). Chemicals under investigation were 1,1,1,2-tetrachloroethane, 1,1,2,2-tetrachloro-ethane, 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%). 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) was observed in rats administered pentachloroethane, 1,1,1,2-tetrachloroethane, and HCE. RDS, indicated by PCNA labeling index, was increased in male rats administered pentachloroethane, 1,1,1,2-tetrachloroethane, HCE, pentabromoethane, and 1,1,2,2-tetrachloroethane. The increase in cell proliferation in the kidneys observed with 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.

Crebelli et al. ([1988](#)) evaluated three chloromethanes and eight chlorinated ethanes (including HCE) for the induction of chromosome malsegregation in the fungus *A. nidulans* (see Section 4.5.1). Although 8 of the 11 compounds tested provided positive results, HCE was negative for chromosome malsegregation induction. Analyses of relationships between biological and chemical variables indicated 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, the authors argued 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 fungus, *A. nidulans* (see Section 4.5.1). Out of the 24 chemicals, 19 were negative for the induction of chromosome malsegregation; 5 chemicals produced reproducible increases in the frequency of euploid whole chromosome segregants. HCE was negative for the induction of chromosome malsegregation. 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. 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](#); [Crebelli et al., 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 that 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

No epidemiology studies of HCE carcinogenicity were identified. Case studies in humans have demonstrated HCE exposure; however limitations of these studies include lack of information on the source and route of HCE exposure, co-exposures, and small sample size (Loh et al., 2008; Loh et al., 2006; Younglai et al., 2002; Seldén et al., 1997; Seldén et al., 1994; Seldén et al., 1993; Allen et al., 1992; Seldén et al., 1989). Oral toxicity studies in laboratory animals are summarized in Table 4-19. The primary noncancer effects observed in animal studies included decreased body weight or body weight gain, increased absolute and relative kidney weights, increased absolute and relative liver weights, 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.

Table 4-19   Oral toxicity studies for HCE

Species	Concentration (mg/m^3)/ duration[a]	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Effect	Reference
NZW 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, granular casts) 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: decreased organ weights, kidney effects in all dose groups Female: decreased organ weights, 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: proximal tubule degeneration at highest dose	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; 78 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: 7 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 Developmental: 167	Maternal: 167 Developmental: 500	Maternal: decreased weight gain and motor activity Fetal: reduced body weight increased incidence of skeletal variations; decreased ossification	Shimizu et al. (1992)

Acute and short-term toxicity tests in animals reported liver necrosis and tubular nephrosis in male rabbits (Weeks et al., 1979; Weeks and Thomasino, 1978), 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 (NTP, 1989). Oral LD_{50} values in rats ranged from 4,460 to 7,690 mg/kg (Weeks et al., 1979).

4.6.1.1 **Nephrotoxicity**

Three short-term studies have reported nephrotoxic effects following oral HCE exposure (see Section 4.4.2). In a 16-day study in F344/N rats, 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 in male F344/N rats, increased absolute and relative kidney weights, tubular regeneration and granular casts, and increased PCNA labeling index in kidneys at doses of 146 and 293 mg/kg-day HCE (NTP, 1996). In a 12-day study with male NZW rabbits, tubular nephrosis and tubular nephrocalcinosis were observed following exposure to 320 and 1,000 mg/kg-day HCE (Weeks et al., 1979). Compared with rabbits, 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 short-term studies makes it difficult to evaluate if the observed renal effects were sex-specific. In addition, subchronic and chronic exposure studies reported nephrotoxic effects of HCE exposure in female rats.

Two subchronic exposure studies have reported nephrotoxic effects of oral HCE exposure (see Section 4.2.1.1). Male F344/N rats administered 34–536 mg/kg-day HCE for 13 weeks (NTP, 1989) exhibited hyaline droplet formation, tubular regeneration, and tubular casts. Male rats in the 536 mg/kg-day dose group also exhibited renal papillary necrosis and degeneration and necrosis of renal tubule epithelium. Female rats did not display kidney effects. Another subchronic exposure study (Gorzinski et al., 1985) in F344 rats reported slight hypertrophy and dilation of the renal tubules in males rats, as well as renal tubule atrophy and degeneration in male and female rats, following exposure to 15-62 mg/kg-day HCE for 16 weeks. Kidney effects in female rats were observed only at the 62 mg/kg-day HCE dose.

Two chronic studies have reported nephrotoxic effects of oral HCE exposure (see Section 4.2.1.2). Chronic toxicity studies were conducted by 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. The chronic toxicity test in B6C3F$_1$ mice (NCI, 1978) was the only study conducted in this species.

Nephropathy (characterized as tubular cell degeneration and regeneration, dilation and atrophy, glomerulosclerosis, interstitial fibrosis, and chronic inflammation) was observed in both male and female F344/N rats following HCE exposure for 103 weeks (NTP, 1989). 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. Nephropathy was reported in both control and exposed groups, likely as result of a spontaneous syndrome known as chronic progressive nephropathy (CPN) associated with aged rats (see Section 4.7.3.1). To

examine the effects of chronic HCE exposure separate from CPN, nephropathy incidence in terms of severity was evaluated. Male rats exposed to HCE showed a dose-related increase in the severity of nephropathy compared to controls: 36%, 48%, and 60% in control, 7, and 14 mg/kg-day HCE dose groups, respectively. Additional dose-related nephrotoxic effects in male rats included linear mineralization of the renal papillae and hyperplasia of pelvic transitional epithelium in the kidney (see Table 4-5). In female rats, increases in both incidence and severity of nephropathy were dose-related (see Table 4-4). Increased linear of renal papillae was also noted in female rats, but only at 57 mg/kg-day dose (see Table 4-5).

Osborne-Mendel rats of both sexes displayed chronic inflammatory kidney lesions in both control and exposed groups following 78 weeks of HCE exposure, 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 (NCI, 1978). There were dose-related increases in incidences of nephropathy in males and females administered 113 and 227 mg/kg-day HCE (see Section 4.2.1.2). B6C3F$_1$ mice of both sexes displayed chronic kidney inflammation and tubular nephropathy following HCE exposure for 78 weeks (see Section 4.2.1.2). In male B6C3F$_1$ mice, chronic kidney inflammation was observed at higher levels in the controls and low dose group than in the high dose mice; 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. In female B6C3F$_1$ mice, chronic kidney inflammation was observed only in vehicle controls and the 227 mg/kg-day HCE dose group (2%), whereas tubular nephropathy was observed in both exposure groups of both sexes at high incidences (see Section 4.2.1.2).

The available information for HCE-induced nephrotoxicity indicates that the male rat is the most sensitive sex/species to HCE-induced renal toxicity. Limited information available in other species indicates that nephrotoxic doses 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. Similarly, subchronic and chronic exposure studies in rats (NTP, 1989; Gorzinski et al., 1985) indicated that nephrotoxic doses in female rats were at least 4-fold greater than the lowest nephrotoxic dose in male rats. These toxicity data are consistent with the tissue distribution data (see Section 3.2) and demonstrating that the male kidney accumulated higher HCE concentrations than the female kidney and potential sex differences in the distribution and metabolism of HCE and support the kidney as the primary target organ following oral exposure to HCE.

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 ≥ 300 mg/kg-day HCE (see Section 4.4.2). Male F344 rats exhibited significantly increased relative liver weights at 293 mg/kg-day, and significantly increased AST and NAG serum activity. 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 NZW rabbits administered 320 and 1,000 mg/kg-day HCE 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 at least twofold more controls, indicating reduced hepatic function.

Two subchronic studies (NTP, 1989; Gorzinski et al., 1985) reported hepatotoxic effects in male and female rats exposed to HCE (see Section 4.2.1.1). Females were more sensitive than males, with severity and statistically significant increases in hepatoxicity occurring at lower doses than male rats. Liver weight to body weight ratios (mg/g) increased in a dose-related manner for both male and female rats exposed to HCE (Table 4-3). Hepatocellular necrosis was noted in females exposed to 134-536 mg/kg-day and in males exposed to 268-536 mg/kg-day (NTP, 1989). Gorzinski et al. (1985) reported a slight swelling of the hepatocytes in the 15 and 62 mg/kg-day dose groups. 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. Other than a statistically significant increase in liver weight at 62 mg/kg-day HCE, the females were not affected. This result contrasts the hepatocellular effects noted in female rats in the 13-week NTP study (1989). However, the highest dose used by Gorzinski et al. (1985), 62 mg/kg-day, is below the NOAEL(67 mg/kg-day) for female rats in the NTP (1989) study, indicating that exposure doses in Gorzinski et al. (1985) may have been too low to cause hepatotoxicity in female rats.

Although hepatocellular necrosis was observed in the subchronic study, no noncancerous liver effects observed in the rats and mice administered HCE for chronic durations. The range of doses in the subchronic assay [34 - 536 mg/kg-day in F344 rats (NTP, 1989)] encompassed the doses used in the chronic assays for female F344 rats and both sexes of Osborne-Mendel rats (57-227 mg/kg-day) (NTP, 1989; NCI, 1978). The LOAEL for hepatocellular necrosis in female F344/N rats [134 mg/kg-day (NTP, 1989)] in the subchronic study exceeded the highest dose of the chronic study (NTP, 1989) of F344/N rats, 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 at 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.

HCE-induced liver effects were only observed in animals in short-term and subchronic studies. 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. Female rats exhibited a greater sensitivity to liver effects as evidenced by the effects observed at lower doses compared with males (NTP, 1989). These data suggest that the female rat is the most sensitive sex/species to HCE hepatotoxicity.

4.6.1.3   **Developmental Toxicity**

Two developmental studies in rats (Shimizu et al., 1992; Weeks et al., 1979) indicated that oral HCE exposure induced teratogenicity in the presence of maternal toxicity (see Section 4.3.1). In the Shimizu et al. (1992) study, pregnant Wistar rats (gavaged with HCE) displayed decreased motor activity, piloerection, and subcutaneous hemorrhage. Fetuses from dams exposed to 500 mg/kg HCE displayed decreased body weight, skeletal variations such as rudimentary lumbar ribs, and ossification effects, but no skeletal malformations were observed. In Weeks et al. (1979), pregnant Sprague-Dawley rats gavaged with 500 mg/kg HCE displayed pulmonary effects, but fetuses did not exhibit any skeletal or soft tissue anomalies. These results indicate that oral exposure HCE resulted in teratogenic effects at doses that were also maternally toxic.

4.6.2   **Inhalation**

The database of inhalation toxicity studies on HCE is limited. Reports on HCE-induced human health effects are limited and confounded by co-exposure to multiple solvents or other toxicants (e.g., HCE-zinc oxide smoke). Studies observed HCE exposure in smoke bomb production workers, but the sample sizes were too small to provide definitive conclusions on health effects. The inhalation toxicity database of HCE in animals is limited to a single publication (Weeks et al., 1979). This study conducted acute exposure (see Section 4.4.1.2), subchronic (see Section 4.2.2.1), and developmental (see Section 4.3.2) toxicity studies with HCE. The data from this study are summarized in Table 4-20. Neurological effects, such as tremors and ataxia, were observed in Beagle dogs and in pregnant and nonpregnant female 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. 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). Overall, changes in body or organ weight and neurotoxic effects (e.g., tremors) were consistently observed across several species at the highest exposure dose (2,517 mg/m^3 HCE).

Table 4-20   Summary of data from the Weeks et al. (1979) inhalation toxicity study 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; 11 days	Maternal: 465 Developmental: 2,517	Maternal: 2,517 Developmental: Not established	Maternal: tremors[b], decreased body weight gain 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/m3 correspond to concentrations reported by Weeks et al. (1979) as 15, 48, and 260 ppm, respectively
[b]Incidence data on tremors was not reported by the study authors

4.6.3   Mode-of-Action Information

The mode of action for HCE-induced toxicity is unknown. The available data on mode of action for HCE toxicity is limited to animal studies. 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 (see Figure 3-1). It is unknown whether 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 (Gorzinski et al., 1985). This study did not quantify metabolite concentrations, so these estimations are of limited.

Neurological effects have been consistently observed following oral and inhalation exposure to HCE (Weeks et al., 1979) (Shimizu et al., 1992; Fowler, 1969; Southcott, 1951); however, these data for neurological effects of HCE exposure are limited are inadequate to determine a mode of action. Thus, the mode of action for HCE-induced neurotoxicity is unknown

Although HCE-induced nephropathy has been observed in both sexes of rats and mice (see Section 4.6.1.1), the mode of action for HCE-induced kidney toxicity is unknown. Some data suggest an

α_{2u}-globulin mode of action could contribute to HCE-induced nephropathy (see Section 4.7.3.1). 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 immunohistochemical data demonstrating α_{2u}-globulin in the hyaline droplets, (2) the hyaline droplet accumulation (caused by excessive protein load) may not be exclusively related to α_{2u}-globulin accumulation, and (3) the existence of renal toxicity in female rats, as well as 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 CPN (see Section 4.7.3.1) could contribute to the observed nephrotoxicity following HCE exposure. However, changes in the severity of the nephropathy were greater in HCE exposed male rats compared with controls, indicating that HCE exposure exacerbated effects in the kidney. Additionally, HCE-exposed male rats demonstrated dose-dependent increases in incidences 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 in the HCE-exposed female rats, suggesting that CPN is not solely responsible for the nephropathy observed by NTP (1989). Insufficient data are available that support an α_{2u}-globulin mode of action and the data indicate that CPN is not solely responsible for the observed nephropathy. Therefore, the data are unavailable to support exacerbation of CPN by α_{2u}-globulin nephropathy as a mode of action for renal nephropathy following HCE exposure.

The liver has been demonstrated to be a target organ of HCE exposure (see Section 4.6.1.2), but the mode of action of the hepatotoxicity of HCE is unknown. Studies of TCA (a potential metabolite of HCE) indicate that free radical generation may play a role in mediating toxicity, particularly in the liver. Town and Leibman (1984) reported lipid peroxidation following treatment with HCE, which the authors suggested involved a free radical. However, no data were available that demonstrated generation of free radicals following exposure to HCE.

Although in vitro and in vivo experiments on HCE genotoxicity were predominantly negative, in vivo binding studies suggested that HCE can bind to DNA, RNA, and protein (see Section 4.5). In the rat, higher levels of DNA, RNA, and protein binding were observed in the kidney and liver. 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 indicated 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 free radicals. Binding HCE to macromolecules was interpreted by the presence of radiolabeled carbon; however, radiolabeled carbon may have been incorporated into these macromolecules from intermediary HCE metabolites.

4.7 Evaluation of Carcinogenicity

4.7.1 Summary of Overall Weight of Evidence

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), HCE is "likely to be carcinogenic to humans" based on evidence of statistically significant increased incidences of multiple tumor types in male rats and both sexes of mice (NTP, 1989; NCI, 1978). Specifically, NTP (1989) reported dose-dependent increases in the combined incidence of renal adenomas or carcinomas in male F344/N rats (see Table 4-6). NTP (1989) also reported increases in the incidence of pheochromocytomas in male F344/N rats, although the increase was not dose-related (see Table 4-7). NCI (1978) observed statistically significant increases in the incidence of hepatocellular carcinomas in male and female B6C3F$_1$ mice (see Table 4-10). The male mice demonstrated a dose-related increase in hepatocellular carcinomas, although increases in hepatocellular carcinomas in female mice were not dose-related.

Some data suggest that HCE-induced kidney tumors in male rats may involve a male rat-specific α_{2u}-globulin-mediated mode of action. As this mode of action is unique to the male rats, there is some uncertainty regarding the human relevance of these tumors for human health assessment. The available data on the role of α_{2u}-globulin-mediated mode of action in the carcinogenic effects of HCE were considered (see Section 4.7.3.1). EPA concluded that there is insufficient evidence to attribute HCE-induced kidney tumors in male rats to an α_{2u}-globulin mode of action and that the mode of action for renal tumors is unknown.

The available data are considered insufficient to describe the mode of action for the carcinogenic effects of HCE in the liver (see Section 4.7.3.2). 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. These processes could potentially lead to cytotoxicity, inflammation, and regenerative cell proliferation. However, these potential key events have not been evaluated for HCE.

The relevance of rodent pheochromocytomas as a model for human cancer risk has been the subject of discussion in the scientific literature (Greim et al., 2009; Powers et al., 2008). Although more common in laboratory rats, evidence suggests that rat pheochromocytomas may have similarity to human pheochromocytomas and that they may be produced by the same mechanism of action (Greim et al., 2009; Eisenhofer et al., 2004; Lehnert et al., 2004; Elder et al., 2003; Goldstein et al., 1999). Data are lacking to describe the mode of action for pheochromocytomas following HCE exposure (see Section 4.7.3.3).

The descriptor"likely to be carcinogenic to humans" is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "carcinogenic to humans." An example provided in the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b) is "an agent that has tested positive in animal

experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans." As is discussed in Section 4.2.1.2 of this assessment the results from several rodent bioassays indicate that HCE exposure can cause tumors in two species, both sexes of animals, and multiple sites. On this basis, these data support the cancer descriptor "likely to be carcinogenic to humans." However, there are uncertainties associated with relating the observed tumors in animals following exposure to HCE to human carcinogenicity. Additional mechanistic data, particularly related to the formation of the renal tumors in male rats, would inform the uncertainty associated with the assumption that these tumors are relevant to humans. If these tumors were determined to not be relevant to humans, then the weight of evidence regarding human carcinogenic potential would be reduced.

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b) 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 information (e.g., toxicokinetic data) that absorption does not occur by other routes. Information available on the carcinogenic effects of HCE via the oral route demonstrated that tumors occurred in tissues remote from the site of absorption. Information on the carcinogenic effects of HCE via the inhalation and dermal routes in humans or animals was absent. Based on the observance of systemic tumors following oral exposure, and in the absence of information to indicate otherwise, it was assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, the data are sufficient to conclude that HCE 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 on HCE carcinogenicity. Carcinogenic effects of chronic oral HCE exposure have been reported in animal bioassays (see Section 4.2.1.2). These animal bioassays provided evidence of renal adenomas and carcinomas and pheochromocytomas in male rats and hepatocellular carcinomas in male and female mice following HCE exposure. The available information indicates that the male rat kidney is the most sensitive sex/species/organ for HCE-induced carcinogenicity. In vitro data provide evidence of macromolecule binding of HCE and suggests that HCE is capable of tumor promotion, but not tumor initiation in the liver.

Male F344/N rats chronically exposed to 7 or 14 mg/kg-day HCE (NTP, 1989) exhibited a dose-related increase in the incidence of combined renal adenomas or carcinomas (see Table 4-6). In addition, NTP (NTP, 1989) reported increased incidence of combined pheochromocytomas (benign, malignant, and complex pheochromocytomas) at all exposure doses in male F344/N rats, although only the increased incidence was not dose-related (see Table 4-7). NTP (NTP, 1989) did not observe renal or adrenal tumors in female rats chronically exposed to 57 or 114 mg/kg-day HCE for 103 weeks. The study authors concluded that the renal adenomas and carcinomas in male rats were evidence of carcinogenicity, based on incidence in historical controls. NTP (NTP, 1989) also stated that the increased incidence of pheochromocytomas male rats were possibly exposure related. NCI (1978; Weisburger, 1977) conducted

chronic exposure studies in Osborne-Mendel rats and B6C3F$_1$ mice. Male and female Osborne-Mendel rats chronically exposed to 113 or 227 mg/kg-day HCE had increased incidences of several tumor types including kidney tubular cell adenoma and hartoma, pituitary chromophobe adenoma, thyroid follicular cell adenoma or carcinoma, testicular interstitial cell tumors, mammary gland fibroadenoma, and ovary granulosa cell tumors in rats (see Table 4-8 and Table 4-9); however, no statistical differences in tumor frequencies were observed between exposed and control rats. In addition, these tumor types are considered to be spontaneous lesions in Osborne-Mendel rats. Therefore NCI concluded that there was no evidence of carcinogenicity in this rat study. Male and female B6C3F$_1$ mice chronically exposed to 360 or 722 mg/kg-day HCE exhibited statistically significant increases in incidences of hepatocellular carcinomas (see Table 4-10). Male mice demonstrated a dose-related increased tumor response for hepatocellular carcinomas, whereas, female mice demonstrated an increased tumor response that was not dose related. NCI concluded that HCE was carcinogenic in both sexes of B6C3F$_1$ mice.

The animal carcinogenicity bioassays have provided evidence of renal tumors in male rats and liver tumors in male and female mice following HCE exposure. Notably, the HCE dose resulting in a statistically significant increase in hepatocellular carcinoma in B6C3F$_1$ mice (NCI, 1978) was approximately 26 times greater than doses producing a statistically different incidence in renal tumors in F344 male rats (NTP, 1989). In vitro experiments also provide some evidence of HCE carcinogenicity. Evidence of HCE carcinogenic promotion, but not initiation, potential was observed in the liver of male Osborne-Mendel rats (Milman et al., 1988; Story et al., 1986). Lattanzi et al. (1988) reported species differences for binding to DNA, RNA, and protein (see Table 4-17), with the mouse exhibiting higher levels of DNA binding in the liver than the rat.

4.7.3   Mode-of-Action Information

Hepatocellular carcinoma, 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. The mechanistic data available for HCE is limited; however, there are data suggesting that induction of kidney tumors in male rats involves the accumulation of α_{2u}-globulin in the kidney and induction of liver tumors in male and female mice may involve increased cytotoxicity, inflammation, and regenerative cell proliferation in the liver.

