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Provisional Peer-Reviewed Toxicity Values for

Cyclohexane
(CASRN 110-82-7)

Superfund Health Risk Technical Support Center
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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMD	benchmark dose
BMCL	benchmark concentration lower bound 95% confidence interval
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
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure
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 CYCLOHEXANE (CASRN 110-82-7)

BACKGROUND

HISTORY

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) EPA's Integrated Risk Information System (IRIS)
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in EPA's Superfund Program
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR);
   - California Environmental Protection Agency (CalEPA) values; and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by a panel of six EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

DISCLAIMERS

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

QUESTIONS REGARDING PPRTVS

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may 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), or OSRTI.

INTRODUCTION

Cyclohexane is a cycloalkane used as a nonpolar solvent in the chemical industry as well as a feedstock for production of nylon. The IRIS (U.S. EPA, 2009a) database includes an RfC value of 6 mg/m^3 for cyclohexane based on a two-generation reproduction study in rats (i.e., Kreckmann et al., 2000) that identified a NOAEL of 6886 mg/m^3 and a LOAEL of 24,101 mg/m^3 for developmental effects (reduced pup weight in F1 and F2 generation offspring). IRIS includes discussions on oral toxicity and cancer, but, due to inadequate data, no RfD value or quantitative cancer risk estimates were derived (U.S. EPA, 2009a). The source document for the IRIS assessment is a *Toxicological Review of Cyclohexane* that was published in August 2003 (U.S. EPA, 2003). Cyclohexane is currently undergoing review as part of the *IRIS Track Report for Alkylates Assessment*, which began in November 2008 (U.S. EPA, 2009b). See Figure 1 for the chemical structure of cyclohexane.

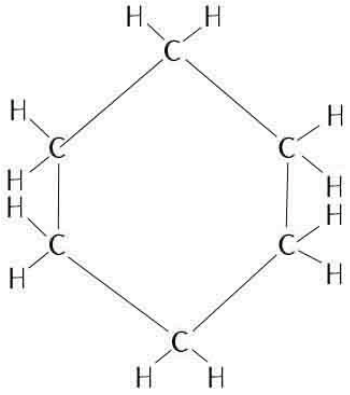

Figure 1. Chemical Structure of Cyclohexane

No RfD, RfC, or cancer assessment for cyclohexane is available on the HEAST (U.S. EPA, 1997) or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). No relevant documents were located in the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994, 1991). ATSDR (2009) has not published a Toxicological Profile for cyclohexane, and no Environmental Health Criteria Document is available from the World Health Organization (WHO, 2009). The carcinogenicity of cyclohexane has not been assessed by the International Agency for Research on Cancer (IARC, 2009) or the National Toxicology Program (NTP, 2009, 2005). The American Conference for Governmental Industrial Hygienists (ACGIH, 2008) has adopted a threshold limit value-time-weighted average (TLV-TWA) of 100 ppm as protective against central nervous system (CNS) impairment. The National Institute for Occupational Safety and Health (NIOSH, 2009) recommended exposure limit (REL) is 300 ppm based on irritation of eyes, skin, and respiratory system and CNS effects. The Occupational Safety and Health Administration (OSHA, 2009) permissible exposure limit (PEL) is 300 ppm.

Literature searches were initially conducted from the 1960s through July 2010 for studies relevant to the derivation of provisional toxicity values for cyclohexane. Databases searched included MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (February through July 2010).

REVIEW OF PERTINENT DATA

HUMAN STUDIES

Oral Exposure

No information was located regarding the subchronic or chronic oral toxicity of cyclohexane in humans.

Inhalation Exposure

Two small studies have been conducted among workers primarily exposed to cyclohexane (Yasugi et al., 1994; Yuasa et al., 1996). Both of these studies were reviewed by EPA in the 2003 *Toxicological Review of Cyclohexane* (U.S. EPA, 2003) and are summarized below.

Yasugi et al. (1994) surveyed women workers (20–60 years old) from a Japanese factory where glue containing at least 75% cyclohexane was applied to surfaces by automated sprayers. During the survey, each worker was equipped with a personal air monitor at the start of each shift. Each worker was administered a questionnaire on subjective symptoms experienced within the last 3 months both at home and at work. Health effects were compared between 38 exposed (mean age 25.2) workers who were either directly involved in glue application or worked in the vicinity of glue application for at least 1 year, and 9 nonexposed (mean age 25.5) clerical workers employed at the same factory but located in a different building. Blood and urine samples were collected from all women at the end of each shift on four separate occasions. In addition, 14 exposed workers provided a preshift urine sample the morning following the study day. Venous blood samples were submitted for hematology (white blood cell count [WBC], red blood cell count [RBC], hemoglobin concentration [Hgb], and hematocrit [Hct]) and serum

chemistry (total protein, blood urea nitrogen [BUN], creatinine, uric acid, total cholesterol, HDL-cholesterol, triglyceride, aspartate aminotransferase [AST], alanine aminotransferase [ALT], γ-glutamyl transpeptidase [γ-GTP], alkaline phosphatase [ALP], leucine aminopeptidase, and lactate dehydrogenase [LDH]). The concentration of cyclohexane in blood was also determined. Urine samples were analyzed to measure metabolite (cyclohexanone and cyclohexanol) levels. In addition, blood samples from nine exposed and nine nonexposed workers (five smokers and four nonsmokers from each group) were evaluated for the possible effects of cyclohexane on the sister chromatid exchange (SCE) rates of peripheral lymphocytes.

There was a discrepancy in reporting of exposure levels in Yasugi et al. (1994); the geometric mean measured cyclohexane concentration was reported as 27 ppm (93 mg/m^3) in the text but as 18.2 ppm (63 mg/m^3) in Table 1 of the report. The maximum air concentration of cyclohexane vapor was reported to be 274 ppm (943 mg/m^3). Concentrations of cyclohexane in blood and cyclohexanol in urine appeared to correlate with measured exposure levels. Based on questionnaire responses, there was no difference in the prevalence of subjective symptoms experienced at work between the exposed workers and controls. However, nonexposed subjects complained of 57 symptoms while not at work, significantly more than exposed workers ($p < 0.01$). There were no significant differences based on individual symptoms. Hematology results revealed a difference in the prevalence of leukocytopenia between nonexposed (1/9) and exposed (3/17 low exposure, 1/16 high exposure) workers that achieved marginal statistical significance ($0.05 < p < 0.10$). No other statistically significant differences between exposed workers and controls were observed based on hematology or serum chemistry (data not shown). There were no statistically significant differences in SCE rates between exposed and nonexposed workers. Although no significant adverse health effects were reported that could be associated with occupational exposure to cyclohexane, a small cohort size, discrepancies in reporting of measured air levels, and lack of reporting details limit the interpretation of these results.

In another Japanese study, neurophysiological effects were analyzed in female luggage factory workers exposed to glue containing 75% cyclohexane, 12% toluene, and 0.9% *n*-hexane (Yuasa et al., 1996). Eighteen women who had worked at the plant for at least half of a year and were exposed primarily to cyclohexane were chosen for the study. In the past, *n*-hexane was the primary solvent used at the factory, but it was gradually discontinued and replaced with cyclohexane. Twelve of the 18 women included in the study were previously exposed to *n*-hexane (duration of exposure ranged from 0.3 to 20 years) within 0.7–2.6 years of the start of the study. Because of these past exposures, Yuasa et al. (1996) also performed a follow-up study 1 year later on nine workers who remained in the same positions since the end of the first study. Eighteen medical students and clerical workers matched based on sex and age to the cyclohexane-exposed workers served as control subjects. Health effects were assessed based on responses to a survey of 61 symptoms including 20 general symptoms (e.g., weight loss, general fatigue, etc.) and neurological symptoms. Urine samples collected at the end of each shift were analyzed for levels of cyclohexanol as an indication of cyclohexane exposure. Neurophysiological parameters evaluated included maximum nerve conduction velocity (MNCV), motor distal latency (MDL), proximal conduction velocity (SNCVp), distal conduction velocity (SNCVd), amplitude of maximum sensory nerve action potential, and duration of sensory nerve action potential.

Table 1. Summary of Responses to an Auditory Stimulus During Inhalation Exposure to Cyclohexane Vapor for 90 Days

Response	Exposure Concentration (mg/m^3)			
	0	**1721**	**6886**	**24,101**
90-Day rat study				
Normal response	65/65[a]	61/65	0/65	0/65
Diminished response	0/65	4/65	16/65	1/65
No response	0/65	0/65	49/65	64/65
90-Day neurotoxicity rat study				
Normal response	71/71	71/71	4/71	0/71
Diminished response	0/71	0/71	32/71	3/71
No response	0/71	0/71	35/71	68/71
90-Day mouse study				
Normal response	67/67	67/67	7/67	3/67
Diminished response	0/67	0/67	47/67	17/67
No response	0/67	0/67	13/67	2/67
Hyperactivity	0/67	0/67	4/67	2/67
Abnormal behavior[b]	0/67	0/67	0/67	61/67

[a]Number of exposures when a given response was observed/total number of exposures. Animals in each exposure group were observed together in the exposure chamber, and the response of the group as a whole to a standardized auditory stimulus (prior to exposure, after 2, 4, and 6 hours of exposure, and 30 minutes after exposure ended) was subjectively characterized as normal, diminished, absent, or hyperresponsive; observers were not blind to exposure status of the animals.
[b]Clinical signs of abnormal behavior in mice at 24,101 mg/m^3 included hyperactivity, jumping, hopping, circling, flipping, rear leg kicking, standing on front legs, and excessive grooming, These behaviors frequently prevented determination of response to a sound stimulus in this group.

