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Provisional Peer-Reviewed Toxicity Values for

Cyclohexanone
(CASRN 108-94-1)

Superfund Health Risk Technical Support Center
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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 CYCLOHEXANONE (CASRN 108-94-1)

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

There is an RfD assessment but no RfC or carcinogenicity assessments for cyclohexanone (chemical structure shown in Figure 1) on IRIS (U.S. EPA, 2009). The RfD of 5 mg/kg-day was derived based on a NOAEL for body-weight depression in rats exposed to cyclohexanone in the drinking water for 2 years (Lijinsky and Kovatch, 1986). There are no entries for cyclohexanone in the HEAST (U.S. EPA, 1997), Drinking Water Standards and Health Advisories list (U.S. EPA, 2006), or Chemical Assessments and Related Activities (CARA) database (U.S. EPA, 1994a, 1991).

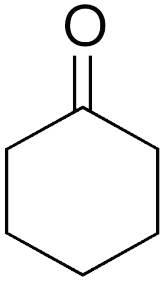

Figure 1. Chemical Structure of Cyclohexanone.

Occupational health guidelines and standards are available for cyclohexanone. The American Conference of Governmental Industrial Hygienists (ACGIH, 2007, 2003) recommends a Threshold Limit Value-time-weighted average (TLV-TWA) of 20 ppm (80 mg/m^3) and TLV-Short-Term Exposure Limit (STEL) of 50 ppm (200 mg/m^3), mainly to minimize the potential for eye, nasal, and throat irritation. The TLV recommendations are accompanied by a skin irritancy notation and an A4 carcinogenicity notation (*Not Classifiable As a Human Carcinogen*). The National Institute for Occupational Safety and Health (NIOSH, 2005) lists a

Recommended Exposure Limit (REL) of 25 ppm (100 mg/m^3) TWA with a skin notation, as well as an Immediately Dangerous to Life or Health (IDLH) concentration of 700 ppm (2814 mg/m^3). The Occupational Safety and Health Administration (OSHA, 2009) has promulgated a Permissible Exposure Limit of 50 ppm (200 mg/m^3) TWA.

There is no ATSDR (2009) Toxicological Profile or World Health Organization (WHO, 2009) Environmental Health Criteria Document for cyclohexanone. CalEPA (2009a,b,c) has not derived chronic oral or inhalation RELs or a cancer potency factor for cyclohexanone. The National Toxicology Program (NTP) tested the oral carcinogenicity of cyclohexanone in rats and mice with negative results (Lijinsky and Kovatch, 1986), and has not included the chemical in its annual Report on Carcinogens (NTP, 2005), which lists known and likely human carcinogens. The International Agency for Research on Cancer (IARC, 1999, 1989) classified the carcinogenicity of cyclohexanone in Group 3 (*Not Classifiable As to Human Carcinogenicity*) based on lack of human cancer data and inadequate evidence of carcinogenicity in animals.

Literature searches were conducted for studies relevant to the derivation of provisional toxicity values for cyclohexanone. Databases searched include MEDLINE, TOXLINE (BIOSIS and NTIS), TOXCENTER, CCRIS, DART/ETIC, DTIC, TSCATS/TSCATS 2, GENETOX, HSDB, RTECS, and Current Contents. The time period covered by most of the searches ranged from the 1960s through early January 2009, although some searches covered earlier years.

REVIEW OF PERTINENT DATA

HUMAN STUDIES

There are no available human data from oral exposure to cyclohexanone. Limited data are available regarding inhalation exposure of humans to cyclohexanone. Nelson et al. (1943) exposed volunteers to cyclohexanone at 25, 50, or 75 ppm (100, 201, or 301 mg/m^3) in an inhalation chamber for 3−5 minutes. No signs of discomfort were reported at 25 ppm (100 mg/m^3), but at 50 ppm (201 mg/m^3), subjects reported throat irritation, and at 75 ppm (301 mg/m^3), subjects exhibited pronounced irritation of the eyes, nose, and throat. In another study, volunteers exposed to cyclohexanone for 7 minutes reported marked eye irritation and slight skin irritation at 160 ppm (642 mg/m^3) (Esso Research and Engineering Co., 1965).

IARC (1989) reviewed a Russian study by Bereznyak (1984) that found no effect on nervous system function, blood, or respiration in a group of 100 production workers exposed to cyclohexanone by both inhalation (3.7 mg/m^3) and skin contact (10^{-4} mg/m^2 on the hands), relative to 49 controls. The primary study was not available for this review, and the IARC (1989) review does not provide any further detail on this study other than to mention that there was some indication of liver disorders among a subgroup of workers, 30−39 years old, with more than 5 years of exposure to cyclohexanone.

A more recent study found that clinical symptoms (i.e., ocular, upper respiratory tract, and cutaneous irritation; mood disorders; irritability; memory difficulties; sleep disturbances; headache; numbness; muscular pains; abdominal pains; and irregular bowel movements) were more frequently reported in a group of 75 furniture factory workers with known exposure to a

wood coating product containing cyclohexanone than in a group of 85 matched controls without known chemical exposures (Mitran et al., 1997). It is unclear what other chemicals these workers may have been exposed to in the wood coating product. Cyclohexanone exposure levels over an 8-hour shift were reported to range from 162 to 368 mg/m^3, and the duration of exposure averaged 14 years. The basis of these air measurements is unclear, as Mitran et al. (1997) do not provide any further information regarding this cohort, methodologies used to collect air measurements in the factory, or conditions at the factory, which may have changed over time due to the implementation of engineering controls or changes in processes. There were no significant differences between the exposed and control groups in urinary excretion of cyclohexanone sulfate metabolites or in serum chemistry tests. Motor nerve conduction velocity testing revealed statistically significant ($p < 0.05$) differences between exposed and control groups—most notably increased distal latency in peripheral nerves (see Table 1). Cyclohexanone-exposed workers also showed significantly delayed reaction times to visual and auditory stimuli (data not shown). Mitran et al. (1997) noted that although these results demonstrated peripheral nerve disturbances following cyclohexanone exposure, the results should not be overinterpreted, given the difficulties interpreting peripheral electrodiagnostic studies.

A possible neurological effect of cyclohexanone is also suggested by a worker with known occupational exposure to a mixture of organic solvents (cyclohexanone, white spirit, and isopropanol) who experienced a long history of temporal epileptic seizures (Jacobsen et al., 1994). This worker also often complained of headache, nausea, and vertigo. The seizures disappeared shortly after exposure ceased, but reappeared following short-term re-exposure to high levels of cyclohexanone. Subsequent to this last seizure, the subject had no further exposure to organic solvents and no further epileptic seizures.

Table 1. Motor Nerve Conduction Velocity Among Furniture Factory Workers Exposed to Cyclohexanone at 162−368 mg/m^3 for an Average of 14 Years[a]

Parameter	Median Nerve	Ulnar Nerve	Peroneal Nerve
Proximal			
Latency (msec)			
Exposed	7.69 ± 0.95	9.30 ± 2.05[b]	12.92 ± 1.59
Controls	6.91 ± 1.20	7.01 ± 1.99	11.64 ± 2.69
Amplitude (mV)			
Exposed	6.01 ± 1.20	6.75 ± 1.97	3.55 ± 1.33[b]
Controls	7.15 ± 2.24	8.01 ± 1.01	5.99 ± 2.01
Duration (msec)			
Exposed	6.20 ± 1.44	7.15 ± 2.40	6.08 ± 2.56
Controls	5.70 ± 1.03	6.28 ± 1.03	5.10 ± 1.08
Distal			
Latency (msec)			
Exposed	4.12 ± 0.90[b]	4.33 ± 1.01[b]	5.96 ± 0.88[c]
Controls	2.90 ± 1.01	3.01 ± 0.93	2.11 ± 1.06
Amplitude (mV)			
Exposed	7.01 ± 2.61	7.99 ± 0.90	2.40 ± 2.03[c]
Controls	9.05 ± 2.33	8.95 ± 1.10	6.55 ± 2.99
Duration (msec)			
Exposed	5.90 ± 0.80	6.02 ± 1.44	6.02 ± 3.02
Controls	5.05 ± 0.99	5.42 ± 1.75	5.86 ± 2.77
Nerve conduction velocity (msec)			
Exposed	52.01 ± 8.03	46.10 ± 0.93[b]	44.03 ± 2.33
Controls	55.12 ± 7.91	55.60 ± 0.99	46.70 ± 1.45

[a]Values presented as mean ± SD.
[b]Significantly different from controls by Student's *t*-test ($p < 0.05$).
[c]Significantly different from controls by Student's *t*-test ($p < 0.01$).

Source: Mitran et al. (1997).