4.7.3.1   Kidney Tumors

Some data suggested that HCE-induced kidney tumors in male rats may involve an α_{2u}-globulin-mediated mode of action. This mode of action is unique to the male rat; female rats and other laboratory mammals administered the same chemicals do not accumulate α_{2u}-globulin in the kidney and do not subsequently develop renal tubule tumors (Doi et al., 2007; IARC, 1999; U.S. EPA, 1991b). An analysis of the data, outlined below, indicates that there is insufficient evidence to attribute HCE-induced kidney

tumors in male rats to an α_{2u}-globulin mode of action. Specifically, no immunohistochemical data demonstrated the presence of α_{2u}-globulin in hyaline droplets. Furthermore, reported renal toxicity in female rats and male and female mice exposed to HCE suggests a mode of action other than α_{2u}-globulin-associated nephropathy. In the absence of sufficient information demonstrating the involvement of α_{2u}-globulin processes, male rat renal toxicity/tumors are considered relevant for risk assessment purposes.

Description of the Hypothesized Mode of Action

Hypothesized mode of action. Generally, kidney tumors observed in cancer bioassays are assumed to be relevant for assessment of human carcinogenic potential. However, male rat-specific kidney tumors caused by the α_{2u}-globulin accumulation are not considered relevant to humans. Accumulation of α_{2u}-globulin in hyaline droplets initiates a sequence of events that leads to renal nephropathy and, eventually, renal tubular tumor formation.

Identification of key events

The role of α_{2u}-globulin accumulation in the development of renal nephropathy and carcinogenicity observed following HCE exposure was evaluated using the U.S. EPA (1991a) Risk Assessment Forum Technical panel report. This report (U.S. EPA, 1991a) 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 to 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 (Doi et al., 2007; IARC, 1999; U.S. EPA, 1991a).

The hypothesized mode of action ascribed to α_{2u}-globulin-associated nephropathy is defined by a progressive sequence of events in the male rat kidney, often culminating in renal tumors. The involvement of hyaline droplet accumulation in renal tubules, in the early stages of nephropathy (associated with excessive accumulation of the urinary protein, α_{2u}-globulin) is an important difference from the sequence of events observed with classic carcinogens. The pathological changes that precede the proliferative sequence for classic 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 unique 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 summarizing available data on HCE for each step of this sequence):

- Excessive accumulation of hyaline droplets in renal proximal tubules

- Immunohistochemical evidence that α_{2u}-globulin is the protein accumulating in the hyaline droplets

- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium;

- Sustained regenerative tubule cell proliferation (with continued exposure);

- 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; and

- Renal tubule tumors

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

Progressive sequence of events leading to renal tubule tumors	Study, Dose (mg/kg-day [Sex]), Exposure Duration (weeks or days)											
	NTP (1989) 7 or 14 [M]; 57 or 114 [F] 103 weeks		NCI (1978) 113 or 227 111-112 weeks		Gorzinski et al., (1985) 1; 15; or 62 16 weeks		NTP (1989) 34; 67; 134; 268; or 536 13 weeks		NTP (1996) 146 or 293 21 days		NTP (1989) 140; 281; or 563 16 days	
	M	F	M	F	M	F	M	F	M	F	M	F
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		
Renal tubule tumors	X											

NT = not tested; X = presence of effect; M = male; F = female

In addition to this histopathological sequence, U.S. EPA (1991a) 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 in nephrotoxicity and carcinogenicity following HCE exposure and, if so, to what extent α_{2u}-globulin-associated events, rather than other processes, account for the tumor increase.

As outlined in the U.S. EPA Risk Assessment Forum Technical Panel report (U.S. EPA, 1991a), the following information from 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 desired. The three categories of information and criteria are as follows:

- *Increased number and size of hyaline droplets in the renal proximal tubule cells of exposed 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; 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.

In the absence of sufficient 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.

Experimental Support for the Hypothesized Mode of Action: Strength, consistency, and specificity of association

The oral database for HCE consists of short-term exposure studies in male and female rats (NTP, 1989, 1986), subchronic exposure studies in male and female rats (NTP, 1989; Gorzinski et al., 1985), chronic exposure studies in male and female rats (NTP, 1989; NCI, 1978), and chronic exposure studies in male and female mice (NCI, 1978). These studies have reported the accumulation of hyaline droplets in male rats, as well as aspects of the pathological sequence associated with α_{2u}-globulin nephropathy. However, there is insufficient evidence to attribute HCE-induced kidney tumors in male rats to an α_{2u}-globulin mode of action because none of the available studies demonstrated the accumulation of α_{2u}-globulin in the hyaline droplets.

Accumulation of hyaline droplets in male rats has been demonstrated following short-term and subchronic exposure to HCE. In one short-term exposure study, NTP (1989) reported hyaline droplets in the cytoplasm of the renal tubular epithelium in all males surviving exposure to 140-563 mg/kg-day HCE for 16 days. Female rats exposed to HCE at the same doses did not display accumulation of hyaline droplets. In a second short-term exposure study, NTP (1996) reported marked hyaline droplet accumulation in male rats, characterized as two severity grades above the control rats, following exposure to 146 or 293 mg/kg-day HCE for 21 days. Hyaline droplet accumulation was also reported in male rats, but not female rats, following subchronic exposure to 34-536 mg/kg-day HCE (NTP, 1989). These data provide evidence that HCE exposure leads to hyaline droplet accumulation in male rats.

Although hyaline droplet accumulation has been demonstrated following HCE exposure, hyaline droplet accumulation is a nonspecific response to protein overload. Based upon the criteria for evaluating the role of α_{2u}-globulin mode of action in male rat nephropathy (U.S. EPA, 1991a), it is necessary to demonstrate that the protein in the droplet is α_{2u}-globulin. None of the available studies demonstrated that the protein in the hyaline droplets was α_{2u}-globulin (see Table 4-21). This lack of α_{2u}-globulin immunohistochemical data represents an important data gap.

The available short-term, subchronic, and chronic HCE exposure studies have reported histopathological effects consistent with an α_{2u}-globulin mode of action. Tubular cell regeneration and eosinophilic granular casts of cell debris in the tubule lumina at the corticomedullary junction male, but not female, rats exposed to 140 or 281 mg/kg-day HCE for 16 days (NTP, 1989). Increased incidence of tubular regeneration and eosinophilic granular casts in the outer medullary tubules were also reported in male rats exposed to 146 and 293 mg/kg-day HCE for 21 days (NTP (1996). Tubular cell regeneration, eosinophilic granular casts of cell debris in the tubular lumina at the corticomedullary region (with associated tubular dilatation), and renal degeneration and necrosis were reported in male, but not female, rats exposed to 34-536 mg/kg-day HCE 13 weeks (NTP (1989). Similarly, Gorzinski et al. (1985) reported increased incidences of renal degeneration and slight hypertrophy/dilation of the renal proximal tubules in male rats following exposure to 15 or 62 mg/kg-day HCE for 16 weeks. Interestingly, Gorzinski et al. (1985) observed renal degeneration in female rats subchronically exposed to 62 mg/kg-day HCE. Chronic exposure studies also reported histopathological effects in both male and female rats (see Table 4-21), although the male rats were more sensitive to HCE exposure. Both sexes of rats chronically exposed to 113 or 227 mg/kg-day HCE for 78 weeks (NCI, 1978) had increased incidence of nephropathy (described as tubular degeneration and necrosis and the presence of large hyperchromatic regenerative epithelial cells). NCI (1978) also reported additional histopathological effects in male and female rats, some of which are consistent with an α_{2u}-globulin mode of action: focal pyelonephritis, tubular ectasia, cast formation, chronic interstitial nephritis and fibrosis, and focal glomerulosclerosis. In another chronic HCE exposure study, nephropathy (characterized as tubular cell degeneration and atrophy, tubular dilatation, tubular cell regeneration, glomerulosclerosis, interstitial fibrosis, and chronic inflammation) was reported in both male and female rats (NTP, 1989). Cast formation was also reported in both male and female rats. Additional histopathological effects consistent with an α_{2u}-globulin mode of action were also reported in male rats, including linear mineralization of the renal papillae, pelvic epithelium hyperplasia, and renal tumors. NTP (1989) noted that the hyperplasia and tumors of the renal tubules represented a morphologic continuum. A sex difference was noted in (NTP (1989), as males were more sensitive to HCE-exposure-related nephropathy than females . This sex specificity is associated with both the incidence of nephrotoxicity and severity of nephropathy observed in both control and HCE-exposed groups. 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 to female rats at a dose one eighth of the dose administered to female rats.

Summary of evidence for strength, specificity, and consistency

The criteria for demonstrating the α_{2u}-globulin-related mode of action for risk assessment purposes have been defined (U.S. EPA, 1991a): (1) an increase in hyaline droplets in the renal proximal tubule cells; (2) the determination that the accumulating protein in the droplets is α_{2u}-globulin; and (3) the presence of additional pathological lesions associated with α_{2u}-globulin.

Hyaline droplets in renal proximal tubule cells were reported following short-term and subchronic exposure to HCE. However, hyaline droplets accumulation was not reported following chronic exposure (NTP, 1989). It is possible that hyaline droplets were present, but were obscured by the prevalence of the other lesions. Therefore, the available data on hyaline droplet accumulation are consistent with an α_{2u}-globulin mode of action for HCE.

Although hyaline droplets observed following administration of HCE (NTP, 1989, 1986), none of the HCE studies performed immunohistochemical assays to confirm the presence of α_{2u}-globulin protein within. It is unclear whether HCE is binding to α_{2u}-globulin or to other proteins during the formation of hyaline droplets. Therefore, there is insufficient immunohistochemical data to determine if HCE renal nephropathy occurs through α_{2u}-globulin accumulation or if another mechanism is operating.

Pathological lesions consistent with an α_{2u}-globulin mode of action have been reported following short-term, subchronic, and chronic HCE exposure. In male rats, each histopathological event in the progressive sequence leading to renal tubule tumors has been observed (see Table 4-21). In addition, data are available indicating histopathological events in female rats and male and female mice following chronic HCE exposure (NTP, 1989; NCI, 1978). NTP (1989) reported dose-dependent increases incidence and severity of renal nephropathy in female rats, while NCI (1978) reported dose-related nephropathy in female rats that was not apparent in the controls (see Section 4.2.1.2). Nephropathy was also reported in male and female mice chronically administered HCE (NCI, 1978). NCI (1978) reported the appearance of renal tubular effects in almost all ($\geq$ 92%) of the HCE- exposed male and female mice following chronic HCE exposure, but the mice did not develop renal tubule tumors (see Section 4.2.1.2). Considering that α_{2u}-globulin nephropathy is male rat-specific, the appearance of nephrotoxic effects in the female rats, as well as the male and female mice, and the identification of other histopathological effects not specifically associated with α_{2u}-globulin (i.e., glomerulosclerosis and interstitial fibrosis) suggest that at least some of renal effects of HCE may not be the result of α_{2u}-globulin accumulation. Therefore, although some of the histopathological data in male rats is consistent with an α_{2u}-globulin protein, the available data in HCE-exposed female rats and male and female mice is inconsistent with an α_{2u}-globulin mode of action.

Limitations do exist in studies describing 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 only 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 to 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 more fully characterize the dose response of HCE-exposure-related nephropathy.

Dose-response concordance

The accumulation of α_{2u}-globulin in hyaline droplets is expected to occur at lower doses than subsequent α_{2u}-globulin-related effects. Because none of the HCE studies confirmed the presence of α_{2u}-globulin protein within the hyaline droplets observed following administration of HCE (NTP, 1996, 1989), dose-response concordance of the α_{2u}-globulin accumulation in hyaline droplets cannot be determined.

Most of the histopathological events in epithelial cells of the proximal tubules leading to renal tumors (Doi et al., 2007; IARC, 1999; U.S. EPA, 1991a) increased in incidence with increasing doses of HCE in the short-term and subchronic exposure studies. Histopathological effects associated with α_{2u}-globulin nephropathy (i.e., tubular cell degeneration and atrophy, tubular dilatation, and tubular cell regeneration) were noted in almost all of the exposed and unexposed animals. 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 (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. 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 subchronic studies (Gorzinski et al., 1985). Although nephropathy was noted in the shorter duration studies (NTP, 1989, 1986; Gorzinski et al., 1985), the only evidence of carcinogenicity was from the chronic exposure studies (NTP, 1989; NCI, 1978).

Temporal relationship

The initial 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. As immunohistochemical evidence of α_{2u}-globulin protein

within the hyaline droplets was available for HCE, the temporal relationship for the accumulation of α_{2u}-globulin could not be determined.

Histopathological effects associated with α_{2u}-globulin-related nephropathy were observed in animals exposed 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; 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) led to the reported renal tubule tumors because the animals were only examined at the end of the 103-week study period. A temporal relationship between renal nephropathy and renal carcinogenicity cannot be determined from available data.

Biological plausibility and coherence

Generally, the kidney toxicity and tumor formation was observed in rats and mice are biologically plausible effects that could potentially occur in humans. If the tumor formation in male rats is due to accumulation of α_{2u}-globulin mode of action, then these tumors would not be considered to be relevant to human health risk assessment. An α_{2u}-globulin mode of action was evaluated as a hypothesized mode of action for HCE-induced carcinogenicity and nephropathy (U.S. EPA, 1991c). The α_{2u}-globulin related 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 ((Doi et al., 2007; IARC, 1999; Hard et al., 1993; U.S. EPA, 1991a). Evidence of nephrotoxic effects in female rats in two chronic studies (NTP, 1989; NCI, 1978), in female rats in a subchronic study (Gorzinski et al., 1985), and in male and female mice in one chronic study (NCI, 1978) precludes the conclusion that HCE is acting through an α_{2u}-globulin-associated mode of carcinogenic action.

Other Possible Modes of Action

There is insufficient evidence to support an α_{2u}-globulin-related mode of action for renal tumors in male rats following HCE exposure. It is possible that advanced CPN may play a role in the incidence of nephrotoxicity and kidney tumors in aged male rats. CPN is associated with aged rats, especially F344, Sprague-Dawley, and Osborne-Mendel strains. CPN is frequently more severe in males compared with females. Several of the CPN histopathological effects are similar to, and can obscure, 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, 1991a). However, Webb et al. (1990) 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. Hard et al. ([1993](#)) reported the histopathologic 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 in 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); and

- Accumulation of protein droplets in sporadic proximal tubules (in some advanced cases).

With the exception of atrophy of the 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. 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](#)).

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 in female rats ([NTP, 1989](#)), as well as in male and female mice ([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](#)). Another potential mode of action is the exacerbation of CPN by α_{2u}-globulin nephropathy. Insufficient data are available that support an α_{2u}-globulin mode of action and the data indicate that CPN is not solely responsible for the observed nephropathy. Furthermore, additional data gaps identified prevent attributing the renal effects of HCE to the exacerbation of chronic progressive nephropathy by α2u-globulin accumulation (i.e., categorization of end stage renal failure in either the control or HCE-exposed animals, presences of foci of atypical hyperplasia were present, or if renal adenomas were within the areas of

chronic progressive nephropathy). Therefore, there is insufficient data for this potential mode of action for the renal effects of HCE.

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. As tumors were observed in the male rat kidney following chronic HCE exposure (NTP, 1989), the available data were evaluated for a potential α_{2u}-globulin mode of action. The lack of α_{2u}-globulin immunohistochemical data, as well as data demonstrating kidney effects in female rats and mice of both sexes (NTP, 1989; NCI, 1978), prevented attribution of the renal tumors to an α_{2u}-globulin mode of action. Although the age and strain of rats suggested a potential role for CPN in the observed histopathological effects, treatment-related effects in male and female rats suggest that CPN is not solely responsible for the observed nephropathy. While it is possible that an α_{2u}-globulin-related mode of action may be responsible for the male rat kidney tumors whereas more than one mode of action may be responsible for the nephropathy, there is insufficient data to support this hypothesized mode of action. Therefore, the mode(s) of action for the renal adenomas and carcinomas in male rats is unknown.

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 demonstrates that the response results from α_{2u}-globulin accumulation, the tumor data are not used in the cancer assessment (U.S. EPA, 1991a). 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. Therefore, the renal adenomas and carcinomas observed in male rats administered HCE (NTP, 1989) were considered relevant for human health risk assessment.

4.7.3.2   **Liver Tumors**

Significantly increased incidences of hepatocellular carcinomas were observed in male and female B6C3F$_1$ mice in a chronic oral bioassay conducted by NCI (1978). Tumor incidences in male mice demonstrated a dose response, whereas female mice did not demonstrate a dose response. 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-exposed mice. The investigators did not find nonneoplastic liver effects (such as organized

thrombus, inflammation, fibrosis, necrosis, infarctions, amyloidosis, or hyperplasia) in either sex. However, hepatocellular necrosis of the centrilobular area was observed in rats following subchronic HCE exposure (NTP, 1989). It is unknown if hepatocellular necrosis could be a key event in the carcinogenic process because rats in chronic exposure studies (NTP, 1989; NCI, 1978) have not displayed hepatocellular neoplastic endpoints

The mode of action for the carcinogenic effects of HCE in the liver is unknown. Metabolism studies of HCE indicated that the major enzymes involved are phenobarbital-inducible CYP450s, which are primarily localized in the liver, and the majority of HCE metabolism is presumed to occur in the liver. Comparisons of HCE metabolism rates indicated that mice metabolize HCE at twice the rate of rats (Mitoma et al., 1985). In vivo assays have demonstrated that macromolecule binding of HCE (or its metabolites) is consistently higher in mice than in rats, with moderate oncogenic potential in the liver (see Section 4.5.2). Cellular damage leading to cytotoxicity, inflammation, and regenerative cell proliferation is a possible consequence of this binding in the liver. Regenerative cell proliferation has been evaluated in the kidney, but not in the liver of HCE-exposed rats (NTP, 1996). RDS in hepatocytes was evaluated in mice exposed to 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 provided 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 radiolabel binding studies provided a possible explanation for the difference in carcinogenic target organs between rats and mice. The in vivo radiolabel binding data suggest that HCE (or its metabolites) 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, 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.

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. While some data suggest 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. Therefore, the mode of action for liver carcinogenicity is unknown.

4.7.3.3 **Pheochromocytomas**

Pheochromocytomas are catecholamine-producing neuroendocrine tumors. The relevance of rodent pheochromocytomas as a model for human cancer risk has been the subject of discussion in the scientific literature (e.g., Greim et al., 2009; Powers et al., 2008). In humans, pheochromocytomas are rare and usually benign, but may also present as, or develop into, a malignancy (Eisenhofer et al., 2004; Lehnert et al., 2004; 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). Pheochromocytomas are more common in laboratory rats, though evidence suggests that certain rat pheochromocytomas may have similarity to human pheochromocytomas (Powers et al., 2008). Furthermore, mechanisms of action inducing pheochromocytomas in rats are expected to occur in humans as well (Greim et al., 2009). Therefore, adrenal gland tumors in rodents are considered relevant to human health risk assessment.

No studies were identified to determine a mode of action for HCE-induced tumors of the adrenal gland. 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 have addressed possible childhood susceptibility to HCE-induced toxicity or carcinogenicity. CYP450 enzymes of the 2A, 2B, and 3A subfamilies, and CYP450 1A2 are involved in HCE metabolism, suggesting that age-related differences in CYP450 activity could lead to age-related susceptibility in HCE toxicity. Although Dorne (2004) reported 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 , 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). Similarly, Blanco et al. (2000) compared liver microsomal CYP450 activities of humans <10 years old with those >10-60 years old and concluded that factors other than maximal CYP450 catalytic activities may be responsible for age-related differences. Therefore, the extent to which variable age-related expression of CYP450 contributes to childhood susceptibility is unknown.

4.8.2   Possible Gender Differences

Male rats were more sensitive to HCE-induced nephrotoxicity than females (NTP, 1989; Gorzinski et al., 1985; NCI, 1978), whereas 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 (see Table 3-3), sex hormone differences, and/or sex-specific differences in CYP450 activities. No additional studies were located that addressed possible sex-specific differences for HCE-induced toxicity or carcinogenicity.

4.8.3   Other

CYP450 enzymes, which have been shown to metabolize HCE, are polymorphic in the human population. Polymorphisms result in CYP450 enzymes with variant catalytic activity for substrates such as HCE. This enzyme polymorphism could potentially result in decreased HCE detoxification or increased HCE bioactivation. Detoxification enzymes such as the glutathione-S-transferase (GST) family are also polymorphic in the human population, with variant catalytic activities that could affect the detoxification of HCE. No studies were located that addressed possible interindividual differences in CYP450 polymorphisms in HCE-induced toxicity or carcinogenicity.

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 were not available. The oral exposure database for HCE includes a 103-week gavage study in F344 rats (NTP, 1989), a 111-112-week gavage study in Osborne-Mendel rats (NCI, 1978), a 111-112-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). Reported effects included 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). Chronic and subchronic studies in rats and mice indicated that the kidney and liver are target organs of HCE oral toxicity in rodents. The incidence of kidney and liver effects from the studies considered for selection as the principal study are summarized in Table 5-1.

Specifically, nephropathy was observed in both chronic studies (NTP, 1986; NCI, 1978); however, the animals in the chronic NTP study (1989) 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.

Table 5-1   Incidences of noncancerous 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 moderate 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 epithelium	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 (10%)
				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 (10%)
				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%)
				34	0/10 (0%)
				67	0/10 (0%)
				134	0/10 (0%)
				268	1/10 (10%)
				536	2/5 (40%)
		F344 female rat	Hepatocellular necrosis	0	0/10 (0%)
				34	0/10 (0%)
				67	0/10 (0%)
				134	2/10 (20%)
				268	4/10[a] (40%)
				536	8/10[a] (80%)

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

The short-term studies were not considered in the selection of the principal study for the derivation of the RfD because the database contains dose-response data from studies of subchronic and

chronic durations. However, data from short-term studies in rats (NTP, 1996, 1989) were used to support findings in the chronic (see Section 4.2.1.2) and subchronic (see Section 4.2.1.1) studies.