Source: Malley et al. (2000).

Urinary cyclohexanol measurements ranged from 0.12 to 8.23 mg/L (geometric mean of 0.55 mg/L) and were highly correlated to ambient cyclohexane levels in the workplace (Yuasa et al., 1996). Responses from the health effects questionnaire included complaints of fatigue in 9/18 exposed workers and 4/15 controls, headaches in 10/18 exposed workers and 7/15 controls, and dizziness in 7/18 exposed workers and 4/15 controls. In the neurophysiological examination, no significant differences in nerve conduction velocities (NCVs) were observed between exposed workers and controls, but the ulnar and peroneal MDLs were significantly shorter in exposed workers than in controls ($p < 0.05$). During the follow-up study 1 year later, significant improvements in NCV and MDL of exposed workers were observed. These results suggest that past *n*-hexane exposures may have impacted the initial results greater than in the follow-up study. Based on limitations of this study, including past exposures to *n*-hexane, small group sizes, and poorly matched controls, the effects of cyclohexane exposure in these workers cannot be adequately assessed.

Lammers et al. (2009) conducted a physiologically based pharmacokinetic (PBPK) modeling study that involved human volunteers. The neurobehavioral effects of inhaled cyclohexane in rats and humans were investigated to define relationships between internal doses and acute CNS effects. Cyclohexane concentrations in blood were measured to assess internal exposure. Human volunteers were exposed for 4 hours to 86 or 860 mg/m^3 in two test sessions. Neurobehavioral effects were measured using a computerized neurobehavioral test battery. In rats, slight reductions in psychomotor speed in the high-exposure group—but minimal CNS effects—are evident. In humans, there are no significant treatment-related effects at the levels tested. While a NOAEL of 86 mg/m for neurobehavioral outcomes may be suggested from these data, the small number of volunteers and the number of replicates make this determination uncertain. Additionally, because these are acute studies (4 hours), this value would not necessarily be protective of additional outcomes from longer exposures.

EPA (2003) considered these studies, as well as additional studies of occupational exposure to a mixture of solvents where the primary exposures were to solvents other than cyclohexane, to be inadequate for dose-response assessment because of limitations in study design and potential confounding by coexposures to other compounds.

ANIMAL STUDIES

Oral Exposure

No data were located on the subchronic and/or chronic oral effects of cyclohexane in animals.

Inhalation Exposure

Subchronic-duration Studies—There are four subchronic-duration studies in the literature; one in rats, one in mice, and two in rabbits. All of the studies of subchronic-duration inhalation exposure of cyclohexane in animals identified in the literature search were reviewed by EPA (2003), except for an older, 40-day French study in rabbits (Fabre et al., 1952). The subchronic-duration inhalation studies for cyclohexane are summarized below.

Two unpublished, 90-day inhalation toxicity studies conducted with cyclohexane in Crl:CD BR rats and CD-1 mice (Haskell Laboratory, 1996a,b) were later summarized and published as parts of Malley et al. (2000). In these studies, groups of rats and mice (20/gender/species/concentration for control and high-concentration groups and 10/gender/species/concentration for low- and intermediate-concentration groups) were exposed to cyclohexane (99.9% purity) vapor at 0, 500, 2000, or 7000 ppm (0, 1721, 6886, or 24,101 mg/m^3) 6 hours/day, 5 days/week, for 13–14 weeks, for a total of at least 65 exposures. Exposure concentrations were selected based on the results of 2-week range-finding studies in rats and mice (Haskell Laboratories, 1995) and knowledge of the explosive properties of cyclohexane. All rats and mice were monitored during and immediately after the daily exposure periods for clinical signs of distress and for their response to an auditory-alerting stimulus, and weekly for changes in body weight and food consumption. Blood samples were collected from 10 rats/sex/concentration at 45 and 90 days for hematology (RBC, platelet count, Hgb, Hct, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and total and differential WBC) and clinical chemistry (ALP, ALT, AST, sorbitol dehydrogenase [SDH], γ-GTP, creatine phosphokinase, LDH, BUN, glucose, calcium, phosphate, bilirubin, cholesterol, creatinine, triglycerides, total protein,

albumin, globulin, sodium, potassium, and chloride). Blood samples from mice were evaluated for the same hematology parameters as evaluated in the rat and for plasma protein. Urine samples were also collected from rats for urinalysis (volume, osmolality, urobilinogen, pH, Hgb, glucose, protein, bilirubin, and ketone). Both rats and mice were subjected to ophthalmological examination both prior to the start of each study and just prior to the end of the 90-day exposures. At the end of 90 days, 10 rats and 10 mice/species/gender/concentration were sacrificed and necropsied. After a 1-month recovery period, 10 remaining rats and mice/species/sex from the control and high-dose groups were sacrificed and necropsied. The lungs, brain, heart, liver, spleen, kidneys, ovaries, adrenal glands, and testes were weighed, and a complete histological examination was conducted.

There were no treatment-related deaths, and no significant effects were observed based on body weight or food consumption in cyclohexane-treated rats (Malley et al., 2000). Rats exposed at 6886 or 24,101 mg/m^3 demonstrated a diminished or absent alerting response each day in the exposure chamber, as detailed in Table 1. The researchers considered the effect on alerting response to represent a compound-related sedative effect. The effect was transient, as no clinical signs of compromised neurological function were evident when the rats were observed individually upon removal from the exposure chamber 30 minutes after the end of the daily exposure. The only observations in rats at that time were transient signs of stained and/or wet fur, which the researchers attributed to salivation in response to the taste of residual cyclohexane experienced by the rats while grooming themselves upon removal from the inhalation chambers. Although there were a small number of instances of diminished alerting response at the low exposure level of 1721 mg/m^3, the researchers did not consider them to be treatment related due to the low frequency, the lack of time-dependent pattern, and the possibility of misclassification due to the subjective nature of the observations (see Table 1).

No significant treatment-related effects were observed in rats based on ophthalmology or hematology (Malley et al., 2000). Decreases in the activity of some serum enzymes related to hepatic function (AST, SDH, LDH, and creatine phosphokinase) were statistically significant ($p < 0.05$), (Cochran-Armitage trend test) but these parameters generally did not exhibit a dose-response relationship. Although increases in such enzyme activities can indicate tissue damage, the biological significance of decreases in these enzyme activities is not known. Males exposed to 24,101 mg/m^3 demonstrated a significant increase in mean relative liver weights (relative to body and brain weights) both at 90 days and after the 1-month recovery period, as shown in Table 2. Histology revealed centrilobular hepatocellular hypertrophy in these rats at the end of the 90-day exposure period (see Table 2) at the high dose. Although livers of female rats were not significantly enlarged compared to controls following cyclohexane treatment, 50% of female rats exposed to 24,101 mg/m^3 exhibited similar pathological changes as reported in the male rats. Similar liver changes were not observed microscopically in rats of either sex at the end of the 1-month recovery period. Malley et al. (2000) considered the liver changes observed in treated rats to represent an adaptive response rather than an adverse effect. No other significant changes in organ weights or histology were reported in rats.

Table 2. Summary of Liver Changes in Rats Exposed to Cyclohexane Vapor for 90 Days

Parameter	Test Day	Exposure Concentration (mg/m^3)			
		0	1721	6886	24,101
Males					
Mean absolute liver weight (g)	90	18.81 ± 1.97[a]	17.64 ± 3.40	16.86 ± 2.11	19.91 ± 2.53
	123	20.23 ± 2.49	ND[b]	ND	22.37 ± 3.58
Mean relative liver weight (% of body weight)	90	3.65 ± 0.21	3.56 ± 0.30	3.52 ± 0.24	4.00 ± 0.27[c]
	123	3.78 ± 0.24	ND	ND	4.01 ± 0.31
Mean relative liver weight (% of brain weight)	90	8.70 ± 0.71	8.30 ± 0.95	8.03 ± 0.87	9.39 ± 1.09
	123	9.22 ± 1.00	ND	ND	10.37 ± 1.31[c]
Incidence of hepatomegaly	90	0/10	0/10	0/10	10/10
	123	0/10	0/10	0/10	4/10
Incidence of hypertrophy	90	0/10	0/10	0/10	9/10
	123	0/10	ND	ND	0/10
Females					
Incidence of hepatomegaly	90	0/10	0/10	0/10	0/10
	123	0/10	0/10	0/10	0/10
Incidence of hypertrophy	90	0/10	0/10	0/10	5/10
	123	0/10	ND	ND	0/10

[a]Mean ± standard deviation.
[b]Not determined at this time point.
[c]Significantly different from controls ($p < 0.05$, Dunnett's *t*-test).

Source: Malley et al. (2000).