ANIMAL STUDIES

Oral Exposure

Subchronic Studies—In a study by the National Cancer Institute (NCI), groups of five male and five female F344 rats were treated with cyclohexanone (96% purity) in acidified (with HCl to pH 2.5) drinking water at 190, 400, 800, 1600, 3300, 4700, or 6500 ppm for 25 weeks (Lijinsky and Kovatch, 1986; NCI, 1979). Water was acidified to suppress bacterial growth. An additional group of 5 rats (number/sex not specified) served as the untreated control group. Based on EPA (1988) reference values for body weight and water consumption for F344 rats in a subchronic study, using water intake factors of 0.156 and 0.169 L/day for males and females respectively, the estimated daily doses are 0, 30, 62, 124, 249, 513, 731, and 1010 mg/kg-day for males, and 0, 32, 68, 135, 271, 559, 796, and 1100 mg/kg-day for females. Evaluations were limited to survival, body weight, gross pathology, and histopathology (organs and tissues not specified). No mortality occurred during the study. All rats, including controls, displayed signs of moderate chronic respiratory disease, for which the study authors do not provide a reason or

rationale. High-dose rats exhibited a 10% decrease in weight gain compared to controls (data not provided). The only other effect reported was a mild degenerative change in the thyroid gland of two male rats given 4700-ppm cyclohexanone. This pathological change was not observed in female rats or high-dose male rats, indicating that it is not likely related to treatment with cyclohexanone. No other treatment-related effects were reported. Based on decreased weight gain in high-dose rats, the NOAEL values are identified as 731 and 796 mg/kg-day (4700 ppm) for males and females, respectively, and the LOAEL values are identified as 1010 and 1100 mg/kg-day (6500 ppm), respectively.

In a companion mouse study, groups of 10 male and 10 female $B6C3F_1$ mice were administered cyclohexanone (96% purity) also in acidified drinking water at 0, 400, 2300, 6500, 13,000, 25,000, 34,000, or 47,000 ppm for 13 weeks (Lijinsky and Kovatch, 1986; NCI, 1979). Mice were observed for the same endpoints as described above for the rat subchronic study: survival, body weight, gross pathology, and histopathology. Based on EPA (1988) reference values for body weight and water consumption for $B6C3F_1$ mice in a subchronic study, the estimated daily doses are 0, 99, 568, 1600, 3210, 6170, 8390, and 11,600 mg/kg-day for males and 0, 106, 608, 1720, 3430, 6610, 8980, and 12,400 mg/kg-day for females. At the highest dose, 3/10 females and 6/10 males died. One male died at the next highest dose (i.e., 8390 mg/kg-day). Depression in weight gain was observed among females at 8980 mg/kg-day (15%), and among males at 6170 mg/kg-day (19%) and 8390 mg/kg-day (24%). However, specific body-weight data were not provided, and body-weight changes among high-dose animals were not described. At the high-dose, the author reported that some mice showed coagulative liver necrosis, and two female mice showed hyperplasia of the thymus (pathology data not provided). NOAELs of 3210 mg/kg-day (13,000 ppm) and 6610 mg/kg-day (25,000 ppm) and LOAELs of 6170 mg/kg-day (25,000 ppm) and 8980 mg/kg-day (34,000 ppm) are identified for males and females, respectively, based on depression in body-weight gain.

Chronic Studies—Lijinsky and Kovatch (1986) also conducted 2-year drinking water studies in rats and mice that are the principal studies used in the derivation of the EPA IRIS RfD value (U.S. EPA, 2009). In the rat study, groups of 52 male and 52 female F344 rats were treated with cyclohexanone (96% purity) in acidified drinking water at 0, 3300, or 6500 ppm for 2 years. Based on EPA (1988) reference values for body weight and water consumption of F344 rats in a chronic study, using water intake factors of 0.129 and 0.144 L/day for males and females respectively, the estimated doses are 0, 426, and 838 mg/kg-day for males and 0, 476, and 937 mg/kg-day for females. Evaluations included survival, body weight, gross pathology, and histopathology. Survival among high-dose rats of both sexes was reported as >85% at 90 weeks and 70% at study termination (data plotted as probability of survival over time). Survival among low-dose rats and controls of both sexes was >90% at 90 weeks and >70% at termination. Lijinsky and Kovatch (1986) reported that high-dose rats exhibited significant decreases in weight gain compared to controls. Based on the weight curves reported by the authors, high-dose rats of both sexes experienced an estimated body-weight deficit of >30% (in comparison to controls) at study termination. No change in weight gain was noted in the lower dose group. No treatment-related nonneoplastic lesions were observed among either treatment group. Based on the decreases in body-weight gain in male and female rats at the highest dose, the EPA IRIS evaluation of this study identified the NOAEL as 462 mg/kg-day (3300 ppm) and the LOAEL as 910 mg/kg-day (6500 ppm) (U.S. EPA, 2009).

In the companion chronic mouse study, groups of B6C3F$_1$ mice (group size was 41 and 47 for high-dose females and males, respectively, and 50 or 52 for all other groups) were treated with cyclohexanone (96% purity) in acidified drinking water for 2 years at 0, 6500, or 13,000 ppm for males and at 0, 6500, 13,000, or 25,000 ppm for females (Lijinsky and Kovatch, 1986). Based on EPA (1988) reference values for body weight and water consumption for B6C3F$_1$ mice in a chronic study, the estimated daily doses are 0, 1530, and 3070 mg/kg-day for males and 0, 1570, 3130, and 6020 mg/kg-day for females. The same endpoints that were evaluated in the chronic rat study described above were evaluated in mice. Survival among high-dose males was reported as 80% at 90 weeks and 70% at study termination. Among females, however, survival was less than 20% in the high-dose group and only 40% in the mid-dose group at 90 weeks. Survival among low-dose mice of both sexes was comparable to controls, with approximately 90% survival at 90 weeks and >85% survival at termination (data plotted as probability of survival over time). Body weights of high-dose mice of both sexes were decreased by approximately 15−20% compared to controls during most of the study. Body weights were only slightly depressed among mid-dose female mice and were comparable to controls among low-dose mice of both sexes. Lymphoid hyperplasia and lymphocytic infiltrates were common in lymph nodes, spleen, salivary gland, kidneys, pancreas, lungs, and meninges of the brain and spinal cord of most control and treated female mice in this study. Lijinsky and Kovatch (1986) remarked that these changes often involved more than a single organ system, and no cause was found histologically. Chronic effect levels for mice were not described in the EPA evaluation of this study on IRIS (U.S. EPA, 2009). For this review, the lymphatic lesions in control and treated females were considered a potentially confounding observation, and effect levels for females were not defined. For male mice, the low dose of 1530 mg/kg-day (6500 ppm) is identified as a NOAEL, and 3070 mg/kg-day (13,000 ppm) is identified as the LOAEL based on a biologically significant decrease in body-weight gain.

The chronic studies in rats and mice conducted by Lijinsky and Kovatch (1986) summarized above also evaluated the carcinogenic potential of cyclohexanone. Any lesions or tissue masses among control and treated animals at the end of the 2-year study were examined histologically. Table 2 contains the tumor incidence for rats and mice in this study. In rats, there was an increased incidence of adenomas of the adrenal cortex among low-dose males compared to concurrent and historical controls. However, there was no increased incidence in adrenocortical adenomas among high-dose males. Survival of high-dose males in this study was adequate to evaluate carcinogenicity, so this finding suggests that the tumors in the low-dose group were not related to treatment. The only other finding in rats was a marginal increase in the incidence of follicular cell adenoma-carcinomas of the thyroid gland among high-dose males compared to concurrent controls. In mice, the incidence of combined benign and malignant hepatocellular neoplasms was significantly higher among low-dose males compared to concurrent controls, and the incidence of malignant lymphoma was significantly higher among low-dose females compared to controls. However, no significant increases in tumor incidence were observed among high-dose males or among mid- and high-dose females. Survival in high-dose males was adequate to evaluate carcinogenicity, but poor survival in the mid- and high-dose female mice may have compromised the study in female mice. Lijinsky and Kovatch (1986) noted that although the results in low-dose animals are suggestive of a response to cyclohexanone, the absence of a dose-related trend indicates that the evidence for carcinogenic activity is marginal, and the effect, if any, is weak.