The subchronic exposure studies (NTP, 1989; Gorzinski et al., 1985) and chronic exposure studies (NTP, 1989; NCI, 1978) are well designed studies, with animals exposed to at least two HCE doses and an unexposed control group, and examined for a wide range of toxicological endpoints in both sexes of the rodents. One limitation of the Gorzinski et al. (1985) study is potential sublimation of HCE from the food. While the authors did consider HCE sublimation and eating patterns when reporting the administered doses, the potential for inhalation exposure was not considered.

Limitations of the NTP (1989) subchronic study include the lack of incidence and severity data for the reported kidney effects in male rats and the need for additional exposure concentration(s) between the control and low dose for better characterization of the exposure-response curve in male rats. Limitations of the NTP (1989) chronic study are the need for additional exposure concentration(s) between the control and low dose for better characterization of the exposure-response curve for renal effects in male and female rats, as well as the high incidence of renal nephropathy observed in both the control and exposure groups.

Limitations of the NCI (1978) chronic exposure study in Osborne-Mendel rats include alterations in the dosing regimen during the study, cyclical dosing periods, and the need for additional exposure concentration(s) between the control and low dose for better characterization of the exposure-response curve in male and female rats. Limitations of the NCI (1978) chronic exposure study in B6C3F$_1$ mice include alterations in the dosing regimen during the study, cyclical dosing periods, low survival rates in control and low dose males, and the need for additional exposure concentration(s) between the control and low dose for better characterization of the exposure-response curve in male and female rats.

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 were not further considered for POD determination because of the lack of incidence data for the control groups. In addition, the HCE doses administered were more than fourfold 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 the HCE doses that induced kidney effects were more than sevenfold higher than doses associated with kidney effects in rats following subchronic (Gorzinski et al., 1985) or chronic (NTP, 1989; NCI, 1978) exposure.

Further consideration was given to the renal endpoints including, 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) as candidate 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 in male rats, data from the NTP (1989) 13-week subchronic study on incidence of hepatocellular necrosis from the female rats were also

considered as candidate critical effects for comparison purposes. The hepatocellular necrosis data on the male rat liver effects were not considered for comparison purposes because incidence was not significantly elevated above controls at any HCE dose.

5.1.2   Methods of Analysis—Including Models

The benchmark dose (BMD) modeling approach (U.S. EPA, 2000c) was employed to identify the candidate POD following subchronic (Gorzinski et al., 1985) and chronic (NTP, 1989; NCI, 1978) HCE exposure (Table 5-2 and B-1). A benchmark response (BMR) of 10% extra risk was considered appropriate for derivation under the assumption that it represents a minimally biologically significant response level. 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 (NTP, 1989; Gorzinski et al., 1985; NCI, 1978), as well as the incidence data for hepatocellular necrosis in female rats (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 95% lower bound confidence limit on the BMD (U.S. EPA, 2009) modeling outcomes for a BMR of 5 and 1% are also 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 kidney effects data at the 10% BMR level and the candidate PODs (BMDL$_{10}$ values) are identified for each effect.

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

Study	Endpoint	Sex/species (group size)	Duration (route)	"Best-fit" model	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 Quantal-linear, and Weibull	1.22	0.710
Gorzinski et al. (1985)	Atrophy and degeneration of renal tubules	Male rats (n = 10)	16 weeks (dietary)	Gamma, Multistage 1°, and Quantal-linear	1.34	**0.728**
		Female rats (n = 10)		Probit	16.10	10.51
NCI (1978)	Tubular Nephropathy	Male rats (n ≈ 50)	78 weeks (gavage)	Gamma, Multistage 1°, and Weibull	21.23	16.99
		Female rats (n ≈ 50)		Multistage 2°	80.63	41.89
NTP (1989)	Increased incidence of moderate to marked tubular nephropathy	Male rats (n ≈ 50)	103 weeks (gavage)	Probit	3.81	2.60
	Increased incidence of mild to moderate tubular nephropathy	Female rats (n ≈ 50)		Gamma, Quantal-linear, and Weibull	15.17	10.72
NTP (1989)	Linear mineralization	Male rats (n ≈ 50)	103 weeks (gavage)	Probit	3.98	3.22
NTP (1989)	Hyperplasia of the pelvic transitional epithelium	Male rats (n ≈ 50)	103 weeks (gavage)	LogLogistic	7.05	4.48

The most sensitive effect observed in male rats exposed to HCE was slight hypertrophy and/or dilation of proximal convoluted renal tubules (Gorzinski et al., 1985); however, the candidate 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 critical effect for male rats exposed to HCE. The tubular nephropathy in male rats observed in the chronic exposure studies (NTP, 1989; NCI, 1978) 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). 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.

As shown in Appendix B, the gamma, multistage 1°, logistic, probit, quantal-linear, and 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., 1985) 16-week study (Table B-1), as assessed by a χ^2 goodness-of-fit p-values. BMD$_{10}$ and BMDL$_{10}$ estimates from these models were within a factor of three of each other, suggesting no appreciable model dependence. The models with the lowest Akaike's information criterion (AIC; a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint) values were for the gamma, multistage 1°, and quantal-linear models; therefore, the model with the lowest BMDL$_{10}$ was selected. These models had identical BMD$_{10}$ and

BMDL$_{10}$ values. Therefore, the BMDL$_{10}$ of 0.728 mg/kg-day associated with a 10% extra risk for nephropathy in male rats was selected as the candidate POD for these data. The BMDL$_{10}$ of 0.728 mg/kg-day 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. Based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), uncertainty factors, addressing five areas of uncertainty resulting in a composite UF of 1,000, were applied to the selected POD to derive an RfD.

- An interspecies uncertainty factor, UF$_A$, of 10 was applied to account for uncertainty in extrapolating from laboratory animals to humans in the absence of information to characterize the toxicokinetic or toxicodynamic differences between rats and humans after oral HCE exposure. Although the toxicokinetics have been minimally evaluated in animals, the toxicokinetics of HCE have not been sufficiently characterized in either rats or humans to identify the active compound or determine dose metrics.

- An intraspecies uncertainty factor, UF$_H$, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to oral HCE exposure in the human population.

- A subchronic-to-chronic UF (UF$_S$) of 3 was applied. The study selected as the principal study was the 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 (i.e., two year chronic bioassay). Some chronic data (NTP, 1989; Gorzinski et al., 1985; NCI, 1978) were available to inform the nature and extent of effects that would be observed with a longer duration of exposure to HCE. The chronic data identified the kidney is the target organ of HCE toxicity, consistent with the findings from the Gorzinski et al. (1985) study. Increases in severity of tubular nephropathy in the NTP (1989) chronic study was reported at similar doses as atrophy and degeneration of renal tubules in the Gorzinski et al. (1985) subchronic study, suggesting consistency in dose response relationships between chronic and subchronic studies. In addition, data from the NCI (1978) chronic study suggested that an increase in duration of HCE exposure may not increase the incidence of nephropathy. However, the lowest dose tested in the chronic exposure studies (NTP, 1989; NCI, 1978) represented a LOAEL, limiting the ability of these studies to inform the impact of increased exposure duration on renal effects observed at the lowest dose in the subchronic study (Gorzinski et al., 1985). For these reasons, a UF$_S$ of 3 was used to account for extrapolation from subchronic-to-chronic exposure duration.

- A LOAEL to NOAEL uncertainty factor, UF$_L$, of 1 was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% increase in the

incidence of renal tubule atrophy and degeneration was selected under an assumption that it represents a minimal biologically significant change.

- A database uncertainty factor, UF_D, of 3was applied to account for database deficiencies due to 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.

The chronic RfD of 7×10^{-4} mg/kg-day for HCE was calculated as follows:

$$RfD = BMDL_{10} \div UF$$
$$= 0.728 \text{ mg/kg-day} \div 1,000$$
$$= 7 \times 10^{-4} \text{ mg/kg-day}$$

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 potential PODs for nephrotoxicity in male rats with applied UF values and potential reference values. 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 with females. Figure 5-1 provides a graphical display of dose-response information from three studies considered in the selection of a POD for the oral RfD. As discussed in Section 5.1.1 and Section 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 derived from the other studies are presented for comparison purposes.

Table 5-3 Potential PODs for nephrotoxicity in male rats with applied UF values 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 (111-112 week)	16.99	300	10	10	1	3[a]	0.0566	NCI ([1978](#))
Hyperplasia of pelvic transitional epithelium; BMDL (103 week)	4.48	300	10	10	1	3[a]	0.0149	NTP ([1989](#))
Linear mineralization; BMDL (103 week)	3.22	300	10	10	1	3[a]	0.0107	
Moderate to marked tubular nephropathy; BMDL (103 week)	2.60	300	10	10	1	3[a]	0.0087	
Slight hypertrophy and/or dilation of proximal convoluted renal tubules; BMDL (16-week)	0.710	1,000	10	10	3[a]	3[a]	0.0007	Gorzinski et al. ([1985](#))
Atrophy and degeneration of renal tubules; BMDL (16-week)	0.728	1,000	10	10	3[a]	3[a]	0.0007	

[a]3: ($10^{\frac{1}{2}}$ = 3.16, rounded to 3)

Figure 5-1   Array of potential PODs with applied UF values and potential reference values for nephrotoxic effects in male rats, from the three 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 a NOAEL/LOAEL approach. 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. In the current assessment, the atrophy and degeneration of renal tubules in rats reported by Gorzinski et al. (1985) also served as the basis for the RfD; however, BMD modeling was used to derive a POD and, in accordance with current EPA practices and guidance (U.S. EPA, 2002), an additional uncertainty factor was applied to account for database deficiencies.

5.1.6   Confidence in the RfD

Confidence in the principal study (Gorzinski et al., 1985), is high. The 16-week diet study was a well-conducted study that used three dose groups plus a control. NTP (1989) also conducted 16-day, 13-week, and 103-week gavage studies that supported the results observed in the 16-week diet study. Application of BMD modeling provided a POD upon which to base the derivation of the RfD. The critical effect on which the RfD was based is supported by other oral short-term, subchronic, and chronic studies. Confidence in the database was low to medium because the database included acute, short-term, subchronic, and chronic toxicity studies and developmental/teratogenic 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.

5.2 Inhalation Reference Concentration (RfC)

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

The database of inhalation toxicity studies on HCE is limited. Data on HCE-induced human health effects are limited and confounded by co-exposure (e.g., HCE-zinc oxide smoke). Studies observed HCE exposure in smoke bomb production workers, but the sample sizes were too small to provide definitive conclusions on health effects. No chronic inhalation exposure studies were available, and only a single subchronic inhalation study in animals was identified. Weeks et al. (1979) exposed Sprague-Dawley rats, male Beagle dogs, male Hartley guinea pigs, and Japanese quail to HCE, and examined a number of endpoints including toxicology, neurotoxicity, pulmonary, and teratology (see Section 4.2.2, Section 4.3.2, Section 4.4.1.2, Section 4.4.3.2, and Section 4.4.4). The 6-week subchronic inhalation study by Weeks et al. (1979) was considered for derivation of an RfD.

Weeks et al. (1979) was a well-conducted subchronic bioassay which used three concentrations of HCE plus an unexposed control, and incorporated a variety of endpoints across a range of species. The authors evaluated portal of entry effects by gross examination of lungs, trachea, and nasal turbinates following necroscopy on animals that died during the study or were sacrificed at 12 weeks post-exposure. In addition, Weeks et al. (1979) evaluated upper respiratory effects by examining histological sections of the nasal turbinates and evaluated upper respiratory inflammation by the presence of polymorphonuclear leukocytes in close association with excess mucus within the lumens of the nasal passages. 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 exposure concentration would have allowed for better characterization of the exposure-response curve. As the only repeat exposure study available, the Weeks et al. (1979) study was selected as the principal study for the derivation of the RfC.

Weeks et al. (1979) identified excess mucopurulent exudate, upper and lower respiratory tract irritation, statistically significant decreases in body weight gain, and neurotoxicity following inhalation exposure to HCE (see Table 5-4). The responses were generally observed only following exposure to the highest concentration. Body weight changes, pulmonary effects, and neurological effects were also reported following acute exposure to HCE (Weeks and Thomasino, 1978).

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) Subchronic	0, 145, 465, or 2,517 mg/m^3 6 weeks	465	2,517	Males: neurotoxic effects (tremors and ruffled pelt), reduced body weight gain, increased relative, spleen, and testes weights Non-pregnant females: neurotoxic effects (tremors and ruffled pelt), increased relative liver weight
Male Beagle dogs (4/dose) Subchronic		465	2,517	Tremors, ataxia, hypersalivation, head bobbing, facial muscular fasciculations
Male Hartley guinea pigs (10/dose) Subchronic		465	2,517	Reduced body weight, increased relative liver weight
Pregnant Sprague-Dawley rats (22/dose) Teratogenic/ Developmental	0, 145, 465, or 2,517 mg/m^3 GDs 6–16 (11 days)	Maternal: 465	Maternal: 2,517	Maternal: neurotoxic effects (tremors [GD12-16]); reduced body weight gain Developmental: no effects

Source: Weeks et al. (1979).

Weeks et al. (1979) attributed the increased incidence of respiratory lesions in rats to an endemic mycoplasma infection, based on the histopathological observation of an increased incidence and severity of mycoplasma-related lesions in the nasal turbinates (mucopurulent exudate), trachea (lymphoid hyperplasia in the lamina propria), and lung (pneumonitis) in male and female rats. Lesions characteristic of respiratory mycoplasmosis in rodents were also detected in the oral developmental/teratogenic study in pregnant rats. The presence of mycoplasma infection in the rats in both the oral and inhalation studies and in the controls of the oral study suggested that respiratory tract effects were due to a potentiation of the underlying infection rather than a result of HCE exposure.

Reduced weight gain in the rats could also be related to mycoplasma, as 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 guinea pigs, but mycoplasma infection was not reported (Weeks et al., 1979). Like rats and mice, guinea pigs can carry the mycoplasma organism; however, they are not clinically affected (Fox et al., 1984; Holmes, 1984). No data were 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 HCE. The male and non-pregnant female rats exhibited tremors and ruffled pelt. The inhalation-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. Considering the consistent observation of neurotoxic effects across experiments in rats and dogs, neurotoxic effects were selected as the critical effect for determination of the POD for the RfC.

5.2.2 Methods of Analysis—Including Models

The subchronic Weeks et al. (1979) inhalation study included three exposure groups (145, 465, and 2,517 mg/m^3) plus a control. Neurological effects were observed in male and non-pregnant female Sprague-Dawley rats, male Beagle dogs, and pregnant Sprague-Dawley rats only at the highest dose tested. Incidence data were not reported, which precluded application of BMD modeling; therefore, the NOAEL of 465 mg/m^3 served as the POD. Although the NOAELs for neurological effects in dogs and rats are the same, the male and female rats were selected as the study animals upon which to base the POD because the pregnant dams in the teratology study were only exposed for 11 days and only four male dogs were exposed to HCE.

The POD from this study was based on repeated, intermittent HCE inhalation exposures (in male and non-pregnant female rats) for 6 hours/day, 5 days/week, for 6 weeks. Thus, prior to deriving the RfC, this POD was adjusted for continuous exposure (24 hours/day, 7 days/week). The duration-adjusted POD (POD$_{[ADJ]}$) was derived using the following equation (U.S. EPA, 1994a):

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

The *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (referred to as the RfC Methodology) recommends converting the POD$_{[ADJ]}$ to a human equivalent concentration (HEC) (U.S. EPA, 1994a). The RfC Methodology separates gases into three categories based on their water solubility and reactivity with tissues in the respiratory tract. Category 1 gases are highly water soluble and/or rapidly irreversibly reactive in the surface-liquid/tissue of the respiratory tract, such that they do not significantly accumulate in blood. Category 2 gases are moderately water soluble and rapidly reversibly reactive or moderately to slowly irreversibly metabolized in respiratory tract tissue, such that they have the potential for significant accumulation in the blood and potential for respiratory and systemic toxicity. Category 3 gases are relatively water insoluble and unreactive in the surface-liquid/tissue of the respiratory tract.

Categorizing HCE into one of these three gas categories is difficult because data regarding the inhalation effects of HCE are limited. HCE is a slightly water soluble, non-directly reactive gas, and has an unknown blood:air partition coefficient. Inhalation exposure to HCE produces a variety of systemic

effects and no noted respiratory tract effects. HCE has been observed in blood following oral exposures to HCE, but it is unknown whether HCE accumulates in blood following inhalation exposure. Thus, HCE appears to exhibit characteristics most concordant with Category 3 gases whose uptake occurs primarily in the pulmonary region and site of toxicity is generally remote to the site of absorption. In view of the fact that neurotoxicity is a systemic effect, the methods for Category 3 gases were used to derive the HEC.

Consequently, for dosimetric purposes, the human equivalent concentration (HEC) for HCE was calculated by applying the appropriate dosimetric adjustment factor (DAF) for systemic acting gases (i.e. Category 3 gases) to the duration-adjusted exposure level ($POD_{[ADJ]}$), in accordance with the U.S. EPA RfC methodology (1994a). 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 animal and human blood:gas partition coefficients for HCE are not known. In accordance with the RfC Methodology (U.S. EPA, 1994a) when the partition coefficients are unknown or $(H_{b/g})_A$ is greater than $(H_{b/g})_H$, a RGDR of 1 is used. The partition coefficients were unknown for HCE; resulting in a $POD_{[HEC]}$ of 83.0 mg/m^3.

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

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. Based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), uncertainty factors, addressing five areas of uncertainty resulting in a composite UF of 3,000, were applied to the selected POD to derive an RfC.

- An interspecies uncertainty factor, UF_A, of 3 was applied to account for uncertainty in extrapolating from laboratory animals to humans in the absence of information to characterize the toxicodynamic differences between rats and humans after oral HCE exposure. This value is adopted by convention, where an adjustment from an animal-specific POD_{ADJ} to a POD_{HEC} has been incorporated as described in the RfC methodology (U.S. EPA, 1994a).

- An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to oral HCE exposure in the human population.

- A subchronic-to-chronic, UF_S, of 10 was applied to account for extrapolation from a subchronic exposure duration study to a chronic RfD. The study selected

as the principal study was a 6 week study by Weeks et al. (1979). No chronic inhalation studies were identified for HCE; therefore, there were no data to inform the effects that might be observed with increased exposure duration.

- A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because this assessment utilized a NOAEL as the POD.

- A database uncertainty factor, UF_D, of 10 was applied to account for deficiencies in the toxicity database for inhalation exposure to HCE. The toxicity data for inhalation exposure to HCE is limited and largely restricted to one subchronic (6-week) inhalation study (Weeks et al., 1979) in rats, male dogs, male guinea pigs, and quail. The same investigators performed a developmental/teratogenic study and an acute study (single 6 or 8 hour inhalation exposures) in rats. Although maternal toxicity was reported in the developmental/teratogenic study, fetuses of HCE-exposed dams did not exhibit any significant skeletal or soft tissue anomalies. The toxic effects observed in the dams in the developmental/teratogenic study (11-day exposure) were similar to those observed in the rats exposed for 6 weeks, although additional effects were observed in the rats exposed for the longer duration. The database lacks a long-term study and a multigeneration reproductive toxicity study. In addition, the database lacks studies of neurotoxicity and developmental neurotoxicity, endpoints of concern based on the available inhalation data demonstrating neurotoxicity in rats and dogs.

The chronic RfC of 3×10^{-2} mg/m^3 for HCE was calculated as follows:

$$RfC = NOAEL_{[HEC]} \div UF$$
$$= 83 \text{ mg/m}^3 \div 3,000$$
$$= 0.028 \text{ mg/m}^3 \text{ or } 3 \times 10^{-2} \text{ mg/m}^3$$

5.2.4   RfC Comparison Information

The predominant noncancer effect of subchronic inhalation exposure to HCE based on the available data was 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. Because of the consistent observation across species, neurotoxic effects were considered the most sensitive effects and were selected to serve as the basis for the derivation of the RfC for HCE (Weeks et al., 1979). Based on the lack of alternative endpoints that could be considered for the basis of the RfC, a graphical display of dose-response information from the subchronic inhalation study was not provided.

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.

However, no discussion was presented in the 1987 IRIS Summary describing why this study was not used to develop an RfC.

5.2.6 Confidence in the RfC

Confidence in the principal study, Weeks et al. (1979), is low. The study was limited by the relatively short exposure duration (6 weeks) and minimal reporting of effects, especially quantitative changes. Confidence in the database is low because the database included one acute and one subchronic inhalation toxicity study in multiple species and one inhalation developmental/teratogenic toxicity study in rats. The database lacks studies by another laboratory and a multigenerational reproductive study. Overall confidence in the RfC is low.

5.3 Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration

The following discussion identifies uncertainties in the quantification of the RfD and RfC for HCE beyond those discussed during application of the UFs (U.S. EPA, 1994b) to the POD values for derivation of the RfD (see Section 5.1.3) and RfC (see Section 5.2.3).

The 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 that might provide adequate fit, and other models could be selected to yield different results, both higher and lower than those included in this assessment. Uncertainty also exists in the selection of the BMR level utilized in the BMD modeling of the critical effect to estimate the POD. In the absence of information to identify the level of change in atrophy and degeneration of renal tubules in male F344 rats related to a biologically significant change, a BMR of 10% was selected for the modeling of the increased incidence to represent a minimally biologically significant change.

The RfC was based on the NOAEL from a subchronic inhalation study. 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 BMD modeling.

