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Provisional Peer-Reviewed Toxicity Values for

Cyclohexene
(CASRN 110-83-8)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR CYCLOHEXENE (CASRN 110-83-8)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (http://www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVs

Questions regarding the contents and appropriate use of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

Cyclohexene (see Figure 1), CAS No. 110-83-8, is used as an industrial solvent as well as an intermediate in a variety of industrial processes. Cyclohexene has a high vapor pressure (119 hPa at 25ºC), suggesting it has high volatility; however, it is moderately soluble in water (0.250 g/L at 25ºC) (OECD SIDS, 2002). A table of physicochemical properties is provided below (see Table 1).

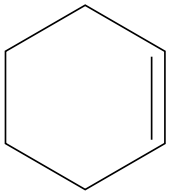

Figure 1. Cyclohexene Structure

Table 1. Physicochemical Properties for Cyclohexene (CASRN 110-83-8)[a]

Property (unit)	Value
Boiling point (ºC)	83
Melting point (ºC)	−103.5
Density (g/cm^3 at 20°C)	0.810
Vapor pressure (hPa at 25ºC)	119
Solubility in water (g/L at 25ºC)	0.250
Relative vapor density (air = 1)	No data
Molecular weight (g/mol)	82.15

[a]OECD SIDS (2002).

No Reference Dose (RfD), Reference Concentration (RfC), or cancer assessment for cyclohexene is included in the United States Environmental Protection Agency (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2011a) or on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2009). No RfD or RfC values are reported in the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 2011b). The Chemical Assessments and Related Activities (CARA) list does not include a Health and Environmental Effects Profile (HEEP) for cyclohexene; there are no noncancer toxicity values (U.S. EPA, 1994). The toxicity of cyclohexene has not been reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2011) or the World Health Organization (WHO, 2011). The California Environmental Protection Agency (CalEPA, 2008, 2009) has not derived toxicity values for exposure to cyclohexene. An 8-hour time-weighted average exposure limit of

300 ppm (1015 mg/m^3) for cyclohexene has been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2008) and is considered a threshold exposure limit by ACGIH. The same value has been adopted by the Occupational Safety and Health Administration (OSHA, 2010) as a permissible exposure limit. A 10-hour time-weighted average exposure limit of 300 ppm (1015 mg/m^3) has been recommended (i.e., the recommended exposure limit) by the National Institute of Occupational Safety and Health (NIOSH, 2010).

The HEAST (U.S. EPA, 2011b) does not report any values for cancer or a cancer weight-of-evidence classification for cyclohexene. The International Agency for Research on Cancer (IARC, 2011) has not reviewed the carcinogenic potential of cyclohexene. Cyclohexene is not included in the *12th Report on Carcinogens* (NTP, 2011). CalEPA (2008) has not prepared a quantitative estimate of carcinogenic potential for cyclohexene.

Literature searches were conducted on sources published from 1900 through November 2011 for studies relevant to the derivation of provisional toxicity values for cyclohexene, CAS No. 110-83-8. Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI, and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for relevant health information: ACGIH, ATSDR, CalEPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, U.S. EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides an overview of the relevant database for cyclohexene and includes all potential repeated short-term-, subchronic-, and chronic-duration studies. Principal studies are identified in bold and through the notation "PS". In this document, unless otherwise noted, "statistical significant" denotes a $p < 0.05$.

Table 2. Summary of Potentially Relevant Data for Cyclohexene (CASRN 110-83-8)								
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Critical Effects**	**NOAEL[a]**	**BMDL/ BMCL[a]**	**LOAEL[a]**	**Reference (Comments)**	**Notes[b]**
Human								
1. Oral (mg/kg-d)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	ND							
Chronic[f]	ND							
2. Inhalation (mg/m^3)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	ND							
Chronic[f]	ND							
Animal								
1. Oral (mg/kg-d)[a]								
Subchronic	**12/12, Crj:CD(SD)IGS rat, gavage, 7 d/wk for 48 d in males or 43−53 d in females**	**0, 50, 150, or 500 (Adjusted)**	**Total bilirubin and total bile acid**	**NDr**	**19.71**	**50**	**MHLW (2001a)**	**PS, PR**
Chronic	ND							

Table 2. Summary of Potentially Relevant Data for Cyclohexene (CASRN 110-83-8)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Developmental	12/12, Crj:CD(SD)IGS rat, gavage, 7 d/wk for 48 d in males or 43−53 d in females, from 14 d prior to mating through LD4	0, 50, 150, or 500	No effects observed on developmental parameters	500	NDr	NDr	MHLW (2001b)	PR
Reproductive	12/12, Crj:CD(SD)IGS rat, gavage, 7 d/wk for 48 d in males or 43−53 d in females, from 14 d prior to mating through LD4	0, 50, 150, or 500 (Adjusted)	No effects observed on reproductive parameters	500	NDr	NDr	MHLW (2001b)	PR
Carcinogenicity	ND							
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	20/0, rat (strain not specified), inhalation, 6 hr/d, 5 d/wk, 6 mo	0, 45, 90, 180, or 360[g]	Increase in alkaline phosphatase[h]	NDr	NDr	NDr	Laham (1976a)	NPR
	50/50, F344 rat, inhalation, 6 hr/d, 5 d/wk, 104 wk	**0, 360, 720, or 1440[g]**	**Increase incidence of spongiosis hepatis**	**360**	**NDr**	**720**	**MHLW (2003a)**	**PS, NPR**
	50/50, Crj:BDF1 mouse, inhalation, 6 hr/d, 5 d/wk, 104 wk	0, 45, 90, or 180[g]	Data is lacking on many endpoints, no critical effect can be determined	NDr	NDr	NDr	MHLW (2003b)	NPR
	10/0, guinea pig, inhalation, 6 hr/d, 5 d/wk, 6 mo	0, 45, 90, 180, or 360[g]	No adverse effects reported	360	NDr	NDr	Laham (1976b)	NPR

Table 2. Summary of Potentially Relevant Data for Cyclohexene (CASRN 110-83-8)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Chronic	6/0, rabbit, inhalation, 6 hr/d, 5 d/wk, 6 mo	0, 45, 90, 180, or 360[g]	No adverse effects reported	360	NDr	NDr	Laham (1976c)	NPR
Developmental	ND							
Reproductive	ND							
Carcinogenicity	50/50, F344 rat, inhalation, 6 hr/d, 5 d/wk, 104 wk	0, 360, 720, or 1440[g]	No increases in tumors	NDr	NDr	NDr	MHLW (2003c)	NPR
	50/50, Crj:BDF1 mouse, inhalation, 6 hr/d, 5 d/wk, 104 wk	0, 45, 90, or 180[g]	No increases in tumors	NDr	NDr	NDr	MHLW (2003d)	NPR

[a]Dosimetry: All exposure values of long-term exposure (4 weeks and longer) are converted from a discontinuous to a continuous (daily) exposure. Values for inhalation (cancer and noncancer), and oral (cancer only) are further converted to an HEC/HED. Values from animal developmental studies are not adjusted to a continuous exposure.
[b]Notes: PS = Principal study, PR = Peer reviewed, NPR = Not peer reviewed, NA = Not applicable.
[c]Acute = Exposure for 24 hours or less (U.S. EPA, 2002).
[d]Short-term = Repeated exposure for >24 h ≤30 d (U.S. EPA, 2002).
[e]Long-term = Repeated exposure for >30 d ≤10% lifespan (based on typical lifespan of 70 years) (U.S. EPA, 2002).
[f]Chronic = Repeated exposure for ≥10% lifespan (U.S. EPA, 2002).
[g]HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × blood:gas partition coefficient.
[h]Adversity of this endpoint cannot be determined.

ND = No data, NDr = Not determinable.

HUMAN STUDIES

Oral Exposures

No suitable subchronic- or chronic-duration exposure studies are available.

Inhalation Exposures

No suitable subchronic- or chronic-duration exposure studies are available.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to cyclohexene have been evaluated in one subchronic-duration study (i.e., MHLW, 2001a) and one reproductive/developmental screening study (MHLW, 2001b), which were run concurrently.