Table 2. Neoplasms in Rats and Mice Given Cyclohexanone in Drinking Water for 2 Years

Neoplasm	Dose (mg/kg-d)					
	Male			Female		
Rat	**0**	**426**	**838**	**0**	**476**	**937**
Adrenal cortex: adenoma	1/52	7/52[a,b]	1/51	8/52	4/51	4/52
Thyroid gland: follicular cell adenoma-carcinoma	1/52	0/51	6/51[c]	0/52	1/52	1/52
Mammary gland: fibroadenoma	2/52	1/52	0/52	13/52	10/52	4/52[a]
Uterus: endometrial stromal polyp	–	–	–	5/52	6/52	1/51
Liver:						
Carcinoma	2/52	0/52	0/51	0/52	0/52	0/52
Carcinomas plus neoplastic nodules	6/52	5/52	4/51	3/52	4/52	5/52

Neoplasm	Dose (mg/kg-d)						
	Male			Female			
Mouse	**0**	**1530**	**3070**	**0**	**1570**	**3130**	**6020**
Lung: alveolar-bronchiolar adenoma or carcinoma	13/52	7/51	3/47	3/52	2/50	2/50	1/41
Lymphoma or leukemia	6/52	2/52	4/47	8/52	17/50[b]	4/50	0/41
Liver: adenoma or carcinoma	16/52	25/51[a]	13/46	3/52	6/50	3/50	2/41
Harderian gland: adenoma	0/52	4/52	0/47	0/52	1/50	1/50	0/41

[a]Significantly different from control by incidental and/or life table tests ($p < 0.05$).
[b]Historical control incidence of adrenocortical adenomas in male F344 rats = 1%.
[c]Marginally different from control ($p = 0.053$).

Source: Lijinsky and Kovatch (1986).

Reproductive/developmental Studies—In a developmental study from the French literature, pregnant mice (strains TB and MNRI, unknown group size) were fed a diet containing 1% cyclohexanone throughout pregnancy and lactation (Gondry, 1973). Based on EPA (1988) reference values for body weight and food consumption of $B6C3F_1$ mice (default strain used in absence of values for TB and MNRI strains), using a food intake factor of 0.195 kg food/kg BW/day, the estimated dose is 2000 mg/kg-day. A second group of pregnant mice fed a diet containing no cyclohexanone served as a control group. The researchers evaluated the developmental effects of treatment after the first generation and following continuous treatment across multiple generations. Offspring were evaluated only for mortality and growth. No evaluations of clinical signs, skeletal abnormalities, or other developmental endpoints were conducted. Increased neonatal mortality was observed during the first 21 days of life among offspring from treated dams compared to controls. A depression in growth was observed among first-generation offspring from treated dams. This effect was more pronounced in female offspring than male offspring. Following the discontinuation of treatment after the first generation, growth was comparable to controls by the second generation. However, when cyclohexanone was administered without interruption to multiple successive generations, the inhibiting effect on growth was maintained. No numerical or statistical information is presented for these findings. Based on these findings, a LOAEL of 2000 mg/kg-day is identified based on increased neonatal mortality and depressed offspring growth.

Chernoff and Kavlock (1983) developed a screening system for identifying teratogens and tested this system for a variety of chemicals. In this study, 24 pregnant CD-1 mice were administered cyclohexanone (purity not given) via gavage in corn oil at 800 mg/kg-day on Gestation Days (GDs) 8−12. A second group receiving only corn oil served as a control. Evaluations included maternal body weight, survival and clinical signs, as well as litter size, offspring survival, birth weights, and body weights on Day 3. Evaluations of visceral and skeletal abnormalities and other prenatal developmental endpoints were not conducted. Two of 24 treated mice died during the study, and one control mouse died. The data showed no significant effects on maternal body-weight gain, litter size, offspring survival, or pup body weights. Based on these findings, a NOAEL of 800 mg/kg-day is identified for both maternal reproductive and developmental toxicity.

Gray and Kavlock (1984) followed the animals evaluated by Chernoff and Kavlock (1983, described above) for 250 days to assess postnatal effects of cyclohexanone treatment. Endpoints included body weights at 22 days of age (males and females) and at 57 days of age (males only), behavioral testing for locomotor activity, reproductive function, gross developmental abnormalities, organ weights (males only; liver, testes, seminal vesicles, and right kidney), and gross pathology. There were no significant effects on viability, growth, morphology, locomotor activity, reproductive function, organ weights, or gross pathology in offspring (Gray and Kavlock, 1984; Gray et al., 1986). Based on these findings, a NOAEL of 800 mg/kg-day is identified.

Another teratogenic screening test conducted by Seidenberg et al. (1986) administered cyclohexanone (purity not reported) to a group of 28 time-pregnant ICR/SIM mice via gavage in corn oil at 2200 mg/kg-day on GDs 8−12. A second group receiving only corn oil served as the control. Evaluations included maternal body weight, survival, and clinical signs, as well as litter size and offspring survival, birth weights, and body weights on Day 3. Evaluations of visceral

and skeletal abnormalities and other prenatal developmental endpoints were not conducted. Six of the 28 treated dams died, and mean maternal body weight was significantly reduced compared to controls. There was no effect on litter size or pup survival, but mean neonatal body weights were significantly depressed both at birth and at Day 3 compared to controls. Based on these findings, a LOAEL of 2200 mg/kg-day is identified for both maternal and developmental toxicity.

Inhalation Exposure

Subchronic Studies—Groups of four rabbits (sex not specified) were exposed by inhalation to 0 (untreated), 0 (sham-exposed), 190, 309, 773, 1414, or 3082 ppm (converted by EPA to 0, 763, 1241, 3103, 5677, or 12,373 mg/m^3 using the adjustment calculation of mg/m^3 = ppm × [molecular weight ÷24.45]; the molecular weight of cyclohexanone = 98.15 g/mol) of cyclohexanone (purity not reported) vapor, 6 hours/days, 5 days/week, for 3 weeks (for the highest dose level only, 12,373 mg/m^3) or 10 weeks (all other groups) (Treon et al., 1943). In addition to the rabbits, one Rhesus monkey was exposed to 608 ppm (2441 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 (e.g. erythrocyte and leukocyte counts and hemoglobin concentration). Gross pathology and histopathology (tissues not specified) were conducted following the 2-month postexposure observation period. At the highest concentration, 2/4 rabbits died, and clinical signs such as narcosis, labored breathing, loss of coordination, weight loss, and hypothermia were observed after 3 weeks of exposure. At concentrations ≥773 ppm (3103 mg/m^3), rabbits exhibited salivation, conjunctival congestion and irritation, lacrimation, and lethargy. Rabbits exposed to 309-ppm (1241-mg/m^3) cyclohexanone only exhibited very slight conjunctival congestion, and no clinical signs or effects on body weight were observed among rabbits exposed at the lowest concentration. Incidence of clinical signs was not reported. The monkey exhibited slight salivation and slight conjunctival congestion. No significant hematological changes were observed at any concentration of the 10-week exposure protocol. Two months after the end of exposure, pathology revealed "barely demonstrable" degenerative changes in liver and kidneys (not further described, incidence not reported) of rabbits exposed to 190 ppm (763 mg/m^3). Histological observations in rabbits exposed at higher concentrations or in controls were not described. Extensive injury to the heart, lungs, liver, and kidneys was found in the treated monkey; however, these effects were confounded by a concurrent, chronic broncho-pulmonary infection in this animal. Treon et al. (1943) concluded that the maximum safe concentration of cyclohexanone was "slightly below" 190 ppm (763 mg/m^3) based on the liver and kidney lesions. However, due to the "barely demonstrable" nature of the undescribed lesions, the 2-month separation between examination and the end of exposure, and the lack of evidence of progression of these changes with exposure concentration (no discussion of histopathology at higher exposures was provided), the reported liver and kidney lesions were not used to identify effect levels for this review. Based on these findings, a NOAEL of 309 ppm (1241 mg/m^3) and a LOAEL of 773 ppm (3103 mg/m^3) are identified based on clinical signs of toxicity (i.e., salivation, conjunctival congestion and irritation, lacrimation, and lethargy) following 10 weeks of inhalation exposure to cyclohexanone.

A series of subchronic studies examined the effects of cyclohexanone exposure on olfactory bulb development in young rats. These studies showed that rat pups exposed to cyclohexanone at low concentrations (ranging from 1−4 ppm [or 4−16 mg/m^3]) over about

1−4 months exhibit alterations of mitral cells (Panhuber and Laing, 1987; Laing et al., 1985; Laing and Panhuber, 1980, 1978; Pinching and Doving, 1974) and spine density of granule cell dendrites (Rehn et al., 1988) of the olfactory bulb. The alterations in mitral cells were morphologically similar to transneuronal degeneration and appeared to occur in a chemical-specific pattern. The affected cells are characterized by a darkening of the nucleus and cytoplasm and are smaller than in normal rats. Panhuber et al. (1987) evaluated whether prolonged exposure to cyclohexanone in adult rats would produce similar results to those seen in rat pups. Significant shrinkage of mitral cells of the olfactory bulb was observed in adult rats exposed to 8-ppm (32-mg/m^3) cyclohexanone for 10 weeks. However, significant shrinkage of mitral cells was also observed in adult rats exposed to deodorized air for 10 weeks (although the severity of shrinkage was less than that seen in cyclohexanone-exposed rats). Experiments performed by these researchers suggest that these effects are ultimately reversible and do not alter learning rates or olfactory acuity for cyclohexanone. These studies did observe lowered sensitivity to other similar but novel odorants following prolonged exposure to cyclohexanone. These researchers acknowledge that the interpretation and functional significance of altered mitral cells remain unclear. In addition, although epidemiology data on other chemicals (NRC, 1979) have shown evidence of olfactory sensitivity following prolonged exposure to an odorant, there are no additional data to suggest that altered mitral cells result in decreased olfactory sensitivity in humans.

