FOREWORD INTRODUCTION

Cyclohexene

CAS N°:110-83-8

SIDS Initial Assessment Report

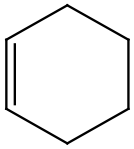
For

SIAM 15

Boston, US, 22-25 October, 2002

1. Chemical Name:	Cyclohexene
2. CAS Number:	110-83-8
3. Sponsor Country:	Japan National SIDS Contact Point in Sponsor Country: Ms. Mizuho Hayakawa, Ministry of Foreign Affairs, Japan
4. Shared Partnership with:	
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	
• Process used	
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	The original draft documents were prepared by the Japanese government. **Tests:** No testing ( ) Testing (x) log P_{ow}, Water solubility, Hydrolysis, Biodegradation, Bioconcentration, Acute toxicity to fish, daphnia and algae, Chronic toxicity to daphnia, Acute toxicity, Combined repeated and reproductive/developmental toxicity, Ames test and Chromosomal aberration test
7. Review Process Prior to the SIAM:	
8. Quality check process:	
9. Date of Submission:	August 13, 2002
10. Date of last Update:	
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	110-83-8
Chemical Name	Cyclohexene
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

An oxidation of cyclohexene at the allylic position has been shown by *in vitro* studies but there is no detailed information *in vivo* regarding absorption, metabolism and excretion.

The oral acute toxicity of cyclohexene is low: the LD_{50} in rats is 1,000-2,000 mg/kg [OECD TG 401]. Some clinical signs including hypoactivity were observed. Dermal acute toxicity is negligible: the LD_{50} in guinea pigs is >16,220 mg/kg. Acute inhalation toxicity is very low: exposure of rats to 21,388 mg/m^3 produced no deaths. There is no reliable information on eye and skin irritation and sensitization.

According to a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [OECD TG 422], SD rats received gavage doses of 0, 50, 150 and 500 mg/kg b.w./day for 48 days in males and for 42-53 days from 14 days before mating to day 4 of lactation throughout the mating and pregnancy period in females. Salivation was observed for about 5 minutes after dosing in 3 of 12 males and 2 of 12 females at 150 mg/kg b.w./day and up to 6 hours after dosing all of 12 males and 12 females at 500 mg/kg b.w./day. Blood chemical examination showed a decrease in triglyceride in males at 150 and 500 mg/kg b.w./day, and increases in total bilirubin in males at 500 mg/kg b.w./day and in total bile acid in both sexes at 150 mg/kg b.w./day and more. In males of the 500 mg/kg b.w./day group, there was an increase in relative kidney weight. On histopathological examinations, no dose-related changes were observed. Therefore, the NOAEL for repeated dose toxicity was considered to be 50 mg/kg b.w./day for both sexes.

In a reverse gene mutation assay [OECD TG 471], this chemical was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *urv*A with and without exogenous metabolic activation. In a chromosomal aberration test [OECD TG 473], structural chromosomal aberrations and polyploidy were not induced with and without exogenous metabolic activation in cultured Chinese hamster lung (CHL/IU) cells.

There is no data available on carcinogenicity.

Regarding the effects on the reproductive/developmental parameters, in the above-mentioned combined study [OECD TG 422], no effects of this chemical were observed on mating, pregnancy, delivery, lactation of parent animals and viability, body weight, general appearance and the autopsy finding of offspring. The NOAEL for reproductive/developmental toxicity was considered to be 500 mg/kg b.w./day.

Environment

Cyclohexene has a vapour pressure of 119 hPa (25degree C), a water solubility of 250 mg/L, a LogPow of 2.99. Its Henry's law constant is 4.55E-2 atm.m3/mol.

Cyclohexene is not readily biodegradable and its BCF is less than 45. In the air, this chemical is expected to be photodegraded ($T_{1/2}$= 1.4 hours) by ozone. Hydrolysis is not expected to occur.

In acute toxicity to aquatic organisms, for daphnid a 48hEC50 of 2.1 mg/L (*Daphnia magna*, OECD TG202, closed system) and for fish a 96hLC50 of 5.8 mg/L (*Oryzias latipes*, OECD TG 203 semistatic) and a 96hLC 50 of 12.4 mg/L (*Poecilia reticulata,*semistatic) have been reported.

Three chronic toxicity values from two trophic level species were available: a NOErC of 0.67 mg/L and a 72hNOEbC of 1.8 mg/L in algae (*Selenastrum capricornutum,* OECD TG 201, closed system) and a 21dNOEC of 0.53 mg/L in the daphnid (*Daphnia magna*, OECD TG 211, semistatic) on reproduction.