5.4 Cancer Assessment

There were no available studies on cancer in humans associated with exposure to HCE. The carcinogenic data reported in chronic animal studies included: (1) dose-dependent, statistically significant increases in the incidence of renal adenoma or carcinoma (combined) in male F344/N rats; (2) statistically significant increases in the incidence of pheochromocytomas/malignant pheochromocytomas (combined)

in male F344/N rats (NTP, 1989); and (3) statistically significant increases in the incidence of hepatocellular carcinomas in male and female B6C3F$_1$ mice (NCI, 1978). Additionally, HCE was shown to be a tumor 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, 2005b), data indicating cancer in more than one animal species, more than one sex, and more than one site of cancer supported the cancer descriptor "likely to be carcinogenic to humans" for HCE; even in the absence of evidence of carcinogenicity in humans.

5.4.1   Choice of Study/Data—with Rationale and Justification

Two chronic 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 or carcinomas (combined), and pheochromocytomas/malignant pheochromocytomas and complex pheochromocytomas (combined) in male F344 rats administered HCE for 103 weeks. Female rats received higher doses in this study, yet 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 for 78 weeks. Male mice in this study demonstrated a dose-response relationship, whereas female mice did not.

Both NTP (1989) and NCI (1978) were well-designed studies, conducted in both sexes of two species with 50 animals/sex/dose. Each study utilized two dosage groups of HCE and an unexposed 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 included changes to the dosing regimen 9 weeks into the study, cyclical dosing periods, and decreased survival 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.

5.4.2   Dose-response Data

The cancer incidence data are summarized in Table 5-5. Dose-related increases were reported in renal adenomas or carcinomas (combined) following chronic exposure to HCE (NTP, 1989). Male rats also exhibited increased incidences of pheochromocytomas/malignant pheochromocytomas (combined), although these increases were not dose-related (NTP, 1989). No HCE-related tumors were observed in female rats. In male mice, dose-related increases in hepatocellular carcinomas were reported following chronic exposure to HCE (NCI, 1978). Statistically significant increases in the incidence of hepatocellular carcinomas were also reported in female mice following chronic exposure to HCE, although these

increases were not dose-related (NCI, 1978). These data indicate that male rats are more sensitive to HCE-related carcinogenicity than male or female mice.

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	2/50 (4%)
			14	7/50 (14%)[b]
NTP (1989)	Male F344 rats	Pheochromocytomas/ malignant pheochromocytomas	0	14/50 (28%)
			7	26/45 (58%)[b]
			14	19/49 (39%)
NCI (1978)	Male B6C3F$_1$ mice	Hepatocellular carcinoma	0	3/20 (15%)[a]
			360	15/50 (30%)[b]
			722	31/49 (63%)[b]
NCI (1978)	Female B6C3F$_1$ mice	Hepatocellular carcinoma	0	2/20 (10%)[a]
			360	20/50 (40%)[b]
			722	15/49 (31%)[b]

[a]Incidence data are for the matched vehicle controls rather than the pooled controls from NCI (1978).
[b]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, 2011, 2005b). 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 were 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:

Scaled human dose (HED) = animal dose × (animal body weight/human body weight)^{¼}

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

Scaled human dose (HED) = 7 mg/kg-day × (0.483 kg/70 kg)^{¼}

= 2.05 mg/kg-day

Scaled human dose (HED) = 14 mg/kg-day × (0.471 kg/70 kg)^{¼}

= 4.10 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:

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

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

These scaled human doses were 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:

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

where:

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

$q_i = $ parameters estimated in fitting the model, $i = 1, \ldots, k$

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

The multistage model in BMDS ([U.S. EPA, 2009](#)) was fit to the incidence data (Table 5-5) using the calculated HEDs in order to derive an oral slope factor for HCE. In the NCI ([1978](#)) data, the low survival rates in the vehicle and unexposed male control groups led the authors to compare tumor incidences in the dosed males and females to vehicle control data pooled from bioassays for hexachloroethane, trichloroethane, and 1,1,2-trichloroethane. For BMD modeling, the incidence of hepatocellular carcinoma in the exposed group was compared to the incidence of hepatocellular carcinoma in the matched vehicle controls rather than the pooled controls. The BMR selected was the default value of 10% extra risk recommended for dichotomous models ([U.S. EPA, 2000c](#)). No data were excluded from the BMD multistage modeling.

The multistage model was fit to the incidences of renal adenomas or carcinomas (combined) in male rats and hepatocellular carcinomas in male mice. In all cases, the 2° multistage model provided the best fit. The multistage model was also fit to the incidence of pheochromocytomas/malignant pheochromocytomas (combined) in male rats and the incidence of hepatocellular carcinomas in female mice. The multistage model exhibited a significant lack of fit for the pheochromocytomas (in male rats) and the hepatocellular carcinomas in female mice (according to the χ^2 statistic with $p < 0.1$, see Appendix B for modeling outputs). Thus, these datasets were not useful for dose-response assessment because the tumor incidences are not a monotonic increasing function of dose, as demonstrated by the Cochran-Armitage Trend Test. Therefore, only the BMD modeling results for the kidney and liver tumors in male rats and male 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	BMR	BMD_{10}	$BMDL_{10}$ or POD	Oral slope factor $(mg/kg-day)^{-1}$
NTP (1989)	Male F344 rats	Renal adenomas/ carcinomas combined	2° Multistage	0.1	3.74	2.45	0.041
NCI (1978)	Male B6C3F$_1$ mice	Hepatocellular carcinomas	2° Multistage	0.1	38.09	13.80	0.007

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005b) 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, 2005b). As summarized in Section 4.7.3.1 and 4.7.3.2, the mode of carcinogenic action of HCE in the rat kidney and mouse liver is unknown. Although data was evaluated for with the involvement of the male rat-specific α_{2u}-globulin mode of action in the kidney, two principal factors contributed to the conclusion that the available data were insufficient support an α_{2u}-globulin mode of action for the development of renal tumors in male rats: (1) the lack of information identifying the α_{2u}-globulin protein in HCE-exposed rats, and (2) evidence of nephropathy in female rats as well as male and female mice (see Section 4.7.3.1). Some data suggest that metabolism and binding in mice are involved in the development of liver tumors. However, the role of DNA binding in the mode of action for HCE-induced hepatotoxicity and carcinogenesis is not known. 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 candidate oral slope factors were derived by linear extrapolation to the origin from the POD by dividing the BMR by the $BMDL_{10}$ (the lower bound on the exposure associated with a 10% extra cancer risk). The oral slope factor represents an upper bound estimate on cancer risk associated with a continuous lifetime exposure to HCE. In accordance with the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005b), an oral slope factor for renal tumors in male rats of 0.04 $(mg/kg-day)^{-1}$ was calculated by dividing the BMR of 0.1 by the human equivalent $BMDL_{10}$ of 2.45 mg/kg-day (Appendix B). An oral slope factor for hepatocellular tumors in male mice of 0.007 $(mg/kg-day)^{-1}$ was calculated by dividing the BMR of 0.1 by the human equivalent $BMDL_{10}$ of 13.80 mg/kg-day (Appendix B). 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 $(mg/kg-day)^{-1}$ for HCE.**

In the absence of data on the carcinogenicity of HCE via the inhalation route, an inhalation unit risk was not derived.

5.4.5 Uncertainties in Cancer Risk Values

The largest sources of uncertainty in the HCE cancer risk estimates are interspecies extrapolation and low-dose extrapolation. 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	Decision	Justification
Human relevance of rodent 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	It was assumed that rodent tumors are relevant to humans; tumor 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, 2005b)
Bioassay	Alternatives could ↑ or ↓ oral slope factor by an unknown extent	NTP study (1989)	Alternative bioassays in rats were unavailable. A 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 a lower risk estimate than rats. No increase of kidney tumors was observed 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. If the responsible metabolites are generated in proportion to administered dose, the estimated slope factor is an unbiased estimate.
Low-dose extrapolation procedure	Alternatives could ↑ or ↓ oral slope factor by 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 area under the curve, $BW^{3/4}$ 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.

↑ = increase; ↓ = decrease

5.4.5.1 Sources of Uncertainty

Relevance to humans. As described in Section 4.7.3, 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. The human relevance of the renal tumor mode of action was considered in Section 4.7.3.1. An evaluation of the available data concluded that there were insufficient data to support an α_{2u}-globulin mode of action for the development of renal tumors in male rats. Additional information on key data gaps (e.g., immunohistochemical data identifying α_{2u}-globulin in the hyaline droplets, data on the incidence of end stage renal failure or high severe nephropathy for controls and HCE-exposed animals, presence of foci of atypical hyperplasia, and if the location of renal adenomas were within the areas of chronic progressive nephropathy) would inform the human relevance of the observed kidney tumors.

The human relevance of the liver tumor mode of action was considered in Section 4.7.3.2. Experimental animal studies have demonstrated that oral exposure to HCE induces liver tumors in male and female mice. A potential mode of action for HCE-induced hepatocellular carcinomas in mice was 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 evaluated for HCE. Additional data distinguishing the similarities and differences between experimental animals and humans in terms of HCE metabolism or toxicity would inform the human relevance of the reported liver tumors.

The human relevance of the adrenal gland tumor mode of action was considered in Section 4.7.3.3. Pheochromocytomas occur in both humans and rats, although they are more common in laboratory rats. Evidence suggests that certain rat pheochromocytomas may have similarity to human pheochromocytomas (Powers et al., 2008). Furthermore, mechanisms of action inducing pheochromocytomas in rats are expected to occur in humans as well (Greim et al., 2009). The relevance of rodent pheochromocytomas as a model for human cancer risk has been the subject of discussion in the scientific literature (Greim et al., 2009; Powers et al., 2008). Additional data distinguishing the similarities and differences between pheochromocytoma induction in animals and humans would inform the human relevance of the reported adrenal gland tumors.

Bioassay selection. Of the two chronic animal bioassays selected for BMD analysis and subsequent quantitative cancer assessment, the NTP (1989) study was used for the development of an oral slope factor because male rats exhibited greater sensitivity to HCE-induced carcinogenicity than mice.

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 were 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. Evidence suggesting the kidney is a target organ of HCE toxicity in both species lends strength to the concern for human carcinogenic potential.

Dose metric. HCE is potentially 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 administered exposure is not the most relevant dose metric, then the impact on the human equivalent slope factor is unknown. Consequently; the low-dose cancer risk value may be higher or lower than that estimated, by an unknown amount. In the absence of data identifying the carcinogenic moiety for HCE, the administered exposure was selected as the dose metric.

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. In the absence of mode of action information to inform the dose-response at low doses, a linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with HCE exposure. The overall uncertainty in low-dose risk estimation would be reduced if the mode of action for HCE was more fully characterized.

Etiologically different tumor types were not combined across sites prior to modeling, 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. 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 additional mechanistic data, the human relevance of the rodent cancer results 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 suggested that HCE is potentially carcinogenic to humans. 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, 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, 2005b) and extrapolating from a POD in the observable range is designed in part to minimize model dependence. Measures of statistical uncertainty require assuming that the underlying model and associated assumptions are valid for the data under consideration. The multistage model used provided an adequate fit to all the datasets for kidney and liver tumors. For the multistage model applied to the incidence of tumors, the BMDL values should generally be within a factor of 3 of the BMDs. This indicates that there is a reasonably typical degree of uncertainty at the 10%

extra risk level. A large difference between the BMD and BMDL raises concern that the algorithm for the calculation of the BMDL is not accurate (U.S. EPA, 2005b). The ratios of the BMD_{10} values to the $BMDL_{10}$ values did not exceed a value of 2.6, indicating that the estimated risk was not influenced by any unusual variability in the model and associated assumptions.

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 U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2011, 2005b). 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 responses 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 genetically heterogeneous than inbred strains of laboratory animals (Calderon, 2000), 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 1987 HCE cancer assessment was based on the incidence of hepatocellular carcinomas in male mice in the NCI (1978) study. The current risk value was derived from the incidence of renal adenomas or carcinomas in male rats (NTP, 1989) and resulted in an oral slope factor approximately 2.8-fold higher than the previous assessment.

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

Scaled human dose = animal dose × (animal weight/human body weight)1/3 × (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 United States 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 FDA withdrew approval for this use in 1971. HCE has also been used as a polymer additive, a moth repellant, a plasticizer for cellulose esters, and an 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; Town and Leibman, 1984; Nastainczyk et al., 1982a; Nastainczyk et al., 1982b; Salmon et al., 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., 1982a; Nastainczyk et al., 1982b; 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 rather than 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 subchronic (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.

Under EPA's *Guidelines for Carcinogen Risk Assessment* (2005b), 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 a quantitative cancer risk assessment 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 HCE by oral exposure relates 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 in Gorzinski et al. (1985), using EPA's BMDS, resulted in a POD of 0.728 mg/kg-day. A composite UF of 1,000 was applied to the POD to derive an oral RfD of 7×10^{-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 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 oral subchronic studies. Confidence in the database is low because the database includes one acute and one subchronic 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, 2005b), 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) and 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, consistent with U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b). 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, while there are data to indicate that the the mechanism leading to the formation of the kidney tumors may be due to α_{2u}-globulin accumulation, important information is lacking and data indicating nephrotoxicity in other species and sexes confound any conclusions. 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 4×10^{-2}(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.

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 (U.S. EPA, 2011, 2005b) 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 HCE via the inhalation route are unavailable, and route-to-route extrapolation was not possible due to the lack of a PBPK 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

The Toxicological Review of Hexachloroethane (dated May, 2010) has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a). An external peer-review workshop was held September 21, 2010. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers, and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in the development of Appendix A. EPA did not receive any scientific comments from the public on the Toxicological Review of HCE.

External Peer Review Comments

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

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

General Charge Questions

Charge Question 1

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

Comment 1 The majority of the reviewers commented that the Toxicological Review was comprehensive and logically presented; however, all of the reviewers commented that the Toxicological Review was repetitious. The reviewers recommended including more synthesis, particularly in Section 5, as a way to improve clarity and conciseness of the Toxicological Review. Individual reviewers provided suggestions for improving clarity. One reviewer commented that the Toxicological Review did not provide adequate justification for using a NOAEL/LOAEL approach to model the inhalation effects. One reviewer disagreed with the rationale for the application of some of the uncertainty factors. One reviewer requested additional consideration of sublimation on the estimates of oral exposure dose when comparing the subchronic dietary exposure study (Gorzinski et al., 1985) and the subchronic gavage study (NTP, 1989). One reviewer commented that the available data on renal cancer following

HCE exposure is consistent with a mode of action that is a combination of α_{2u}-globulin nephropathy and exacerbation of chronic progressive nephropathy. One reviewer recommended that conclusions regarding the evaluation of the α_{2u}-globulin mode of action be stated in a more positive manner.

Response   The Toxicological Review in general, and Section 5 in particular, have been revised to include more synthesis and less repetition in the text to improve clarity and conciseness.

A NOAEL/LOAEL approach was selected for the inhalation data because the Weeks et al. ([1979](#)) study did not provide incidence data for the neurological effects, which precluded application of BMD modeling. Text in Section 5.2.2 has been modified to clarify this justification.

The rationale for the application of uncertainty factors is presented in Section 5.1.3 and Section 5.2.3. Text has been added to Section 5.1.3 and Section 5.2.3 to clarify the rationale for applying individual uncertainty factors. Further explanation of the rationale for applying individual uncertainty factors is provided in response to Charge Question A-4 and Charge Question B-4.

Text has been revised in Section 5.1.1 to clarify the selection of the principal study for the derivation of the RfD. The effect of sublimation on dietary HCE exposure has been considered in Gorzinski et al. ([1985](#)); however, potential inhalation effects from sublimation were not discussed by the study authors. The potential for inhalation effects from sublimation has been identified as a weakness in the study in Section 5.1.1. While the NTP ([1989](#)) 13 week study administered HCE by gavage, thus eliminating potential inhalation effects, the study had limitations. The NTP ([1989](#)) 13 week study did not provide incidence data for the kidney effects and administered higher doses of HCE than the subchronic study ([Gorzinski et al., 1985](#)) and the NTP ([1989](#)) chronic study. Therefore, the NTP ([1989](#)) 13 week study was considered, but not selected as the principal study.

Chronic progressive nephropathy and the potential exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation were discussed in Section 4.6.3 and Section 4.7.3.1. EPA concluded that HCE-related effects in male and female rats indicated that chronic progressive nephropathy is not solely responsible for the reported effects. In addition, EPA concluded that there is insufficient evidence to attribute the kidney effects of HCE exposure to an α_{2u}-globulin mode of action. Therefore, there was insufficient data to determine if the HCE-related nephropathy results from the exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation.

The evidence needed to attribute the kidney effects of HCE exposure to an α_{2u}-globulin mode of action were outlined in Section 4.7.3.1. Although there were data suggesting an α_{2u}-globulin mode of action for HCE-related nephropathy, none of the available studies identified α_{2u}-globulin in the hyaline droplets (see Table 4-21). Lack of immunohistochemical data prevented attributing the kidney effects of HCE exposure to an α_{2u}-globulin mode of action. Text was modified in Section 4.6.3, Section 5.4.3, and Section 5.4.5.1 to reiterate the conclusion reached in Section 4.7.3.1 that the available data were insufficient to support the α_{2u}-globulin mode of action.

Charge Question 2

Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Comment 2   Four of the reviewers were unaware of published studies that would significantly impact the Toxicological Review. Two reviewers commented that an immunohistochemical assessment of kidneys from the NTP 90-day study animals (NTP, 1989) would inform the mode of action for male rat kidney lesions. One reviewer provided a review on the exacerbation of chronic progressive nephropathy following chemical exposure as support for a combination of α_{2u}-globulin accumulation and exacerbation of chronic progressive nephropathy as the mode of action for renal tumors following HCE exposure. One reviewer provided a reference [Hemmila et al., Mutat. Res 701(2), 137-144] on the cytotoxicity, genotoxicity, and irritation potency of two red phosphorus-based pyrotechnic smokes, but commented that this study was unlikely to provide significant insight into HCE-induced toxicity.

Response   The lack of immunohistochemical evidence of α_{2u}-globulin in the hyaline droplets was identified in Section 4.7.3.1 and Section 5.4.5.1 as a data gap. This data gap would be addressed by the experiments recommended by the reviewers; however, immunohistochemical data were unavailable.

Section 4.6.3 and 4.7.3.1 discussed both chronic progressive nephropathy and potential for the exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation. EPA concluded that (1) chronic progressive nephropathy was not solely responsible for the reported kidney effects of HCE exposure and (2) lack of immunohistochemical data prevented attributing the kidney effects of HCE exposure to an α_{2u}-globulin mode of action. Therefore, the available data was insufficient to support an exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation as a mode of action for the renal effects of HCE exposure. EPA appreciates the identification of this review article; however the review describes other primary literature demonstrating exacerbation of chronic progressive nephropathy following exposures to other chemicals. In the case of HCE, nephropathy was also observed in female rats (NTP, 1989), as well as in male and female mice (NCI, 1978). Changes in severity of the nephropathy were also observed to be greater in male rats exposed to HCE compared to controls, indicating that HCE exposure exacerbated effects in the kidney of male rats. 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). The study recommended by the peer reviewer does not specifically address HCE-induced renal toxicity and therefore was not included in the Toxicological Review.

The human health effects of pyrotechnic munition smokes were briefly considered in Section 4.1. These studies (Seldén et al., 1994; Seldén et al., 1993) demonstrated HCE exposure in the smoke bomb production workers, but the sample sizes of the health effects studies were too small to reach definitive conclusions. Furthermore, the smoke produced by pyrotechnic smoke bombs is a mixture of chemicals consisting primarily of zinc oxychloride and zinc chloride. Therefore, the study recommended by the peer reviewer is unlikely to provide insight into HCE-induced toxicity and was not included in the Toxicological Review.

Chemical Specific Charge Questions

Oral Reference Dose (RfD) for HCE

Charge Question A.1

A 16-week dietary exposure study of HCE in F344 rats by Gorzinski et al. (1985) was selected as the basis for the derivation of the RfD. Kidney effects were observed in male rats in this study at doses below the range of exposure tested in the available chronic NTP (1989) study. Please comment on the scientific justification for the use of the subchronic Gorzinski et al. (1985) study as the principal study for the derivation of the RfD. Is the rationale for this selection clearly described? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comment All of the reviewers agreed with the selection of Gorzinski et al. (1985) as the principal study; however, some reviewers suggested clarifications to improve transparency in the selection of the Gorzinski et al. (1985) study. One reviewer requested additional discussion regarding the observation that kidney effects in the subchronic study were observed at doses lower than estimated by BMD modeling of data from the chronic studies. One reviewer requested further discussion of the selection of atrophy and degeneration of renal tubules from the subchronic study over other kidney effects (i.e., increased severity of tubular nephropathy or linear mineralization) reported in the chronic study. One reviewer recommended that the rationale for selecting the subchronic Gorzinski et al. (1985) study over the chronic NTP (1989) study should include discussion of both duration and the need for extrapolation below the lowest dose tested. One reviewer suggested that a better rationale for selecting the subchronic study was that kidney toxicity was observed after only 16 weeks of exposure and the subchronic study produced the lowest BMD/BMDL values.

Response Kidney effects in the 16 week subchronic study (Gorzinski et al., 1985) were observed at doses lower than estimated by BMD modeling of the available chronic data; however, these reported kidney effects were not statistically different from controls. The doses utilized in the subchronic study which reported statistically significant increases in kidney effects are consistent with the $BMDL_{10}$ for the kidney effect data from chronic studies.