Reproductive/developmental Studies—In a multigeneration study, groups of 30 male and 30 female CD Sprague-Dawley rats were exposed by inhalation to 0, 250, 500, or 1000 [F0]/1400 [F1] ppm (0, 1004, 2007, or 4015/5621 mg/m^3) cyclohexanone (purity not reported) vapor, 6 hours/day through two consecutive generations (ABC, 1986a). Evaluations included clinical signs, growth, urinalysis (i.e., volume, glucose, pH, protein, ketone, bilirubin, occult blood, and urobilinogen), mating and fertility indices, progeny survival and body weight, pre- and postweaning neurologic performance and neuropathology, and histopathology (reproductive organs, liver, kidneys, brain, and eyes). Additional information on the protocol used, such as age of mating and number of pregnant dams is not reported in the ABC (1986a) study. Lacrimation, ataxia, and irregular breathing were noted in high-dose F0 animals following the first two exposures, but these clinical signs dissipated, and the animals appeared normal thereafter. No significant effects were observed on growth or reproductive performance in F0 animals. Six of 60 high-dose F1 animals died, including three during the first week of increased exposure (from 1000 ppm [4015 mg/m^3] in the parental animals to 1400 ppm [5621 mg/m^3] in the F1 animals). Only one other postweaning death occurred in the entire study, suggesting that the F1 deaths at 1400 ppm (5621 mg/m^3) were probably exposure related. Both F1 males and females exposed to 1400 ppm (5621 mg/m^3) and males exposed to 500 ppm (2007 mg/m^3) exhibited significant decreases in body weights compared to controls during the first week of increased exposure. In addition, males demonstrated significant differences in body weights during 31 of the 34 weeks of 1400-ppm (5621-mg/m^3) exposure. Females only demonstrated additional significant decreases in body weights through the first 3 weeks of exposure to 1400 ppm (5621 mg/m^3). Terminal body weights were comparable to controls among both sexes at all exposure concentrations. F1 animals from the 1400-ppm (5621-mg/m^3) group also exhibited purportedly adverse clinical signs characterized by urine-soaked fur, lacrimation, irregular breathing, ataxia, and lethargy. No significant depressions were observed based on reproductive indices for any treatment group compared with controls—although male fertility indices at 1400 ppm (5621 mg/m^3) using all males paired were approximately 20% less

than controls, and when using only males paired with fertile females, were 24 to 29% less than controls. Table 3 shows that progeny weights among the F1a litter were significantly different from controls during the lactation period. However, the dose-response pattern is not clearly defined across all time points during the lactation period, and effects seen at the high-dose appear to resolve by Lactation Day 28.

Table 3 summarizes significant findings observed in the F2a and F2b litters. The total numbers of viable progeny born to F1 animals were not significantly different among exposure groups compared to controls, but during the lactation period, there were significant decreases in the mean numbers of viable progeny from 1400-ppm (5621-mg/m^3) F1 animals compared to controls in both the F2a and F2b generations (ABC, 1986a). Progeny survival was significantly decreased at 1400 ppm (5621 mg/m^3) during the first 4 days of the lactation period in both the F2a and F2b generations. Mean litter weights were significantly reduced for most of the lactation period at 1400 ppm for both F2a and F2b litters. In the F2a litters, pup body weights were also significantly reduced at 250 and/or 500 ppm (1004 and/or 2007 mg/m^3) during the latter half of lactation. However, this was not seen with the F2b litters. The researchers did not consider maternal exposure to 250- or 500-ppm (1004- and/or 2007-mg/m^3) cyclohexanone to adversely affect pup body weights because statistical weight differences noted for the 250- and 500-ppm (1004- and/or 2007-mg/m^3) progeny were 'minimal' (5−17%) compared to controls, and similar effects were not seen for the F2B progeny. Furthermore, effects on fetal weight in developmental toxicity studies (described below) were observed only at 1400 ppm (5621 mg/m^3) and not at lower concentrations. For the purposes of this review, a LOAEL of 1400 ppm (5621 mg/m^3) and NOAEL of 500 ppm (2007 mg/m^3) are identified for effects in F1 animals including mortality, clinical signs, decreased body weights, and effects on reproduction (reduced viability and body weight of F2 pups).

A subsequent study conducted during the postexposure recovery period evaluated the reversibility of reproductive effects in the F1 male CD Sprague-Dawley rats treated with 1400-ppm (5621-mg/m^3) cyclohexanone (ABC, 1986b). Males were rested (unexposed) following the last exposure for 2 days prior to the start of mating trials that occurred for 4 consecutive weeks (Weeks 1−4), Week 6, and Week 8. ABC (1986b) determined that the decrease in second-generation male fertility in the 1400-ppm group was reversible (ABC, 1986b).

Table 3. Summary of Progeny Observations from F1 Generation Rats Exposed to Cyclohexanone Vapor								
Exposure Concentration (ppm)	**Lactation Day**							
	0 (birth)	**1**	**4**	**7**	**14**	**21**	**28**	
F1A Litter								
Mean progeny body weights (g)							Male	Female
0	5.4 ± 0.8	–	7.8 ± 1.5	10.8 ± 2.7	19 ± 4.5	31.6 ± 7.3	61.6 ± 13.1	55.7 ± 11.3
250	5.4 ± 0.7	–	7.9 ± 1.3	10.3 ± 2.1	17.8 ± 3.1[a]	28.2 ± 5.4[b]	55.0 ± 9.8[b]	52.7 ± 9.8
500	5.4 ± 0.8	–	7.6 ± 1.6	9.7 ± 2.5[b]	16.0 ± 4.7[b]	26.9 ± 7.0[b]	50.9 ± 14.0[b,c]	48.8 ± 13.9[b]
1000	5.5 ± 1.0	–	8.3 ± 1.6[b]	10.8 ± 2.3	17.6 ± 4.0[a]	28.9 ± 5.9[b]	56.5 ± 11.7	53.8 ± 10.2
F2A Litter								
Mean number of viable progeny from F1 generation rats								
0	11.8 ± 2.83	11.7 ± 2.83	11.5 ± 2.74	7.6 ± 0.99	7.2 ± 1.96	7.2 ± 1.95	7.2 ± 1.95	
250	12.0 ± 3.48	11.8 ± 3.47	11.6 ± 3.47	7.4 ± 1.27	6.9 ± 2.05	6.6 ± 2.52	6.6 ± 2.52	
500	11.8 ± 2.89	11.2 ± 3.35	10.9 ± 3.69	7.3 ± 1.81	7.3 ± 1.81	7.3 ± 1.80	7.3 ± 1.80	
1400	9.1 ± 4.76	7.1 ± 5.83[b]	6.8 ± 5.74[b]	4.7 ± 3.65[b]	3.9 ± 3.82[b]	3.9 ± 3.82[b]	3.5 ± 3.89[b]	
Percent progeny survival (at birth: relative to total delivered; on Days 1 and 4: relative to number born alive; on Days 7−28: relative to number retained on Day 4)								
0	96.7	99.6	97.4	98.7	93.5	92.9	92.9	
250	99.3	98.9	97.1	98.8	91.3	87.9	87.9	
500	95.2	95.4	92.7	99.4	99.4	98.8	98.8	
1400	85.6[b]	77.9[b]	75.3[b]	96.4	79.5	79.5	72.3	
Mean progeny body weights (g)							Male	Female
0	5.9 ± 0.9	–	7.9 ± 1.5	11.7 ± 2.4	19.7 ± 3.7	32.2 ± 6.2	62.7 ± 10.4	56.0 ± 11.4
250	5.6 ± 0.8[b]	–	8.2 ± 1.8	11.4 ± 2.4	17.8 ± 3.9[b]	28.4 ± 6.0[b,d]	53.1 ± 13.1[b]	51.0 ± 12.2
500	5.5 ± 0.8[b]	–	8.1 ± 1.6	11.3 ± 2.1	17.9 ± 3.5[b]	27.2 ± 5.7[b,d]	52.2 ± 12.8[b,d]	49.5 ± 10.7[b]
1400	5.3 ± 0.8[b,d]	–	7.8 ± 1.1	9.1 ± 2.0[b,d]	14.3 ± 2.0[b,d]	20.8 ± 4.6[b,d]	38.8 ± 9.3[b,d]	37.5 ± 9.9[b,d]