Subchronic-Duration Studies

Ministry of Health, Labor, and Welfare (MHLW), 2001a

The subchronic component of the peer-reviewed rat study by MHLW (2001a) is selected as the principal study for derivation of the subchronic and chronic p-RfD. MHLW (2001a) conducted a subchronic oral toxicity study that also examined reproductive and developmental effects that will be discussed separately (MHLW, 2001b). This study appears to be proprietary (may have been part of a Japanese toxicity assessment conducted by MHLW) and is in Japanese. OECD SIDS (2002) peer-reviewed and summarized the study (cited as MHLW, 2002) and EPA subsequently had the document translated. The internal and external peer reviewers of this PPRTV document also concurred that the MHLW (2001a) study was suitable for deriving a provisional toxicity value. This study was conducted as a combined repeated dose toxicity study with reproduction/developmental toxicity screening according to OECD test guideline 422 and was stated by OECD to be GLP compliant (no GLP statement was provided in the study report). Crj:CD(SD)IGS rats (12 animals/sex/treatment group) were administered 0, 50, 150, or 500 mg/kg-day of cyclohexene (98.6% pure) in corn oil via gavage. Dose formulations were tested for concentration and stability. Males were dosed for 48 days and females for 43−53 days beginning 14 days before mating, throughout the mating and gestational period, to Day 4 of lactation. Animals were observed for clinical signs of toxicity daily. Body weight and food consumption were measured weekly and at necropsy. Urinalysis was conducted on 5 males/treatment group at 43−48 days of treatment. At sacrifice (on Day 49 for males and 5 days after delivery for females), blood was collected for hematology and clinical chemistry in all animals. The brain, liver, kidney, spleen, adrenal glands, thymus, testis, and epididymis were weighed. Tissues and organs were examined histologically in at least the control and high-dose group. Statistical analyses performed included Bartlett's test for homogeneity of variance, Dunnett's multiple comparison test (if equal variance), and Steel's test for unequal variances. The χ^2 and Fisher's exact probability tests were also used where appropriate.

Salivation was observed at 150 (for about 5 minutes in 3/12 males and 2/12 females) and 500 mg/kg-day (all animals for 30−60 minutes in males and 6 hours in females). Lacrimation was observed in 2/12 males at 500 mg/kg-day and females at ≥150 mg/kg-day (1/12 for each dose group). There were some small—but statistically significant—hematological changes at 500 mg/kg-day. Increased were the number of reticulocytes and bilirubin in males and prothrombin time and total bile acids in females. Decreased was the level of APTT in males. There were no treatment-related significant changes in body weight, or food consumption, in

either sex or in the urinalysis findings for males (females not measured). There was a dose-dependent decrease in triglyceride in males (see Table B.1). Even though triglyceride in the 500 mg/kg-day group males was 43% lower than in the controls, the results were not statistically significant nor was this effect noted in the females. There was an increase in total bilirubin in both sexes; reanalysis of the data indicates that there are statistically significant increases at all doses in males and in high-dose females. Total bile acid was increased by >10% in all dose groups. However, the results were highly variable and not dose dependent. Only the 150-mg/kg-day males and the 50- and 500-mg/kg-day females showed statistically significant changes above the controls. High-dose males had a statistically significant increase in relative kidney weight that was not accompanied by any histopathological changes and did not reach 1SD (standard deviation) above the control (see Table B.2). OECD SIDS (2002) reported a NOAEL of 50 mg/kg-day for the repeated dose toxicity portion of the test based on transient salivation observed in both sexes at 150 mg/kg-day. Transient salivation is not considered sufficiently adverse to identify as a critical effect. Although the bile acid increase was not dose dependent and was variable, the data taken together may indicate bile duct blockage. Bile duct blockage is also consistent with the statistically significant increase in alkaline phosphatase in rats noted by Laham (1976) following inhalation exposure. Based on the statistically significant increase in total bile acid in females and total bilirubin in males at the lowest dose, no NOAEL can be determined and the LOAEL is 50 mg/kg-day.

Chronic-Duration Studies

No studies were identified.

Reproductive/Developmental Studies

MHLW, 2001b

MHLW (2001b) conducted a subchronic oral combined reproductive/developmental toxicity study that also examined subchronic oral effects that were discussed separately above (MHLW, 2001a). This study appears to be proprietary (part of a Japanese toxicity assessment that was conducted by the MHLW), and is in Japanese. OECD SIDS (2002) peer-reviewed and summarized the study (cited as MHLW, 2002) and EPA subsequently had the report translated. This study was conducted as a combined repeated dose toxicity study with reproduction/developmental toxicity screening according to OECD test guideline 422 and was stated by OECD to be GLP compliant (no GLP statement was provided in the study report). Crj:CD(SD)IGS rats (12 animals/sex/treatment group) were administered 0, 50, 150, or 500 mg/kg-day of cyclohexene (98.6% pure) in corn oil via gavage. Males were dosed for 48 days and females for 43−53 days beginning 14 days before mating, throughout the mating and gestational period, to Day 4 of lactation. Animals were observed for clinical signs of toxicity daily. Body weight and food consumption were measured on a routine basis. The parameters examined as part of the repeat dose portion of the study are reported above (MHLW, 2001a). Reproductive and developmental parameters examined included successful copulation, number of pregnant females, copulation index (number of pairs with successful copulation/number of pairs mated × 100), fertility index (number of pregnant animals/number of animals with successful copulation × 100), estrous cycle, number of dams delivering live pups, duration of gestation, total number of corpora lutea, total number of implants, total number of pups born, total number of live pups born, sex ratio, total number of dead pups, total number of cannibalized pups, gestation index (number of females with live pups/number of pregnant females × 100), implantation index (number of implants/number of corpora lutea × 100), delivery index (number

of pups born/number of implants × 100), live birth index (number of live pups/number of pups born × 100), and viability index on Day 4 (number of live pups on Day 4 after birth/number of live pups born × 100). Pups were examined for external abnormalities and weighed on Days 0 and 4 after birth. Gross necropsy was also performed on pups in this study.

There were no statistically significant changes in body weight or in food consumption in either males or females. No effects were reported on reproductive performance in the male and female rats (see Table B.3). Similarly, there were no effects reported on the developmental parameters examined. Although the mean estrous cycles were similar, it was stated that one (8.3%) of 150 mg/kg-day females and two (16.7%) of 500 mg/kg-day females had an irregular estrous cycle. This did not appear to affect reproduction. The reproductive and developmental NOAEL is 500 mg/kg-day, the highest dose tested, in both sexes. No LOAEL can be determined from the data.

Carcinogenicity Studies

No studies were identified.

Inhalation Exposures

The effects of inhalation exposure of animals to cyclohexene have been evaluated in five chronic-duration studies (i.e., Laham, 1976a,b,c; MHLW, 2003a,b). Carcinogenicity data are also provided by MHLW (2003c,d).

Subchronic Studies

No studies were identified.

Chronic Studies

Laham, 1976 a

Laham (1976a) conducted a chronic-duration inhalation study in which adult male rats (strain not specified; 20/treatment group) were exposed to 0, 75, 150, 300, or 600 ppm of cyclohexene (purity not reported) 6 hours/day, 5 days/week, for 6 months. The exposure concentrations adjusted for continuous exposure and unit conversion are 0, 45, 90, 180, and 360 mg/m^3. The only information, available for review is a published abstract that has not been peer-reviewed. Cyclohexene levels were stated to be continuously monitored using an automatic sampling system connected to a Carlo Erba gas chromatograph. The blood:gas partition coefficient is assumed to equal one. Humidity, temperature, and pressure were also stated to be monitored. None of the results, however, were provided. Body weight was obtained weekly. Blood was collected for hematology (white blood cell counts [WBC], red blood cell counts [RBC], platelets, hemoglobin, hematocrit, and differential WBC counts) before, during (timing not specified), and after exposure. Clinical chemistry (glucose, blood urea nitrogen [BUN], cholesterol, alanine aminotransferase [ALT], aspartate aminotransferase [AST], lactate dehydrogenase [LDH], alkaline phosphatase, electrolytes, and other unspecified endpoints) and gross pathology of hemopoietic organs were conducted at sacrifice after 6 months of exposure. High-dose rats had significantly decreased body weight gain compared to controls, but no data were provided. All exposure groups had a statistically significant higher alkaline phosphatase level than the controls. However, all other parameters were within normal range (data were not provided), no changes were observed in the bone marrow, and there is no indication that the liver was examined; therefore, the biological significance of the elevated alkaline phosphatase cannot

be determined. The data are not sufficient to determine adversity of the effects noted. Therefore, no NOAEL/LOAEL can be determined. However, based on the increase in alkaline phosphatase a lowest-observed-effect-level (LOEL) of 45 mg/m^3 is determined and a no-observed-effect-level (NOEL) cannot be determined.