Atrophy and degeneration of renal tubules was consistently observed in both subchronic and chronic studies, leading to its selection as the candidate critical effect for male rats exposed to HCE. Section 5.1.2 describes the selection of the subchronic Gorzinski et al. (1985) study over the chronic NTP (1989) and NCI (1978) studies, including consideration of dose. As stated in the Toxicological Review, U.S. EPA selected the Gorzinski et al. (1985) study as the principal study for derivation of the RfD because kidney effects were observed in male rats at doses below the range of exposure tested in the NTP (1989) study and the tubular nephropathy in male rats in the chronic exposure studies (NTP, 1989; NCI, 1978) resulted in higher PODs than the subchronic study (Gorzinski et al., 1985).

Study duration was considered during the application of uncertainty factors, as described in Section 5.1.3.

Charge Question A.2

Nephrotoxicity as indicated by atrophy and degeneration of renal tubules in male rats (Gorzinski et al., 1985) was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Comment   All of the reviewers supported the selection of atrophy and degeneration of renal tubules in male rats as the critical effect. One reviewer commented that the moderate-to-marked renal nephropathy from the chronic NTP (1989) study should be considered during the application of the subchronic-to-chronic uncertainty factor. One reviewer commented that spontaneous chronic progressive nephropathy may be a confounding factor and noted that the Gorzinski et al. (1985) study did not score the extent of chronic progressive nephropathy. This reviewer questioned if it was possible to distinguish effects of chronic progressive nephropathy and effects related to HCE exposure, suggesting that chemical-specific renal injury separate from chronic progressive nephropathy may occur at higher doses.

> **Response**   Section 5.1.3 was revised to discuss the available chronic data in the application of the subchronic-to-chronic uncertainty factor. The application of the subchronic-to-chronic uncertainty factor is further discussed in response to Charge Question A-4.

Section 4.7.3.1 discussed chronic progressive nephropathy and acknowledged that chronic progressive nephropathy can obscure the lesions characteristic of α_{2u}-globulin-related nephropathy. Although the Gorzinski et al. (1985) study did not provide data on chronic progressive nephropathy, the authors reported dose-dependent renal effects in both male and female rats and identified NOAELs for the renal effects in both sexes. These data suggested that chronic progressive nephropathy was not solely responsible for the reported renal effects.

Charge Question A.3

Benchmark dose (BMD) modeling was applied to the atrophy and degeneration of renal tubules data in male rats to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of atrophy and degeneration of renal tubules) scientifically justified and clearly described?

Comment   The majority of reviewers commented that BMD modeling was appropriately conducted and clearly described. One reviewer commented that mode of action considerations could lead to a higher point of departure.

Response   EPA concluded that while there were data to indicate that the mode of action for renal effects may be specific for male rats, there were confounding issues as indicated in Section 4.6.3.

Charge Question A.4

Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically justified and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale

Comment   All of the reviewers agreed with the application of an uncertainty factor of 10 for the interspecies extrapolation. One reviewer recommended expanding the justification for the application of this uncertainty factor to include availability of data on the active form of HCE and appropriate dose metrics.

All of the reviewers agreed with the application of an uncertainty factor of 10 for the intraspecies extrapolation.

One reviewer agreed with the application of an uncertainty factor of 10 for the subchronic-to-chronic extrapolation, while the remaining reviewers recommended changes to this uncertainty factor. One reviewer commented that the findings from the chronic NTP ([1989](#)) study supported the findings from the subchronic Gorzinski et al. ([1985](#)) study and, therefore, application of an uncertainty factor of 2-4 for the subchronic-to-chronic extrapolation would be adequate. One reviewer stated that the application of an uncertainty factor of 10 to account for subchronic-to-chronic extrapolation was debatable. Specifically, this reviewer provided a comparison of RfDs derived from both the chronic and subchronic data. This reviewer stated that if EPA were to apply a composite UF of 300 (keeping the currently applied UFs, except 1 for subchronic-to-chronic extrapolation) to the subchronic Gorzinski et al. (1985) study and a composite UF of 1000 (10 for inter- and intraspecies differences, 10 for LOAEL to NOAEL extrapolation, 1 for subchronic-to-chronic extrapolation, and 3 for database deficiencies) to the chronic NTP (1989) study the RfDs would be the same . Two reviewers recommended application of a subchronic-to-chronic uncertainty factor of 3 because the available chronic data do not suggest that prolonged exposure would exacerbate the renal tubule effects observed in the subchronic Gorzinski et al. ([1985](#)) study. One reviewer questioned whether the renal effects observed in the chronic NTP ([1989](#)) study were more severe than the renal effects in the subchronic Gorzinski et al. ([1985](#)) study and commented that a subchronic-to-chronic uncertainty factor of 10 was debatable; however, the reviewer did not recommend a value for the uncertainty factor.

The majority of reviewers agreed with the application of an uncertainty factor of 1 for the LOAEL-to-NOAEL extrapolation because the Gorzinski et al. ([1985](#)) study identified a NOAEL. One reviewer commented that the $BMDL_{10}$ is more reflective of a LOAEL than a NOAEL and, therefore, suggested application of an uncertainty factor of 3 for LOAEL-to-NOAEL extrapolation.

The majority of the reviewers agreed with the application of an uncertainty factor of 3 for the database uncertainty factor because of the lack of a multigeneration reproductive toxicity study. One reviewer recommended expanding the justification for the application of the database uncertainty factor to more transparently describe which studies were missing from the database and include

considerations of related chemicals and metabolites. One recommended either not applying the database uncertainty factor or equivalently applying a database uncertainty factor of 1. This reviewer stated that in practice either the database is adequate to derive an RfD or it is inadequate and no RfD is developed. This reviewer also suggested that the toxic effects observed in the developmental toxicity studies for HCE were at higher doses than the doses that induce renal toxicity in the subchronic and chronic studies.

Response   The discussion of the interspecies uncertainty factor in Section 5.1.3 was modified to indicate that the available toxicokinetic data for HCE was insufficient to identify the active compound or determine dose metrics for extrapolation.

The available data were reconsidered in the selection of the subchronic-to-chronic uncertainty factor and, as a result, the subchronic-to-chronic uncertainty factor has been reduced from 10 to 3. The Gorzinski et al. (1985) study duration was minimally longer than the standard subchronic (90-day) study and falls well short of a standard lifetime study (i.e., two year chronic bioassay), although chronic data were available for comparison. These chronic data suggest: (1) incidence of nephropathy may not increase with prolonged exposure and (2) consistency in dose response relationships with the subchronic studies. However, the lowest dose tested in the chronic exposure studies (NTP, 1989; NCI, 1978) represented a LOAEL, limiting the ability of these studies to inform the impact of increased exposure duration on renal effects observed at the lowest dose in the subchronic study (Gorzinski et al., 1985). Reduction of the subchronic-to-chronic uncertainty factor resulted in a composite uncertainty factor of 1,000. Section 5.1.3 has been modified to reflect the reduction of the subchronic-to-chronic uncertainty factor.

Section 5.1.3 has been modified to indicate that a two-generation reproduction study is absent from the database, resulting in the application of a database uncertainty factor of 3. Available data on HCE metabolism are limited and there is insufficient evidence to determine if the reported effects are due to the parent compound or the metabolites. In the absence of metabolism data, additional discussion of potential HCE metabolites and the impact on the database uncertainty factor is not warranted. Application of a database uncertainty factor does not indicate that the available data are insufficient to derive a reference value, as suggested by one reviewer. Rather, the database uncertainty factor accounts for the potential to underestimate noncancer hazard as a result of data gaps. The database for HCE does not contain a two-generation reproductive study, indicating an incomplete characterization of HCE toxicity. Therefore, the database uncertainty factor of 3 was applied in the derivation of the RfD.

B. Chronic Inhalation Reference Concentration (RfC) for HCE

Charge Question B.1

A 6-week inhalation exposure study in rats by Weeks et al. (1979) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Is the rationale for this selection clearly described? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comment   All the reviewers commented that in light of the limited database, the Weeks et al. (1979) data was the most appropriate study for deriving the RfC. One reviewer requested additional discussion regarding the adequacy of the Weeks et al. (1979) study for consideration as a principal study. One reviewer recommended an expanded discussion of why the Weeks et al. (1979) study was not used for RfC derivation in the previous 1987 IRIS assessment. One reviewer requested additional discussion of the human relevance of the reported neurobehavioral effects, given that these effects were observed only at a high dose of HCE.

Response   The Weeks et al. (1979) study is a well-conducted subchronic inhalation bioassay that evaluated an array of endpoints and established NOAELs and LOAELs for HCE in a number of different species. The authors evaluated portal of entry effects on lungs, trachea, and nasal turbinates by gross examination as well as histological sectioning. Weeks et al. (1979) examined sections of the nasal turbinates for upper respiratory effects and evaluated upper respiratory inflammation by the presence of polymorphonuclear leukocytes in close association with excess mucus within the lumens of the nasal passages. Section 5.2.1 has been revised to indicate the examination of the portal of entry effects by the study authors.

An RfC for HCE was not previously derived. In the 1987 IRIS Summary, Weeks et al. (1979) was briefly summarized in the Additional Studies/Comments section for the oral RfD. The 1987 IRIS Summary concluded that the Gorzinski et al. (1985) study is a better basis for the oral RfD. The 1987 IRIS Summary did not discuss why an inhalation RfC was not derived; therefore, it is unclear if the Weeks et al. (1979) data were considered for the derivation of the inhalation RfC. This information has been added to the Section 5.2.5.

The Weeks et al. (1979) study identified neurobehavioral effects in animals, but did not provide sufficient data on pharmacokinetic or mechanistic considerations to inform the human relevance of these effects. In the absence of pharmacokinetic or mechanistic data, the neurobehavioral effects in animals were assumed to be relevant to humans.

Charge Question B.2

Neurobehavioral effects in Sprague-Dawley rats (Weeks et al., 1979) were selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Comment   All of the reviewers agreed that neurobehavioral effects were supported by the available data and were appropriately chosen as the critical effect. One reviewer recommended clarifications to the discussion of critical effect selection, including (1) considerations of structure-activity relationships with related chemicals to support the selection of neurobehavioral effects as the critical effect; (2) discussion of the differences in target organ between oral and inhalation exposure, and (3) clarification that the respiratory effects were not selected as the critical effect because these effects were considered by the authors (Weeks et al., 1979) as attributable to mycoplasma infection. Two other reviewers also requested additional discussion on the difference in effects between oral and inhalation exposure to HCE, particularly the absence of nephrotoxicity following inhalation exposure. One reviewer commented that body weight changes were also observed in multiple species after

exposure to the highest dose and could have been selected as the critical effect. One reviewer recommended more discussion of dose relevance with respect to human exposures, as well as the implications of neurobehavioral effects that only occurred at the high exposure doses.

Response   A literature search did not identify any structure-activity relationships relevant to neurobehavioral effects of HCE exposure. Although oral and inhalation exposure to HCE affects different target organs, data were unavailable to inform the observed differences. As stated in Section 4.2.2.1, Weeks et al. (1979) attributed the increased incidence of respiratory lesions in rats to an endemic mycoplasma infection; however, the Weeks et al. (1979) study did not provide data demonstrating the presence of mycoplasma in the lungs. This data gap prevented exclusion of the respiratory tract effects from consideration as a potential critical effect. Rather, the consistent observation of neurotoxic effects across experiments was the rationale for selecting neurobehavioral effects as the critical effect. Text has been modified in Section 5.2.1 to clarify these conclusions.

The Weeks et al. (1979) study identified neurotoxicity, statistically significant decreases in body weight gain, and upper and lower respiratory tract irritation as effects of inhalation exposure to HCE. Of these effects, neurobehavioral effects and changes in body weight gain were consistently observed in multiple species. Neurobehavioral effects were assumed to pose a potential hazard to humans and therefore selected as the critical effect.

The Weeks et al. (1979) study did not provide data to inform pharmacokinetic considerations of the human relevance of the exposure dose; however, the available human exposure data for HCE (see Section 4.1) reported HCE levels lower than the neurotoxic dose reported in the Weeks et al. (1979) study. In the absence of pharmacokinetic or mechanistic data, the neurobehavioral effects in animals were assumed to be relevant to humans.

Charge Question B.3

The NOAEL/LOAEL approach was used to derive the POD for the RfC. Please comment on whether this approach is scientifically justified and clearly described.

Comment 9   All of the reviewers agreed that the NOAEL approach was justified for deriving the POD for the RfC. Two reviewers recommended clarification that it was the lack of individual responses at all exposure doses, not the 100% response at the high dose, which prevented BMD modeling of the Weeks et al. (1979) data. One reviewer suggested additional discussion of the human equivalent concentration derivation, particularly the categorization of HCE as a Category 2 gas.

Response   Section 5.2.2 has been modified to indicate that the lack of incidence data prevented BMD modeling of the Weeks et al. (1979) neurotoxicity data.

Section 5.2.2 has also been modified to clarify the gas categories for deriving a human equivalent concentration and the classification of HCE as a Category 3 gas.

Charge Question B.4

Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs scientifically justified and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

Comment   Three reviewers agreed with the application of an uncertainty factor of 3 for interspecies extrapolation, whereas the remaining three reviewers recommended an interspecies uncertainty factor of 10. One reviewer commented that the derivation of a human equivalent concentration did not adequately cover the interspecies toxicity uncertainty. One reviewer commented that derivation of a human equivalent concentration partially addressed toxicokinetics, but requested additional explanation for how the human equivalent concentration derivation addressed toxicodynamic considerations. Two reviewers recommended additional discussion for reducing the interspecies uncertainty factor to 3 when the regional gas dose ratio defaulted to 1 because of the absence of data. One reviewer suggested additional discussion of the blood:air partition coefficients in rats and humans for related chemicals, as well as discussion that the regional gas dose ratio defaults to 1 because the animal coefficient is usually larger than the human value. One reviewer requested additional clarification for the human equivalent concentration derivation.

All of the reviewers agreed with the application of an uncertainty factor of 10 for the intraspecies extrapolation.

All of the reviewers agreed with the application of an uncertainty factor of 10 for the subchronic-to-chronic exposure extrapolation. One reviewer recommended clarifying that the subchronic-to-chronic uncertainty factor was applied in the absence of any longer-term studies.

All of the reviewers agreed that no uncertainty factor was necessary for LOAEL-to-NOAEL extrapolation.

Two reviewers agreed with the application of an uncertainty factor of 10 for the limitations in the inhalation database, whereas the remaining three reviewers recommended a database uncertainty factor of 3. One of the reviewers stated that the available toxicity studies were sufficiently diverse and supported a database uncertainty factor of 3. Another reviewer commented that arguments could be made to support both an uncertainty factor of 3 for the database limitations as well as a database uncertainty factor of 10. Specifically, this reviewer recommended that EPA describe the key study types and remaining uncertainty in greater depth. This reviewer stated that a factor of 3 is sufficient given the lack of a multi-generation reproductive toxicity study. However, this reviewer believed that an argument could be made for the application of a factor of 10 due to the observed neurotoxicity (tremors) and lack of evaluation of more sensitive measures of neurological effects at the NOAEL (citing lack of post exposure sacrifice and histopathology). A third reviewer believed that even though a chronic inhalation study was not available, the availability of a developmental study supports an uncertainty factor of 3. Another reviewer recognized the lack of a developmental neurotoxicity study and multigenerational reproductive toxicity was of concern, but recommended a database uncertainty factor of 3 because the available literature included exposure in multiple species, a general toxicity study, a reproductive study, and a neurobehavioral study.

One reviewer noted that the overall uncertainty factor for the RfC was similar to the RfD, despite the comparatively larger data gaps in the inhalation database.

Response   As described in Section 5.2.3, an interspecies uncertainty factor of 3 is applied when incorporating an animal-specific NOAEL$_{ADJ}$ to a human equivalent NOAEL$_{HEC}$ dosimetric adjustment. This dosimetric adjustment reduces uncertainty by accounting for the variability in toxicokinetics; however, this dosimetric adjustment does not account for species differences in toxicodynamics. Therefore, in the absence of sufficient toxicodynamic data, an interspecies uncertainty factor of 3 is retained to account for toxicodynamic differences between animals and humans. Text was modified in Section 5.2.3 to clarify that toxicokinetic component of interspecies uncertainty is addressed by the dosimetric adjustment, whereas insufficient data exist to inform the toxicodynamic component of the intraspecies uncertainty factor. A regional gas dose ratio of 1 is also recommended if the animal blood:gas coefficient is greater than the human blood:gas coefficient or the animal and human partition coefficients are unknown. In accordance with current practices, a regional gas dose ratio of 1 was used because the animal and human blood:gas partition coefficients are unknown. Text has been added to the Section 5.2.2 to clarify the application of the default regional gas dose ratio as well as the derivation of the human equivalent concentration.

Text has also been added in Section 5.2.3 to clarify the subchronic Weeks et al. (1979) was the only repeat exposure study available.

Weeks et al. (1979) was a subchronic inhalation bioassay that evaluated an array of endpoints and established NOAELs and LOAELs. The Weeks et al. (1979) study represents the minimum database for deriving an RfC. In applying the database uncertainty factor, Section 5.2.3 indicated the deficiencies for the inhalation database. Specifically, the database is lacking a long-term study, a multigeneration reproductive toxicity study, and neurotoxicity and developmental neurotoxicity studies. Because of these data gaps, a database uncertainty factor of 10 was applied for the RfC derivation. Text was modified in Section 5.2.3 to clarify these data gaps.

Generally, uncertainty factors for the RfD and RfC are independently determined. The available information for the RfD and RfC (e.g., resulting in database deficiencies, use of NOAEL/LOAEL approach versus BMD modeling) was taken into account in the application of the individual uncertainty factors.

C. Carcinogenicity of HCE

Charge Question C.1

Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgrd.html), HCE is likely to be carcinogenic to humans by all routes of exposure. Is the cancer weight of evidence characterization scientifically justified and clearly described?

Comment   Five of the six reviewers agreed with the cancer descriptor "likely to be carcinogenic to humans." Several reviewers provided qualifying comments related to the carcinogenic mode of action and potential human relevance. One reviewer suggested that the narrative should capture the uncertainties associated with the cancer descriptor including more discussion of the human relevance of the kidney tumors and pheochromocytomas in the male rats and liver tumors in mice. This reviewer stated that

while the data indicate that HCE is "likely to be carcinogenic to humans," the weight of the evidence is on the low end of the spectrum for this descriptor. Another reviewer stated that the classification of HCE as "likely carcinogenic to humans" appeared to be excessive, but when evaluating the data and the U.S. EPA *Guidelines for Carcinogen Risk Assessment* the descriptor was inevitable.

One of the six reviewers did not comment specifically on the choice of the cancer descriptor but stated that in his opinion both an α_{2u}-globulin mechanism and chronic progressive nephropathy are involved in kidney tumor development, the mechanism for liver tumor development appears to be unknown, and the relevance of the increase in pheochromocytomas at low dose was questionable because the response was not dose-related.

A reviewer recommended collecting additional data to help evaluate the human relevance of the renal tubule tumors, including: the incidence of end stage renal failure or high severe nephropathy for controls and HCE-exposed animals, the presence of foci of atypical hyperplasia, if the location of renal adenomas were within the areas of chronic progressive nephropathy, and the presence of α_{2u}-globulin protein in the hyaline droplets. One reviewer commented that pentachloroethane, a potential metabolite of HCE, causes α_{2u}-globulin nephropathy.

Response EPA agrees with the majority of the reviewers that the cancer descriptor of "likely to be carcinogenic to humans" is an appropriate characterization of the weight of the evidence and is in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Text in Section 4.7.1 was revised to discuss uncertainties in the data, including the insufficient evidence for an α_{2u}-globulin mode of action for the kidney effects, as well as the human relevance of the kidney, adrenal gland, and liver tumors. Additionally, text related to these and other uncertainties is discussed in Section 5.4.5 and summarized in Table 5-7.

The specific comments on the mode of action for kidney, liver and adrenal tumors are addressed in the response to Charge Question C.4. Regarding the pheochromocytoma dose-response comment, these tumors were significantly increased in the low dose group, but not the high dose group (see Table 4-7). This dataset was not used in the dose-response assessment because the tumor incidence was not a monotonic increasing function of dose. Pheochromocytomas were considered relevant to humans (see Section 4.7.3.3); therefore, the observation of pheochromocytomas was considered as supporting evidence for the cancer descriptor of "likely to be carcinogenic to humans" for HCE.

Chronic progressive nephropathy and the potential exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation were discussed in Section 4.7.3.1. The severity of nephropathy was considered in the evaluation of the NTP (1989) study in Section 4.2.1.2 (summarized in Table 4-4); however, data were unavailable to categorize end stage renal failure in either the control or HCE-exposed animals. Similarly, data were unavailable to determine if foci of atypical hyperplasia were present, if renal adenomas were within the areas of chronic progressive nephropathy, or confirming the presence of α_{2u}-globulin protein in the hyaline droplets. These data gaps prevent attributing the renal effects of HCE to the exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation. Text in Section 5.4.5.1 was revised to indicate the additional data gaps that would inform the human relevance of the kidney tumors.

EPA acknowledges that additional mechanistic data, as well as additional human carcinogenicity data, would inform the uncertainty in the human relevance of renal and adrenal tumors following HCE exposure of the observed tumors and potentially impact selection of the cancer descriptor.

Also, one reviewer suggested that data for pentachloroethane may have potential relevance to the toxicity of HCE. Pentachloroethane is a putative metabolite of HCE (see Figure 3-1), but the available data on HCE metabolism are limited. The putative metabolites are briefly discussed in the Toxicological Review as support for the toxicological effects reported following HCE exposure; however, there is insufficient evidence to determine if the reported effects are due to the parent compound or the metabolites.

Charge Question C.2

A two-year oral gavage cancer bioassay in F344 rats (NTP, 1989) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantitation is scientifically justified and clearly described. Please identify and provide the rationale for any other studies that should be selected.