Table 3. Summary of Progeny Observations from F1 Generation Rats Exposed to Cyclohexanone Vapor

Exposure Concentration (ppm)	Lactation Day							
	0 (birth)	**1**	**4**	**7**	**14**	**21**	**28**	
F2B Litter								
Mean number of viable progeny from F1 generation rats								
0	12.3 ± 3.33	12.3 ± 3.33	12.2 ± 3.26	7.6 ± 1.23	7.6 ± 1.23	7.6 ± 1.23	7.5 ± 1.28	
250	12.8 ± 2.67	12.1 ± 3.95	12.0 ± 3.92	7.4 ± 1.95	7.0 ± 2.55	7.0 ± 2.55	7.0 ± 2.55	
500	10.9 ± 3.76	10.8 ± 3.71	10.4 ± 4.03	6.9 ± 2.47	6.8 ± 2.73	6.8 ± 2.73	6.8 ± 2.73	
1400	9.3 ± 5.58	7.0 ± 6.34[a]	6.4 ± 6.66[b]	3.9 ± 3.75[b]	3.8 ± 3.87[b]	3.8 ± 3.87[b]	3.8 ± 3.87[b]	
Percent progeny survival (at birth: relative to total delivered; on Days 1 and 4: relative to number born alive; on Days 7−28: relative to number retained on Day 4)								
0	98.1	100.0	99.0	99.2	99.2	99.2	98.5	
250	99.6	98.5	94.1	99.3	94.6	94.6	94.6	
500	99.5	95.0	94.9	96.9	95.3	95.3	95.3	
1400	97.4	75.2[b]	69.1[b]	96.9	92.3	92.3	92.3	
Mean progeny body weights (g)							Male	Female
0	5.9 ± 0.8	–	8.9 ± 1.4	13.1 ± 1.5	22.5 ± 3.7	35.5 ± 6.9	67.8 ± 11.0	64.6 ± 9.3
250	6.2 ± 0.9[a]	–	9.3 ± 1.3[a]	12.9 ± 2.2	23.3 ± 2.8	36.8 ± 5.2	69.2 ± 9.0	66.2 ± 7.9
500	6.2 ± 1.0[a]	–	9.5 ± 1.5[b]	13.5 ± 1.8	22.0 ± 3.4	34.6 ± 6.5	69.2 ± 11.3	62.1 ± 10.9
1400	5.8 ± 1.2	–	8.3 ± 1.7[a]	10.8 ± 2.3[b,c]	17.3 ± 4.0[b,c]	26.0 ± 6.3[b,c]	51.9 ± 15.7[b]	47.9 ± 11.5[b,c]

[a]Significantly different from controls ($p < 0.05$); progeny body-weight data based on individual weights analyzed by analysis of variance (ANOVA) and Scheffe's multiple comparison.
[b]Significantly different from controls ($p < 0.01$); progeny body-weight data based on individual weights analyzed by ANOVA and Scheffe's multiple comparison.
[c]Significantly different from controls based on mean litter weight data analyzed by ANOVA and Dunnett's *t*-test ($p < 0.01$).

Source: ABC (1986a).

Groups of 10 pregnant Sprague-Dawley rats were exposed by inhalation to 0, 100, 250, or 500 ppm (0, 402, 1004, or 2007 mg/m^3) of cyclohexanone (99.8% purity) vapor, 7 hours/day on GDs 5−20 (Samimi et al., 1989). Concurrent control groups of five pregnant rats exposed to room air (negative controls) and five pregnant rats exposed to 2-ethoxyethanol (positive control) were run with each exposure concentration. Dams were sacrificed on GD 21 and evaluated for body weight, gravid uterus weights, mean number of corpora lutea per litter, mean number and placement of early and late resorption sites per litter, mean percentage of resorption sites, and placement of live and dead fetuses. Fetuses were examined for body weight and sex ratio as well as for evidence of external, visceral, and skeletal malformations, and skeletal variations. No mortality was reported. Dams exhibited only a slight reduction in body-weight gain compared to controls. Gross examination revealed a grey mottling of the lungs in several dams exposed to 250 or 500 ppm (1004 or 2007 mg/m^3) cyclohexanone (data not provided). The interpretation of this finding is unclear because incidence was not provided, histology was not conducted, and no other respiratory endpoints were evaluated. The data showed no treatment-related effects on the number of corpora lutea per dam, number of implants, resorption percentage, fetal weight, viability, or sex ratio. Table 4 shows developmental malformations and variations. Three rats treated with ≥250 ppm (1004 mg/m^3) exhibited visceral malformations in the form of a right subclavian artery arising off of the aortic arch or absence of the innominate artery. Few external or skeletal malformations or variations were observed among treated rats. Slight skeletal developmental variations were noted in fetuses from rats treated with ≥250 ppm (1004 mg/m^3) characterized as rudimentary 14th ribs and incompletely ossified sternebrae numbers 5 and 6. None of these findings were significantly different from controls. In the absence of other conventional signs of embryotoxicity, Samimi et al. (1989) concluded that inhalation exposure to up to 500-ppm (2007-mg/m^3) cyclohexanone was not developmentally toxic in rats. In the absence of significant systemic or developmental effects observed in rats in this study, a NOAEL of 500 ppm (2007 mg/m^3) is identified for maternal and developmental toxicity.

Table 4. Mean Percentages of Fetuses with External, Visceral, and Skeletal Malformations and Skeletal Variations per Litter in Cyclohexanone-Exposed Rats and Negative Controls[a]

Exposure Concentration (ppm)	External Malformations		Visceral Malformations		Skeletal Malformations		Skeletal Variations	
	NC	CH	NC	CH	NC	CH	NC	CH
100	1.5 ± 3.4	0	0	0	0	0	13.7 ± 6.4	24.2 ± 23.4
250	0	0.8 ± 2.2	0	5.0 ± 14.1	0	1.6 ± 4.4	27.3 ± 20.4	37.3 ± 20.5
500	0	0	0	2.9 ± 6.4	0	1.0 ± 2.7	26.3 ± 27.4	23.5 ± 22.8

[a]Values reported as percentage ± standard deviation.

NC = negative control (exposed to room air); CH = cyclohexanone exposed.

Source: Samimi et al. (1989).

Biodynamics (1984a), (range-finding data in Biodynamics 1983), exposed groups of 26 pregnant CD rats by inhalation to cyclohexanone (99.9% purity) vapor at mean measured concentrations of 0 (sham-exposed), 303, 657, or 1410 ppm (0, 1216, 2638, or 5661 mg/m^3), 6 hours/day, on GDs 6−19. Dams were evaluated for survival, body weight, clinical signs, behavioral response, uterus weights, number of corpora lutea, and gross pathology. Developmental endpoints included fetus viability, resorptions, implantations, fetal sex ratio, and external, visceral, and skeletal malformations and skeletal variations. No mortality was observed among treated dams, and no significant effects were observed on the number of corpora lutea or pregnancy rate. Effects noted in dams were limited to the high-dose group and included adverse clinical signs (lacrimation, lethargy, and nasal and vaginal discharge), abolition of a startle response, and a significant decrease in body-weight gain during gestation (see Table 5). Developmental effects were also limited to fetuses from high-dose dams and included decreased fetal body weight and increased incidence of fetal skeletal alterations, including incompletely ossified cranial bones, hyoid, sternebrae, metatarsals, and phalanges (see Table 5). The increased incidence in skeletal variations was only significantly different from controls on a per fetus-basis, not on a litter-basis, because 100% of the control and treated litters exhibited some form of a skeletal variation. Based on clinical signs, abolition of startle response and decreased body-weight gain, a NOAEL of 657 ppm (2638 mg/m^3) and a LOAEL of 1410 ppm (5661 mg/m^3) are identified for maternal toxicity. Based on decreased fetal body weights and increased skeletal variations at the high-dose, a NOAEL of 657 ppm (2638 mg/m^3) and a LOAEL of 1410 ppm (5661 mg/m^3) are also identified for developmental toxicity.