Exposure

Cyclohexene is used as an intermediate for the production of cyclohexanol and cyclohexeneoxide and as a solvent. The fugacity model (Mackay level III) suggests that if cyclohexene is released to one of the compartments of air, water and soil, it has a tendency to remain in the original compartment.

Occupational exposure to cyclohexene through inhalation and dermal routes is possible.

No information is available on consumer exposure.

RECOMMENDATION

Human Health: The chemical is currently of low priority for further work.

Environment: The chemical is a candidate for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemical is currently of low priority for further work based on a low hazard potential.

Environment: There is no information available on the production volume or use as a solvent, and since the substance shows chronic toxicity to aquatic organisms, an environmental exposure assessment is recommended.

FULL SIDS SUMMARY

CAS NO: 110-83-8		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point		Unknown	- 103.5 °C
2.2	Boiling Point		Unknown	83 °C
2.3	Density		Unknown	0.810 g/cm^3 at 20°C
2.4	Vapour Pressure		Unknown	119 h Pa at 25 °C
2.5	Partition Coefficient (Log P_{ow})		OECD TG 107	2.99
2.6 A.	Water Solubility		OECD TG 105	250 mg/L at 25 °C
B.	pH			None
	pKa			None
2.7	Flash point		Unknown	-12°C
2.12	Oxidation: Reduction Potential			None
2.12	Viscosity		Unknown	0.625mPaS
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation		Calculated	$T_{1/2}$ =1.4 hours (ozone) $T_{1/2}$ =2.1 hours (hydroxyl radical) $T_{1/2}$ =0.83 hours (ozone and hydroxyl radical)
3.1.2	Stability in Water		OECD TG 111	Stable at pH 4, 7 and 9 at 50 °C for five days
3.2	Monitoring Data		-	No data is available.
3.3	Transport and Distribution		Calculated (Level III Fugacity Model)	(Release 100 % to air) Air Water Soil Sediment 99.7 % 0.1 % 0.2 % 0.0 % (Release 100 % to water) Air Water Soil Sediment 0.6 % 94.2 % 0.0 % 5.2 % (Release 102 % to soil) Air Water Soil Sediment 0.9 % 0.2 % 98.9 % 0.0 %
3.5	Biodegradation		OECD TG 301C	Not readily biodegradable
3.7	Bioaccumulation		OECD TG 305	12–38 at 100 µg/L of test water for 28 days 23– 45 at 10 µg/L of test water for 28 days
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oryzias latipes*	OECD TG 203	96h LC_0 = 4.0 mg/L 96h LC_{50} = 5.8 mg/L 96h LC_{100} = 17 mg/L
		Poecilia reticulate		96h LC_0 = 3.1 mg/L 96h EC_{50} = 4.7 mg/L (mortality and paralysis) 96h LC_{50} = 12.4 mg/L
4.2	Acute Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 202	48h EC_0 = 1.5 mg/L 48h EC_{50} = 2.1 mg/L
4.3	Toxicity to Aquatic Plants e.g. Algae	*Selenastrum capricornutum*	OECD TG 201 Closed system	72h NOErC = 0.67 mg/L 72h NOEbC = 1.8 mg/L
4.5.2	Chronic Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 211	21d Reproduction EC_{50} = 1.0 mg/L LOEC = 0.74 mg/L NOEC = 0.53 mg/L

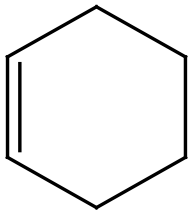
CAS NO: 110-83-8		SPECIES	PROTOCOL	RESULTS
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	OECD TG 401	LD_{50} = 1,000 – 2,000 mg/kg b.w.
5.1.2	Acute Inhalation Toxicity	Rat	Other	LD_{50} > 21 388 mg/m^3.
5.1.3	Acute Dermal Toxicity	Guinea pig	Other	LD_{50} > 16 220 mg/kg b.w.
5.2.1	Skin Irritation			No reliable data
5.2.2	Eye Irritation			No data
5.3	Sensitization			No data
5.4	Repeated Dose Toxicity	Rat	OECD TG 422	NOAEL = 50 mg/kg b.w./day
5.5	Genetic Toxicity In Vitro			
A.	Bacterial Test (Gene mutation)	**Salmonella Typhimurium, E. coli**	OECD TG 471	- (With metabolic activation) - (Without metabolic activation)
B.	Non-Bacterial In Vitro Test (Chromosomal aberrations)	CHL cells	OECD TG 473	- (With metabolic activation) - (Without metabolic activation)
5.6	Genetic Toxicity In Vivo			No data
5.7	Carcinogenicity			No data
5.8	Toxicity to Reproduction	Rat	OECD TG 422	NOAEL = 500 mg/kg b.w./day
5.9	Developmental Toxicity/ Teratogenicity	Rat	OECD TG 422	NOAEL = 500 mg/kg b.w./day
5.10	Other Relevant Information			No data
5.11	Experience with Human Exposure			No data