In the corresponding mouse study, there were no treatment-related deaths, and no significant effects were observed based on body weight or food consumption (Malley et al., 2000; Haskell Laboratories, 1996b). Similar to rats, mice exposed to 6886 or 24,101 mg/m^3 generally demonstrated a diminished or absent alerting response while in the exposure chamber, although hyperactivity in response to alerting stimulus was observed near the end of the experiment (Exposures 64–67) in these groups (see Table 1). Furthermore, group observations during exposure showed that mice exposed to 24,101 mg/m^3 had marked CNS stimulation characterized by circling, jumping/hopping, excessive grooming, kicking of the rear legs, standing on the front legs, and an occasional flipping behavior that persisted for a short period after the end of each daily exposure. These clinical observations were apparent by the fourth exposure and persisted throughout the remaining exposures. The abnormal behavior frequently prevented assessment of auditory stimulus response in this group. In the individual observations for clinical signs performed 30 minutes after the end of exposure, mice in the 24,101-mg/m^3 group showed increases in abnormal gait/mobility, excessive grooming, hyperactivity, hyperreactivity, leg spasms, and ruffled fur (see Table 3). These clinical signs were not observed during the recovery period.

Table 3. Incidence of Clinical Signs in Mice Exposed to Cyclohexane Vapor for 90 Days

Clinical Observation	Exposure Concentration (mg/m^3)			
	0	1721	6886	24,101
Males				
Abnormal gait or mobility	0/20[a]	0/10	0/10	2/20[b]
Excessive grooming	0/20	0/10	0/10	2/20[b]
Hyperactive	0/20	0/10	0/10	3/20[b]
Hyperreactive	1/20	1/10	0/10	8/20[b]
Spasms rear leg(s)	0/20	0/10	0/10	2/20[b]
Ruffled fur	9/20	6/10	1/10	4/20
Females				
Abnormal gait or mobility	0/20	0/10	0/10	1/20
Excessive grooming	0/20	0/10	0/10	0/20
Hyperactive	0/20	0/10	1/10	1/20
Hyperreactive	1/20	0/10	2/10	6/20[b]
Spasms rear leg(s)	0/20	0/10	0/10	1/20
Ruffled fur	1/20	1/10	0/10	5/20[b]

[a]Number of responders/number in group. Based on individual animal observations performed each exposure day upon removal from the exposure chamber 30 minutes after the end of the exposure period.
[b]Significantly different from controls ($p < 0.05$, Cochran-Armitage test for trend).

Source: Malley et al. (2000).

Hematology revealed statistically significant increases in RBC and Hct among all exposed male groups at 90 days and among females of the highest exposure group at 90 days and after the 1-month recovery period, as shown in Table 4 (Malley et al., 2000). Hgb concentrations were also elevated among both sexes at the highest exposure level. In addition, plasma protein was significantly elevated in males from the highest exposure group. The pattern of changes suggests a possible hemoconcentration effect, perhaps secondary to dehydration. However, the changes were small in magnitude, not clearly related to dose, and within the range of biological variation for control animals, and so not considered biologically relevant by the researchers. As shown in Table 5, increased mean liver weights were observed in male mice from the 24,101-mg/m^3 exposure group. However, no corresponding histological changes were observed. Malley et al., 2000 considered the liver-weight changes observed in treated mice to represent an adaptive response rather than an adverse effect. No other gross or histological changes were observed in mice.

Table 4. Summary of Changes in Hematology and Clinical Pathology Parameters in Mice Exposed to Cyclohexane Vapor for 90 Days

Parameter	Test Day	Exposure concentration (mg/m^3)			
		0	1721	6886	24,101
Males					
RBC (×10^6/ul)	45	9.29 ± 0.54[a]	9.78 ± 0.83	9.58 ± 0.70	9.96 ± 0.86
	90	9.28 ± 0.68	10.14 ± 0.71[b]	10.35 ± 0.86[b]	10.16 ± 0.74[b]
	123	8.99 ± 0.73	ND[c]	ND	9.54 ± 0.61
Hct (%)	45	44 ± 2	46 ± 3	45 ± 2	48 ± 4
	90	44 ± 3	48 ± 4[b]	48 ± 3[b]	49 ± 3[b]
	123	42 ± 2	ND	ND	45 ± 3[b]
Hgb (g/dl)	45	16.0 ± 0.7	16.7 ± 0.9	16.6 ± 0.9	17.9 ± 1.4[b]
	90	16.5 ± 0.7	17.5 ± 1.1	17.6 ± 1.5	17.6 ± 1.5
	123	15.1 ± 0.4	ND	ND	15.9 ± 0.9[b]
Plasma protein	45	6.2 ± 0.3	6.2 ± 0.3	6.3 ± 0.5	6.8 ± 0.5[b]
	90	6.1 ± 0.4	6.7 ± 0.3	6.5 ± 0.5	6.5 ± 0.4
	123	6.4 ± 0.4	ND	ND	6.5 ± 0.4
Females					
RBC (×10^6/uL)	45	9.39 ± 0.52	9.53 ± 0.74	9.26 ± 0.94	10.26 ± 1.57
	90	9.14 ± 0.84	9.14 ± 0.80	9.11 ± 0.61	9.98 ± 0.73[b]
	123	8.99 ± 0.86	ND	ND	9.79 ± 0.73[b]
Hct (%)	45	45 ± 3	46 ± 3	45 ± 4	50 ± 7[b]
	90	43 ± 3	44 ± 3	44 ± 3	49 ± 3[b]
	123	43 ± 4	ND	ND	45 ± 4
Hgb (g/dL)	45	16.1 ± 0.8	16.6 ± 1.3	16.1 ± 1.4	18.2 ± 2.8[b]
	90	16.1 ± 0.9	16.1 ± 0.7	15.8 ± 0.9	17.4 ± 1.2[b]
	123	15.8 ± 1.3	ND	ND	16.6 ± 1.1
Plasma protein	45	5.9 ± 0.3	6.2 ± 0.2	5.9 ± 0.3	6.2 ± 0.6
	90	5.8 ± 0.3	6.1 ± 0.3	6.1 ± 0.3	6.1 ± 0.3
	123	6.0 ± 0.3	ND	ND	6.1 ± 0.4

[a]Mean ± standard deviation.
[b]Significantly different from controls ($p < 0.05$, Dunnett's *t*-test).
[c]Not determined at this time point.

Source: Malley et al. (2000).

Table 5. Summary of Liver Changes in Mice Exposed to Cyclohexane Vapor for 90 Days

Parameter	Test Day	Exposure concentration (mg/m^3)			
		0	1721	6886	24,101
Males					
Mean absolute liver weight (g)	90	1.28 ± 0.16[a]	1.46 ± 0.21	1.42 ± 0.22	1.50 ± 0.10[b]
	123	1.52 ± 0.18	ND[c]	ND	1.50 ± 0.14
Mean relative liver weight (% of body weight)	90	4.15 ± 0.41	4.65 ± 0.50[b]	4.55 ± 0.53	4.82 ± 0.31[b]
	123	4.52 ± 0.43	ND	ND	4.43 ± 0.27
Mean relative liver weight (% of brain weight)	90	2.58 ± 0.39	2.90 ± 0.39	2.87 ± 0.46	3.06 ± 0.21[b]
	123	3.18 ± 0.37	ND	ND	3.12 ± 0.34
Females					
Mean absolute liver weight (g)	90	1.03 ± 0.18	1.06 ± 0.15	1.04 ± 0.17	1.12 ± 0.09
	123	1.22 ± 0.19	ND	ND	1.29 ± 0.19
Mean relative liver weight (% of body weight)	90	4.27 ± 0.39	4.39 ± 0.30	4.53 ± 0.40	4.73 ± 0.31[b]
	123	4.61 ± 0.47	ND	ND	4.87 ± 0.60
Mean relative liver weight (% of brain weight)	90	2.14 ± 0.37	2.16 ± 0.28	2.23 ± 0.32	2.31 ± 0.18
	123	2.5 ± 0.30	ND	ND	2.69 ± 0.38

[a]Mean ± standard deviation.
[b]Significantly different from controls ($p < 0.05$, Dunnett's *t*-test).
[c]Not determined at this time point.

Source: Malley et al. (2000).

For the purposes of this review, NOAEL and LOAEL values of 1721 mg/m^3 (500 ppm) and 6886 mg/m^3 (2000 ppm), respectively, are identified for cyclohexane in these studies in rats and mice based on neurobehavioral effects (diminished response to a sound stimulus, and in mice at 24,101 mg/m^3, hyperreactivity and other behavioral changes). Mild liver changes (increased relative liver weight, and in rats, centrilobular hypertrophy) were found at 24,101 mg/m^3 in both species.

In the third subchronic-duration study, Treon et al. (1943) exposed groups of four white rabbits (gender/strain not specified) whole-body to cyclohexane (purity not reported) vapor at 0, 434, 435, 786, 3330, 7444, 9220, 12,574, 18,565, or 56,572 ppm (0, 1494, 1498, 2706, 11,465, 25,629, 31,744, 43,292, 63,918, or 194,820 mg/m^3) 6 hours/day, 5 days/week, for 2, 5, 10, or 26 weeks. In addition to the rabbits, one Rhesus monkey was exposed to cyclohexane vapor at 1243 ppm (4,730 mg/m^3) by the same schedule for 10 weeks. Evaluations during exposure and up to 2 months after exposure termination included clinical signs, survival, body weight, and hematology (RBC, Hgb, WBC). Gross pathology and histopathology (tissues not specified) were conducted following the 2-month postexposure observation period.