MHLW, 2003a

The nonpeer-reviewed inhalation study in rats by MHLW (2003a) is selected as the principal study for derivation of the screening chronic p-RfC. There is no information to indicate where the study was conducted and it is not considered to be peer-reviewed. The internal and external peer reviewers of this PPRTV document concurred that the MHLW (2003a) study was suitable for deriving a screening provisional toxicity value. The study is in Japanese; therefore, EPA had the study translated on February 17, 2011. Male and female F344/DuCrj (Fischer) rats (50/sex/treatment group) were administered 0, 600, 1200, or 2400 ppm cyclohexene (purity not specified) via inhalation 6 hours/day, 5 days/week, for 104 weeks. Duration adjusted HECs based on extra-respiratory effects with a blood gas partition coefficient of 1 are 0, 360, 720, and 1440 mg/m^3, respectively. Details of the inhalation procedure were not provided. Animals were observed for general condition. Body weight and food consumption were routinely measured. Blood counts, blood biochemistry, and urinalysis were measured at unspecified times. Unspecified organs were stated to have been weighed. Histopathology was stated to have been conducted, but details of the procedure were not provided. The results of statistical tests were not specified, but tables indicate that Peto test, Cochran-Armitage test, and Fisher test were conducted on tumor incidence data.

There were no treatment-related effects on survival. However, survival in high-dose females was slightly lower than the controls (approximately 72% compared to 94%; data digitized from the figure provided in the study report). The body weight was stated to be decreased in high-dose males and females. Results were only provided in figure form, and statistical significance was not specified. The difference from control, however, was less than 10% (data digitized from the figure provided in the study report). Although hematology, clinical chemistry, urinalysis, and organ weights were stated to be assessed, there were no data provided on any of these endpoints. The study authors specify that there was an increase in the incidence of spongiosis hepatis at ≥720 mg/m^3 (presumably in both sexes), but specific incidence data were not provided. Increases in the following lesions were also stated to occur: chronic kidney disease in males, focal follicular cell hyperplasia in both sexes, and cerebellar granule cell degeneration in both males and females; however, no details were provided in the report. Based on the increases in the incidence of spongiosis hepatis, the NOAEL is 360 mg/m^3 and the LOAEL is 720 mg/m^3. However, Karbe and Kerlin (2002) did not consider it a preneoplastic lesion and claim that it is a spontaneous liver lesion that occurs in aging rats (males more often than females) with an unknown cause.

MHLW, 2003b

There is no information to indicate where this study was conducted. Although the web site is in Japanese, EPA had the information translated on February 17, 2011. Male and female Crj:BDF1 mice (50/sex/treatment group) were administered 0, 75, 150, or 300 ppm cyclohexene (purity not specified) via inhalation 6 hours/day, 5 days/week, for 104 weeks. Duration-adjusted HECs based on extra-respiratory effects with a blood gas partition coefficient of 1 are 0, 45, 90, and 180 mg/m^3, respectively. Details of the inhalation procedure were not provided. Animals

were observed for general condition. Body weight and food consumption were routinely measured. Blood counts, blood biochemistry, and urinalysis were measured at unspecified times. Unspecified organs were stated to have been weighed. Histopathology was stated to have been conducted, but details of the procedure were not provided. The results of statistical tests were not specified, but tables indicate that Peto test, Cochran-Armitage test, and Fisher test were conducted on tumor incidence.

There were no treatment-related effects on survival, body weight, or food consumption. Although hematology, clinical chemistry, urinalysis, and organ weights were stated to be conducted, there were no data provided for any of these endpoints. Based on the lack of data provided, no NOAEL or LOAEL can be determined.

Laham, 1976b,c

Laham (1976) conducted identical studies in guinea pigs (i.e., Laham, 1976b) and rabbits (i.e., Laham, 1976c) as was conducted in rats (i.e., Laham, 1976a), which is detailed above. Because the blood:gas partition coefficient is 1 for all these species, the dose conversion is also the same. There were no adverse effects reported for either the guinea pigs or the rabbits. Therefore, the NOAEL for the guinea pig (i.e., Laham, 1976b) and rabbit (i.e., Laham, 1976c) studies is 360 mg/m^3, the highest dose tested.

Developmental Studies

No studies were identified.

Reproductive Studies

No studies were identified.

Carcinogenicity Studies

MHLW, 2003c

There is no information to indicate where this study was conducted. Although the web site is in Japanese, EPA had the information translated on February 17, 2011. Male and female F344/DuCrj(Fischer) rats (50/sex/treatment group) were administered 0, 600, 1200, or 2400 ppm cyclohexene (purity not specified) via inhalation 6 hours/day, 5 days/week, for 104 weeks. Duration-adjusted HECs based on extra-respiratory effects with a blood gas partition coefficient of 1 are 0, 360, 720, and 1440 mg/m^3, respectively. Details of the inhalation procedure were not provided. Animals were observed for general condition. Body weight and food consumption were routinely measured. Nonneoplastic endpoints were measured and are detailed above under chronic information. Histopathology was stated to have been conducted, but details of the procedure were not provided. The results of statistical tests were not specified, but tables indicate that Peto test, Cochran-Armitage test, and Fisher test were conducted on tumor incidence data.

There were no treatment-related effects on survival. The body weight was stated to be decreased in high-dose males and females, but is <10% different from the control (data digitized from the figure provided in the study report). There was a slight increase in the combined incidence of hepatocellular adenoma and carcinoma in males (see Table B.4). These results were only statistically significant with the Peto test (tests for trend). They were not statistically significant with Fisher test or the Cochran-Armitage trend test. Separately, the tumors

(hepatocellular adenoma or hepatocellular carcinoma) were not statistically significantly different from the control. Only one carcinoma is observed in the treated animals. The rest of the combined incidence is composed of adenomas. The study authors specify that there was an increase in the incidence of eosinophilic foci (a pretumorous lesion) in the livers of high-dose males and an increased incidence of spongiosis hepatis at ≥720 mg/m^3 (presumably in both sexes), but specific incidence data were not provided. There were no increases in tumors found in the female rat. The study authors concluded that cyclohexene was not carcinogenic in rats.

MHLW, 2003d

There is no information to indicate where this study was conducted. Although the web site is in Japanese, EPA had the information translated on February 17, 2011. Male and female Crj:BDF1 mice (50/sex/treatment group) were administered 0, 75, 150, or 300 ppm cyclohexene (purity not specified) via inhalation 6 hours/day, 5 days/week for 104 weeks. Duration adjusted HECs based on extra-respiratory effects with a blood gas partition coefficient of 1 are 45, 90, and 180 mg/m^3, respectively. Details of the inhalation procedure were not provided. Animals were observed for general condition. Nonneoplastic endpoints were measured and are detailed above, under chronic studies. Histopathology was stated to have been conducted, but details of the procedure were not provided. The results of statistical tests were not specified, but tables indicate that Peto test, Cochran-Armitage test, and Fisher test were conducted on tumor incidence.

There were no treatment-related effects on survival, body weight, or food consumption. There were no increases in tumors found in either sex of mice. In male mice, there was a significant decrease in hepatocellular carcinomas and combined incidence of hepatocellular adenomas and carcinomas in contrast to the male rat controls (see Table B.4).

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

The genotoxicity of cyclohexene has been studied in several in vitro test systems (i.e., Sycheva et al., 2000; BOZO Research Center, 2000a,b,c; De Mik and De Groot, 1978). Table 3A summarizes the genotoxicity studies on cyclohexene. Table 3B provides information on the toxicokinetics of cyclohexene (i.e., James et al., 1971; Leibman and Ortiz, 1970, 1971, 1978; Maples and Dahl, 1993). Table 3B also provides summaries of the two mechanistic studies available for cyclohexene (i.e., Nesnow et al., 1985; Ortiz de Montellano and Mico, 1980).

