Cyclohexene

(CAS No: 110-83-8)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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1 Introduction

The present document contains the assessment of the health hazard of cyclohexene by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by H Stouten, M.Sc. (TNO Nutrition and Food Research, Zeist, The Netherlands).

The evaluation of the toxicity of cyclohexene has been based on the review by the American Conference of Governmental Industrial Hygienists (ACG98). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in April 1999, literature was searched in the on-line databases Medline, Toxline, and Chemical Abstracts, starting from 1966, 1965, and 1967, respectively, and using the following key words: cyclohexene, 110-83-8, and 33004-06-7.

In July 2000, the President of the Health Council released a draft of the document for public review. No comments were received.

An additional search in Toxline and Medline in November 2003 did not result in information changing the committee's conclusions.

2 Identity

name	:	cyclohexene
synonyms	:	cyclohex-1-ene; benzenetetrahydride; tetrahydrobenzene; hexanaphthylene
molecular formula	:	C_6H_{10}
structural formula	:	
CAS number	:	110-83-8

3 Physical and chemical properties

molecular weight	:	82.1
boiling point	:	83°C
melting point	:	-104°C
flash point	:	-6°C (closed cup)
vapour pressure	:	at 20°C: 8.9 kPa
solubility in water	:	insoluble
log $P_{octanol/water}$	:	2.86 (experimental); 2.96 (estimated)
conversion factors	:	at 20°C, 101,3 kPa: 1 ppm = 3.42 mg/m^3 1 mg/m^3 = 0.29 ppm

Data from ACG98, http://esc.syrres.com.

Cyclohexene is a highly flammable, colourless liquid, with a sweet odour. An odour threshold of 0.18 ppm (0.6 mg/m^3) has been reported (Amo83).

4 Uses

Cyclohexene is used in oil extraction, as a catalytic solvent, and in organic synthesis and in the manufacture of adipic acid, maleic acid, and tetrahydrobenzoic acid and aldehyde (ACG98, NLM03).

5 Biotransformation and kinetics

The committee did not find human data on the kinetics of cyclohexene.

In vitro experiments with microsomal liver preparations showed that cyclohexene can be metabolised by cytochrome P450 monooxygenases. Binding experiments resulted in difference spectra characteristic of Type I substrates (Can74, Jam71). The experiments indicated that under the applied conditions cyclohexene is converted into its *trans*-diol derivative via an epoxide (Lei70, Lei71). Formation of 2-cyclohexen-1-ol and 2-cyclohexen-1-one was found as well (Lei78). Cyclohexene was not a suicide substrate for cytochrome P450 (Ort80).

No cyclohexene oxide could be detected in the blood of rats during a 1-hour exposure to 600 ppm (2040 mg/m^3) while cyclohexene levels increased to ca. 2 µg/g blood, in an experiment performed to investigate the phenomenon that exposure to alkenes that inactivate cytochrome P450 result in an early peak in epoxide blood levels. Cyclohexene did not affect cytochrome P450

concentrations in livers sampled 20 or 360 minutes after starting exposure (Map93). Following oral administration of 250 mg/kg bw to rats, a rapid decrease in total glutathione content in the liver was measured. When an oral dose of 165 mg/kg bw was given to rats, 12.7% of the dose was excreted in the urine as 3-hydroxycyclohexylmercapturic acid (both the *cis*- and *trans*-isomer) while there were only traces present of cyclohexylmercapturic acid and *cis*-2-hydroxycyclohexylmercapturic acid (but no *trans*-isomer). In rabbits, 3-hydroxycyclohexylmercapturic acid (25.4%), cyclohexylmercapturic acid (traces), and *cis*-2-hydroxycyclohexylmercapturic acid (traces) were found as well, in addition to glucuronides (20%) and sulphates (3.5 %). This experiment was mainly aimed at identifying (possible) mercapturic acid metabolites. Glucuronides and sulphates were not further specified and the remaining fraction was not accounted for (Jam71). In a separate experimented only investigating the possible hydroxylation at the allylic position in rats, 0.1% of an oral dose of ca. 60 mg/kg bw was excreted as 2-cyclohexen-1-one in the 24-hour urine while no 2-cyclohexen-1-ol (free or glucuronidated) could be detected (Lei78).