Comment Five reviewers agreed with the selection of the NTP (1989) study for the derivation of an oral slope factor, based on the sensitivity of male rats to HCE exposure. One reviewer recommended characterizing the available data as "insufficient" to support an α_{2u}-globulin mode of action. One reviewer requested the BMD modeling output for hepatocellular carcinomas in female mice be added to Appendix B and recommended to not include the hepatocellular carcinoma endpoint in Table 5-6. One reviewer questioned the use of linear low-dose extrapolation in the derivation of the oral slope factor. One reviewer noted that measurements of α_{2u}-globulin in the NTP (1989) study and consideration of the α_{2u}-globulin exacerbation of chronic progressive nephropathy mode of action would inform the human relevance of the observed renal tumors in male rats.

One reviewer disagreed with the derivation of an oral slope factor based on renal tubule tumors in male rats from the NTP (1989). This reviewer recommended deriving an oral slope factor based on hepatocellular carcinomas in male mice reported in the NCI (1978) study.

Response Sections 5.4.3 and 5.4.5.1 were modified to reiterate the conclusions in Section 4.6.3 and Section 4.7.3.1, that the available data were insufficient to support an α_{2u}-globulin mode of action.

The multistage cancer BMD modeling output for the hepatocellular carcinomas in female mice was added to Appendix B of the Toxicological Review. Also, because the BMD modeling output indicated that the multistage model exhibited significant lack of fit for the hepatocellular carcinomas in female mice, the data have been removed from Table 5-6, and the corresponding text has been modified.

Determination of α_{2u}-globulin protein in the hyaline droplets could inform the role of an α_{2u}-globulin mode of action in the renal effects of HCE exposure reported in the NTP (1989) study; however, these data are unavailable. In addition, the nephrotoxic effects of HCE observed in male and female mice confounded the determination and indicates that there may be more than one mode of action for renal toxicity. Similarly, data were unavailable to inform the potential for exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation. Currently, there are insufficient data to conclude that the renal effects observed following HCE exposure are attributable to either an α_{2u}-globulin mode of action or exacerbation of chronic progressive nephropathy by α_{2u}-globulin accumulation.

The oral slope factor was derived based on the renal tubule tumors in male rats because (1) the renal effects were considered relevant to humans, and (2) the rats exhibited greater sensitivity to HCE-induced carcinogenicity than the mice.

Charge Question C.3

The renal tubule tumor data in male rats from the NTP (1989) two-year oral gavage cancer bioassay were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

Comment   Four reviewers agreed with the selection of renal tubule tumor data in male rats from the NTP (1989) study as the basis for the quantitative cancer assessment. Two reviewers questioned the human relevance of the renal tubule tumors and recommended selecting hepatocellular carcinomas in mice as the basis for the quantitative cancer assessment.

Response   As discussed in Section 4.7.3.1, two principal factors contributed to the conclusion that there are insufficient data to support an α_{2u}-globulin mode of action for the development of renal tumors in male rats. First, the presence of kidney effects in HCE-exposed male and female mice and female rats suggests a mode of action other than α_{2u}-globulin nephropathy. Second, none of the HCE studies confirmed the presence of α_{2u}-globulin protein within the hyaline droplets. As stated in Section 5.4.4, the renal tubule tumors were selected for the basis of the cancer slope factor because the rats exhibited greater sensitivity to HCE-induced carcinogenicity than the mice. Human relevance of the renal tubule tumors is discussed in Section 4.7.3.1. EPA concluded that in the absence of sufficient information demonstrating the involvement of α_{2u}-globulin processes, male rat renal toxicity/tumors were considered relevant for risk assessment purposes.

Charge Question C.4

EPA concluded that the mode of action for renal tubule tumors observed following oral exposure to HCE is unknown. An analysis of the mode of action data for renal tumors is presented in the Toxicological Review. Based on this analysis, EPA determined that HCE-induced renal tumors could not be attributed to the accumulation of α_{2u}-globulin. Please comment on the scientific support for these conclusions. Please comment on whether the analysis is scientifically justified and clearly described.

Comment   Four reviewers agreed with the determination that HCE-induced renal tumors could not be attributed to the accumulation of α_{2u}-globulin. Two reviewers disagreed with the conclusion that HCE-induced renal tumors were not attributable to α_{2u}-globulin accumulation.

One reviewer requested additional discussion of the mouse hepatic tumors and pheochromocytomas in rats in the overall cancer risk assessment of HCE, particularly in the context of determining the cancer descriptor and the U.S. EPA *Guidelines for Carcinogen Risk Assessment*. This reviewer also recommended inclusion of a discussion of the similarities and differences related to the data for renal

proliferative lesions produced by α_{2u}-globulin nephropathy and advanced chronic progressive nephropathy for tetrahydrofuran.

One reviewer commended EPA on the clarity and concise presentation of Section 4.7.3.1. This reviewer stated that EPA correctly concluded that the criteria for an α_{2u}-globulin mode of action for HCE-induced renal tumors were not met under EPA's guidance. This reviewer suggested that the data do indicate a dose-response and temporal relationship for key events in an α_{2u}-globulin mode of action and requested that EPA include more synthesis in the dose response section (i.e., table showing at which doses the key events in the α_{2u}-globulin mode of action occur). This reviewer also requested discussion of the α_{2u}-globulin mode of action conclusions reached by several recent reviews on HCE carcinogenicity.

Another reviewer noted that since renal tubule tumors were only observed in male rats, the occurrence of nephrotoxicity in female rats is not contributing evidence for excluding the α_{2u}-globulin mode of action for HCE carcinogenicity. This reviewer also commented that they did not ultimately disagree with EPA's conclusions regarding mode of action for renal tumors, but stated that the document should be clearer about the rationale and explanation for the conclusion. Specifically, this reviewer suggested that EPA state that the α_{2u}-globulin mode of action for HCE carcinogenicity may in part explain the renal effects, but other modes of action also exist that would not exclude these tumors from consideration in risk assessment. This reviewer did not specifically state what other modes of action may exist.

One reviewer also disagreed with the conclusion that HCE renal carcinogenicity cannot be attributed to an α_{2u}-globulin mode of action, arguing that the available data provide evidence of 6 out of 7 steps in the α_{2u}-globulin mode of action. This reviewer commented that weight of evidence from other chemicals structurally related to HCE support a male rat specific mode of action for HCE renal carcinogenicity. Lastly, the reviewer suggested that exacerbation of chronic progressive nephropathy by HCE further supports a mode of action for HCE renal carcinogenicity that is not relevant to humans.

Response   Section 4.7 has been revised to include additional consideration of the hepatocellular carcinomas and pheochromocytomas in the determination of the cancer descriptor. The presence of statistically significant increases in the incidence of pheochromocytomas/malignant pheochromocytomas (combined) and renal tubule tumors in male F344/N rats and statistically significant increases in the incidence of hepatocellular carcinomas in male and female B6C3F$_1$ mice provided evidence for the cancer descriptor "likely to be carcinogenic to humans" for HCE. Section 5.4 has been modified to clarify the selection of the cancer descriptor "likely to be carcinogenic to humans" for HCE. With respect to the conclusions of review manuscripts on HCE carcinogenicity, EPA conducted an independent mode of action analysis of the relevant primary literature as the basis for the conclusions presented in the Toxicological Review (see Section 4.7.3.1). The available review manuscripts relevant to the α_{2u}-globulin mode of action in renal nephropathy and renal tumors were used as references for the evaluation of the available renal effects data for HCE.

Unlike the tetrahydrofuran studies (Bruner et al., 2010; Chhabra et al., 1998), none of the available HCE studies performed immunohistochemical analysis to identify α_{2u}-globulin in the hyaline droplets (see Table 4-21). Therefore, consideration of the tetrahydrofuran data would not inform the mode of action for the renal effects of HCE exposure. Because of the absence of immunohistochemical data,

the HCE dose at which α_{2u}-globulin accumulates hyaline droplets is unknown. Consequently, there was insufficient data to determine if accumulation of α_{2u}-globulin in hyaline droplets occurs at lower HCE doses than subsequent α_{2u}-globulin-related effects. In the absence of these data, a table showing the HCE doses at which key events in the α_{2u}-globulin mode of action occur was not added to the Toxicological Review. Text was added to Section 4.7.3.1 to clarify that dose-response concordance (of the accumulation of α_{2u}-globulin in hyaline droplets) could not be demonstrated from the available data. As discussed in Section 4.7.3.1, the temporal relationship between renal tumor tubules and the α_{2u}-globulin mode of action could not be established because none of the HCE studies confirmed the presence of α_{2u}-globulin protein within the hyaline droplets. Although renal tubule tumors were only reported in male rats, the occurrence of nephrotoxicity in female rats contributed to the supporting evidence that the renal effect of HCE may not be attributable to α_{2u}-globulin accumulation. Accumulation of α_{2u}-globulin is unique to the male rat, as female rats and other laboratory mammals do not accumulate α_{2u}-globulin in the kidney and do not subsequently develop renal tubule tumors. Therefore, the evidence of nephropathy in female rats, as well as male and female mice, suggested that the HCE-induced renal tumors may not be attributable to an α_{2u}-globulin mode of action, or that more than one mode of action may be operating. The sex-specific differences in carcinogenic effects of HCE exposure may also reflect sex-specific differences in the kidney concentrations of HCE following oral exposure (see Table 3-3).

Some data suggested that the male rat-specific α_{2u}-globulin mode of action could contribute to HCE-induced nephropathy. As summarized in Section 4.7.3.1, the data were insufficient to support an α_{2u}-globulin mode of action in the development of renal tumors in male rats following HCE exposure. Chronic progressive nephropathy was also discussed in Section 4.7.3.1. There was insufficient evidence to attribute the kidney effects of HCE exposure to exacerbation of chronic progressive nephropathy. Lastly, a literature search did not identify any structure-activity relationships relevant to carcinogenic effects of HCE exposure.

Charge Question C.5

The oral cancer slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk for renal tumors in male rats). Has the modeling approach been appropriately conducted and clearly described?

Comment All of the reviewers agreed that the modeling approach was appropriately conducted. One reviewer requested clarification about whether or not EPA used the matched vehicle control data when modeling the hepatocellular carcinoma data.

Response Text was modified in the Section 5.4.3 and Table 5-5 to indicate that the matched vehicle control data were presented and were used for BMD modeling.

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](#)) 78 weeks Gavage	Tubular nephropathy	Male rat Osborne-Mendel	**Gamma**	**0.93**	**133.68**	**21.23**	**16.99**
			Multistage 1°	**0.93**	**133.66**	**21.25**	**17.01**
			Weibull	**0.93**	**133.68**	**21.23**	**16.99**
		Female rat Osborne-Mendel	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](#)) 103 weeks Gavage	Moderate to marked Tubular nephropathy	Male rat F344	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 moderate Tubular nephropathy	Female rat F344	**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](#)) 103 weeks Gavage	Linear mineralization	Male rat F344	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](#)) 103 weeks Gavage	Hyperplasia of the pelvic transitional epithelium	Male rat F344	Gamma	0.42	84.64	7.33	4.87
			LogLogistic	**0.48**	**84.42**	**7.05**	**4.48**
			Multistage 2°	0.42	84.64	7.33	4.87
			Weibull	0.42	84.64	7.33	4.87
			Quantal-linear	0.42	84.64	7.33	4.87
Gorzinski et al. ([1985](#)) 16 weeks Diet	Atrophy and degeneration of renal tubules	Male rat F344	**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 F344	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](#)) 16 weeks Diet	Slight hypertrophy and/or dilation of proximal convoluted tubules	Male rat F344	**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	Female	**Gamma**	**0.93**	**38.62**	**118.04**	**60.18**

13 weeks Gavage	necrosis	rat F344	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 CL ($BMDL_{10}$). Modeling output is provided for models that represent the POD for each of the kidney endpoints; these models are highlighted in bold font.

Table B-1 presents the dose-response modeling results using BMDS (version 2.0) based on non-cancerous kidney and liver effects in rats following oral exposure to HCE. 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 candidate 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 candidate 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°, probit, 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 logistic and probit models were the lowest (and identical); therefore, the probit model with the lowest $BMDL_{10}$, of 2.60 mg/kg-day was selected as the candidate POD for this dataset.

Based on the incidence of mild to moderate 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 candidate 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 3.22 mg/kg-day was selected as the candidate 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 candidate 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$_{10}$ values were identical for these models; therefore, the BMDL$_{10}$ of 0.728 mg/kg-day was selected as the candidate 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 probit BMDL$_{10}$ of 10.51 mg/kg-day was selected as the candidate 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 , 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 gamma, Weibull, and quantal-linear models yielded the lowest (and identical) AICs. All of the BMDL$_{10}$ values were identical for these models; therefore, the BMDL$_{10}$ of 0.710 mg/kg-day was selected as the candidate 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 candidate 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 10, 5, and 1% based on noncancerous 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 and Weibull	21.23	16.99	10.33	8.27	2.02	1.62
			Multistage 1°	21.25	17.01	10.35	8.28	2.03	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	Probit	3.81	2.60	1.93	1.32	0.39	0.27
	Mild to moderate tubular nephropathy	Female rat	Gamma, Quantal-linear, and 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, Multistage 1°, and Quantal-linear	1.34	0.73	0.66	0.35	0.13	0.07
		Female rat	Probit	16.10	10.51	8.89	5.60	1.97	1.18
Gorzinski et al. (1985)	Slight hypertrophy and/or dilation of proximal convoluted tubules	Male rat	Gamma, Weibull, and Quantal-linear	1.22	0.71	0.60	0.35	0.12	0.07
Liver effects									
NTP (1989)	Hepatocellular necrosis	Female rat	Gamma	118.04	60.18	84.66	33.34	41.75	8.60

        Gamma Model
          NCI ([1978](#)) Tubular Nephropathy in Male Rats

 ==
        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 cumulative 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
                            Scaled
   Dose    Est._Prob.  Expected  Observed   Size    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

14:55 04/09 2009

Figure B-1   Male rats, Gamma model, Kidney effect (noncancerous): Tubular Nephropathology. NCI (1978), Osborne-Mendel Strain, 78 weeks exposure by gavage.

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
                               Scaled
   Dose   Est._Prob.  Expected  Observed   Size    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
```

Figure B-2   Male rats, Multistage 1° model, Kidney effect (noncancerous): Tubular Nephropathology. NCI (1978), Osborne-Mendel Strain, 78 weeks exposure by gavage.

Figure B-2   Male rats, Multistage 1° model, Kidney effect (noncancerous): Tubular Nephropathology. NCI (1978), Osborne-Mendel Strain, 78 weeks exposure by gavage.

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
                               Scaled
     Dose    Est._Prob.  Expected  Observed   Size     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
```
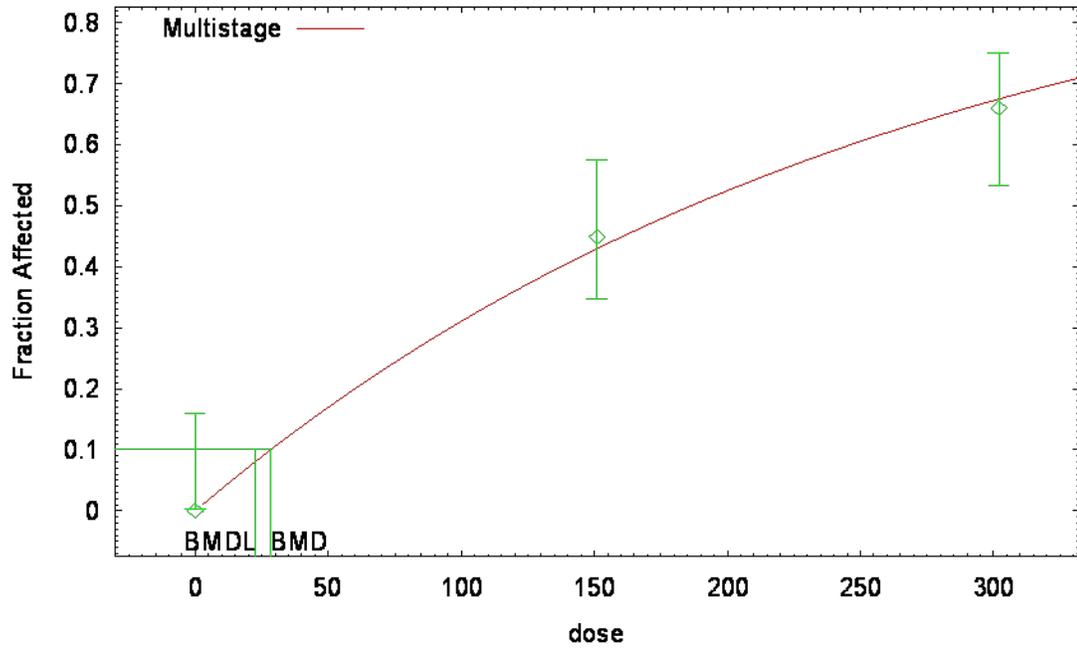
Figure B-3   Male rats, Weibull model, Kidney effect (noncancerous): Tubular Nephropathology. NCI (1978), Osborne-Mendel Strain, 78 weeks exposure by gavage.

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
                            Scaled
   Dose   Est._Prob.  Expected  Observed   Size    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
```

Figure B-4 **Female rats, Multistage 2° model, Kidney effect (noncancerous): Tubular Nephropathology. NCI ([1978](#)), Osborne-Mendel Strain, 78 weeks exposure by gavage.**

NTP ([1989](#)) Male Rat Nephropathy
Probit Model

```
====================================================================
        Probit Model. (Version: 3.1; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpA0E.(d)
        Gnuplot Plotting File: C:\USEPA\BMDS2\Temp\tmpA0E.plt
                                        Wed Apr 08 13:27:38 2009
 ====================================================================

 BMDS Model Run NTP 1989 Tubular Nephropathy 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 = PercentPositiveModerateMarkedTubularNephropathy
  Independent variable = ularNephropathy
  Slope parameter is not restricted

  Total number of observations = 3
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008



          Default Initial (and Specified) Parameter Values
            background =      0  Specified
             intercept =  -0.354714
                 slope =   0.0433259


      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     -0.78

     slope     -0.78        1



                  Parameter Estimates

                         95.0% Wald Confidence Interval
     Variable      Estimate     Std. Err.   Lower Conf. Limit  Upper Conf. Limit
     intercept     -0.35763      0.165052      -0.681127        -0.0341335
       slope      0.0436991    0.0182219      0.00798493        0.0794134



            Analysis of Deviance Table

   Model   Log(likelihood) # Param's Deviance Test d.f.  P-value
   Full model    -100.939       3
  Fitted model   -100.939       2   0.000120944   1       0.9912
```

B-17

```
Reduced model    -103.852     1    5.82641    2      0.0543

      AIC:       205.878


                      Goodness of Fit
                                Scaled
   Dose   Est._Prob.  Expected  Observed   Size    Residual
  ------------------------------------------------------------------
  0.0000   0.3603    18.016   18.000      50     -0.005
  7.0000   0.4794    23.968   24.000      50      0.009
  14.0000  0.6003    30.016   30.000      50     -0.005

 Chi^2 = 0.00   d.f. = 1    P-value = 0.9912


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

Confidence level =      0.95

      BMD =    3.81407

      BMDL =    2.59812
```

Probit Model with 0.95 Confidence Level

13:27 04/08 2009

Figure B-5   Male rats, Probit model, Kidney effect (noncancerous): Tubular Nephropathology (moderate to marked). NTP (1989), F344 Strain, 103 weeks exposure by gavage.

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
                                Scaled
     Dose   Est._Prob.   Expected  Observed   Size     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
```

Gamma Multi-Hit Model with 0.95 Confidence Level

10:19 04/10 2009

Figure B-6   Female rats, Gamma model, Kidney effect (noncancerous): Tubular Nephropathology (mild to moderate). NTP (1989), F344 Strain, 103 weeks exposure by gavage.

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
                                Scaled
    Dose   Est._Prob.  Expected  Observed   Size    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

Figure B-7 Female rats, Quantal-linear model, Kidney effect (noncancerous): Tubular Nephropathology (mild to moderate). NTP (1989), F344 Strain, 103 weeks exposure by gavage.