All rabbits exposed at the highest concentration of 91,486 mg/m^3 died during the 1-hour exposure period (Treon et al., 1943). These rabbits exhibited frank effects including severe,

rapid, extensive rhythmic movements of feet, tremors, rapid narcosis, and opisthotonos prior to death. Treon et al. (1943) observed that mortality appeared to be related more to concentration than exposure duration since prolonged exposures to low concentrations (≤11,465 mg/m^3) did not result in similar effects on survival. Additional fatalities were observed among rabbits at ≥25,629 mg/m^3. Rabbits at these concentrations showed a concentration-related increase in the presence and severity of clinical signs ranging from lethargy, light narcosis, increased respiration, and diarrhea among rabbits exposed to 25,629 mg/m^3 for a total of 60 hours to rhythmic movement of feet, tremors, spasmodic jerking, narcosis, temporary paresis of legs, salivation, conjunctival congestion, and labored respiration among rabbits exposed to 63,918 mg/m^3 for a total of 60 hours. No noteworthy clinical signs were observed among rabbits exposed to ≤11,465 mg/m^3. Rabbits exposed to cyclohexane at ≥31,744 mg/m^3 generally showed decreased body weights (ranging from -188 to -311 g/animal). Treon et al. (1943) reported that the corresponding control group behaved similarly, but these data are not shown. Treon et al. (1943) reported that the monkey exposed to 4730 mg/m^3 survived the duration of the study without demonstrating any noteworthy clinical signs of intoxication other than a reduction in body weight (-333 g/animal). No significant hematological changes were observed at any concentration. Treon et al. (1943) does not specifically report the incidence of pathological changes among treated rabbits. However, the study authors did indicate that gross and microscopic tissue changes produced by inhalation of cyclohexane were not specific for the group as a whole or for individuals within it. This study is limited by small group sizes and insufficient detail in reporting of results. For the purposes of this review, a NOAEL of 3330 ppm (11,465 mg/m^3) and a LOAEL of 7444 ppm (25,629 mg/m^3) are identified based on clinical signs of toxicity.

For the fourth subchronic-duration study, (a French study with an English abstract) groups of white rabbits (strain/gender unspecified) were exposed to cyclohexane vapor (purity unspecified) at concentrations ranging from 2.7 to 22 mg/L (2700–22,000 mg/m^3) 8 hours/day, 6 days/week, for 40 days (Fabre et al., 1952). Rabbits in this study were observed for changes in growth, clinical signs, hematology, and pathological changes in the liver, kidneys, spleen, heart, lungs, adrenal glands, intestines, and brain. No significant effects were observed on growth, clinical signs, hematology, or pathology. Additionally, rabbits received daily cutaneous applications of 10 mL of cyclohexane for an unspecified number of days and subcutaneous injection of 2 mL of cyclohexane daily for 20 days. Rabbits observed for hematological changes following these applications did not exhibit any significant changes in RBCs or WBCs, but the study authors reported observing an increase in the percentage of monocytes among treated rabbits and a slight increase in the coagulation time of the blood. Based on the lack of additional details for this study, these data cannot be used to inform toxicity value derivation.

Reproductive/developmental Studies—there are two developmental studies in the literature: one in rats and one in rabbits. In addition, there is a multigenerational reproductive study in rats. All of the reproductive and developmental toxicity studies of cyclohexane that were identified in the literature search were previously reviewed by EPA (2009a, 2003). Unpublished reports of a two-generation reproduction toxicity study in rats and prenatal developmental toxicity studies in rats and rabbits exposed to cyclohexane by inhalation were submitted by industry (Haskell Laboratories, 1997a,b,c) and later summarized and published by Kreckmann et al. (2000). These studies are summarized below.

As part of a two-generation reproduction study in rats, groups of 30 Crl:CD BR rats/sex/concentration were exposed whole-body to cyclohexane (99.9% purity) vapor at 0, 500, 2000, or 7000 ppm (0, 1721, 6886, or 24,101 mg/m^3) 6 hours/day, 5 days/week, for 10 weeks prior to mating, during mating, and during Gestation Days (GDs) 0–20 (Haskell Laboratories, 1997a; Kreckmann et al., 2000). Exposure concentrations were selected based on a range-finding developmental study in rats (Haskell Laboratories, 1997a). Females were not exposed during the period between GD 21 and Lactation Day (LD) 4. Exposure resumed on LD 5 and continued through weaning. Males continued to be exposed 5 days/week, for up to 106 days, from the start of the exposure period. At least 11 weeks after weaning, 30 F1 rats/sex/concentration were bred with their respective treatment groups to produce the F2 litters. F1 animals were exposed to cyclohexane as described above for the parental animals. Rats in both generations were monitored weekly for changes in body weights and clinical observations, including response to an auditory stimulus. F1 and F2 pup body weights and clinical observations were recorded on Postnatal Days (PNDs) 0, 4, 7, 14, 21, and 25. Following litter production, all parental rats and 20 of the F1 and F2 weanlings/sex/concentration were sacrificed and subjected to gross examination. Reproductive organs and pituitary glands from adult rats in the control and high-exposure groups were collected and examined microscopically.

No significant treatment-related effects on the survival of parental rats were observed (Kreckmann et al., 2000; Haskell Laboratories, 1997a). Clinical observations during the exposure period showed a diminished or absent alerting response to a sound stimulus beginning at Days 16 and 15 in animals exposed to 6886 and 24,101 mg/m^3, respectively. The study authors characterized this observed sedation as transient because the effect was no longer apparent shortly after the rats were removed from the exposure chamber. Adult male rats of both generations from the two highest exposure groups and females of both generations from the high-exposure group also demonstrated clinical signs of toxicity possibly related to sedation including increased salivation, stained perioral area, and wet chin. As described above in the subchronic-duration rat study, such clinical signs are considered to be associated with the propensity of treated rats to groom themselves following removal from the exposure chambers. Kreckmann et al. (2000) did not consider these clinical signs to be toxicologically important.

Table 6 summarizes the effects on mean body weight and body-weight gain in adult P1 and F1 rats. (Kreckmann et al., 2000; Haskell Laboratories, 1997a). As shown, there were no significant reductions in mean body weight or mean body-weight gain during the exposure period among P1 males at any concentration. However, for most data points, mean body weight, and mean body-weight gain were significantly reduced throughout the course of the study among F1 males exposed to 24,101 mg/m^3 compared to controls (6%). Females of both generations exposed to 24,101 mg/m^3 demonstrated significant reductions in both mean body weights at the end of the premating periods (6–8%) and overall mean body-weight gains for the premating period (8–13%). At this concentration, mean female body weights were also significantly reduced during gestation and lactation (7–8%) (LD 25 was not significantly reduced). However, no significant reductions in mean body-weight gains during these periods were observed at any concentration, and in fact, cyclohexane-exposed P1 rats gained significantly more weight than controls during the lactation period. Therefore, the reductions in mean gestation and lactation body weights were most likely due to the preexisting body-weight deficits established during the premating period. No significant changes in food consumption or food efficiency were observed among males of either generation. Food consumption was comparable between treated and

control rats during the premating and lactation periods, but food consumption among the 24,101 mg/m^3 P1 females was significantly less than the controls during GDs 0–7 by approximately 14% ($p < 0.05$). Food consumption among these rats through the remainder of gestation was comparable to controls, and no significant effects on overall food efficiency were observed throughout the gestation period. Food efficiency was significantly reduced in females exposed to 24,101 mg/m^3 from both generations during the premating period (10 and 5% in P1 and F1 females, respectively, $p < 0.05$). In general, effects on food efficiency correlated with the observations in body-weight gains. Overall, although significant body-weight reductions were observed among adult P1 females and F1 males and females, the magnitude of the changes compared to controls was generally <10%, and appeared reversible in some cases, as body-weight gains were increased over controls among treated females in later stages of the study.