6 Effects and mechanism of action

Human data

The committee did not find data on workers occupationally exposed to cyclohexene.

Animal data

Irritation and sensitisation

When 0.1 mL of cyclohexene was instilled into the right eyes of 6 rabbits, the mean Draize score calculated from 6 observation periods was 0.8. When the material was washed out after a 4-second contact period, the mean score was 0.3 (maximum possible score: 8.0) (Moo81a, Moo81b).

When 2 mL/kg (ca. 1600 mg/kg) was applied to the clipped skin of rabbits (n=3/sex) and left covered for 24 hours, erythema, oedema, and induration were observed in all animals, lasting for 3-5, 1, and 14 (i.e., the observation period) days, respectively. Eschar formation or infection were not seen in any of the animals (Moo81c). In guinea pigs (n=3), covered application of undiluted material (5-20 mL/kg) produced slight to moderate oedema, moderate to severe

erythema, and scattered necrosis at 24 hours, eschars over the entire patch after 1 week, and scarring and up to complete alopecia after 2 weeks (Nob80).

The committee did not find data on the possible sensitising effects of cyclohexene.

Acute toxicity

A 4-hour exposure to 6370 ppm (21,660 mg/m^3) was not lethal to rats (n=5/sex). During exposure, depressed activity, ataxia, and squinting in all animals and lachrymation in one male animal were observed. All animals appeared normal within 24 hours after ending exposure. At gross necropsy at the end of the 14-day observation period, multiple pulmonary cysts were seen in 1/5 male and 4/5 female animals. In one female animal, there were multiple haematomas in the thymus (Yat81). Single exposures of unknown duration to 8850 and 14,800 ppm (ca. 30,000 and 50,000 mg/m^3) were stated to seriously affect and kill experimental animals, respectively (ACG98).

Twenty-four-hour covered dermal application of 2 mL/kg (ca. 1600 mg/kg bw) to the clipped skin of rabbits (n=3/sex) did not induce mortality. Apart from the skin effects presented above, 2/3 male and 2/3 female animals showed 'excretory conditions' that lasted for the 14-day observation period. Both in male and female animals, there were weight losses in the second post-exposure observation week. At gross necropsy, dark purple or brown spots were seen on the lungs of 5 animals while discolouration of the liver and spots on the kidney were seen in 2 and one of these animals, respectively. The remaining 6th animal had spots on the kidney (Moo81c).

In male and female rats (Sprague-Dawley), an oral LD_{50} of 3.53 mL/kg bw (ca. 2880 mg/kg bw) was found. At non-lethal doses (2.0 and 2.5 mL/kg bw, ca. 1630 and 2040 mg/kg bw) ruffed fur, docile behaviour, tremors, and blood stained squinting eyes were seen, persisting throughout a significant part or the whole observation period. At lethal doses (3.2 and 4.0 mL/kg bw, ca. 2610 and 3265 mg/kg bw), additional effects seen were amongst others convulsions, paralysis, nasal discharge, salivation, rapid breathing. The surviving animals from the 2 higher dose groups generally gained less weight during the observation period than the animals from the 2 lower dose groups. At gross necropsy, abnormalities were seen in the lungs, liver, and kidneys of the animals of all dose groups, in the stomach, intestine, and spleen of the animals of the 3 higher dose groups, in the adrenals of the animals of the 2 higher dose groups, and in the thymus of the animals of the highest dose group (Moo81d). In a separate study, an LD_{50} of 2.4 mL/kg bw (ca. 1960 mg/kg bw) was found for