Table 5. Maternal and Developmental Observations in Rats Exposed to Cyclohexanone Vapor During Gestation

Endpoint	Exposure Concentration (ppm)			
	0	**303**	**657**	**1410**
Mean gestational body weights (g)				
GD 0	273 ± 20	274 ± 16	273 ± 15	274 ± 16
GD 6	307 ± 20	310 ± 20	309 ± 19	310 ± 16
GD 15	350 ± 20	357 ± 23	346 ± 21	333 ± 19[a]
GD 20	420 ± 24	432 ± 33	418 ± 29	383 ± 29[b]
Mean corrected body weight gain (g)[c]	32.8 ± 12.3	35.5 ± 13.8	29.3 ± 13.7	13.2 ± 10.7[b]
Mean body weight of viable fetuses (g)	3.67 ± 0.28	3.66 ± 0.24	3.68 ± 0.25	2.73 ± 0.43[b]
Males	3.77 ± 0.28	3.8 ± 0.26	3.76 ± 0.27	2.83 ± 0.44[b]
Females	3.56 ± 0.27	3.53 ± 0.23	3.58 ± 0.22	2.64 ± 0.45[b]
Incidence of fetal skeletal variations[d]				
Number of fetuses affected	133 (84.2%)	150 (89.3%)	138 (87.3%)	142 (97.9%)[a]
Number of litters affected	24 (100%)	23 (100%)	24 (100%)	23 (100%)

[a]Significantly different from controls ($p < 0.05$), test not specified.
[b]Significantly different from controls ($p < 0.01$), test not specified.
[c]Corrected body weight = actual body weight − gravid uterine weight.
[d]Values are presented as number (percent).

Source: Biodynamics (1984a).

In a companion mouse study, groups of 30 pregnant CD-1 mice were exposed to cyclohexanone (99.9% purity) vapor at mean measured concentrations of 0 or 1380 ppm (0 or 5541 mg/m^3), 6 hours/day, on GDs 6−17 (Biodynamics, 1984b). Evaluations were similar to those described above for the rat study. Maternal effects included adverse clinical signs (shallow breathing, lacrimation, lethargy), delayed response to stimulus, and reductions in gestational body-weight gain and uterine weights (see Table 6). Based on corrected Day 18-body weights (actual Day 18-body weight corrected by subtracting the weight of the gravid uterus), mean weight gain during the exposure period was significantly higher than controls. Developmental effects included an increase in resorptions, decreased fetal viability, decreased fetal body weight, skeletal variations (retardation in ossification of the cranial bones, cervical vertebral centra, and phalanges), and an increased incidence of visceral malformations (cleft palate and distended renal pelvis) (see Table 6). Both cleft palate and distended renal pelvis have been noted at low incidence in this strain of mouse based on historical controls. Biodynamics (1984b) concluded that cyclohexanone was maternally toxic, fetotoxic, and embryotoxic at 1380 ppm (5541 mg/m^3)—but not teratogenic. Based on clinical signs in dams, decreased maternal body weights and fetal effects including decreased weights and retarded ossification, a LOAEL of 1380 ppm (5541 mg/m^3) is identified for both maternal and developmental effects.

OTHER STUDIES

Genotoxicity

In two independent reverse mutation assays, cyclohexanone tested negative for mutagenicity in bacterial tests using *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 (Haworth et al., 1983; Florin et al., 1980) in the presence and absence of metabolic activation. However, Massoud et al. (1980) observed that cyclohexanone-induced genetic mutations in *Bacillus subtilis* and produced a "large number of revertants" in *S. typhimurium* strain TA98. This study is only available as an abstract and detailed information on the study conditions or concentrations tested are not provided. There is no further elaboration on the results for other *S. typhimurium* strains. A Danish review of this study indicates that the results for *S. typhimurium* were ambiguous, as reversions were observed in controls, and there was no evidence of dose dependency (Miljostyrelsen Institute, 2003). Cyclohexanone-induced DNA damage in *Escherichia coli* in vitro (Rosenkranz and Leifer, 1980).

Cyclohexanone tested negative in a mouse lymphoma cell forward mutation assay in the presence and absence of an exogenous metabolic system (McGregor et al., 1988). In Chinese hamster ovary cells (CHO), cyclohexanone-induced gene mutations at the HGRPT locus and sister chromatid exchanges in the absence of S9 metabolic activation but not in the presence of metabolic activation (Aaron et al., 1985; DuPont, 1984). Under these same test conditions, cyclohexanone did not induce chromosomal aberrations in CHO cells with or without metabolic activation. However, chromosomal aberrations were induced by cyclohexanone in cultured human leukocytes (Collin, 1971; Lederer et al., 1971), and an increased frequency in chromosomal damage characterized by ploidy and structural changes was observed in human lymphocytes (Dyshlovoi et al., 1981, as cited in IARC, 1989).

In vivo, de Hondt et al. (1983) observed an increase in the incidence of chromosomal abnormalities in bone marrow cells of male rats characterized as chromatid gaps, breaks, centric fusions, centromeric attenuation, chromatid exchanges, and polyploidy.

Table 6. Maternal and Developmental Observations in Mice Exposed to Cyclohexanone Vapor During Gestation

Endpoint	Exposure Concentration (ppm)	
	0	**1380**
Mean gestational body weights (g)		
GD 0	25 ± 1	25 ± 2
GD 6	28 ± 2	28 ± 2
GD 12	35 ± 2	34 ± 2
GD 18	48 ± 3	41 ± 6[a]
Mean corrected body weight gain (g)[b]	2.9 ± 2.0	5.1 ± 2.7[a]
Number of resorptions	24	184
Mean ± SD	0.9 ± 1.1	6.3 ± 4.9[c]
Mean % ± SD	7.4 ± 10.1	51.4 ± 38.6
Number of litters with resorptions (%)[c]	15 (53.6%)	26 (89.7%)[a]
Number of viable fetuses	301	170
Mean litter size ± SD	10.8 ± 1.8	5.9 ± 4.7[a]
Mean body weight of viable fetuses (g)	1.31 ± 0.07	1.09 ± 0.08[a]
Males	1.33 ± 0.09	1.09 ± 0.08[a]
Females	1.30 ± 0.08	1.10 ± 0.06[c]
Incidence of fetal visceral malformations[c]		
Number of fetuses affected	0	4 (4.4%)[d]
Number of litters affected	0	4 (19.0%)[d]
Incidence of fetal skeletal variations[c]		
Number of fetuses affected	102 (70.8%)	79 (100%)[a]
Number of litters affected	26 (92.9%)	20 (100%)

[a]Significantly different from controls ($p < 0.01$), test not specified.
[b]Corrected body weight = actual body weight − gravid uterine weight.
[c]Values are presented as number (percent).
[d]Significantly different from controls ($p < 0.05$), test not specified.

Source: Biodynamics (1984b).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR CYCLOHEXANONE

Oral studies of cyclohexanone include subchronic and chronic drinking water studies in rats and mice (Lijinsky and Kovatch, 1986; NCI, 1979), and screening level developmental studies in mice (Gray et al., 1986; Seidenberg et al., 1986; Gray and Kavlock, 1984; Chernoff and Kavlock, 1983; Gondry, 1973). Table 7 summarizes these data. Both the subchronic and chronic studies indicate that cyclohexanone exposure results in decreased body weights in both rats and mice. The developmental screening studies also observe decreased gestational body weights and reduced fetal body weights among mice treated orally with cyclohexanone.

SUBCHRONIC p-RfD

The principal study of Lijinsky and Kavatch (1986) is based on an NCI cancer bioassay oral study with adequate toxicologic endpoints using both mice and rats. It also provided the lowest LOAEL among the subchronic and developmental toxicity studies; the LOAEL of 1010 mg/kg-day for decreased body-weight gain in male rats treated in the drinking water for 25 weeks (Lijinsky and Kavatch, 1986). The rat, rather than the mouse, is chosen as the species to define the critical effect from the study because the rats were exposed for 25 weeks compared to only 13 weeks for the mice, and descriptive details are lacking from the study author's presentation of the mice data. The corresponding NOAEL of 731 mg/kg-day was chosen as the point of departure (POD) for deriving the subchronic p-RfD (Lijinsky and Kovatch, 1986; NCI, 1979). Benchmark dose (BMD) modeling cannot be conducted because the body weight data were not provided in the principal study.

A **subchronic p-RfD** was derived for cyclohexanone by dividing the NOAEL of 731 mg/kg-day for decreased body-weight gain in male rats by a UF of 300, as shown below:

Subchronic p-RfD = NOAEL ÷ UF
= 731 mg/kg-day ÷ 300
= **2 mg/kg-day**

The composite UF of 300 is composed of the following UFs:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating susceptible human response are insufficient.
- UF_A: A factor of 10 is applied for animal-to-human extrapolation because data for evaluating relative interspecies sensitivity are insufficient.
- UF_D: The database for oral exposure to cyclohexanone consists of limited subchronic toxicity studies in two species and several screening level developmental toxicity studies in mice. A factor of 3 ($10^{0.5}$) is applied for database inadequacies because data for evaluating reproductive toxicity are inadequate and limited.
- UF_L: A NOAEL is identified from the database and used as the POD; therefore, a factor of 1 is used.