Table 3A. Summary of Cyclohexene Genotoxicity

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA100, TA98	1000 µg/plate	−	−	Chlorination products of cyclohexene were also tested and found to be mutagenic to the TA100 strain without metabolic activation as well as in a micronucleus test with epithelial cells from mouse urinary bladder and colon.	Sycheva et al., 2000
Reverse mutation	*Salmonella typhimurium* TA100, TA98, TA1535, TA1537	1250 µg/plate (12.5 µg/L)	−, T	−, T	Growth inhibition observed at 625 µg/plate or above with or without activation.	BOZO Research Center, 2000a[c]
Reverse mutation	*Escherichia coli* WP2 uvrA	5000 µg/plate (without activation) 1250 µg/plate (with activation) (12.5 and 50 µg/L respectively)	−	−, T	Growth inhibition observed at 1250 µg/plate or above with activation.	BOZO Research Center, 2000b[c]
DNA damage	*Escherichia coli* MRE 162 (aerosolized into air)	1000 ppb (2 mg/m^3)	−	ND	Vaporized cyclohexene had no effect on the survival of *E. coli* and did not cause damage to the bacterial DNA (measured as loss of reproduction and introduction of breaks in the sedimented DNA). Ozonized cyclohexene decreased survival and induced many breaks in the DNA of *E. coli.*	De Mik and De Groot, 1978
SOS repair induction	ND					

Table 3A. Summary of Cyclohexene Genotoxicity

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	ND					
Recombination induction	ND					
Chromosomal aberration	ND					
Chromosomal malsegregation	ND					
Mitotic arrest	ND					
Genotoxicity studies in mammalian cells—in vitro						
Mutation	ND					
Chromosomal aberrations	Chinese hamster lung (CHL/IU) cells	400 mg/L	−, T	−, T	Structural chromosomal aberration and polyploidy were not induced with either short-term or continuous treatment. Cell toxicity was observed at 400 µg/mL following continuous treatment for 24 and 48 hr.	BOZO Research Center, 2000c[c]
Sister chromatid exchange (SCE)	ND					
DNA damage	ND					
DNA adducts	ND					

Table 3A. Summary of Cyclohexene Genotoxicity

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in mammals—in vivo						
Chromosomal aberrations	ND					
Sister chromatid exchange (SCE)	ND					
DNA damage	ND					
DNA adducts	ND					
Mouse biochemical or visible specific locus test	ND					
Dominant lethal	ND					
Genotoxicity studies in subcellular systems						
DNA binding	ND					

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = Positive, ± = Equivocal or weakly positive, − = Negative, T = Cytotoxicity, ND = No data.
[c]Original citation is in Japanese, but EPA had the reports translated in March of 2011.

Table 3B. Other Studies[a]

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity other than oral/inhalation	ND			
Other toxicity studies (exposures other than oral or inhalation)	ND			
Short-term studies	ND			
Metabolism/ toxicokinetic	In vivo study: Rats (details unspecified) were fed 3 mmol/kg cyclohexene and total liver glutathione levels were measured 0.5, 1, 2, and 4 hr after dosing. In addition, urinary excretion of mercapturic acids was measured in rats and rabbits (details unspecified) dosed with 2 mmol/kg cyclohexene by gavage using paper chromatography separation, methylation, and gas chromatography.	Ingestion of cyclohexene resulted in a rapid drop in the total liver glutathione level in rats. The main mercapturic acid urinary metabolite following administration of cyclohexene to rats and rabbits was found to be 3-hydroxycyclohexylmercapturic acid, with traces of cyclohexylmercapturic acid and 2-hydroxycyclohexylmercapturic acid also detected.	These results suggest that cyclohexene undergoes conjugation with glutathione in rats and rabbits.	James et al., 1971
Metabolism/ toxicokinetic	In vitro study: Hepatic microsomes and supernatant fractions of liver extracts (male New Zealand rabbits pretreated with phenobarbital) were incubated with 20 mM of cyclohexene for up to 20 min. Reaction mixtures were analyzed by both gas and thin layer chromatography.	Cyclohexene oxide was detected with a maximum concentration after 10 min followed by a decline until no longer detectable at the end of incubation.	Cyclohexene oxide is likely an intermediate in the oxidation of cyclohexene to a glycol.	Leibman and Ortiz, 1970
Metabolism/ toxicokinetic	In vitro study: Hepatic microsomes and supernatant fractions of liver extracts (male Hotzmann rats and male New Zealand White rabbits pretreated with phenobarbital) were incubated with 10% cyclohexene in ethanol for up to 1 hr. Reaction mixtures were analyzed by both gas and thin layer chromatography.	Cyclohexene was oxidized to *trans*-1,2-cyclohexanediol. There was no evidence of the formation of *cis*-1,2-cyclohexanediol.	Oxidation of cyclohexene by liver microsomes results predominantly or solely in the diol product of the *trans*-configuration.	Leibman and Ortiz, 1971

Table 3B. Other Studies[a]

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	In vitro study: Hepatic microsomes and supernatant fractions of liver extracts (male Hotzmann rats and male New Zealand White rabbits pretreated with phenobarbital) were incubated with 40 mM cyclohexene for up to 1 hr. Reaction mixtures were analyzed by both gas and thin layer chromatography. In vivo study: 2 male Holtzman rats were administered 0.1 mL of cyclohexene by gavage and their urine was analyzed (with and without β-glucuronidase treatment).	In vitro study: 2-Cyclohexen-1-ol, *trans*-cyclohexanediol, and to a lesser extent, cyclohexene oxide, were all detected at the end of the incubation period. The formation of 2-cyclohexen-1-ol required a NADPH generating system. Microsomes from rats pretreated with phenobarbital induced cyclohexene oxidation as much as 5 times greater than microsomes from rats that were not pretreated. In vivo study: The 24 hr urine samples for both rats contained 2-cyclohexen-1-one (0.1% of the oral dose) but 2-cyclohexen-1-ol was not detected.	Cyclohexene is hydroxylated at the allylic position in the presence of liver microsomal preparations and NADPH through a typical drug metabolizing, mixed-function oxygenase-catalyzed reaction.	Leibman and Ortiz, 1978
Metabolism/ toxicokinetic	In vivo study: 15/0, F344/N rat, nose-only inhalation study. Rats were exposed to 600 ppm gaseous cyclohexene for 20 or 360 min. Blood samples collected at approximately 1.5, 2.5, 25, and 51.5 min were cryogenically distilled and then analyzed using gas chromatography. Hepatic cytochrome P-450 concentrations were measured following sacrifice at 20 or 360 min.	During exposure, blood levels of cyclohexene increased to approximately 2 µg/g after 51.5 min. Blood levels of cyclohexene oxide were below limit of detection. Hepatic cytochrome P-450 levels in treated rats were unchanged from controls.	Cyclohexene is absorbed into the blood stream following inhalation. Metabolism of cyclohexene in vivo to the epoxide is slow. Exposure to 600 ppm cyclohexene resulted in no change in hepatic cytochrome P-450.	Maples and Dahl, 1993
Mode of action/ mechanistic	In vitro study on the potential for several chemicals to inhibit or enhance the oncogenic/malignant cell transformations in *C3H10T1/2CL8* mouse embryo fibroblasts (C3H cells).	Cyclohexene enhanced cell transformation in mouse embryo fibroblasts.	Cyclohexene inhibited epoxide-hydratase activity allowing arene oxides to accumulate in the cells, which led to an enhancement of malignant cell transformations.	Nesnow et al., 1985 (abstract only)

Table 3B. Other Studies[a]

Test	Materials and Methods	Results	Conclusions	References
Mode of action/ mechanistic	In vitro study: Hepatic microsomes (rats pretreated with phenobarbital) were incubated with 10 mM cyclohexene for up to 30 min. The loss of cytochrome P-450 was measured with spectroscopy. In vivo study: 3−5 male Sprague Dawley rats were injected intraperitoneally daily for 4 d with 80 mg/kg phenobarbital followed by a single intraperitoneal injection of 400 µL/kg cyclohexene. 4 hr later, the rats were sacrificed and the livers excised to be tested for porphyrin-substrate adducts (hepatic pigments).	No cytochrome P-450 loss was observed after incubation of hepatic microsomes with cyclohexene. Abnormal hepatic pigments were not found after the administration of cyclohexene to phenobarbital-pretreated rats.	These results suggest that steric and electronic factors are at play with cyclohexene that can suppress the destructive interaction with cytochrome P-450 that other olefins demonstrate.	Ortiz de Montellano and Mico, 1980
Immunotoxicity	ND			
Neurotoxicity	ND			

[a]ND = no data.

Tests Evaluating Carcinogenicity, Genotoxicity, and/or Mutagenicity

The genotoxicity of cyclohexene has been studied in several in vitro test systems (i.e., Sycheva et al., 2000; BOZO Research Center, 2000a,b,c; De Mik and De Groot, 1978). These studies indicate that cyclohexene is not mutagenic or clastogenic in vitro. Studies investigating the genotoxic potential of cyclohexene in vivo have not been identified. Table 3A summarizes the genotoxicity studies on cyclohexene.

Other Toxicity Studies (Exposures Other than Oral or Inhalation)

No studies were identified.