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
                         Scaled
  Dose   Est._Prob.  Expected  Observed   Size    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
```
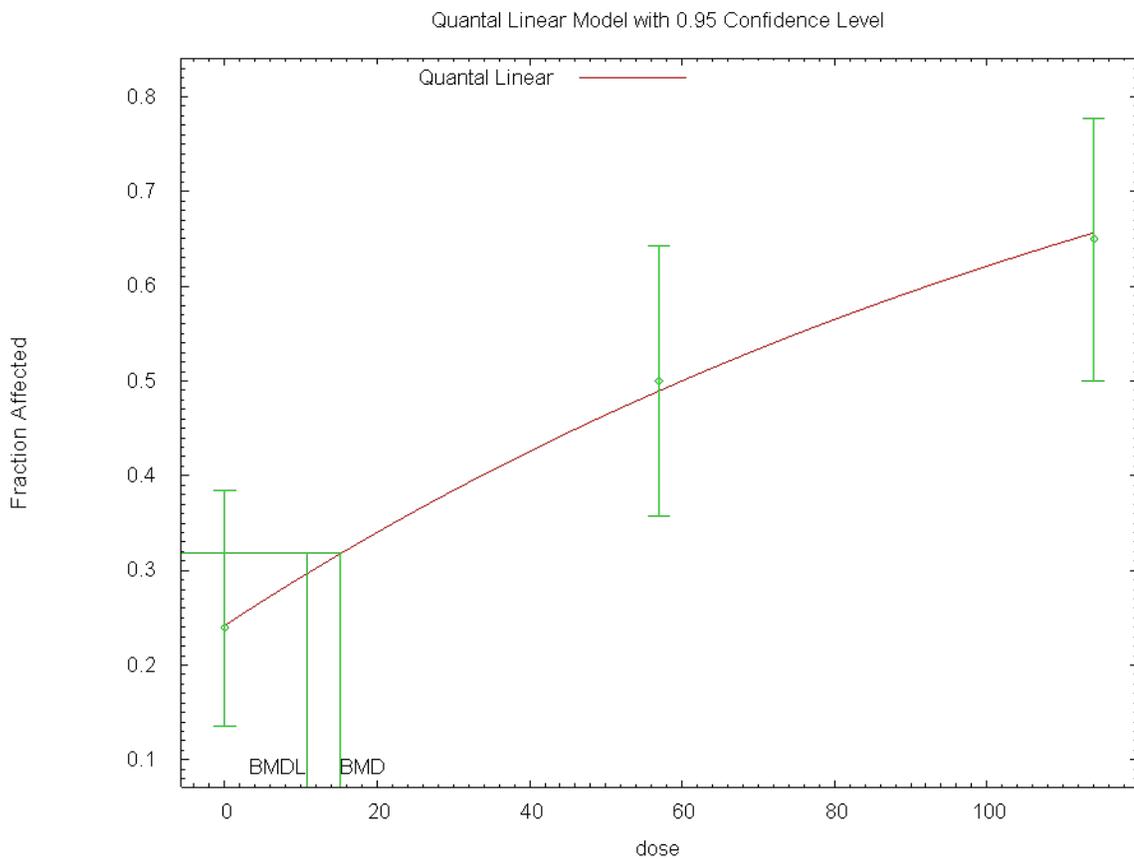
Weibull Model with 0.95 Confidence Level

10:34 04/10 2009

Figure B-8 **Female rats, Weibull model, Kidney effect (noncancerous): Tubular Nephropathology (mild to moderate). NTP (1989), F344 Strain, 103 weeks exposure by gavage.**

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
                          Scaled
  Dose   Est._Prob.  Expected  Observed  Size    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

14:24 04/08 2009

Figure B-9   Male rats, Probit model, Kidney effect (noncancerous): Linear minerialization. NTP (1989), F344 Strain, 103 weeks exposure by gavage.

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 1989 – 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
                           Scaled
  Dose   Est._Prob.  Expected  Observed   Size     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

14:26 08/12 2009

Figure B-10 Male rats, Log-logistic model, Kidney effect (noncancerous):
Hyperplasia of the pelvic transitional epithelium. NTP (1989),
F344 Strain, 103 weeks exposure by gavage.

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
 ====================================================================

 Gamma AtropyandDegenRenalTubulesDataNoSeverityMaleRat.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
                              Scaled
   Dose    Est._Prob.  Expected  Observed   Size     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
```
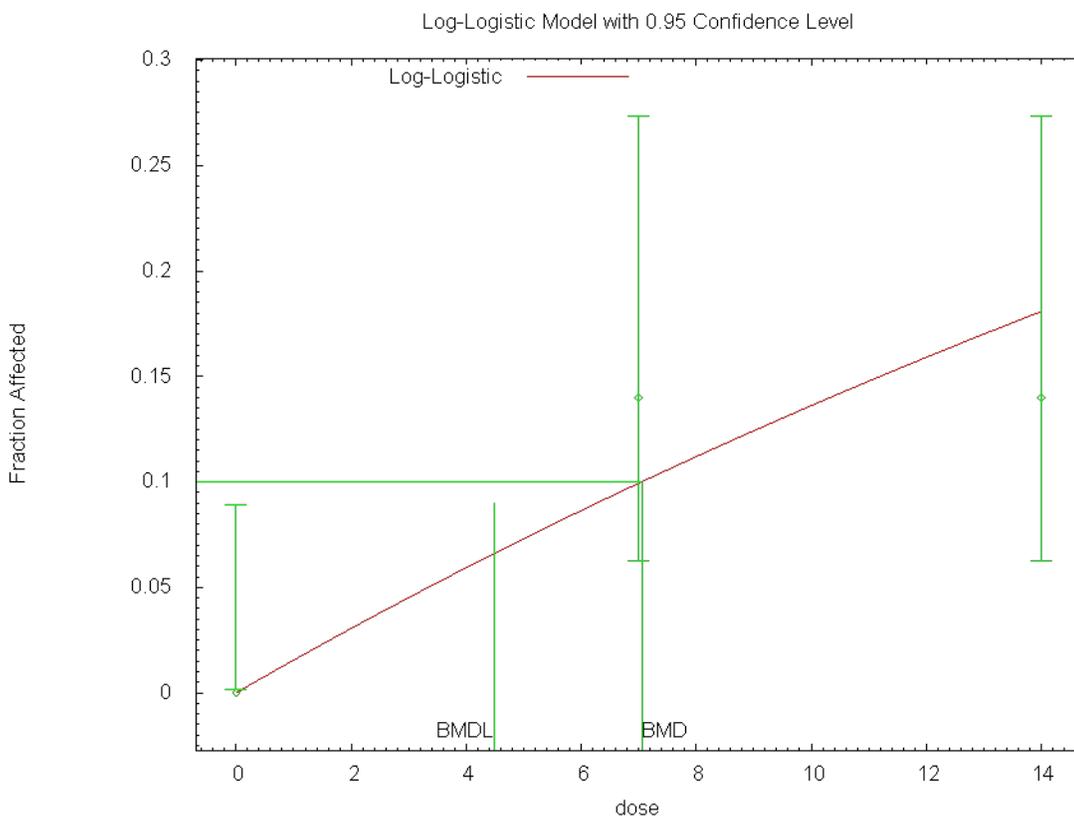

Gamma Multi-Hit Model with 0.95 Confidence Level

08:59 10/08 2009

**Figure B-11 Male rats, Gamma model, Kidney effect (noncancerous):
Atrophy and degeneration of renal tubules. Gorzinski et al.
(1985), F344 Strain, 16 weeks exposure by diet.**

Multistage 1° 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
 ====================================================================

 MS1° AtropyandDegenRenalTubulesDataNoSeverityMaleRat.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
                               Scaled
    Dose    Est._Prob.   Expected  Observed   Size    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
```
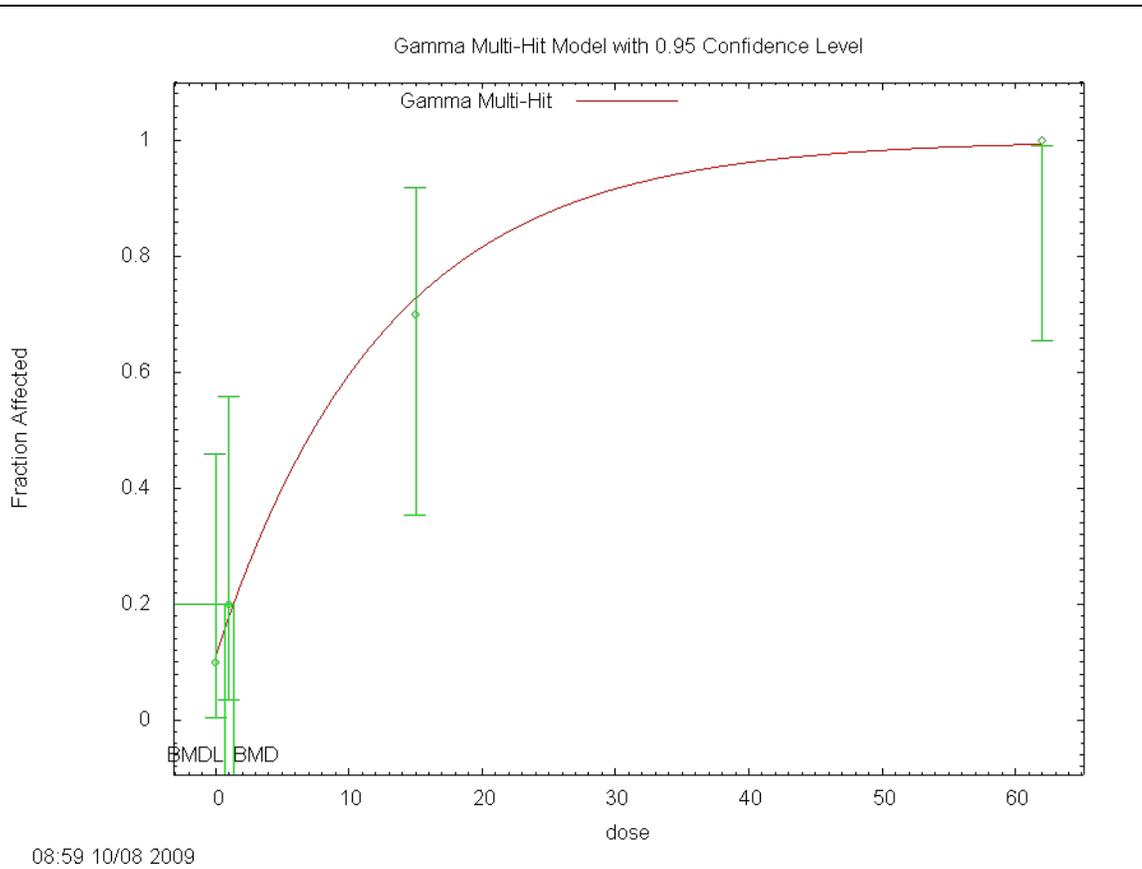
Multistage Model with 0.95 Confidence Level
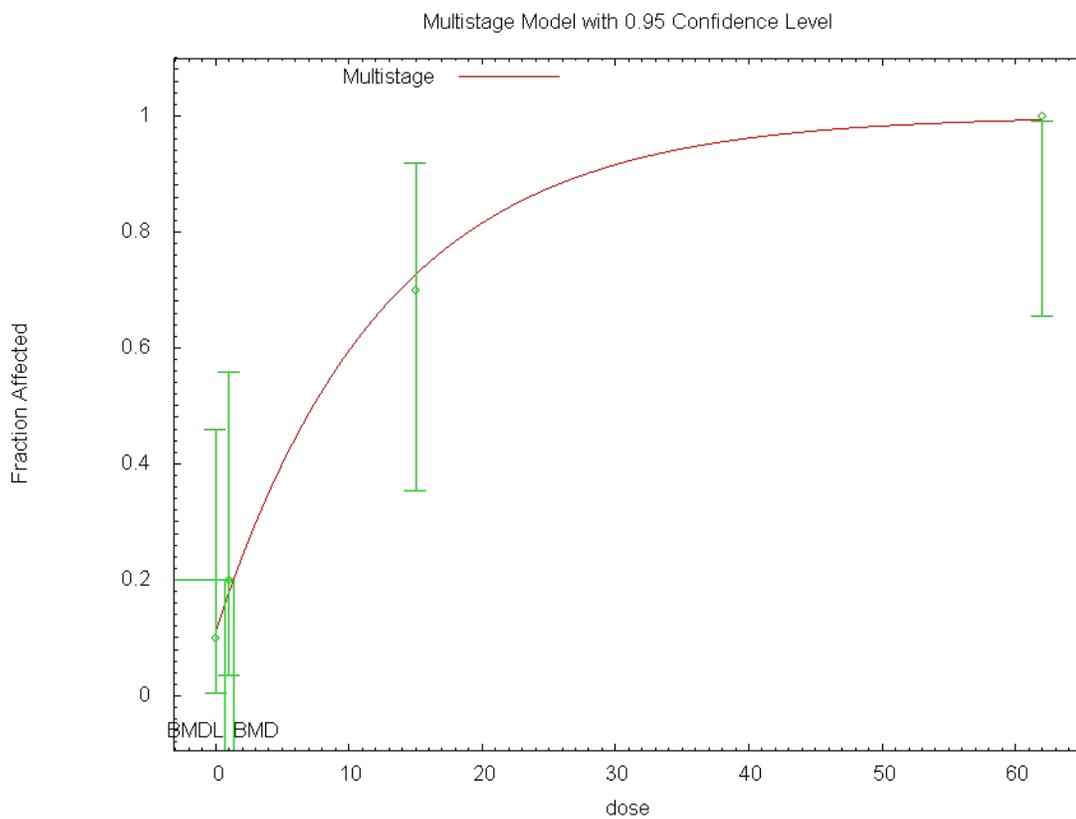
09:00 10/08 2009

Figure B-12 Male rats, Multistage 1° model, Kidney effect (noncancerous): Atrophy and degeneration of renal tubules. Gorzinski et al. (1985), F344 Strain, 16 weeks exposure by diet.

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
====================================================================

 QL AtropyandDegenRenalTubulesDataNoSeverityMaleRat.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
                              Scaled
   Dose   Est._Prob.  Expected  Observed   Size    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
```


Quantal Linear Model with 0.95 Confidence Level

09:02 10/08 2009

Figure B-13 Male rats, Quantal-linear model, Kidney effect (noncancerous): Atrophy and degeneration of renal tubules. Gorzinski et al. (1985), F344 Strain, 16 weeks exposure by diet.

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

```
==================================================================
        Probit Model. (Version: 3.1; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmpF0E.(d)
        Gnuplot Plotting File: C:\USEPA\BMDS2\Temp\tmpF0E.plt
                                        Thu May 06 10:06:12 2010
 ==================================================================

 Probit AtropyandDegenRenalTubulesDataNoSeverityFemaleRat.dax
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

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

  where CumNorm(.) is the cumulative normal distribution function


  Dependent variable = Effect
  Independent variable = DOSE
  Slope parameter is not restricted

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



         Default Initial (and Specified) Parameter Values
            background =       0  Specified
            intercept =    -1.21184
              slope =  0.0236401


       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     -0.69

   slope     -0.69       1



                 Parameter Estimates

                          95.0% Wald Confidence Interval
    Variable     Estimate      Std. Err.    Lower Conf. Limit  Upper Conf. Limit
    intercept     -1.26508      0.324595        -1.90127          -0.628881
      slope    0.0246481     0.00871343      0.00757005         0.0417261



           Analysis of Deviance Table

   Model   Log(likelihood) # Param's Deviance Test d.f.  P-value
    Full model    -18.2358      4
  Fitted model   -18.2465      2    0.0214055    2       0.9894
 Reduced model  -22.4934      1    8.51521     3       0.03648
```

```
    AIC:      40.493


              Goodness of Fit
                               Scaled
    Dose   Est._Prob.  Expected  Observed   Size    Residual
  -----------------------------------------------------------------------
   0.0000    0.1029      1.029     1.000      10     -0.030
   1.0000    0.1074      1.074     1.000      10     -0.076
   15.0000   0.1853      1.853     2.000      10      0.120
   62.0000   0.6038      6.038     6.000      10     -0.024

  Chi^2 = 0.02   d.f. = 2    P-value = 0.9893


   Benchmark Dose Computation

 Specified effect =      0.1

 Risk Type     =   Extra risk

 Confidence level =      0.95

       BMD =     16.0998

      BMDL =     10.5128
```
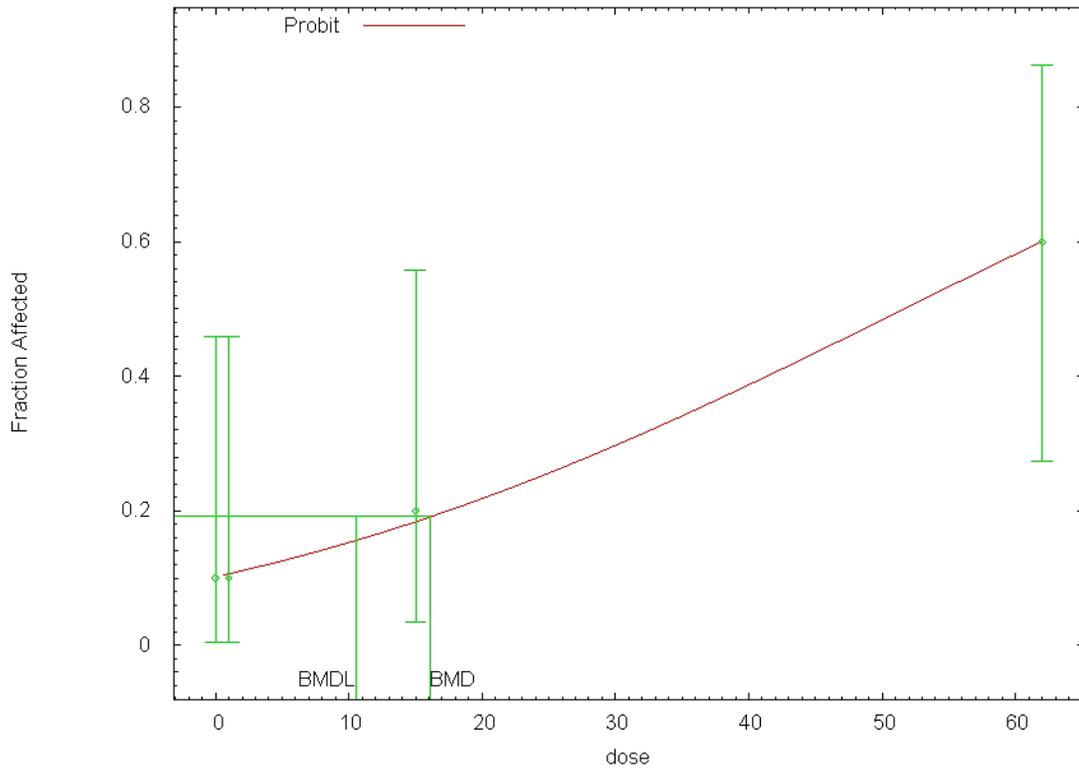
Figure B-14 Female rats, Probit model, Kidney effect (noncancerous): Atrophy and degeneration of renal tubules. Gorzinski et al. (1985), F344 Strain, 16 weeks exposure by diet.

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
                             Scaled
   Dose   Est._Prob.  Expected  Observed   Size    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

14:31 08/12 2009

Figure B-15 Male rats, Gamma model, Kidney effect (noncancerous): Slight hypertrophy and/or dilation of proximal convoluted tubules. Gorzinski et al. (1985), F344 Strain, 16 weeks exposure by diet.

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
                          Scaled
   Dose   Est._Prob.  Expected  Observed   Size    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
```

Weibull Model with 0.95 Confidence Level

14:35 08/12 2009

Figure B-16 Male rats, Weibull model, Kidney effect (noncancerous): Slight hypertrophy and/or dilation of proximal convoluted tubules. Gorzinski et al. (1985), F344 Strain, 16 weeks exposure by diet.

Quantal-linear Model

```
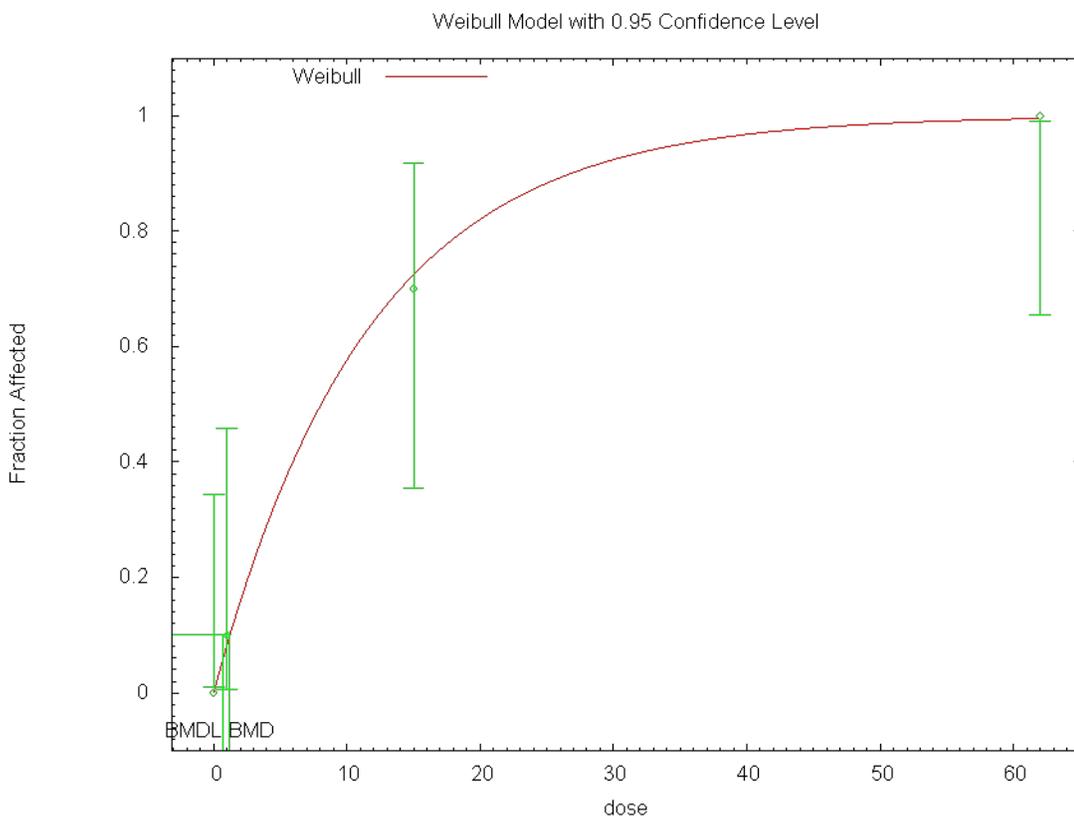 ====================================================================
        Quantal-linear Model using Weibull Model (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp4DA.(d)
        Gnuplot Plotting File: C:\USEPA\BMDS2\Temp\tmp4DA.plt
                                       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
                                Scaled
   Dose    Est._Prob.  Expected  Observed  Size    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
```

14:37 08/12 2009

Figure B-17 Male rats, Quantal-linear model, Kidney effect (noncancerous): Slight hypertrophy and/or dilation of proximal convoluted tubules. Gorzinski et al. (1985), F344 Strain, 16 weeks exposure by diet.