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 110-83-8
IUPAC Name: Cyclohexene
Molecular Formula: C_6H_{10}
Structural Formula:



Synonyms: Benzenetetrahydride
Tetrahydrobenzene
Cyclohexene
Hexanaphthylene
1,2,3,4-Tetrahydrobenzene

1.2 Purity/Impurities/Additives

Substance type: organic

Physical status: liquid

Purity: 99.8 % w/w

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Melting point	- 103.5 °C	Unknown
Boiling point	83 °C	Unknown
Relative density	0.810 g/cm^3 at 20 °C	Unknown
Vapour pressure	119 h Pa at 25 °C	Unknown
Water solubility	250 mg/L at 25°C	OECD TG 105
Partition coefficient n-octanol/water (log value)	2.99	OECD TG 107
Viscosity	0.625 mPaS	Unknown
Flash point	-12 °C	Unknown

Cyclohexene is a colorless and flammable liquid with a specific odor. It is moderately soluble in water (250 mg/L at 25 °C).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

This chemical is not released to the water compartment in Japan.

This chemical is used as an intermediate for the production of cyclohexanol and cyclohexeneoxide, and as a specific solvent.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Cyclohexene is not readily biodegradable as 0 % of BOD was observed in a test after 28 days according to OECD TG301C (METI Report on Hazard data collection for HPV chemicals (2001)). The substance was hydrolytically stable at pH 4, 7 and 9 at 50 degrees C for five days in a test according to OECD TG 111. The substance is therefore not expected to hydrolyse in the environment. The bioconcentration potential seems to be low as the BCF in carp was less than 45 at test concentrations of 10 and 100 µg/L after 28 days in a test according to OECD TG305, which is confirmed by a measured $LogP_{ow}$ of 2.99 with OECD TG107 (METI Report on Hazard data collection for HPV chemicals (2001)).

The potential environmental distribution of Cyclohexene obtained from a generic level III fugacity model under three emission scenarios is shown in Table 2. The result shows that if Cyclohexene is released to one of the compartments of air, water and soil, it has a tendency to remain in the original compartment.

Volatilization to the atmosphere is expected to be rapid according to its physical chemical properties. This chemical has an estimated half-life in the atmosphere of 2.1 hours by the reaction with OH radicals (rate constant $6.1x10^{-11}$ cm^3 $molecule^{-1}$ sec^{-1}) and an estimated half-life of 1.4 hours by reaction with ozone (rate constant $2.0x10^{-16}$ cm^3 $molecule^{-1}$ sec^{-1}). The overall half life with ozone and hydroxyl radicals is 0.83 hours. The Henry's Law constant is $4.55x10^{-2}$ atm m^3/mol. .

Table 2: Environmental distribution of Cyclohexene using a generic level III fugacity model under three emission scenarios

Compartment	Release: 100 % to air	Release: 100 % to water	Release: 100 % to soil
Air	99.7 %	0.6 %	0.9 %
Water	0.1 %	94.2 %	0.2 %
Soil	0.2 %	0.0 %	98.9 %
Sediment	0.0 %	5.2 %	0.0 %

Data used

Melting point: -103.5 °C, Vapour pressure: 119 hPa, Water solbility: 250 mg/L, $LogP_{ow}$: 2.99, Half-life time in air: 0.83 hours, Half-life times in water, soil and sediment are 240,000, 240,000 and 720,000 hours, because this chemical is not readily biodegradable.

2.3 Human Exposure

2.3.1 Occupational Exposure

Cyclohexene is a volatile liquid and worker exposure through inhalation and dermal routes is possible. In Japan, cyclohexene is synthesized in a closed system by catalytic hydrogenation of benzene, isolated by fractionation, stored temporarily in storage tanks and used solely as an intermediate for cyclohexanol synthesis in the same factory. Workers take quality control samples of fractionated cyclohexene once a day and stored cyclohexene once a month. The duration of these sampling operations is about two minutes. The sampling ports are enclosed and have ventilation systems. The estimated exposure concentration of cyclohexene is 10-50 ppm, using the EASE model. The EHE_{inh} of a worker with a body weight of 70 kg is 0.1 mg/kg/day, if the worker operates all the sampling work in a month. Workers wear goggles and protecting gloves during sampling, exposure through dermal contact is therefore expected to be minimal.