Short-Term Studies

No studies were identified.

Metabolism/Toxicokinetic Studies

Little information on the toxicokinetics of cyclohexene is available (James et al., 1971; Leibman and Ortiz, 1970, 1971, 1978; Maples and Dahl, 1993). Results of the available studies indicate that cyclohexene is absorbed to some extent following inhalation or oral exposure. Metabolism of cyclohexene occurs in the liver via two metabolic pathways; namely, conjugation with glutathione or oxidation at the allylic position. Cyclohexene oxide was shown to be an intermediate in the oxidation of cyclohexene to *trans*-1,2-cycloheanediol. Table 3B summarizes the toxicokinetics/metabolism studies on cyclohexene.

Mode of Action/Mechanistic Studies

Two mechanistic studies are available for cyclohexene (i.e., Nesnow et al., 1985; Ortiz de Montellano and Mico, 1980). Unlike ethylene and other olefins, cyclohexene was not shown to cause a loss in hepatic cytochrome P-450 concentrations when tested in vitro. In an in vitro cell transformation test, cyclohexene enhanced oncogenic cell transformation in mouse embryo fibroblasts through the inhibition of epoxide-hydratase activity, which allowed arene oxides to accumulate in the cells (Nesnow et al., 1985). Table 3B summarizes the mechanistic studies on cyclohexene.

Immunotoxicity

No studies were identified.

Neurotoxicity

No studies were identified.

DERIVATION OF PROVISIONAL VALUES

Table 4. Summary of Reference Values for Cyclohexene

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD_{HED}	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Total bilirubin	5×10^{-2}	$BMDL_{1SD}$	4.81	100	MHLW (2001a)
Chronic p-RfD (mg/kg-d)	Rat/M	Total bilirubin	5×10^{-3}	$BMDL_{1SD}$	4.81	1000	MHLW (2001a)
Subchronic p-RfC (mg/m^3)	NDr						
Screening Chronic p-RfC (mg/m^3)	Rat/M+F	Spongiosis hepatis	1×10^{0}	NOAEL	360	300	MHLW (2003a)

NDr = Not determinable.

Table 5. Summary of Cancer Values for Cyclohexene

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	NDr			
p-IUR	NDr			

NDr = Not determinable.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

There is a single repeat-dosing study available, which includes reproductive/developmental toxicity screening and an assessment of subchronic toxicity (MHLW, 2001a,b). The only finding in the study was observations of transient salivation and changes in clinical chemistry parameters (e.g., triglyceride, total bilirubin, and total bile acid levels). Although the transient salivation was considered an endpoint for developing a NOAEL by OECD SIDS (2002), it is not a sufficient endpoint for deriving a p-RfD due to the lack of any data indicating that the salivation was not just an effect of unpalatability. With the exception of total bilirubin and total bile acids, the clinical chemistry parameters did not appear to have a dose response and were variable. The increases in total bilirubin combined with increases in total bile acid, however, indicate a possible blockage of the bile duct and was considered for POD selection.

The study by MHLW (2001a) is selected as the principal study for derivation of the subchronic p-RfD. The critical effect is a statistically significant elevation of total bilirubin in male rats. The effect is linked to a possible blockage of the bile duct and is consistent with an elevation of alkaline phosphatase observed by Laham (1976a) in rats following inhalation exposure. Details of the MHLW (2001a) study are provided in the "Review of Potentially Relevant Data" section. Benchmark dose (BMD) analyses were conducted on the data for total bilirubin and total bile acids in both male and female rats. None of the female data had an adequate fit for any of the models nor does the male total bile acid data. The only data with an adequate fit is the total bilirubin in male rats. The remaining observed endpoints did not show a clear dose-response (see Tables B.1. and B.2.). Among the available, acceptable studies, this was the only study that identified an effect from cyclohexene exposure and also includes the lowest POD for developing a subchronic p-RfD.

All available continuous models in the EPA Benchmark Dose Software (BMDS version 2.1.2; U.S. EPA, 2010) were fit to the data on total bilirubin in male rats following exposure to cyclohexene for approximately 48 days (see Table 6). In the absence of any cogent basis for selecting a benchmark response (BMR) for the elevated total bilirubin data, a BMR of 1 standard deviation (SD) from the control mean can be used as the standardized reporting level for comparisons for continuous data (U.S. EPA, 2000).

Table 6. Total Bilirubin for Male Crj:CD(SD)IGS Rats Treated with Cyclohexene for Approximately 48 Days—Used for BMD Analysis[a]

Average Daily Dose (mg/kg-d)[b]	Number of Subjects	Response[c]
0	12	0.03 ± 0.01
50	11	0.04 ± 0.01*
150	12	0.05 ± 0.01**
500	12	0.05 ± 0.01**

[a]MHLW (2001a).
[b]Dose was as administered.
[c]Mean ± standard deviation.
*Statistically significant difference from control ($p \leq 0.05$) using two sample *t*-test was determined for this review because the results reported by the study author were inconsistent (e.g., total bilirubin in males was significant in the high-dose group, but not the mid-dose group even though all information was the same);
** $p \leq 0.01$.

Table 7 summarizes the BMD modeling results for the total bilirubin data in male Crj:CD(SD)IGS rats. The curve and BMD output for the selected model are provided in Appendix C. The BMD Exponential (M4) (constant variance) model with a BMDL of 19.71 mg/kg-day is selected because there is a good visual fit to the data and because it is the only model that provides an adequate fit (using goodness-of-fit test; $p \geq 0.1$).

Table 7. Model Predictions for Total Bilirubin in Male Crj:CD(SD)IGS Rats[a]

Model	Goodness of Fit *p*-Value[b]	AIC for Fitted Model	BMD_{1SD} (mg/kg-day)	$BMDL_{1SD}$ (mg/kg-day)	Conclusions
Hill (constant variance)	N/A	−380.07	49.56	16.64	Fails *p*-value criteria
Exponential (M4) (constant variance)	**0.508**	**−381.63**	**39.96**	**19.71**	**Lowest AIC[c] Lowest BMDL[c]**
Exponential (M5) (constant variance)	N/A	−380.07	48.77	20.93	Fails *p*-value criteria
Linear (constant variance)	0.001	−369.82	356.22	242.73	Fails *p*-value criteria
Polynomial (constant variance)	0.001	−369.82	356.22	242.73	Fails *p*-value criteria
Power (constant variance)	0.001	−369.82	356.22	242.73	Fails *p*-value criteria
Exponential (M2) (constant variance)	0.001	−368.92	400.13	288.58	Fails *p*-value criteria
Exponential (M3) (constant variance)	0.001	−368.92	400.13	288.58	Fails *p*-value criteria

[a]MHLW (2001a).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Lowest values with acceptable Goodness of Fit.

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose.

The POD in this study is a $BMDL_{1SD}$ of 19.71 mg/kg-day based on elevated total bilirubin in male rats (MHLW, 2001a). Comparatively, 50 mg/kg-day was a LOAEL for total bilirubin in male rats and for total bile acids in females and a NOAEL for most other effects. No dosimetric adjustments for duration of exposure are made because the doses in the principal study were administered via gavage in mg/kg-day, 7 days/week for the study duration.

In EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches may include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In lieu of chemical-specific models or data to inform the derivation of human equivalent oral exposures, EPA endorses body weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving a RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving a RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite, but not for portal-of-entry effects.

The selected critical effect of elevated bilirubin is associated with the parent compound or a stable metabolite. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for this effect.

Following U.S. EPA (2011c) guidance, the POD based on elevated bilirubin in adult animals is converted to a HED through application of a dosimetric adjustment factor (DAF)[1] derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.244. Applying this DAF to the elevated bilirubin, identified as the critical effect in mature rats yields a $BMDL_{HED}$ as follows:

$$\begin{aligned} BMDL_{HED} &= BMDL_{1SD} \times DAF \\ &= 19.71 \text{ mg/kg-day} \times 0.244 \\ &= 4.81 \text{ mg/kg-day} \end{aligned}$$

The subchronic p-RfD for cyclohexene is derived as follows:

$$\begin{aligned} \textbf{Subchronic p-RfD} &= \textbf{BMDL}_{\textbf{HED}} \div \textbf{UF}_{\textbf{C}} \\ &= 4.81 \text{ mg/kg-day} \div 100 \\ &= \mathbf{5 \times 10^{-2} \text{ mg/kg-day}} \end{aligned}$$

Table 8 summarizes the uncertainty factors (UFs) for the subchronic p-RfD for cyclohexene.