Table 7. Summary of Oral Noncancer Dose-Response Information for Cyclohexanone

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/kg-day)[a]	LOAEL (mg/kg-day)[a]	Responses at the LOAEL	Comments	Reference
Rat (5/sex/group)	Continuously in the drinking water for 25 weeks at 0, 30, 62, 124, 249, 513, 731, or 1010 mg/kg-day in males and 0, 32, 68, 135, 271, 559, 796, or 1100 mg/kg-day in females.	731 (males) 796 (females)	1010 (males) 1100 (females)	Decreased weight gain.	Weight-gain data were not provided other than to state that the magnitude of difference from controls was 10%. Moderate chronic respiratory infection among control and treated animals.	Lijinsky and Kovatch, 1986
Mouse (10/sex/group)	Continuously in the drinking water for 13 weeks at 0, 99, 568, 1600, 3210, 6170, 8390, or 11,600 mg/kg-day in males and 0, 106, 608, 1720, 3430, 6610, 8980, or 12,400 mg/kg-day in females.	3210 (males) 6610 (females)	6170 (males) 8980 (females)	Decreased body weights.	Weight-gain data were not provided other than the percentage differences from controls in selected groups. Body-weight changes among high-dose animals were not described.	Lijinsky and Kovatch, 1986
Rat (52/sex/group)	Continuously in the drinking water for 2 years at 0, 426, or 838 mg/kg-day for males, 0, 476, or 937 for females, and 0, 462, or 910 mg/kg-day for both sexes.	462	910	Depression in body-weight gain.	Principal study used as the basis of the EPA IRIS RfD value. No significant dose-related trend in tumor incidence was observed.	Lijinsky and Kovatch, 1986
Mouse (41−52/sex/group)	Continuously in the drinking water for 2 years at 0, 1530, or 3070 mg/kg-day for males and at 0, 1570, 3130, or 6020 mg/kg-day for females.	1530 (males) NA (females)	3070 (males) NA (females)	Decreased body weights.	NOAEL/LOAEL was not derived for females due to confounding lymphatic lesions in control and treated mice. No significant dose-related trend in tumor incidence was observed.	Lijinsky and Kovatch, 1986

Table 7. Summary of Oral Noncancer Dose-Response Information for Cyclohexanone

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/kg-day)[a]	LOAEL (mg/kg-day)[a]	Responses at the LOAEL	Comments	Reference
Mouse (unknown group size)	Diet containing 2000 mg/kg-day fed continuously throughout pregnancy and lactation for multiple generations.	NA	2000	Neonatal mortality within the first 21 days of life and depressed offspring growth.	Study report written in French with English abstract.	Gondry, 1973
Mouse (24/group)	Gavage treatment at 0 or 800 mg/kg-day on GDs 8−12.	800	NA	No significant effects on dams or offspring.		Chernoff and Kavlock, 1983
Mouse (10/group)	Gavage treatment at 0 or 800 mg/kg-day on GDs 8−12.	800	NA	No significant effects on dams or offspring.		Gray et al., 1986; Gray and Kavlock, 1984
Mouse (28/group)	Gavage treatment at 0 or 2200 mg/kg-day on GDs 8−12.	NA	2200	Decreased gestational body weights in dams; decreased neonatal body weights.		Seidenberg et al., 1986

[a]NOAEL and LOAEL are based on continuous exposure in these studies.

Confidence in the principal study (Lijinsky and Kovatch, 1986) is medium. The subchronic study included multiple dose levels but only five rats per sex at each level. The evaluations are limited to survival, body weight, and gross and microscopic examinations. The results are reported briefly with few details, and no data were shown. All rats, including controls, displayed signs of moderate chronic respiratory disease. However, the corresponding chronic study (Lijinsky and Kovatch, 1986) demonstrated similar results and improved evaluations with 52 rats per sex per dose level. Confidence in the database is medium. In addition to the principal subchronic rat study, there is a subchronic mouse study reported by the same researchers that provides supporting evidence for an effect on body weight but had some of the same shortcomings. In addition, the database includes screening-level developmental studies in mice, which were consistent in finding effects on offspring body weight at high doses and no effects at doses near the POD. Reproductive toxicity has not been adequately studied by oral exposure; Gondry (1973) only evaluated mortality and growth in the offspring and provided no statistical or numerical information. The inhalation database suggests that developmental effects may be an endpoint of concern for cyclohexanone. Overall confidence in the subchronic p-RfD is medium.

The subchronic p-RfD derived herein is lower than the chronic RfD for cyclohexanone on IRIS. This is because the derivation of the subchronic p-RfD includes application of a database UF, which was not EPA practice at the time the chronic IRIS RfD was developed (posted 09/02/1986).

CHRONIC p-RfD

A chronic RfD of 5 mg/kg-day based on the 2-year drinking water study in rats (Lijinsky and Kovatch, 1986) was derived by EPA in 1986 and is available on IRIS (U.S. EPA, 2009). The RfD was calculated from a NOAEL of 462 mg/kg-day for decreased body-weight gain derived from combined male and female data and a composite UF of 100 (10 for interspecies extrapolation and 10 for intraspecies variability among the human population).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR CYCLOHEXANONE

Human studies indicate that inhalation of cyclohexanone can cause eye, nose, and throat irritation (Esso Research and Engineering Co., 1965; Nelson et al., 1943). Interpretation of results suggestive of neurological effects at relatively low concentrations (Mitran et al., 1997) is limited by methodological and reporting problems with the study. The human data are not considered to be suitable for the derivation of the p-RfC values. Inhalation studies in animals include a subchronic study that evaluated various endpoints in rabbits (Treon et al., 1943), a series of subchronic studies that were specifically designed to evaluate the effects on the olfactory bulb in rats (Rehn et al., 1988; Panhuber and Laing, 1987; Panhuber et al., 1987; Laing et al., 1985; Laing and Panhuber, 1980, 1978; Pinching and Doving, 1974), a multigenerational reproduction study in rats (ABC, 1986a,b), and developmental studies in rats (Samimi et al., 1989; Biodynamics, 1984a) and mice (Biodynamics, 1984b). Table 8 summarizes these data. The primary findings were clinical signs indicative of eye irritation and neurological effects and reduced body-weight gain in rabbits with subchronic exposure

Table 8. Summary of Inhalation Noncancer Dose-Response Information for Cyclohexanone						
Species and Study Type (*n*/sex/group)	**Exposure**	**NOAEL (mg/m^3)**	**LOAEL (mg/m^3)**	**Responses at the LOAEL**	**Comments**	**Reference**
Rabbit (4/group)	0, 763, 1241, 3103, 5677, or 12,373 mg/m^3 6 hours/day, 5 days/week for 3 weeks (12,373 mg/m^3) for 10 weeks (all other groups).	1241 HEC: 222	3103 HEC: 554	Clinical signs as salivation, conjunctival congestion and irritation, lacrimation and lethargy.	More severe clinical signs including conjunctival irritation, lacrimation, lethargy, narcosis, labored breathing, loss of coordination, weight loss, and hypothermia were observed at higher concentrations.	Treon et al., 1943
Rat (various group sizes)	0−32 mg/m^3 for 1−4 months.	NA	NA	NA	Alterations in mitral cells and spine density of granule cell dendrites were seen at all concentrations tested, but also in response to deodorized air. Interpretation of these results is unclear. There was no effect on olfactory sensitivity.	Rehn et al., 1988; Panhuber and Laing, 1987; Panhuber et al., 1987; Laing et al., 1985; Laing and Panhuber, 1980, 1978; Pinching and Doving, 1974
Rat (30/sex/group)	0, 1004, 2007, or 4015/5621 mg/m^3 6 hours/day through two consecutive generations.	2007 HEC: 502	5621 HEC: 1405	Effects in F1 animals included mortality, adverse clinical signs, decreases in body weights, decreased fertility in males, and decreases in progeny viability, survival, and body weights.	A subsequent study determined that the decrease in male fertility in this study was reversible in the second generation (ABC, 1986b).	ABC, 1986a
Rat (10/group)	0, 402, 1004, or 2007 mg/m^3 7 hours/day on GDs 5−20.	2007 HEC: 585	NA	NA	Grey mottling of the lungs was observed in rats exposed to 1004, or 2007 mg/m^3 upon gross examination. No histology was performed, and incidence was not reported.	Samimi et al., 1989

Table 8. Summary of Inhalation Noncancer Dose-Response Information for Cyclohexanone

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Responses at the LOAEL	Comments	Reference
Rat (26/group)	0, 1216, 2638, or 5661 mg/m^3 6 hours/day on GDs 6−19.	2638 HEC[a]: 660	5661 HEC[a]: 1415	Adverse clinical signs, abolition of a startle response, and decreased gestational body-weight gain in dams. Decreased fetal body weight and increased incidence of fetal skeletal alterations.		Biodynamics, 1984a
Mouse (30/group)	0 or 5541 mg/m^3 6 hours/day on GDs 6−17.	NA	5541 HEC[a]: 1385	Adverse clinical signs, delayed response to stimulus, and decreased gestational body-weight gains in dams. Increased incidence of resorptions, decreased fetal viability and body weights, and retarded bone ossification.		Biodynamics, 1984b

[a]HEC calculated as follows: $NOAEL_{[HEC]}$ = NOAEL × exposure hours/24 hours × exposure days/7 days × dosimetric adjustment. For systemic effects, the dosimetric adjustment is the ratio of the animal:human blood:gas partition coefficients for cyclohexanone (in the absence of experimental values, a default value of 1 was used).