Table 6. Summary of Changes in Body Weight and Body-Weight Gain in Rats Exposed to Cyclohexane Vapor as Part of a Two-Generation Reproduction Study

Parameter	Test Day	Exposure concentration (mg/m^3)			
		0	1721	6886	24,101
P1 Males					
Mean body weight (g)	1	266.5 ± 18.2[a]	266.5 ± 19.7	266.6 ± 19.5	264.6 ± 17.3
	71[b]	488.3 ± 53.5	489.4 ± 49.8	494.0 ± 48.2	468.0 ± 53.9
	106	519.0 ± 58.5	515.6 ± 55.6	530.8 ± 60.3	509.2 ± 58.7
Mean body-weight gain (g)	1–71	221.8 ± 42.5	222.9 ± 34.0	227.3 ± 42.3	203.4 ± 43.4
	71–106	30.7 ± 15.1	26.2 ± 17.0	38.4 ± 9.6	41.2 ± 20.3
	1–106	252.5 ± 48.6	249.1 ± 40.7	264.1 ± 54.9	244.6 ± 49.2
F1 Males					
Mean body weight (g)	1	69.9 ± 7.9	70.5 ± 6.3	70.0 ± 6.7	63.4 ± 5.5[c]
	78[b]	480.6 ± 47.8	484.9 ± 45.6	487.4 ± 39.9	452.6 ± 45.9[c]
	106	524.1 ± 49.0	524.9 ± 48.6	531.5 ± 41.3	497.5 ± 46.6
	120	547.9 ± 51.4	547.5 ± 52.7	548.0 ± 42.5	513.2 ± 48.2[c]
Mean body-weight gain (g)	1–78	410.8 ± 44.6	414.4 ± 42.2	417.4 ± 37.7	389.1 ± 42.5
	78–120	67.2 ± 16.2	62.9 ± 15.5	59.0 ± 32.1	61.9 ± 10.7
	1–120	478.0 ± 48.7	477.0 ± 48.5	478.2 ± 39.7	449.7 ± 44.5[c]
P1 Females					
Mean body weight (g)	1	190.4 ± 11.3	191.1 ± 12.3	191.8 ± 12.0	186.6 ± 15.4
	71[b]	282.0 ± 26.3	283.5 ± 19.1	280.9 ± 22.3	265.8 ± 21.4[c]
	GD 0	288.4 ± 27.4	288.2 ± 24.3	286.3 ± 23.0	268.6 ± 24.2[c]
	GD 21	418.4 ± 42.7	421.6 ± 25.9	415.8 ± 28.8	391.1 ± 31.2[c]
	LD 0	320.9 ± 34.6	319.1 ± 25.2	320.0 ± 26.2	296.2 ± 26.2[c]
	LD 25	315.3 ± 28.3	321.9 ± 18.7	327.6 ± 26.9	305.9 ± 22.9

Table 6. Summary of Changes in Body Weight and Body-Weight Gain in Rats Exposed to Cyclohexane Vapor as Part of a Two-Generation Reproduction Study

Parameter	Test Day	Exposure concentration (mg/m^3)			
		0	1721	6886	24,101
Mean body-weight gain (g)	1–71	91.5 ± 18.6	92.4 ± 14.7	89.1 ± 15.9	79.2 ± 13.4[c]
	GD 0–21	130.0 ± 23.9	133.4 ± 14.3	129.5 ± 21.6	122.5 ± 13.9
	LD 0–25	-5.6 ± 15.3	2.8 ± 13.8	7.6 ± 19.8[c]	9.6 ± 14.2[c]
F1 Females					
Mean body weight (g)	1	64.8 ± 8.3	64.5 ± 5.3	64.8 ± 7.4	60.1 ± 5.8[c]
	78[b]	290.1 ± 34.1	285.2 ± 24.6	292.4 ± 28.5	267.6 ± 23.4[c]
	GD 0	301.5 ± 28.4	300.5 ± 30.3	310.0 ± 33.3	277.7 ± 27.6[c]
	GD 21	445.2 ± 34.7	439.6 ± 35.7	460.5 ± 42.1	415.6 ± 32.8[c]
	LD 0	339.3 ± 23.1	333.5 ± 29.6	346.9 ± 32.7	309.3 ± 25.2[c]
	LD 25	349.7 ± 16.3	336.9 ± 29.8	349.6 ± 23.8	324.5 ± 23.6[c]
Mean body-weight gain (g)	1–78	225.2 ± 32.0	220.9 ± 21.3	227.6 ± 26.0	207.5 ± 19.1[c]
	GD 1–21	143.7 ± 19.8	139.1 ± 24.4	150.5 ± 21.0	137.9 ± 17.3
	LD 0–25	10.4 ± 16.9	3.4 ± 14.2	2.6 ± 19.7	15.2 ± 13.5

[a]Mean ± standard deviation.
[b]End of premating period.
[c]Significantly different from controls ($p < 0.05$, One-Way ANOVA and Dunnett's test).

GD = gestation day; LD = lactation day.

Source: Haskell Laboratories (1997a); Kreckmann et al. (2000)

There were no significant differences in mating, fertility, or gestation indices, implantation efficiency, or gestation length in either the P1 or the F1 generations (Kreckmann et al., 2000; Haskell Laboratories, 1997a). No significant effects were observed on the mean number of implantation sites or mean number of pups/litter for both F1 and F2 litters. Among F1 litters exposed to 24,101 mg/m^3, the mean percent of pups born alive was significantly lower than controls (see Table 7). However, this observation was not repeated among the F2 litters, and the observed percentage of 98.1% was within the range of historical control data (97.5%; range of 92.5–100%). Mean pup weight was significantly reduced from Postnatal Day 7 through the remainder of the lactation period for both F1 and F2 litters of the high-exposure group (7–15%) (see Table 8). No treatment-related effects on organ weights, gross observations, or microscopic findings were observed. EPA (2003) identified a NOAEL for developmental effects in this reproductive toxicity study of 6886 mg/m^3 (2000 ppm) based on reduced rat pup weights during lactation in the two generations tested. The corresponding LOAEL is 24,101 mg/m^3 (7000 ppm). EPA (2003) identified the diminished alerting response at ≥6886 mg/m^3 as the most sensitive effect in parental rats in this study. Body weights were slightly reduced in parental rats at 24,101 mg/m^3.

Table 7. Mean Pup Numbers and Survival Among F1 and F2 Litters from Rats Exposed to Cyclohexane Vapor During a Two-Generation Reproduction Study

Parameter	Exposure concentration (mg/m^3)			
	0	**1721**	**6886**	**24,101**
F1 Generation				
Number of litters/group	28	27	27	28
Number of implantation sites	13.1	12.5	13.0	13.5
Mean number of pups born/litter	12.7	12.7	12.7	12.4
Mean number of pups born alive/litter	12.7	12.3	12.6	12.2
Sex ratio (males)	0.5	0.55	0.46	0.5
Implantation efficiency (%)	90.2	90.2	94.2	92.1
Gestation Index (%)	100	96.3	100	96.4
Mean % born alive	100	96.3	99	98.1[a]
F2 Generation				
# litters/group	22	25	22	24
# implantation sites	13.6	12.2	14.5	14.3
Mean # of pups born/litter	12.9	13.0	15.0	13.5
Mean # of pups born alive/litter	12.7	12.5	14.7	13.5
Sex ratio (males)	0.54	0.48	0.56	0.51
Implantation efficiency	93.2	96.3	96.1	91.2
Gestation Index	100	96	100	100
Mean % born alive	97.9	95.0	98.7	100

[a]Significantly different from controls ($p < 0.05$, Jonekheere's test).

Source: Kreckmann et al. (2000).

Table 8. Mean Pup Weights Among F1 and F2 Litters from Rats Exposed to Cyclohexane Vapor During a Two-Generation Reproduction Study

Exposure day	Exposure concentration (mg/m^3)			
	0	**1721**	**6886**	**24,101**
F1 Generation				
Number of rats	28	26	27	27
Day 0	6.7 ± 0.6[a]	6.7 ± 0.6	6.7 ± 0.6	6.6 ± 0.5
Day 4 Preculling	11.0 ± 1.6	11.0 ± 1.3	11.2 ± 1.6	10.6 ± 1.1
Day 4 Postculling	11.0 ± 1.6	11.0 ± 1.3	11.3 ± 1.6	10.6 ± 1.1
Day 7	16.2 ± 2.0	16.2 ± 1.8	16.3 ± 2.0	15.1 ± 1.4[b]
Day 14	30.0 ± 3.1	29.9 ± 2.6	29.7 ± 2.9	26.5 ± 2.0[b]
Day 21	48.5 ± 5.0	48.5 ± 3.9	48.3 ± 4.8	43.1 ± 3.9[b]
Day 25	67.5 ± 7.3	67.8 ± 4.6	68.3 ± 5.9	62.2 ± 4.7[b]
F2 Generation				
Number of rats	21–22	21–24	22	24
Day 0	6.4 ± 0.9	6.6 ± 0.5	6.3 ± 0.5	6.3 ± 0.6
Day 4 Preculling	10.8 ± 1.7	10.8 ± 1.3	10.1 ± 1.3	10.2 ± 1.7
Day 4 Postculling	10.9 ± 1.7	10.8 ± 1.4	10.1 ± 1.2	10.1 ± 1.7
Day 7	16.3 ± 2.4	16.0 ± 1.8	15.3 ± 1.8	14.3 ± 2.1[b]
Day 14	31.0 ± 3.2	30.2 ± 3.1	28.9 ± 2.6	26.2 ± 3.4[b]
Day 21	50.0 ± 5.4	48.3 ± 5.5	46.4 ± 5.9	42.8 ± 6.6[b]
Day 25	69.3 ± 6.9	67.1 ± 6.4	65.6 ± 6.9	61.3 ± 7.8[b]

[a]Values reported in grams.
[b]Significantly different from controls ($p < 0.05$, Analysis of Covariance with litter size and sex ratio as covariates).

Sources: Kreckmann et al. (2000); Haskell Laboratories (1997a).