```
 ====================================================================
         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
                           Scaled
   Dose   Est._Prob.  Expected  Observed   Size    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

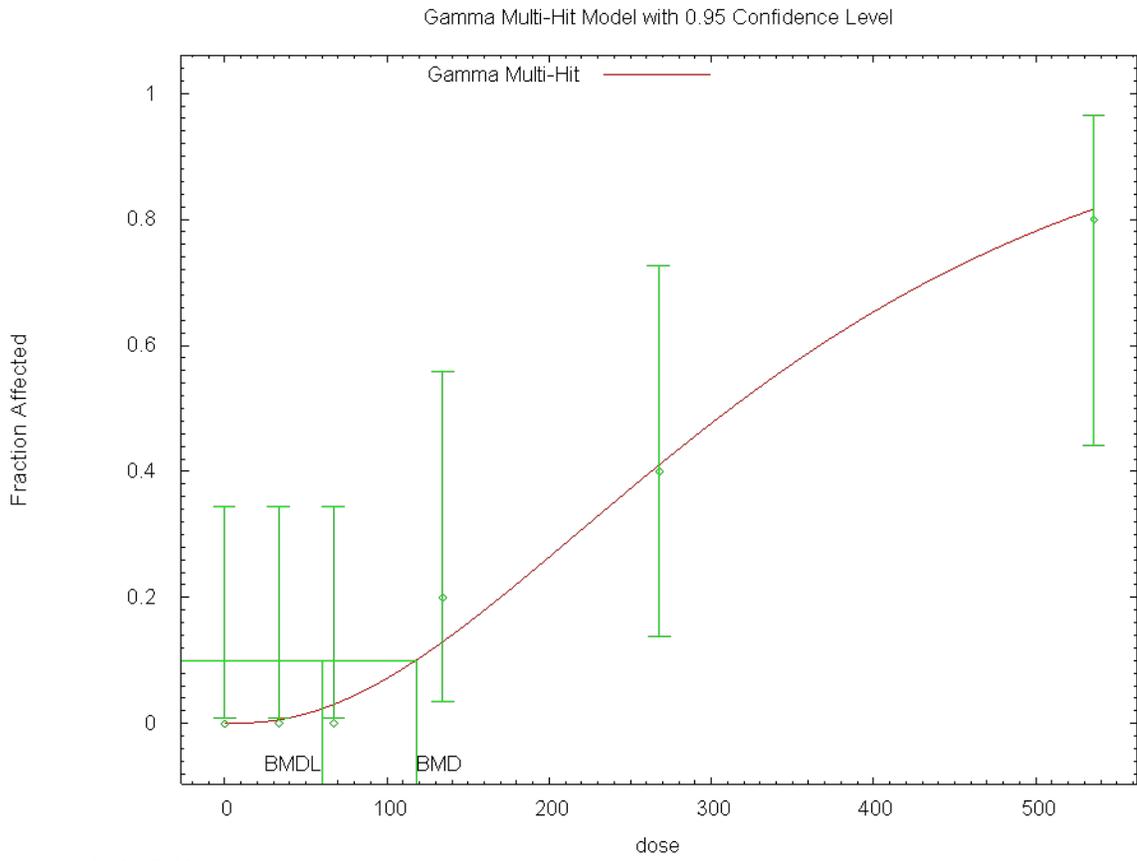 Chi^2 = 0.84   d.f. = 4    P-value = 0.9331


  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

09:14 04/09 2009

Figure B-18 Female rats, Gamma model, Liver effect (noncancerous): Heptocellular necrosis. NTP (1989), F344 Strain, 13 weeks exposure by gavage.

NTP (1989) BMD Modeling of Renal 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

                            95.0% Wald Confidence Interval
      Variable      Estimate     Std. Err.   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
                            Scaled
   Dose    Est._Prob.  Expected  Observed   Size     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
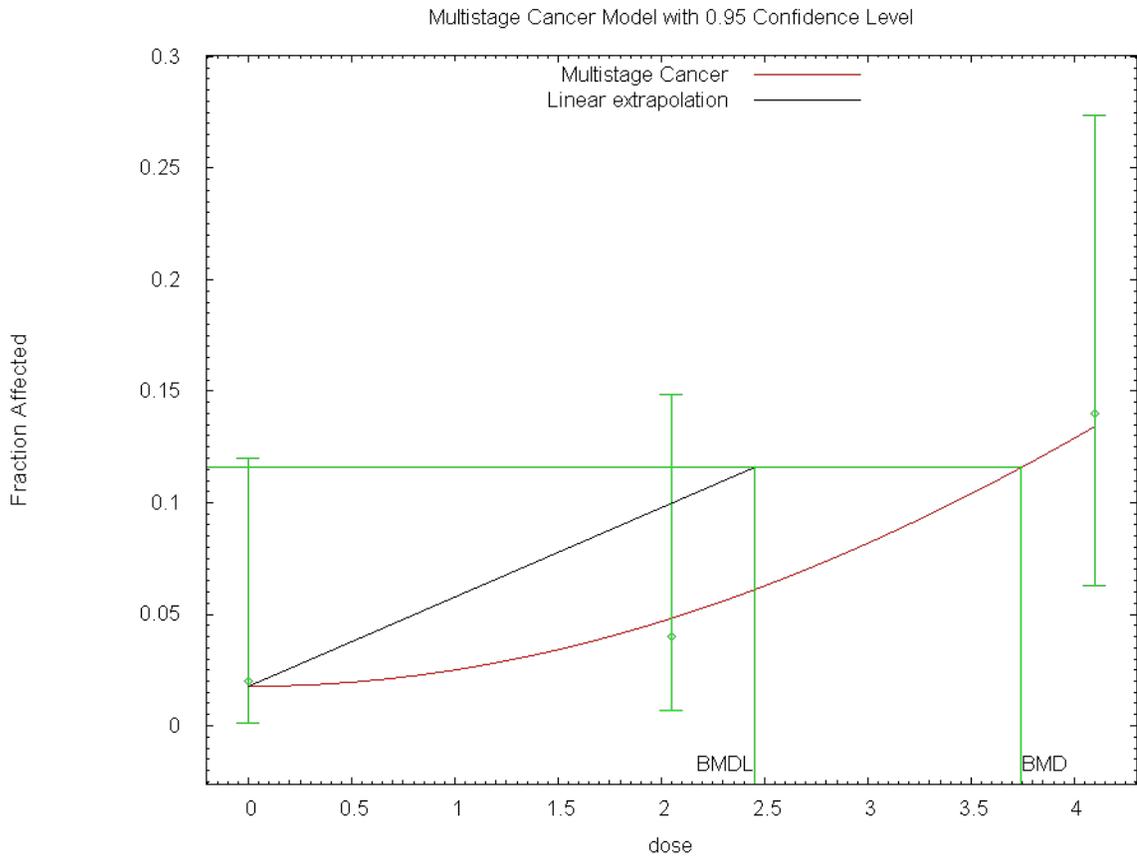
     BMDU =    9.24921

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

14:38 04/13 2009

Figure B-19 Male rats, Multistage 2° model, Kidney effect (cancerous): Renal Adenoma/Carcinoma. NTP (1989), F344 Strain, 103 weeks exposure by gavage.

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
====================================================================

 BMDS Model Run NCI 1978 Hepatocellular Carcinoma Male Mice - 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 = 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

                      95.0% Wald Confidence Interval
```

```
    Variable      Estimate     Std. Err.    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
                             Scaled
    Dose   Est._Prob. Expected Observed   Size   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

08:30 04/14 2009

Figure B-20 Male mice, Multistage 2° model, Liver effect (cancerous):Hepatocellular carcinoma. NCI (1978), B6C3F1 Strain, 78 weeks exposure by gavage.

NCI ([1978](#)) BMD Modeling of Hepatocellular Carcinoma in Female Mice

Multistage 2°

```
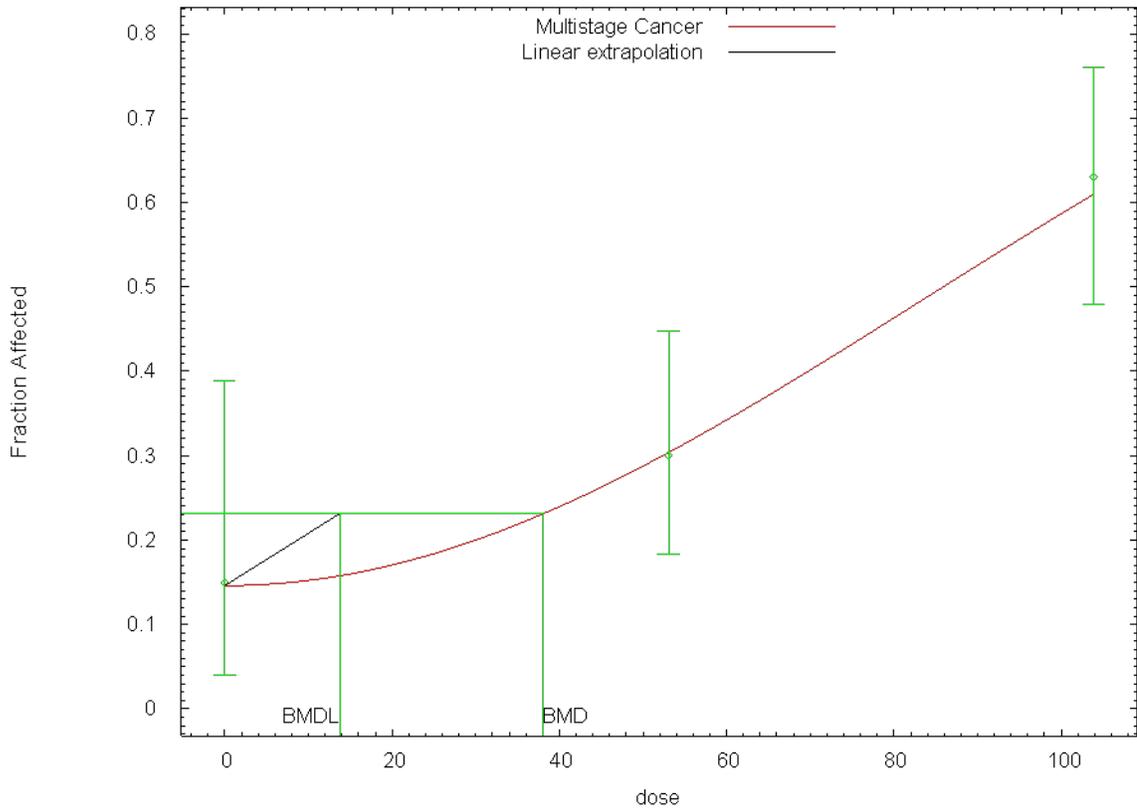====================================================================
        Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp303.(d)
        Gnuplot Plotting File: C:\USEPA\BMDS2\Temp\tmp303.plt
                                        Wed May 20 14:37:03 2009
====================================================================

 BMDS Model Run - NCI 1978 Hepatocellular Carcinoma Female Mice - 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 = 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 =   0.178486
            Beta(1) = 0.000367312
            Beta(2) =       0


      Asymptotic Correlation Matrix of Parameter Estimates

      ( *** The model parameter(s) -Beta(2)
         have been estimated at a boundary point, or have been specified by the user,
         and do not appear in the correlation matrix )

       Background    Beta(1)

Background       1    -0.89

  Beta(1)    -0.89       1



             Parameter Estimates
```

```
                              95.0% Wald Confidence Interval
      Variable      Estimate    Std. Err.    Lower Conf. Limit   Upper Conf. Limit
    Background      0.189829        *             *                  *
     Beta(1)     0.000368083        *             *                  *
     Beta(2)          0            *             *                  *

* - Indicates that this value is not calculated.



               Analysis of Deviance Table

     Model    Log(likelihood) # Param's Deviance Test d.f.  P-value
    Full model    -70.4882        3
   Fitted model   -72.8848        2      4.79332    1       0.02857
  Reduced model   -73.9112        1      6.84615    2       0.03261

        AIC:      149.77


                 Goodness of Fit
                             Scaled
    Dose    Est._Prob.   Expected  Observed   Size     Residual
   ------------------------------------------------------------------------
   0.0000    0.1898      3.797     2.000       20      -1.024
 360.0000    0.2904     14.519    20.000       50       1.708
 722.0000    0.3789     18.566    15.190       49      -0.994

 Chi^2 = 4.95   d.f. = 1    P-value = 0.0260


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

Confidence level =      0.95

     BMD =     286.241
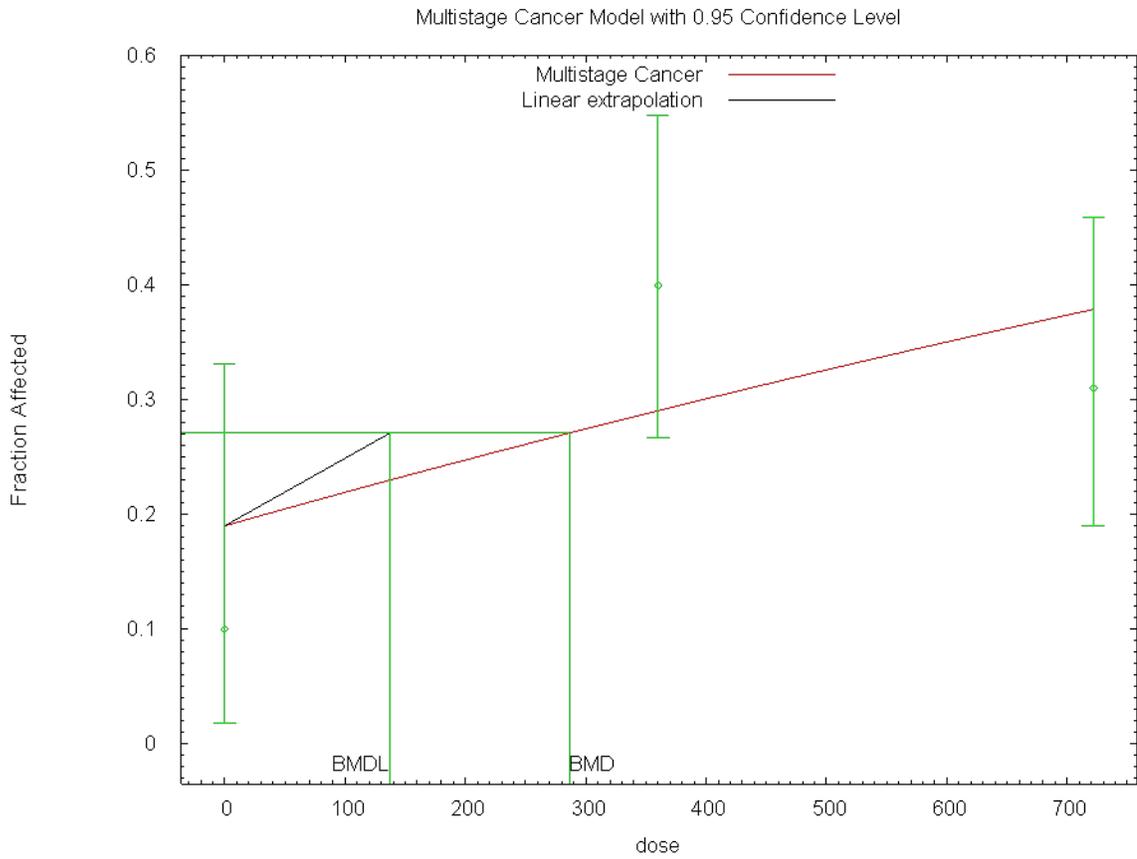
     BMDL =    136.877


BMDU did not converge for BMR = 0.100000
BMDU calculation failed
     BMDU =   1.40207e+009

Multistage Cancer Slope Factor =  0.000730581
```

Multistage Cancer Model with 0.95 Confidence Level

14:37 05/20 2009

Figure B-21 Female mice, Multistage 2° model, Liver effect (cancerous):Hepatocellular carcinoma. NCI (1978), B6C3F1 Strain, 78 weeks exposure by gavage.

NTP (1989) BMD Modeling of Pheochromocytoma/Malignant Pheochromocytomas in Male Rats

Multistage 2°
```
====================================================================
        Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS2\Temp\tmp70C.(d)
        Gnuplot Plotting File: C:\USEPA\BMDS2\Temp\tmp70C.plt
                                        Mon Apr 13 15:55:38 2009
 ====================================================================
BMDS Model Run - NTP 1989 Pheochromocytoma/Malignant Pheochromocytomas Male Mice -
Multistage Cancer 2 degree Model

 PheochromocytomaMaleRat.dax
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  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 = PercentPheochromocytomaMalignantPheochromocytoma
  Independent variable = Pheochromocytoma

 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 =   0.381549
             Beta(1) =  0.0404371
             Beta(2) =      0


       Asymptotic Correlation Matrix of Parameter Estimates

       ( *** The model parameter(s) -Beta(2)
          have been estimated at a boundary point, or have been specified by the user,
          and do not appear in the correlation matrix )

         Background    Beta(1)

Background      1    -0.78

  Beta(1)    -0.78      1



             Parameter Estimates
```

```
                         95.0% Wald Confidence Interval
    Variable      Estimate    Std. Err.   Lower Conf. Limit  Upper Conf. Limit
   Background     0.341708       *            *                 *
    Beta(1)       0.055345       *            *                *
    Beta(2)          0          *            *                *

* - Indicates that this value is not calculated.



           Analysis of Deviance Table

   Model    Log(likelihood) # Param's Deviance Test d.f.  P-value
   Full model    -93.0295      3
 Fitted model    -96.701       2      7.34302    1     0.006732
Reduced model    -97.5291      1      8.99926    2      0.01111

     AIC:      197.402


              Goodness of Fit
                           Scaled
  Dose    Est._Prob.  Expected  Observed   Size     Residual
 ---------------------------------------------------------------------
 0.0000    0.3417    17.085    14.000      50     -0.920
 2.0500    0.4123    18.554    26.100      45      2.285
 4.1000    0.4753    23.292    19.110      49     -1.196

 Chi^2 = 7.50   d.f. = 1    P-value = 0.0062


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

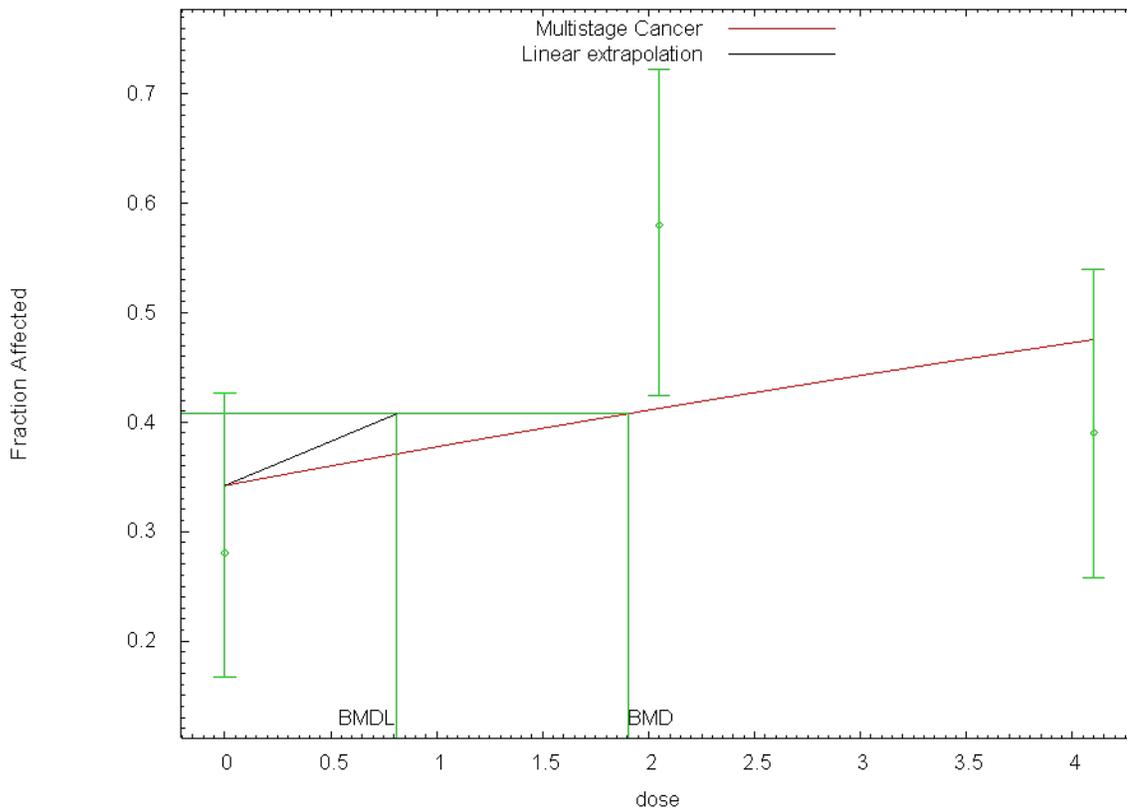Confidence level =      0.95

      BMD =     1.9037

     BMDL =    0.811704


BMDU did not converge for BMR = 0.100000
BMDU calculation failed
     BMDU = Inf
```

Multistage Cancer Model with 0.95 Confidence Level

Figure B-22 Male rats, Multistage 2° model, Adrenal effect (cancerous): Pheochromocytoma/Malignant Pheochromocytomas. NTP (1989), F344 Strain, 103 weeks exposure by gavage.