[1]As described in detail in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011), rate-related processes scale across species in a manner related to both the direct ($BW^{1/1}$) and allometric scaling ($BW^{3/4}$) aspects such that $BW^{3/4} \div BW^{1/1} = BW^{-1/4}$, converted to a $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Table 8. Uncertainty Factors for Subchronic p-RfD of Cyclohexene

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) was applied to account for uncertainty in characterizing the toxicodynamic differences between rats and humans following oral cyclohexene exposure. The toxicokinetic uncertainty was accounted for by calculation of a human equivalent dose (HED) through application of a dosimetric adjustment factor (DAF) as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c).
UF_D	3	A UF_D of 3 is selected because there is limited reproduction and developmental data based on a reproductive/developmental screening study (MHLW, 2001b).
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a BMDL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was utilized.
UF_C	100	Composite uncertainty factor for derivation of the subchronic p-RfD.

The confidence of the subchronic p-RfD for cyclohexene is low as explained in Table 9.

Table 9. Confidence Descriptors for Subchronic p-RfD for Cyclohexene

Confidence Categories	Designation[a]	Discussion
Confidence in study	M	The confidence in the study is medium. Although the Japanese study was conducted according to OECD 422 guidelines with a wide array of endpoints examined and translated by EPA, OECD SIDS (2002) provides a secondary source peer review of the data.
Confidence in database	L	The confidence in the database is low because there is only one oral repeat dose study in a single species available. Although the study conducted reproductive and development screening, there are no two-generation or full developmental studies available.
Confidence in subchronic p-RfD[b]	L	The overall confidence is low because the confidence in the database is low.

[a]L = Low, M = Medium, H = High.
[b]The overall confidence cannot be greater than lowest entry in table.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

There is a single repeat-dosing study available that includes reproductive/developmental screening and subchronic portions (i.e., MHLW, 2001a,b). The subchronic portion of the study by MHLW (2001a) is selected as the principal study for derivation of the chronic p-RfD. The critical effect is total bilirubin in male rats. Details on the MHLW (2001a,b) study are provided in the "Review of Potentially Relevant Data" section. The same justification and BMD analysis applies, as was conducted for the subchronic p-RfD, with details provided in the "Derivation of Subchronic Provisional RfD (Subchronic p-RfD)" section.

The POD in this study is a $BMDL_{1SD}$ of 19.71 mg/kg-day based on elevated total bilirubin in male rats (MHLW, 2001a). No dosimetric adjustments are made because the doses in the principal study were administered via gavage in mg/kg-day, 7 days/week for the study duration. Following U.S. EPA (2011c) guidance, the POD based on elevated bilirubin in adult animals was converted to a HED through application of a DAF as described above. The chronic p-RfD for cyclohexene is derived as follows:

$$BMDL_{HED} = BMDL_{1SD} \times DAF$$
$$= 19.71 \text{ mg/kg-day} \times 0.244$$
$$= 4.81 \text{ mg/kg-day}$$

$$\textbf{Chronic p-RfD} = \textbf{BMDL}_{\textbf{HED}} \div \textbf{UF}_{\textbf{C}}$$
$$= 4.81 \text{ mg/kg-day} \div 1000$$
$$= \mathbf{5 \times 10^{-3} \text{ mg/kg-day}}$$

Table 10 summarizes the UFs for the chronic p-RfD for cyclohexene.

Table 10. Uncertainty Factors for Chronic p-RfD of Cyclohexene

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) was applied to account for uncertainty in characterizing the toxicodynamic differences between rats and humans following oral cyclohexene exposure. The toxicokinetic uncertainty was accounted for by calculation of a human equivalent dose (HED) through application of a dosimetric adjustment factor (DAF) as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c).
UF_D	3	A UF_D of 3 is selected because there is limited reproduction and developmental data based on a reproductive/developmental screening study (MHLW, 2001b).
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a BMDL.
UF_S	10	A UF_S of 10 is applied because a subchronic-duration study was utilized.
UF_C	1000	Composite uncertainty factor used in the derivation of the chronic p-RfD.

The confidence of the chronic p-RfD for cyclohexene is low as explained in Table 11.

Table 11. Confidence Descriptors for Chronic p-RfD for Cyclohexene

Confidence Categories	Designation[a]	Discussion
Confidence in study	M	The confidence in the study is medium. Although the Japanese study was conducted according to OECD 422 guidelines with a wide array of endpoints examined and translated by EPA, OECD SIDS (2002) provides a secondary source peer review of the data.
Confidence in database	L	The confidence in the database is low because there is only one repeat dose oral study in a single species available. Although the study conducted reproductive and development screening, there are no two-generation or full developmental studies available.
Confidence in subchronic p-RfD[b]	L	The overall confidence is low because the confidence in the database is low.

[a]L = Low, M = Medium, H = High.
[b]The overall confidence cannot be greater than lowest entry in table.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

No subchronic p-RfC value can be derived because no subchronic inhalation studies were identified.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

No chronic p-RfC value can be derived because the principal study is not peer reviewed. However, Appendix A of this document contains a screening value that might be useful in certain instances. Please see the attached Appendix for details.

Justification

The only information available for inhalation toxicity was provided in a conference abstract or details on a web site (i.e., Laham, 1976a,b,c; MHLW, 2003). These publications are not considered to be peer reviewed. The abstract by Laham (1976a,b,c) details similar studies conducted in three species (rat, guinea pigs, and rabbits). There was a statistically significant increase in alkaline phosphatase level compared to the controls in rats reported, however, the data were not provided. The increase in alkaline phosphatase is consistent with the increase in bilirubin and total bile acids seen after oral exposure (MHLW, 2001a) as an indicator of bile duct blockage. The Laham (1976a,b,c) abstract, however, is not considered suitable for deriving the chronic p-RfC, due to lacking information. MHLW (2003) reports on a 2-year study in rats and mice. There were no noncancer effects reported for the chronic-duration exposure in mice. Although the web site does not provide sufficient data, it appears that the respiratory tract was examined as incidences of lung tumors were reported. In rats, an increased incidence of spongiosis hepatis was stated to occur at concentrations $\geq$720 mg/m^3, however, no incidence data are provided. In addition, the hematology and clinical chemistry results are not provided. Taking all the data into consideration, the liver appears to be a target organ. Although there is some concern that spongiosis hepatis is a preneoplastic lesion, Karbe and Kerlin (2002) suggest

that it is a sign of hepatotoxicity. They examined 12 oncogenicity studies where spongiosis hepatis occurred and found that it was more predominant in the males and that it was associated more with hepatotoxicity than carcinogenicity. Therefore, this lesion can be considered as a critical effect for p-RfC derivation. A NOAEL of 360 mg/m^3 is used to derive the screening chronic p-RfC from the MHLW (2003a) study (see Appendix A). Because the study is not peer reviewed and there are no data available on all the endpoints stated to have been tested, the p-RfC is relegated to a screening value.

CANCER WEIGHT-OF-EVIDENCE (WOE) DESCRIPTOR

Table 12 identifies the cancer WOE descriptor for cyclohexene.

Table 12. Cancer WOE Descriptor for Cyclohexene

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	NS	NA	There are no human data available.
"Likely to Be Carcinogenic to Humans"	NS	NA	There is not enough evidence to support this statement.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	There is not enough evidence to support this statement.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**No oral carcinogenicity studies were identified. There was a slight increase in combined hepatocellular adenomas and carcinomas at the highest dose in male rats, however, no concentration was significant and tumors analyzed separately were not significant. Male mice had a significant decrease in liver tumors. Female rats and mice were not affected.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	There is not enough evidence to support this statement.

NS = Not selected; NA = Not applicable.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

The lack of oral data on the carcinogenicity of cyclohexene precludes the derivation of quantitative estimates for oral (p-OSF) exposure.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

MHLW (2003) conducted a 2-year inhalation carcinogenicity study in rats and mice. While there was a statistically significant dose-related trend for increased incidence of combined hepatocellular adenomas and carcinomas in male rats noted with the Peto trend test, the results were not significant with the Cochran-Armitage trend test, nor were any of the concentrations

statistically different from the control. This incidence was low and primarily composed of adenomas, which did not achieve statistical significance alone. Additionally, there was a lack of a clear dose response in the data (the low dose incidence is less than the controls). Male rats also had increased incidence of preneoplastic lesions in the liver, but the incidences were not provided. However, male mice had a statistically significant decrease in the incidence of combined hepatocellular adenomas and carcinomas and no effects on liver tumors were noted in females of either species. The increase in liver tumors in male rats was given consideration for deriving a p-IUR. However, the low incidence and the lack of a clear dose response were not conducive to BMD modeling. When the data are applied to the multistage linear model with a 10% extra risk, the BMD is approximately 1700 mg/m^3, which is above the highest dose tested (i.e., 1440 mg/m^3). This is likely due in part because there was no statistically significant increase in the response and because the incidence at the highest dose was only 10%, and not increased above the control by 10% (control had a 4% incidence in combined hepatocellular adenomas and carcinomas). Because the incidence was low, lacked a dose response, and was not supported by data in male mice or females of either species, the available data are inadequate to derive a p-IUR.