male rats (Charles River CD), with no mortality occurring at doses of 0.8 and 1.6 mL/kg bw (ca. 650 and 1305 mg/kg bw). At these latter doses, depressed activity was observed. At necropsy, effects on the stomach, kidneys, and spleens were reported in some of the animals (Fie79). In male mice (Royalhart ICR), the LD_{50} was calculated to be greater than 3.2 mL/kg bw (ca. 2610 mg/kg bw). No mortality was found at single dose of 0.2, 0.4, and 1.6 mL/kw bw (ca. 165, 325, and 1305 mg/kg bw), while doses of 0.8 and 3.2 mL/kg bw (ca. 650 and 2610 mg/kg bw) caused the death of 1/4 animals in both groups. Effects observed were limited to the highest dose group and included depression, ataxia, and loss of righting reflexes, and pale kidneys in one mouse at necropsy (Fie80).

Repeated-dose toxicity

Information on toxicity following repeated dosing is limited to one abstract of a 6-month inhalation study in which male rats (n=20/group), guinea pigs (n=10/group), and rabbits (n=6/group) were simultaneously exposed to 75, 150, 300, and 600 ppm (255, 510, 1020, and 2040 mg/m^3), 6 hours/day, 5 days/week, for 6 months. Examinations included body weight recordings (weekly), haematology (before, during, after exposure), biochemistry (after the 6-month exposure period), and gross pathology of the haematopoietic organs (after the 6-month exposure period). The rats of the highest concentration group showed significantly less body weight gains than the animals of the 300-ppm and the control group (no information on comparison with other groups given). Alkaline phosphatase was significantly increased in all rat exposure groups. Furthermore, it was stated that most of the other parameters were within normal limits for all groups and that there were no significant changes in the bone marrow in any of the exposure groups (Lah76).

In a study designed to investigate the mechanism of the ovotoxicity induced in mice by 4-vinylcyclohexene, neither cyclohexene nor cyclohexene oxide caused ovotoxicity (parameter: follicle counts) following intraperitoneal injections of ca. 615 mg/kg bw and 140 mg/kg bw, respectively, for 30 days (Doe95). The committee did not find other data on the potential reproduction toxicity of cyclohexene.

Mutagenicity and genotoxicity

Cyclohexene was negative when tested both with and without a metabolic activating system (derived from induced rat livers) in *S. typhimurium* strains TA98 and TA100 (at concentrations of 2.5-2500 µg/plate - precipitation at 2500

µg - or 0.1-1000 µg/plate) (Flo80, Syc00) and strains TA1535 and TA1537 (250 µg/plate) (Flo80).

7 Existing guidelines

The current administrative occupational exposure limit (MAC) for cyclohexene in the Netherlands is 1015 mg/m^3 (300 ppm), 8-hour TWA.

Existing occupational exposure limits in some European countries and the USA are summarised in the annex.

8 Assessment of health hazard

The committee did not find human data on effects of exposure to cyclohexene.

Limited kinetic data suggest that cyclohexene is metabolised by cytochrome P450 via an epoxide intermediate. It is excreted conjugated with glutathione, glucuronic acid, and sulphate, depending on the species involved.

Cyclohexene was irritating to the skin but not to the eyes of rabbits. The committee did not find data on potential sensitising properties.

Based on acute lethality data, cyclohexene is of low toxicity following exposure by inhalation, oral administration, and by dermal contact.

Data on repeated exposure were limited to an abstract of a 6-month inhalation study in rats, guinea pigs, and rabbits in which no effects were reported to have been induced in guinea pigs and rabbits while in rats there was a decrease in body weight gain in animals exposed to 2040 mg/m^3 (600 ppm) and increased alkaline phosphatase levels in all dose groups (255-2040 mg/m^3 or 75-600 ppm). Although the significance of the latter is unclear in absence of histological data and 1020 mg/m^3 (300 ppm) could be a NOAEL, the committee is of the opinion that this study cannot be used in deriving a health-based occupational exposure limit in view of the limited reporting and the incomplete post-mortem examinations (no microscopic, only partly gross examination). However, the committee notices that the possible NOAEL (based on decreased body weight gain) and the current occupational exposure limit are identical.