(Treon et al., 1943) and at higher concentrations in rats and mice exposed in reproductive and developmental studies (ABC, 1986a; Biodynamics, 1984a,b). The latter studies also showed effects on offspring viability and development (reduced body weights and increased skeletal variations indicative of developmental delay) at the same concentrations.

The NOAELs and LOAELs in Table 8 were converted to human equivalent concentrations (HECs) in order to facilitate comparisons across studies. First, the concentrations, expressed originally in mg/m^3, were adjusted to continuous exposure ($NOAEL_{ADJ}$). Current EPA practice is to include an adjustment to continuous exposure for developmental effects, as is typically done for other endpoints (see U.S. EPA, 2002). The $NOAEL_{ADJ}$ values were calculated as follows:

$$NOAEL_{ADJ} = (NOAEL) (\# \text{ hours} \div 24 \text{ hours}) (\# \text{ days} \div 7 \text{ days})$$

Because the observed effects of cyclohexanone were systemic in nature, the chemical was treated as a Category 3 gas. The human equivalent concentration ($NOAEL_{HEC}$) is calculated for a Category 3 gas by multiplying the $NOAEL_{ADJ}$ by the ratio of the blood:gas (air) partition coefficients of cyclohexanone in animals and humans. However, partition coefficients for cyclohexanone are not available in humans or animals. In accordance with "Methods of derivation of inhalation reference concentrations and application of inhalation dosimetry" (U.S. EPA, 1994b), the value of 1.0 is used for the ratio, and the $NOAEL_{HEC}$ values shown in Table 8 are calculated as follows:

$$NOAEL_{HEC} = NOAEL_{ADJ} \times (H_{b/g})_A \div (H_{b/g})_H$$

SUBCHRONIC p-RfC

The lowest $LOAEL_{HEC}$ across inhalation studies is 554 mg/m^3 based on adverse clinical signs in rabbits including salivation, conjunctival congestion and irritation, lacrimation, lethargy, narcosis, labored breathing, loss of coordination, weight loss, and hypothermia (Treon et al., 1943). The corresponding $NOAEL_{HEC}$ is 222 mg/m^3. Similar clinical signs were seen at $LOAEL_{HEC}$ values of approximately 1400 mg/m^3 in parental rats and mice in the reproduction (ABC, 1986a) and developmental (Biodynamics, 1984a,b) studies. Treon (1943) evaluated a relatively wide array of clinical and histopathological effects, and the only other available data are reproductive/developmental studies that demonstrated slightly higher NOAELs. The $NOAEL_{HEC}$ of 222 mg/m^3, based on clinical signs in rabbits at the next highest dose (described above; Treon et al., 1943), is chosen as the POD for deriving the p-RfC. BMD modeling cannot be conducted because quantitative data were not provided in the principal study.

A **subchronic p-RfC** for cyclohexanone, based on the $NOAEL_{HEC}$ of 222 mg/m^3 for adverse clinical signs in rabbits (Treon et al., 1943), was derived as follows:

$$\begin{aligned} \textbf{Subchronic p-RfC} &= NOAEL_{HEC} \div UF \\ &= 222 \text{ mg/m}^3 \div 30 \\ &= \mathbf{7 \text{ mg/m}^3} \end{aligned}$$

The composite UF of 30 is composed of the following UFs:

- UF_A: A factor of 3 ($10^{0.5}$) is applied for animal-to-human extrapolation to account for potential pharmacodynamic differences between rabbits and humans. The dosimetric conversion to an HEC accounts for pharmacokinetic differences.
- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating susceptible human response are insufficient.
- UF_D: The database for inhalation toxicity of cyclohexanone consists of a subchronic toxicity study in rabbits, a series of subchronic studies in rats specifically evaluating the effects on cells of the olfactory bulb, a multigenerational reproduction study in rats, and developmental toxicity studies in rats and mice. A factor of 1 is applied for database inadequacies.
- UF_L: A NOAEL is identified from the database and used as the POD; therefore, a factor of 1 is used.

Confidence in the principal study (Treon et al., 1943) is low based on small group sizes (four rabbits/group), examination of limited endpoints, inclusions of a 2-month recovery period prior to histopathological examination, and insufficient detail in reporting of results. Confidence in the database is medium. Aside from the subchronic study in rabbits used to derive the p-RfC, a multigenerational reproduction study in rats and developmental toxicity studies in rats and mice are also available that support the findings in rabbits. However, there are no supporting systemic toxicity studies in other species. Overall confidence in the subchronic p-RfC is low.

CHRONIC p-RfC

To derive the chronic p-RfC in the absence of chronic data, the POD from the subchronic p-RfC (adverse clinical signs in rabbits) is used along with a composite UF that includes the same areas of uncertainty enumerated above for the subchronic p-RfC, as well as additional 10-fold UFs, as follows:

- UF_A: A factor of 3 ($10^{0.5}$) is applied for animal-to-human extrapolation to account for potential pharmacodynamic differences between rabbits and humans. The dosimetric conversion to an HEC accounts for pharmacokinetic differences.
- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating susceptible human response are insufficient.
- UF_D: The database for inhalation toxicity of cyclohexanone consists of a subchronic toxicity study in rabbits, a series of subchronic studies in rats specifically evaluating the effects on cells of the olfactory bulb, a multigenerational reproduction study in rats and developmental toxicity studies in rats and mice. A factor of 1 is applied for database inadequacies.
- UF_L: A NOAEL is identified from the database and used as the POD; therefore, a factor of 1 is used.
- UF_S: A factor of 10 is applied for using data from a subchronic study to assess potential effects from chronic exposure because data for evaluating responses after chronic exposure are not available.

This results in a total UF of 300 for derivation of the chronic p-RfC.

A **chronic p-RfC** for cyclohexanone, based on the $NOAEL_{HEC}$ of 222 mg/m^3 for adverse clinical signs in rabbits (Treon et al., 1943), is derived as follows:

Chronic p-RfC = $NOAEL_{HEC} \div UF$
= $222\ mg/m^3 \div 300$
= **$0.7\ mg/m^3$**

As discussed for the subchronic p-RfC, confidence in the principal study is low. However, unlike the characterization of the database in reference to the subchronic value derivation, confidence in the database, in the context of a chronic value derivation, is reduced to low for the chronic p-RfC due to the absence of a chronic study. Overall confidence in the chronic p-RfC is low.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR CYCLOHEXANONE

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 cyclohexanone. The only available studies that evaluated the carcinogenic potential of cyclohexanone were the 2-year drinking water studies in rats and mice conducted by Lijinsky and Kovatch (1986). These studies found increased incidences of adrenocortical adenomas in low-dose male rats, increased incidence of hepatocellular tumors in low-dose male mice, and increased incidence of malignant lymphoma in low-dose female mice. None of these findings exhibited a dose-related trend, as these neoplasms were not observed at higher doses. In addition, there was a marginal increase in the incidence of follicular cell adenoma-carcinomas of the thyroid gland among high-dose males compared to concurrent controls. Lijinsky and Kovatch (1986) concluded that the evidence of carcinogenic activity is marginal, and the effect, if any, is weak. IARC (1999, 1989) reviewed these studies and characterized cyclohexanone as *Not Classifiable As to Its Carcinogenicity To Humans* based on inadequate data. ACGIH (2007) also considered cyclohexanone to be *Not Classifiable As a Human Carcinogen* (A4). Genotoxicity data are mixed. Assays for mutagenicity were largely negative in bacteria but mixed in mammalian cells, and there is some evidence of clastogenicity in human cells in vitro and in rats in vivo.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

A lack of suitable data precludes derivation of quantitative estimates of cancer risk for cyclohexanone.

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