In the developmental study in rats, groups of 25 assumed-pregnant Crl:CD BR rats/concentration were exposed whole-body to cyclohexane (99.9% purity) vapor at concentrations of 0, 500, 2000, or 7000 ppm (0, 1721, 6886, or 24,101 mg/m^3), for 6 hours/day, on GDs 6–15 (Kreckmann et al., 2000; Haskell Laboratories, 1997b). In addition to the standard control group, a pair-fed control group was included; this group received an amount of food equal to the cumulative average amount of food consumed by the high-concentration group on the corresponding gestation day. Maternal body weights and food consumption were recorded daily during the exposure period, and clinical signs were recorded before and after exposure. Dams were sacrificed on GD 21, and the organs of the thoracic and abdominal cavities were examined grossly. The types of implants observed in the uterus were counted, and their relative positions were recorded. Fetuses were weighed, sexed, and examined for external and skeletal abnormalities; one-half of the fetuses were examined for visceral and head abnormalities.

Mean maternal body-weight gain was significantly reduced during GDs 7–17 (GDs 6–16 as reported in Kreckmann et al., 2000) by approximately 11 and 31% for the 6886 and 24,101 mg/m^3 exposure groups, respectively (see Table 9) (Haskell Laboratories, 1997b; Kreckmann et al., 2000). Kreckmann et al. (2000) attributed the reduction in maternal body-weight gain at 6886 mg/m^3 to biological variation because the mean value (57.5 g) fell above the mean value for historical controls (53.5 g, range of 40.7–65.5 g). Adjusted mean maternal body-weight gain (adjusted based on final body weight minus gravid uterine weight) was also significantly reduced among 24,101 mg/m^3 rats compared to controls (25%). Reductions in food consumption in the 24,101-mg/m^3 exposure group (11%) corresponded with observations of body-weight deficiencies in this group. Mean body-weight gain was also significantly reduced in the pair-fed control group. This suggests that the reduction in body-weight gain in the high-exposure groups is most likely a consequence of diminished food consumption. Similar to the reproductive study, a sedative effect was observed among dams at 6886 and 24,101 mg/m^3 and was characterized by transient, diminished-alerting responses. In addition, dams exposed to 24,101 mg/m^3 exhibited a significant increase in the incidence of fur stains and wetness, likely related to salivation and grooming activity following removal from the exposure chambers as described in the other rat studies. As described in the reproductive toxicity study, although these clinical signs were considered by the study authors to be treatment related, they were not considered to be toxicologically relevant.

Table 9. Mean Maternal Body-Weight Gain Among Rats Exposed to Cyclohexane Vapor During GDs 6–15

GDs	Exposure concentration (mg/m^3)				
	0	0 (pair-fed)	1721	6886	24,101
Number of rats evaluated[a]	21	25	22	23	23
1–7	18.7 ± 6.71	22.4 ± 8.23	22.3 ± 8.09	24.6 ± 7.80	22.1 ± 10.19
7–17	64.2 ± 10.64	32.1 ± 8.03[b]	60.1 ± 11.28	57.2 ± 8.68[c]	44.2 ± 9.58[c]
17–22	80.5 ± 10.21	68.8 ± 12.16[b]	72.1 ± 12.42	75.8 ± 16.50	81.4 ± 11.13
7–22[d]	49.6 ± 7.97	12.5 ± 14.88[b]	43.0 ± 11.94[c]	43.0 ± 9.53[c]	37.1 ± 10.01[c]

[a]Data from females that were not pregnant were excluded.
[b]Significantly different from controls ($p < 0.05$, ANOVA, and Dunnett's test).
[c]Significantly different from controls ($p < 0.05$, linear contrast of means from ANOVA).
[d]Weight changes calculated using the final body weight minus weight of the intact uterus.

Source: Haskell Laboratories (1997b).

There were no significant effects of cyclohexane exposure on the pregnancy rates, delivery rates, abortion rates, resorption rates, mean number of live fetuses per litter, or sex ratio (Kreckmann et al., 2000; Haskell Laboratories, 1997b). A significant reduction in the mean number of implantations for female rats in the 24,101 mg/m^3 group was observed. However, implantation occurred prior to cyclohexane exposure, so this effect is attributed to normal biological variation. Necropsy revealed no gross lesions. No significant effects were observed on mean fetal weights or fetal development. The total incidence of fetal malformations was four fetuses from four litters in the 24,101-mg/m^3 group, one fetus in one litter in the 6886-mg/m^3 group, none in the 1721-mg/m^3 group, two fetuses in two litters from pair-fed controls, and none

in the ad libitum-fed control group. EPA (2003) noted that although finding one defective fetus in all four litters is of greater concern than an observation of four such fetuses in one litter, the malformations in the four fetuses from the 24,101 mg/m^3 were of different types, malformations were observed in fetuses from two litters from the pair-fed controls, and no other signs of developmental toxicity were noted by the study authors. EPA (2003) identified a NOAEL of 24,101 mg/m^3 (7000 ppm) for developmental toxicity in rats for this study. EPA (2003) identified NOAEL and LOAEL values of 1721 (500 ppm) and 6886 mg/m^3 (2000 ppm), respectively, for maternal toxicity on the basis of the transient sedative effect.

In a companion developmental study in rabbits, groups of 20 pregnant New Zealand white rabbits/concentration were exposed whole-body to cyclohexane (99.9% purity) vapor at concentrations of 0, 500, 2000, or 7000 ppm (0, 1721, 6886, or 24,101 mg/m^3), for 6 hours/day, on GDs 6–18 (Kreckmann et al., 2000; Haskell Laboratories, 1997c). Rabbits were observed for the same endpoints as described above in the rat developmental toxicity study. No significant effects on survival, mean body weight or mean body-weight gain, or maternal food consumption were observed. No significant differences in the incidences of clinical observations were noted in exposed rabbits as compared to controls. There were no significant effects of cyclohexane exposure on the pregnancy rates, delivery rates, abortion rates, resorption rates, or mean number of live fetuses per litter. A significant reduction in the mean number of corpora lutea was observed among does in the 6886- and 24,101-mg/m^3 groups (8.9 and 8.8, respectively) ($p < 0.05$). However, these values were within the range of historical controls (7.0–10.9), and this effect occurred prior to cyclohexane treatment. Therefore, these differences are attributed to normal biological variation. There was a significant trend in sex ratio (number of males/total number of pups), with the ratios being higher for the 6886- and 24,101-mg/m^3 groups (0.59 and 0.54, respectively, compared with the control ratio of 0.48). However, Kreckmann et al. (2000) did not consider the changes in sex ratio to be related to cyclohexane treatment based on the disparity between the ratios for the 1721-mg/m^3 (0.42) and the 6886-mg/m^3 (0.59) groups, the absence of a true dose response, and the observation that the reported values generally fell within the historical control range (0.40–0.56). Necropsy revealed no gross lesions. No significant effects were observed on mean fetal weights or fetal development. Based on the absence of significant treatment-related effects on rabbits in this study, EPA (2003) identified a NOAEL of 24,101 mg/m^3 (7000 ppm) for both maternal and developmental toxicity in rabbits.

OTHER STUDIES

Neurotoxicity

Concern about neurotoxic effects exists because of the similarity of cyclohexane to *n*-hexane, a widely recognized neurotoxicant. The neurotoxicity of cyclohexane has been evaluated in rats in an acute operant behavior study (Christoph et al., 2000; Haskell Laboratories, 1996c), a 90-day inhalation neurotoxicity study (Malley et al., 2000; Haskell Laboratories, 1996d), a 30-week inhalation neurotoxicity study (Frontali et al., 1981), and a subchronic-duration Russian study (Khanin, 1969). These studies are summarized below.

The acute operant behavior study and the 90-day inhalation neurotoxicity study have been reviewed by EPA (2009a, 2003). In the acute operant behavior study, groups of 10 male Crl:CD:BR rats were exposed whole-body to cyclohexane (99.97% purity) vapor at 0, 500, 2000, or 7000 ppm (0, 1721, 6886, or 24,101 mg/m^3) for 6 hours (Christoph et al., 2000; Haskell Laboratories, 1996c). During the exposure period, all animals exhibited a normal startle

response, and there were no obvious signs of intoxication. These rats had undergone a 6-week training of food presentation through the pressing of a lever during a fixed feeding schedule. At 24,101 mg/m^3, a transient decrease in the mean fixed-ratio rate of responding by approximately 11% was apparent on the treatment day relative to the pretreatment period. Fixed-ratio pause duration showed a slight, nonsignificant increase after exposure, which is difficult to interpret since the mean fixed-ratio pause duration for the two high-dose groups was considerably lower prior to exposure than for the other groups.