The committee did not find data on the potential carcinogenicity.

Cyclohexene did not induce mutations when tested in a bacteria cell system *in vitro*. The committee did not find data from other *in vitro* or *in vivo* assays.

Cyclohexene was not ovotoxic in mice following intraperitoneal administration to mice; other data on the potential reproduction toxicity were not available.

The committee considers the toxicological database on cyclohexene too poor to justify recommendation of a health-based occupational exposure limit.

In view of the results of a 6-month inhalation study in rats, the committee concludes that the current MAC-value of 1015 mg/m^3 (300 ppm), 8-hour TWA, may be too high.

References

ACG98 American Conference of Governmental Industrial Hygienists (ACGIH). Cyclohexene. In: TLVs® and other occupational exposure values - 1998 [CD-ROM]. Cincinnati OH, USA: ACGIH®, Inc, 1998.

ACG03a American Conference of Governmental Industrial Hygienists (ACGIH). Guide to occupational exposure values - 2003. Cincinnati OH, USA: ACGIH®, Inc, 2003: 37.

ACG03b American Conference of Governmental Industrial Hygienists (ACGIH). 2003 TLVs® and BEIs® based on the documentation of the Threshold Limit Values for chemical substances and physical agents & Biological Exposure Indices. Cincinnati OH, USA: ACGIH®, Inc, 2003: 24.

Amo83 Amoore JE, Hautala E. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol 1983; 3: 272-90.

Arb02 Arbejdstilsynet. Grænseværdier for stoffer og materialer. Copenhagen, Denmark: Arbejdstilsynet, 2002: 21 (At-vejledning C.0.1).

Can74 Canady WJ, Robinson DA, Colby HD. A partition model for hepatic cytochrome P-450-hydrocarbon complex formation. Biochem Pharmacol 1974; 23: 3075-8.

DFG03 Deutsche Forschungsgemeinschaft (DFG): Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. List of MAK and BAT values 2003. Maximum concentrations and Biological Tolerance Values at the workplace Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA, 2003: 45 (rep no 39).

Doe95 Doerr JK, Hooser SB, Smith BJ, et al. Ovarian toxicity of 4-vinylcyclohexene and related olefins in B6C3F$_1$ mice: role of diepoxides. Chem Res Toxicol 1995; 8: 963-9.

EC04 European Commission: Directorate General of Employment and Social Affairs. Occupational exposure limits (OELs); http://europe.eu.int/comm/employment_social/h&s/areas/oels_en.htm.

Fie79 Field WE. Oral LD$_{50}$ in rats. Clarks Summit PA, USA: CDC Research, Inc, 1979; study no CDC-EA-222-79 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0556687).

Fie80 Field WE. Oral LD$_{50}$ in mice. Clarks Summit PA, USA: CDC Research, Inc, 1980; study no CDC-EA-262-79 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0556688).

Flo80 Florin I, Rutberg L, Curvall M, et al. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 1980; 18: 219-32.

HSE02 Health and Safety Executive (HSE). EH40/2002. Occupational Exposure Limits 2002. Sudbury (Suffolk), England: HSE Books, 2002: 15.

Jam71 James SP, Jeffery DJ, Waring RH, et al. Reaction of mono-bromo derivatives of cyclopentane, cyclohexane and cyloheptane and of related compounds with glutathione in vivo and the nature of the sulphur-containing metabolites excreted. Biochem Pharmacol 1971; 20: 897-907.

Lah76 Laham S. Inhalation toxicology of cyclohexene. Toxicol Appl Pharmacol 1976; 37: 155-6.

Lei70 Leibman KC, Ortiz E. Epoxide intermediates in microsomal oxidation of olefins to glycols. J Pharmacol Exp Ther 1970; 173: 242-6.