No information on exposure through the use of cyclohexene as a solvent are available.

A TWA of 300 ppm is recommended by ACGIH.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Studies in Animals

In vivo Studies

Two *in vivo* studies in rats exposed to cyclohexene by oral or inhalation route were available, in addition to several *in vitro* studies.

In an oral study, two male Holtzman rats were each given 0.1 mL (81.1 mg/kg bw) of cyclohexene by stomach tube [Leibman & Ortiz: 1978]. The urine sample for 24 hr contained 2-cyclohexen-1-one (0.1 % of the oral dose) but 2-cyclohexen-1-ol was not detected even by beta-glucuronidase treatment.

In an inhalation study, male F344/N rats were exposed nose-only to gaseous cyclohexene at 600 ppm (2,015 mg/m^3) for 60 minutes [Maples and & Dahl: 1993]. During exposure, the blood levels of cyclohexene increased up to 2 ug/g blood.

In vitro Studies

In *in vitro* studies, the oxidation of cyclohexene at the allylic position has been shown to occur with hepatic microsomes and with supernatant fractions of liver extracts (centrifugation 9000 g) from male Holtzman rats and male New Zealand White rabbits [Leibman & Ortiz: 1970, 1971 & 1978]. 2-Cyclohexen-1-ol (0.93 ± 0.10 umol/g liver), trans-cyclohexanediol (0.89 ± 0.05 umol/g liver) and cyclohexene oxide (0.06 ± 0.02 umol/g liver) were produced after 10-min incubation of supernatant fractions (centrifugation 9000 g) from rats with 40 mM cyclohexene, [Leibman & Ortiz: 1978]. Pretreatment of rats with phenobarbital induced cyclohexene oxidation by more than 3.5 times.

Studies in Humans

There is no information available on humans.

3.1.2 Acute Toxicity

Studies in Animals

Acute toxicity data are reported for rats, mice and guinea pigs, as shown in Table 3. Among these studies, the study by MHLW (2002) was identified as the key study because it was well conducted and used a current protocol. Other studies have low reliabilities because no details could be obtained. The details of the key study are as follows.

The study by MHLW (2002) was conducted according to OECD TG 401 according to GLP. SD rats were given cyclohexene by gavage at doses of 0, 500, 1,000 and 2,000 mg/kg b.w. Three out of five males or females in the 2,000 mg/kg b.w. group showed some clinical signs such as abnormal gait, adoption of a prone position, salivation, piloerection and tremors, and then died within three days. Lacrimation was observed in both sexes just after dosing at 1,000 mg/kg and more. Hypoactivity were observed in both sexes of all dose groups. Necropsy of the dead animals revealed pulmonary congestion. The LD_{50} values were between 1,000 and 2,000 mg/kg b.w. for both sexes.

Table 3: Acute toxicity of cyclohexene in experimental animals

Route	Species	Type	Value	Reference
Oral	Rat	LD_{50}	1,000-2,000 mg/kg b.w.	MHLW, Japan: 2002
	Rat	LD_{50}	2.4 mL/kg b.w. (1,946 mg/kg b.w.)	NTIS: OTS054026
	Mouse	LD_{50}	> 3.2 mL/kg b.w. (2,595 mg/kg b.w.)	NTIS: OTS0546026
Inhalation	Rat	LC_{LO}*	> 6,370 ppm (21,388 mg/m^3)	NTIS: OTS0555329
Dermal	Guinea pig	LD_{50}	> 20 mL/kg b.w. (16,220 mg/kg b.w.)	NTIS: OTS0556686

*Lethal concentration, low

Studies in Humans

There is no available information on human toxicity.

Conclusion

The LD_{50} values were between 1,000 and 2,000 mg/kg b.w. for both sexes.

3.1.3 Irritation

Studies in Animals

In a study on inhibition of warts induced by mustard gas [Berenblum et al.: 1935], a preliminary test was conducted to determine the degree of irritation produced by cyclohexene. In this test, 50% cyclohexene acetone solution was applied to the skin of 4 mice 3 times at weekly intervals, and a week after the last application the skins were examined both macroscopically and microscopically. As a result, marked thickening and loss of hair with little or no evidence of ulceration following repeated applications was reported. However, the validity and reliability of this study is uncertain.