In the 90-day inhalation neurotoxicity study, groups of 12 Crl:CD BR rats/sex/group were exposed whole-body to cyclohexane vapor at 0, 500, 2000, or 7000 ppm (0, 1721, 6886, or 24,101 mg/m^3) 6 hours/day, 5 days/week, for approximately 90 days (Malley et al., 2000; Haskell Laboratories, 1996d). Rats were subjected to a functional observation battery (FOB) and motor activity (MA) assessments prior to exposure and during Weeks 4, 8, and 13 on nonexposure days. Rats were monitored weekly for changes in body weights and food consumption, they were monitored daily for clinical signs and for their response to an auditory-alerting stimulus. After at least 65 days of exposure to cyclohexane, six rats/sex/group were sacrificed and grossly examined. Sections of the brain, spinal cord, muscle, sciatic and tibial nerves, gasserian ganglia, dorsal root fibers and ganglia, and ventral root fibers were collected from rats exposed at 0 or 24,101 mg/m^3 and examined histologically. No treatment-related effects were found on food consumption or body weights at any exposure concentration. Rats exposed to 6886 and 24,101 mg/m^3 demonstrated diminished and/or absent responses to an alerting stimulus. All rats from these exposure groups exhibited an increased incidence in stained and/or wet chins. The study authors characterized these clinical signs as transient because they were only observed immediately following removal from the exposure chambers. No treatment-related effects were observed based on the 34 parameters evaluated during the FOB assessment. Similarly, no treatment-related effects were observed on forelimb or hindlimb grip strength, or hindlimb foot splay. Motor activity was significantly decreased from controls at 6886 mg/m^3 in males at Week 13. Motor activity was also decreased compared to controls at 24,101 mg/m^3 among males at Week 13, but the difference was not statistically significant and was increased over the 6886-mg/m^3 group. Microscopic evaluation revealed no morphological differences from control rats. In summary, no statistically significant, treatment-related effects on FOB, MA, or neuropathology measures were found in rats following exposure to cyclohexane vapor up to 24,101 mg/m^3 for 90 days. However, NOAEL and LOAEL values of 1721 mg/m^3 (500 ppm) and 6886 mg/m^3 (2000 ppm), respectively, are identified based on diminished response to a sound stimulus during exposure.

Frontali et al. (1981) exposed groups of 6–9 male Sprague-Dawley rats to cyclohexane (99.5% purity) at 1500 ppm (5160 mg/m^3), 9 hours/day, 5 days/week, for 7, 14, and 30 weeks, and 2500 ppm (8610 mg/m^3), 10 hours/day, 6 days/week, for 30 weeks. Controls were exposed to room air. Rats were monitored weekly for changes in body weight. Neuropathological response was evaluated based on a "hindlimb spread" test and central-peripheral distal axonopathy, for which sections of the tibial nerve supplying the calf muscles were examined histopathologically at termination. Frontali et al. (1981) compared the results of these evaluations to a control group, although no description of this group is provided. No significant effects on body weight or hindlimb spread were observed. No significant neuropathological alterations were observed among cyclohexane-treated rats. Therefore, for the purposes of this

review, the highest concentration tested of 8610 mg/m^3 is considered a NOAEL for neuropathy in rats in this study.

In addition, a Russian study for which an English abstract is available, apparently observed signs of toxicodystrophic encephalopathy of the CNS and inflammation of internal organs in rats (gender/strain not specified) following inhalation exposure to toxic substances including cyclohexane (purity unspecified) at concentrations of 0.06–0.1 mg/m^3 for 70–82 days (Khanin, 1969). It is unclear from the study abstract if cyclohexane was administered by itself or as a mixture with gasoline and other hydrocarbons. Based on the absence of additional information, these data cannot inform toxicity value derivation.

Toxicokinetics

Hissink et al. (2009) describes a PBPK model for cyclohexane and its use in comparing internal doses in rats and volunteers following inhalation exposures. Parameters describing saturable metabolism of cyclohexane were measured in rats and used along with experimentally determined partition coefficients. The model was evaluated by comparing predicted blood and brain concentrations to data from studies in rats and then allometrically scaled the results to humans. Levels of cyclohexane in blood and exhaled air were measured in human volunteers and compared with model values. The model predicted that exposure of volunteers to cyclohexane at levels of 4100 mg/m^3 (approximately 1200 ppm) would result in brain levels similar to those in rats exposed to 8000 mg/m^3 (the no-effect level for acute CNS effects). There were no acute CNS effects in humans exposed to 860 mg/m^3, consistent with model predictions that current occupational exposure levels for cyclohexane protect against acute CNS effects.

Genotoxicity

The results of standard genotoxicity tests have been mixed. Most of the studies regarding the genotoxicity of cyclohexane identified in the literature search have been reviewed by EPA (2003). Genotoxicity data for cyclohexane are summarized below.

In several independent reverse-mutation assays, cyclohexane tested negative for mutagenicity in bacterial tests using *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA97, and TA100 (Mortelmans et al., 1986; Haskell Laboratories, 1982a; Maron et al., 1981; McCann et al., 1975) in both the presence and absence of metabolic activation. Salmeen et al. (1989) observed a weak positive response in *S. typhimurium* strain TA98 in the absence of metabolic activation but without a clear dose-response relationship. The study authors classified Cyclohexane was as only very weakly mutagenic in this assay. Cyclohexane did induce forward mutations in cultured L51578Y mouse lymphoma cells in the presence of metabolic activation (Haskell Laboratories, 1982b). However, a second study did not observe an increase in forward mutations in mouse lymphoma cells either in the presence or absence of metabolic activation (i.e., Litton Bionetics, 1982). Cyclohexane did not induce sister chromatid exchange (SCE) in cultured Chinese hamster ovary (CHO) cells at exposure levels up to that which inhibited cell growth in the presence or absence of metabolic activation (Haskell Laboratories, 1982c). Perocco et al. (1983) did not observe any effects on DNA synthesis in cultured human lymphocytes as measured by [H] thymidine uptake in the presence or absence of metabolic activation. Kubinski et al. (1981) reported equivocal results in *Escherichia coli* in a DNA cell-binding assay. In vivo, no significant increase in chromosome structural aberration

frequency was observed in bone marrow cells of male or female rats exposed by inhalation for 5 consecutive days to levels of cyclohexane up to 1000 ppm (Litton Bionetics, Inc., 1981).

Cyclohexane was found to be a weak inducer of autosomal recessive lethal mutations and sex-linked recessive lethal mutations in *Drosophila melanogaster* (Shetty and Rangaswamy, 1984).

DERIVATION OF SUBCHRONIC AND CHRONIC PROVISIONAL ORAL RFD VALUES FOR CYCLOHEXANE

No data are available on the effects of cyclohexane in humans or animals exposed orally. The absence of data precludes the derivation of provisional RfD values for cyclohexane.

DERIVATION OF SUBCHRONIC AND CHRONIC PROVISIONAL INHALATION RFC VALUES FOR CYCLOHEXANE

SUBCHRONIC P-RFC

As described above, EPA (2003) determined that the available human data, which are limited to occupational exposures to mixed solvents, or are limited by small numbers of subjects are not useful for the derivation of the p-RfC values. Two 90-day studies in rats and mice (Malley et al., 2000; Haskell Laboratories, 1996a,b), an 10-week study in rabbits (Treon et al., 1943), a two-generation reproduction study in rats (Kreckmann et al., 2000; Haskell Laboratories, 1997a), developmental toxicity studies in rats and rabbits (Kreckmann et al., 2000; Haskell Laboratories, 1997b,c), and neurotoxicity studies in rats (Christoph et al., 2000; Malley et al., 2000; Haskell Laboratories, 1996c,d; Frontali et al., 1981) are, however, available for use in deriving a subchronic p-RfC for cyclohexane. These data are summarized in Table 10, which also includes calculation and presentation of human equivalent concentrations (HECs) for the identified NOAELs and LOAELs.

EPA (2009a, 2003) considered all but one of these same studies in deriving the chronic-RfC for cyclohexane. Frontali et al. (1981) was not reviewed as part of the 2003 Toxicological Review (U.S. EPA, 2003), but this study did not observe any significant neuropathological changes in rats exposed to cyclohexane concentrations up to 8610 mg/m^3 for 30 weeks and supports the findings from the acute (Christoph et al., 2000; Haskell Laboratories, 1996c) and 90-day neurotoxicity (Malley et al., 2000; Haskell Laboratories, 1996d) studies that were reviewed by EPA.

After careful consideration of the available inhalation studies and benchmark concentration (BMC) modeling of a number of endpoints from several different studies, EPA (2009a, 2003) selected the $BMCL_{1SDHEC}$ value of 1822 mg/m^3 based on reduced F2 pup weight gain in the two-generation reproduction toxicity study in rats (Kreckmann et al., 2000; Haskell Laboratories, 1997a) as the point of departure (POD) for the subchronic p-RfC. A duration adjustment was first made to adjust for the 6-hour dosing regimen, by multiplying by 6/24. The air:blood partition coefficients (1.39 for rat, 1.41 for human) were not significantly different and

a value of 1 was used in the calculation of a human equivalent concentration (HEC). The HEC values were modeled and a one-standard deviation benchmark concentration of 1822 mg/m^3 was calculated.