APPENDIX A. PROVISIONAL SCREENING VALUES

For the reasons noted in the main document, it is inappropriate to derive a provisional chronic p-RfC for cyclohexene. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Screening values receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening values should understand that there is considerably more uncertainty associated with their derivation than for a PPRTV. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

DERIVATION OF SCREENING PROVISIONAL INHALATION REFERENCE CONCENTRATIONS

Derivation of Screening Chronic Provisional RfC (Screening Chronic p-RfC)

The study by MHLW (2003a) is selected as the principal study for derivation of the screening chronic p-RfC. The critical effect is spongiosis hepatis observed in male and female rats (MHLW, 2003a). This study was only provided on a web site and was not peer reviewed nor was there information available on GLP compliance. Details are provided in the "Review of Potentially Relevant Data" section. Benchmark dose (BMD) analysis is not possible as no data were provided. Among the available, acceptable studies, the MHLW (2003a) study represents the lowest POD for developing a chronic p-RfC.

The POD in this study is a NOAEL of 360 mg/m^3.

Cyclohexene is likely a category 2 gas. This is based on the moderate solubility and the fact that there is some absorption into the blood stream after inhalation exposure (Maples and Dahl, 1993). Because there is no indication of any adverse respiratory effects, but there is an indication of possible adverse systemic effects, the following dosimetric adjustments have been made for inhalation with a NOAEL for extra-respiratory effects:

$$\begin{aligned} NOAEL_{HEC,\ EXRESP} &= ppm \times (\text{ppm conversion}) \times (\text{average daily dose}) \times (\text{blood gas partition coefficients}) \\ &= ppm \times (MW \div 24.45) \times [(\text{hours exposed} \div 24) \times (\text{days per week exposed} \div 7 \text{ days in a week})] \times (\text{blood gas partition coefficients}) \\ &= 600 \times (82.15 \div 24.45) \times [(6 \div 24) \times (5 \div 7)] \times (1) \\ &= 360\ mg/m^3 \end{aligned}$$

The chronic p-RfC for cyclohexene, based on a $NOAEL_{HEC,EXRESP}$ of 360 mg/m^3 in male rats, is derived as follows:

Screening Chronic p-RfC = $\mathbf{NOAEL_{HEC,EXRESP} \div UF_C}$
= 360 mg/m^3 ÷ 300
= $\mathbf{1 \times 10^0\ mg/m^3}$
= **1 mg/m^3**

Table A.1 summarizes the uncertainty factors for the screening chronic p-RfC for cyclohexene.

Table A.1. Uncertainty Factors for Screening Chronic p-RfC of Cyclohexene

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied for animal-to-human extrapolation to account for the toxicodynamic portion of the UF_A because the toxicokinetic portion ($10^{0.5}$) has been addressed in dosimetric conversions.
UF_D	10	A UF_D of 10 is selected because there are no acceptable two-generation reproduction studies or developmental studies by this route.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	1	A UF_S of 1 is applied because a chronic study was utilized.
UF_C	300	Composite uncertainty factor used in the derivation of the screening chronic p-RfC.

APPENDIX B. DATA TABLES

Table B.1. Select Clinical Chemistry Parameters in Male and Female Crj:CD(SD)IGS Rats After 43−53 Days Oral (Gavage) Exposure to Cyclohexene[a]

Parameter	Average Daily Dose (mg/kg-d)[b]			
	0	50	150	500
Male				
Sample size	12	11	12	12
Triglyceride (mg/dL)	39.2 ± 22.4[c]	36.8 ± 18.8 (94%)	27.7 ± 16.7 (71%)	22.5 ± 7.7 (57%)
Total bilirubin (mg/dL)	0.03 ± 0.01	0.04 ± 0.01 (133%)*	0.05 ± 0.01 (167%)**	0.05 ± 0.01 (167%)**
Total bile acid (µmol/L)	18.8 ± 15.0	20.8 ± 16.6 (111%)	39.9 ± 21.0 (212%)*	32.6 ± 25.5 (173%)
Female				
Sample size	10	10	10	10
Triglyceride (mg/dL)	45.4 ± 24.9	66.6 ± 85.8 (147%)	37.8 ± 9.0 (83%)	39.4 ± 13.9 (87%)
Total bilirubin (mg/dL)	0.04 ± 0.01	0.05 ± 0.02 (125%)	0.05 ± 0.02 (125%)	0.06 ± 0.01 (150%)*
Total bile acid (µmol/L)	19.3 ± 8.6	49.2 ± 28.8 (255%)*	31.2 ± 19.7 (162%)	82.2 ± 81.1 (426%)*

[a]MHLW (2001a).
[b]Dose was as administered.
[c]Mean ± standard deviation (% of control); % is calculated.

Statistically different from the control *$p \leq 0.05$; **$p \leq 0.01$ using two sample *t*-test for the purpose of this review because the results reported by the study author were inconsistent (e.g., total bilirubin in males was significant in the high-dose group, but not the mid-dose group even though all information was the same).

Table B.2. Select Organ Weights in Male Crj:CD(SD)IGS Rats After 43−53 Days Oral (Gavage) Exposure to Cyclohexene[a]

Parameter	Average Daily Dose (mg/kg-d)[b]			
	0	50	150	500
Sample size	12	11	12	12
Absolute kidney weight (g)	3.21 ± 0.33[c]	3.09 ± 0.27 (96%)	3.20 ± 0.27 (100%)	3.31 ± 0.34 (103%)
Relative kidney weight (g%)	0.652 ± 0.057	0.619 ± 0.031 (95%)	0.667 ± 0.059 (102%)	0.705 ± 0.053 (108%)*

[a]MHLW (2001a).
[b]Dose was as administered.
[c]Mean ± standard deviation (% of control); % is calculated.

Statistically different from the control *$p \leq 0.05$.

Table B.3. Reproductive/Developmental Screening Parameters in Male and Female Crj:CD(SD)IGS Rats After 43−53 Days Oral (Gavage) Exposure to Cyclohexene[a]

Parameter		Exposure Group (mg/kg-d)[b]			
		0	50	150	500
Number of pairs mated		12	12	12	12
Number of pairs copulated		12	11	12	12
Copulation index (%)		100.0	91.7	100.0	100.0
Number of pregnant females		11	10	10	10
Fertility index (%)		91.7	90.9	83.3	83.3
Number of dams delivering live pups		11	10	10	10
Gestation index (%)		100.0	100.0	100.0	100.0
Duration of gestation[c]		22.5 ± 0.5	22.2 ± 0.4	22.3 ± 0.5	22.5 ± 0.5
Number of corpora lutea per dam[c]		19.2 ± 2.6	17.4 ± 3.3	18.4 ± 3.2	20.1 ± 3.8
Number of implants per dam[c]		13.7 ± 3.0	14.4 ± 1.6	14.3 ± 1.5	14.3 ± 1.6
Implantation index (%)[c]		73.2 ± 20.3	84.1 ± 9.7	79.0 ± 10.6	72.9 ± 13.1
Number of live pups born per dam[c]		12.8 ± 3.5	13.4 ± 1.6	13.5 ± 2.1	12.5 ± 2.2
Number of dead pups per litter[c]		0.3 ± 0.6	0.1 ± 0.3	0.1 ± 0.3	0.3 ± 0.9
Delivery index (%)[c]		93.2 ± 11.9	93.8 ± 6.1	97.3 ± 4.7	90.0 ± 10.3
Live birth index (%)[c]		98.0 ± 4.7	99.3 ± 2.3	96.9 ± 9.7	97.0 ± 9.5
Sex ratio[c]		0.80 ± 0.23	1.32 ± 0.68	1.14 ± 1.60	0.81 ± 0.56
Number of live pups on Day 4 per litter[c]	Males	5.5 ± 2.2	6.9 ± 2.1	6.7 ± 2.6	5.0 ± 2.1
	Females	6.8 ± 2.8	6.2 ± 1.9	6.7 ± 2.4	7.2 ± 2.0
Viability index on Day 4[c]	Males	90.9 ± 30.2	95.3 ± 10.0	100.0 ± 0.0	96.7 ± 10.5
	Females	88.6 ± 29.8	100.0 ± 0.0	98.3 ± 5.3	98.3 ± 5.3

[a]MHLW (2001b).
[b]Dose was as administered.
[c]Mean ± standard deviation.