Lei71 Leibman KC, Ortiz E. Oxidation of cycloalkenes in liver microsomes. Biochem Pharmacol 1971; 20: 232-6.

Lei78 Leibman KC, Ortiz E. Microsomal metabolism of cyclohexene. Hydroxyaltion in the allylic position. Drug Metab Dispos 1978; 6: 375-8.

Map93 Maples KR, Dahl AR. Levels of epoxide in blood during inhalation of alkenes and alkene oxides. Inhal Toxicol 1993; 5: 43-54.

Moo81a Moon RC. Primary eye irritation test (unwashed) on Phillips cyclohexene. Salt Lake City UT, USA: University of Utah Research Institute, 1981; rep no TR 05450-011 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0556736).

Moo81b Moon RC. Primary eye irritation test (washed) on Phillips cyclohexene. Salt Lake City UT, USA: University of Utah Research Institute, 1981; rep no TR 05450-010 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0556737).

Moo81c Moon RC. Acute dermal toxicity screen on Phillips cyclohexene. Salt Lake City UT, USA: University of Utah Research Institute, 1981; rep no TR 05450-007 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0556738).

Moo81d Moon RC.Acute oral LD_{50} on Phillips cyclohexene. Salt Lake City UT, USA: University of Utah Research Institute, 1981; rep no TR 05450-022 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0556739).

NLM03 US National Library of Medicine (NLM), ed. Cyclohexene. In: Hazardous Substances Data Bank (HSDB) (last revision data cyclohexene file: March 2003; last review date: September 1995); http://toxnet.nlm.nih.gov.

Nob80 Noble M. Toxicity report - Eastman Kodak Company - HS & HFL. Cyclohexene. 1980; available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0556765.

Ort80 Ortiz de Montellano PR, Mico BA. Destruction of cytochrome P-450 by ethylene and other olefins. Mol Pharmacol 1980; 18: 128-35.

Swe00 Swedish National Board of Occupational Safety and Health. Occupational exposure limit values and measures against air contaminants. Solna, Sweden: National Board of Occupational Safety and Health, 2000; Ordinance AFS 2000:3.

Syc00 Sycheva LP, Zholdakova ZI, Polyakova EE, et al. Mutagenic activity of cyclohexene and products of its chlorination. Bull Exp Biol Med 2000; 129: 581-3.

SZW03 Ministerie van Sociale Zaken en Werkgelegenheid (SZW). Nationale MAC-lijst 2003. The Hague, the Netherlands: Sdu, Servicecentrum Uitgevers, 2003: 22.

TRG00 TRGS 900. Grenzwerte in der Luft am Arbeitsplatz; Technische Regeln für Gefahrstoffe. BArbBl 2000; 2.

Yat81 Yates WG, Price NH, Potter RN, et al. Acute inhalation screen on Phillips cyclohexene. Salt Lake City UT, USA: University of Utah Research Institute, 1981; rep no TR 05450-003 (available from National Technical Information Service, Springfield VA, USA; order no NTIS/OTS0555329).

Annex

Occupational exposure limits for cyclohexene in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	300	1015	8 h	administrative		SZW03
Germany						
- AGS	300	1015	8 h			TRG00
	1200	4060	15 min			
- DFG MAK-Kommission	-[c]	-[c]				DFG03
Great Britain						
- HSE	300	1020	8 h	OES		HSE02
Sweden	-	-				Swe00
Denmark	300	1015	8 h			Arb02
USA						
- ACGIH	300	-	8 h	TLV		ACG03b
- OSHA	300	1015	8 h	PEL		ACG03a
- NIOSH	300	1015	8 h	REL		ACG03a
European Union						
- SCOEL	-	-				EC04

[a] S = skin notation; which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Listed among compounds for which studies of the effects in man or experimental animals have yielded insufficient information for the establishment of MAK values.