Table 10. Summary of Inhalation Noncancer Dose-Response Information for Cyclohexane

Species and study type (*n*/sex/group)	Exposure	NOAEL[a] (mg/m^3)	LOAEL[a] (mg/m^3)	Responses at the LOAEL	Comments	Reference
Rat (10–20/sex/group)	0, 1721, 6886, or 24,101 mg/m^3 6 hours/day, 5 days/week for 90 days.	1721 HEC: 307	6886 HEC: 1230	Transient sedative effect	Increased liver wt and centrilobular hepatic hypertrophy at 24,101 mg/m^3	Malley et al., 2000; Haskell Laboratories, 1996a
Mouse (10–20/sex/group)	0, 1721, 6886, or 24,101 mg/m^3 6 hours/day, 5 days/week for 90 days.	1721 HEC: 307	6886 HEC: 1230	Transient sedative effect	Clinical signs of CNS stimulation and increased liver weight at 24,101 mg/m^3.	Malley et al., 2000; Haskell Laboratories, 1996b
Rabbit (4/group)	0, 1494, 1498, 2706, 11,465, 25,629, 31,744, 43,292, 63,918, or 91,486 mg/m^3 6 hours/day, 5 days/week for up to 10 weeks.	11,465 HEC: 2047	25,629 HEC: 4576	Clinical signs such as lethargy, light narcosis, increased respiration, diarrhea	More severe clinical signs including rhythmic movement of the feet, tremors, spasmodic jerking, narcosis, salivation, temporary paresis of legs, and labored respiration were observed at higher concentrations.	Treon et al., 1943
Rat (30/sex/group)	0, 1721, 6886, or 24,101 mg/m^3 6 hours/day, 5 days/week prior to mating, during mating, and through lactation.	Maternal: 1721 HEC: 307 Developmental: 6886 HEC: 1230	Maternal: 6886 HEC: 1230 Developmental: 24,101 HEC: 4304	Transient sedative effect in dams. Reduced body weight in F1 and F2 pups during lactation	Depressed body-weight gain among P1 females during the premating period at 24,101 mg/m^3. Significant decreases were also observed in mean body weights during gestation and lactation among female rats, but since overall mean body-weight gain was not significantly affected, these changes were most likely due to preexisting body weight deficits established during the premating period.	Kreckmann et al., 2000; Haskell Laboratories, 1997a

Table 10. Summary of Inhalation Noncancer Dose-Response Information for Cyclohexane

Species and study type (*n*/sex/group)	Exposure	NOAEL[a] (mg/m^3)	LOAEL[a] (mg/m^3)	Responses at the LOAEL	Comments	Reference
Rat (25/group)	0, 1721, 6886, or 24,101 mg/m^3 6 hours/day on GDs 6–15.	Maternal: 1721 HEC: 430 Developmental: 24,101 HEC: 6025	Maternal: 6886 HEC: 1722 Developmental: NA	Transient sedative effect in dams	No developmental effects at any concentration.	Kreckmann et al., 2000; Haskell Laboratories, 1997b
Rabbit (20/group)	0, 1721, 6886, or 24,101 mg/m^3 6 hours/day on GDs 6–18.	Maternal: 24,101 HEC: 6025 Developmental: 24,101 HEC: 6025	Maternal: NA Developmental: NA	NA	No maternal or developmental effects at any concentration.	Kreckmann et al., 2000; Haskell Laboratories, 1997c
Rat (12/sex/group)	0, 1721, 6886, or 24,101 mg/m^3 6 hours/day, 5 days/week for 90 days.	1721 HEC: 307	6886 HEC: 1230	Transient sedative effect	No significant effects on FOB, motor activity or neuropathology.	Malley et al., 2000; Haskell Laboratories, 1996c
Rat (6–9 males/group)	0, 5160 9 hours/day, 5 days week or 8610 mg/m^3 10 hours/day, 6 days/week for up to 30 weeks.	8610 HEC: 3,075	NA	NA	No significant neuropathological changes were observed.	Frontali et al., 1981

[a]HEC calculated as follows: $NOAEL_{HEC}$ = NOAEL × exposure hours ÷ 24 hours × exposure days ÷ 7 days × dosimetric adjustment. For nonrespiratory effects (no respiratory effects were reported for cyclohexane), the chemical is treated as a Category 3 gas (per U.S. EPA, 1994b) and the dosimetric adjustment is the ratio of the animal:human blood:gas partition coefficients for cyclohexane (as per U.S. EPA, 1994b). A default value of 1 was used because the ratio of measured animal:human blood:gas partition coefficients for cyclohexane is not statistically different than 1[U.S. EPA, 2009a, 2003]).

While the hepatic endpoint described by Malley et al. (2000) is a potential critical effect, it would be less sensitive than the reproductive endpoint. For this review, Kreckmann et al. (2000) data were selected for use as the POD in deriving the subchronic p-RfC for cyclohexane.[1] EPA (2003) stated the following bout the transient sedative effect noted during exposure:

> *The clinical observation of diminished response to a sound stimulus while in the exposure chamber, noted in many of the studies as the most sensitive endpoint, added information to the qualitative assessment of the toxicity of cyclohexane but does not provide data of the quality necessary for the quantitative estimation of an RfC (U.S.EPA, 1994b). These are subjective observations (the observers know which treatment group they are observing), and are made on a per group basis rather than an individual test animal basis (only a few animals in the exposure chamber are visible when the chamber is hit with the rod to produce an alerting stimulus) (Malley et al., 2000).*

Further detail on the selection of the critical study, the modeling efforts, and the POD is available in Section 5 and Appendix B of the 2003 *Toxicological Review of Cyclohexane* (U.S. EPA, 2003).

For derivation of the subchronic p-RfC, the $BMCL_{1SDHEC}$ of 1822 mg/m^3 for reduced body weight of F2 rat pups calculated by EPA (2003) was divided by an UF of 100 to yield the **subchronic p-RfC** for cyclohexane, as shown here:

$$\begin{aligned}\textbf{Subchronic p-RfC} &= BMCL_{1SDHEC} \div UF \\ &= 1822\ mg/m^3 \div 100 \\ &= \mathbf{18\ mg/m^3}\end{aligned}$$

The composite UF of 100 is composed of the following:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating a susceptible human response are insufficient.
- UF_A: A partial UF of 3 ($10^{0.5}$) is applied for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. Two lines of evidence support reducing this UF: First, converting the rat data to human equivalent concentrations by the dosimetric equations accounts for pharmacokinetic differences between rats and humans; thus, an UF of 3 is applied for interspecies extrapolation.
- UF_D: A partial UF of 3 ($10^{0.5}$) is applied for database inadequacies, primarily reflecting the lack of a developmental neurotoxicity study; the database includes subchronic-duration studies in rats and mice, a two-generation reproduction study in rats, and developmental studies in rats and rabbits.
- UF_L: A factor of 1 is applied for extrapolation from a LOAEL to a NOAEL because BMD modeling was used.

[1] No attempt was made to duplicate the modeling efforts conducted for the 2003 *Toxicological Review of Cyclohexane* (U.S. EPA, 2003).

EPA (2003) describes confidence in the principal study (Kreckmann et al., 2000; Haskell Laboratories, 1997a) as high based on adequate numbers of study animals and exposure levels to evaluate an adequate set of endpoints. The database includes subchronic-duration toxicity studies in rats, mice, and rabbits, a two-generation reproduction study in rats, developmental toxicity studies in rats and rabbits, and neurotoxicity studies in rats. EPA (2003) describes confidence in the database for the purposes of deriving a chronic p-RfC value as low-to-moderate. This assessment is based on the lack of data for long-term or lifetime exposures or for developmental neurotoxicity. However, for the purposes of deriving a subchronic p-RfC, confidence in the database is moderate- reflecting primarily the lack of developmental neurotoxicity testing. Moderate confidence in the subchronic p-RfC value follows.

CHRONIC P-RFC

A chronic p-RfC of 6 mg/m^3 is available on IRIS (U.S. EPA, 2009a) based on the two-generation reproduction study in rats (i.e., Kreckmann et al., 2000; Haskell Laboratories, 1997a). The RfC was calculated from a $BMCL_{1SDHEC}$ of 1822 mg/m^3 for decreased pup body weight and a composite UF of 300 (3 [$10^{0.5}$] for interspecies extrapolation, 10 for intraspecies variability among the human population, and 10 for database deficiencies, in particular, the lack of a chronic-duration inhalation study.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR CYCLOHEXANE

WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is "*Inadequate Information to Assess [the] Carcinogenic Potential"* of Cyclohexane. No information was located on the carcinogenicity of cyclohexane in humans or animals. Genotoxicity data provide little evidence to suggest that cyclohexane is mutagenic. In vitro data suggest that cyclohexane was not mutagenic in bacterial mutation assays with *S. typhimurium* (Haskell Laboratories, 1982a; McCann et al., 1975; Mortelmans et al., 1986, Maron et al., 1981). Cyclohexane tested positive for induction of forward mutations in cultured mouse lymphoma cells in the presence of metabolic activation in one study (Haskell Laboratories, 1982b), but was negative in another study (Litton Bionetics, 1982). Cyclohexane tested negative for induction of SCE in CHO cells (Haskell Laboratories, 1982c) and for effects on DNA synthesis in human lymphocytes (Perocco et al., 1983). Equivocal results were reported for a DNA cell-binding assay in *E. coli* (Kubinski et al., 1981). Results were weakly positive in *Drosophila* (Shetty and Rangaswamy, 1984). No signs of genotoxicity were observed in vivo in rats (Litton Bionetics, Inc., 1981).

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

The paucity of suitable data precludes the derivation of quantitative estimates of cancer risk for cyclohexane.

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