Statistically different from the control *$p \leq 0.05$.

Table B.4. Liver Tumors in Male and Female F344 Rats and Crj:BDF1 Mice After a 2-Year Inhalation Exposure[a]

Parameter	Exposure group, ppm (HEC, mg/m^3)[b]			
	0	**600 (360)**	**1200 (720)**	**2400 (1440)**
Male F344 rats				
Sample size	50	50	50	50
Hepatocellular adenoma	2 (4%)[c]	1 (2%)	4 (8%)	4 (8%)
Hepatocellular carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)
Hepatocellular adenoma/hepatocellular carcinoma	2 (4%)	1 (2%)	4 (8%)	5 (10%)[d]
Female F344 rats				
Sample size	50	50	50	50
Hepatocellular adenoma	1 (2%)	1 (2%)	1 (2%)	0 (0%)
Hepatocellular carcinoma	1 (0%)	0 (0%)	0 (0%)	0 (0%)
Parameter	**Exposure group, ppm (HEC, mg/m^3)[b]**			
	0	**75 (45)**	**150 (90)**	**300 (180)**
Male Crj:BDF1 mice				
Sample size	50	50	50	50
Hepatocellular adenoma	8 (16%)	6 (12%)	9 (18%)	5 (10%)
Hepatocellular carcinoma	12 (24%)	7 (14%)	5 (10%)	3 (6%)*[e]
Hepatocellular adenoma/hepatocellular carcinoma	20 (40%)	13 (26%)	12 (24%)	8 (16%)**[e]
Female Crj:BDF1 mice				
Sample size	50	50	50	50
Hepatocellular adenoma	3 (6%)	1 (2%)	3 (6%)	1 (2%)

[a]MHLW (2003c,d).
[b]Doses are converted from ppm to mg/m^3 using the following equation: HEC = ppm × (molecular weight ÷ 24.45) × (hours of exposure per day ÷ 24) × (days dosed ÷ total days) × blood gas partition coefficient.
[c]Number of animals with tumors (%).
[d]Significant increasing trend with the Peto test, but not with the Cochran-Armitage test.
[e]Significant decreasing trend with the Cochran-Armitage test, but not with the Peto test.

Statistically different from controls *$p \leq 0.05$; **$p \leq 0.01$; Fisher test performed by study authors.

APPENDIX C. BMD OUTPUTS

MHLW 2001a_Total bilirubin Male_M4_ExpCV_1

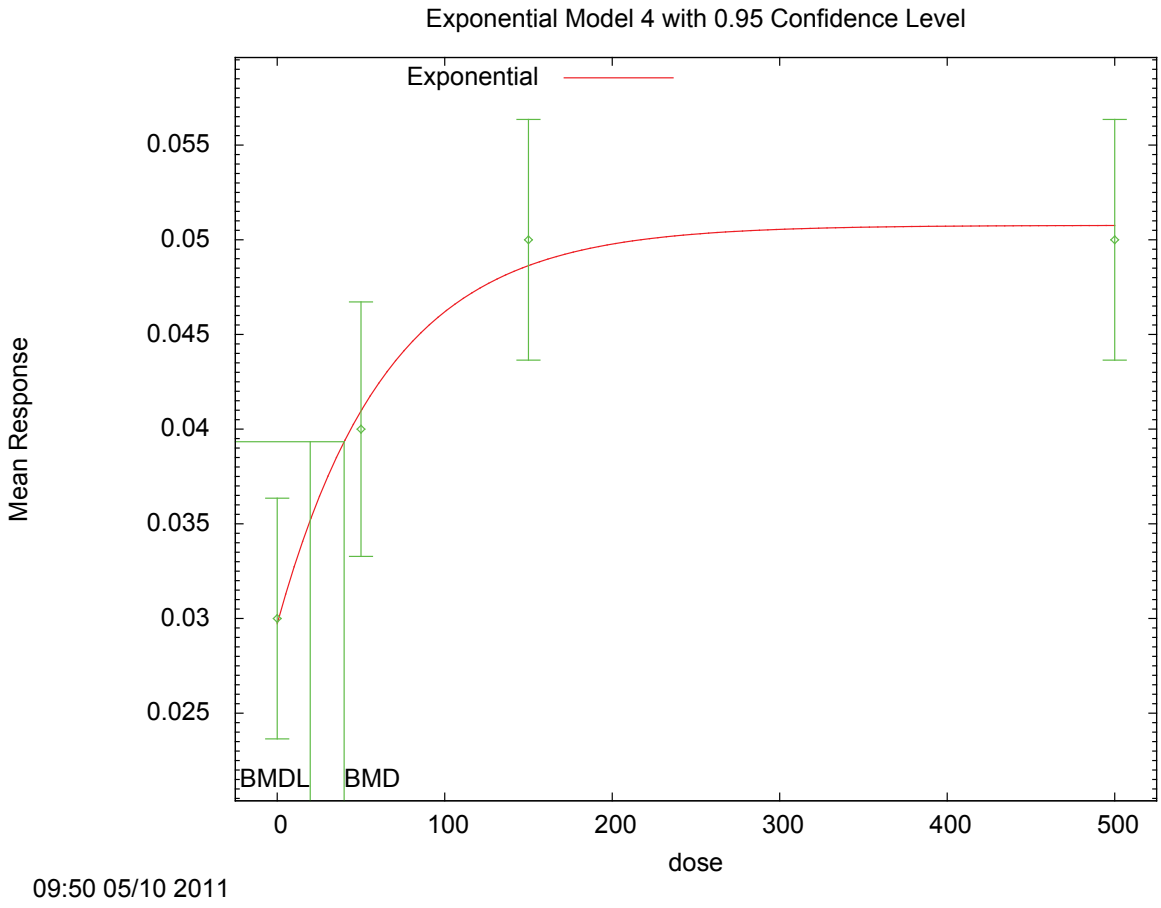

Figure C.1. Exponential (M4) (Continuous Variance) BMD Model for Total Bilirubin Data (MHLW, 2001a)

Text Output for Exponential (M4) (Continuous Variance) BMD Model for Total Bilirubin Data (MHLW, 2001a)

```
 ================================================================================
        Exponential Model. (Version: 1.7;  Date: 12/10/2009)
        Input Data File: C:/1/MHLW 2001a_Total bilirubin Male_ExpCV_1.(d)
        Gnuplot Plotting File:
                                            Tue May 10 09:50:46 2011
 ================================================================================

 [add notes here]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
```

```
   Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
   Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
   Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

 Note: Y[dose] is the median response for exposure = dose;
       sign = +1 for increasing trend in data;
       sign = -1 for decreasing trend.

   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

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

MLE solution provided: Exact


               Initial Parameter Values

               Variable          Model 4
               --------          --------
                 lnalpha            -9.29929
                     rho(S)               0
                       a              0.0285
                       b           0.00546459
                       c              1.84211
                       d                    1

  (S) = Specified


                 Parameter Estimates

                Variable          Model 4
                --------          -------
                 lnalpha            -9.28998
                     rho                   0
                       a           0.0297272
                       b           0.0152693
                       c             1.70773
                       d                   1


         Table of Stats From Input Data
```

Dose	N	Obs Mean	Obs Std Dev
0	12	0.03	0.01
50	11	0.04	0.01
150	12	0.05	0.01
500	12	0.05	0.01

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	0.02973	0.00961	0.09834
50	0.04096	0.00961	-0.3316
150	0.04864	0.00961	0.4916
500	0.05076	0.00961	-0.2724

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	195.0333	5	-380.0665
A2	195.0334	8	-374.0668
A3	195.0333	5	-380.0665
R	181.669	2	-359.338
4	194.8145	4	-381.629

Additive constant for all log-likelihoods = -43.19. This constant added to the
above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	26.73	6	0.0001628
Test 2	0.0002897	3	1
Test 3	0.0002897	3	1
Test 6a	0.4376	1	0.5083

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD = 39.9627

BMDL = 19.7087

APPENDIX D. REFERENCES

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