Studies in Humans

There is no information available on humans.

Conclusion

There is no reliable data on eye and skin irritation and sensitization.

3.1.4 Repeated Dose Toxicity

Studies in Animals

Inhalation

An inhalation study over 6 months using rats, guinea pigs and rabbits was reported [Laham: 1976]. In this study, significant increases in body weight at 600 ppm (2,015 mg/m^3) and in alkaline phosphatase at 75 ppm (252 mg/m^3) and above were noted in rats but no significant changes at up to 600 ppm in guinea pigs and rabbits. However, the validity and reliability of this study was uncertain because only the abstract was available.

Oral

One oral study using rats was reported [MHLW, Japan: 2002]. This study was carried out according to an OECD combined repeated dose toxicity study with the reproduction/developmental toxicity screening test guideline [TG 422] under GLP. This study was identified as a key study and the details are described below.

SD rats (12 animals/sex/dose) received gavage doses of 0 (vehicle: corn oil), 50, 150 and 500 mg/kg b.w./day [MHLW, Japan: 2002]. Males were dosed for 48 days and females for 42-53 days from 14 days before mating to day 4 of lactation throughout the mating and pregnancy period.

Salivation was observed in 3 of 12 males and 2 of 12 females at 150 mg/kg b.w./day and in all of 12 males and 12 females at 500 mg/kg b.w./day. This sign was observed only for about 5 minutes after dosing at 150 mg/kg b.w./day but up to 6 hours after dosing at 500 mg/kg b.w./day. No significant changes of body weight, food consumption and hematological findings for both sexes and urinalysis findings for males were detected. Blood chemical examination showed a decrease in triglyceride in males at 150 and 500 mg/kg b.w./day, and increases in total bilirubin in males at 500 mg/kg b.w./day and in total bile acid in females at 50 mg/kg b.w./day and in both sexed at 150 mg/kg b.w./day and above. In males of the 500 mg/kg b.w./day group, there was an increase in relative kidney weight. On histopathological examinations, no dose-related changes were observed. The increase in total bile acid observed in females at 50 mg/kg b.w./day was not considered to be an adverse effect because of no accompanying changes. Therefore, based on salivation at 150 mg/kg b.w./day, the NOAEL for repeated dose toxicity was considered to be 50 mg/kg b.w./day for both sexes.

Studies in Humans

There is no information available on human toxicity.

Conclusion

In oral repeated dose studies in rats, salivation was observed in both sexes at 150 mg/kg b.w. and above. The NOAEL was considered to be 50 mg/kg b.w./day.

3.1.5 Mutagenicity

In vitro Studies

Bacterial test

The only available result is from a reverse gene mutation assay conducted according to OECD TG 471 & Japanese Guideline for Screening Mutagenicity Testing of Chemicals (Chemical Substances Control Law of Japan) under GLP [MHLW, Japan: 2002]. This study was identified as a key study.

This chemical was not mutagenic with and without S9 mix at concentrations up to 1,250 ug/plate in Salmonella typhimurium TA100, TA98, TA1535, TA1537 and 5,000 ug/plate in Escherichia coli WP2 urvA. Growth inhibition was observed at 625 ug/plate or above with or without S9 mix in Salmonella typhimurium TA100, TA98, TA1535 and TA1537, and at 1,250 ug/plate or above with S9 mix in Escherichia coli WP2 uvrA.

Non-bacterial in vitro test

One chromosomal aberration test using cultured Chinese hamster lung (CHL/IU) cells was reported [MHLW, Japan: 2002]. This study was identified as a key study because it was conducted according to OECD TG 473 under GLP.

Structural chromosomal aberration and polyploidy were not induced up to the maximum concentration of 400 ug/mL, which was established based on the result of a preliminary growth inhibition test. Cell toxicity was observed at 400 ug/mL after continuous treatment for 24 and 48 hrs.

In vivo Studies

There is no information available on genotoxicity *in vivo*.

Conclusion

This chemical was not genotoxic with and without an exogenous metabolic activation in bacterial tests as well as in a chromosomal aberration test *in vitro*.

3.1.6 Carcinogenicity

There is no data available.

3.1.7 Toxicity for Reproduction

Studies in Animals

The only results available are from a study conducted according to the OECD combined repeated dose toxicity study with the reproduction/developmental toxicity screening test guideline [TG 422] [MHLW, Japan: 2002]. This study was identified as a key study.

Cyclohexene was administered to SD(Crj:CD)IGS rats by gavage at doses of 0, 50, 150 and 500 mg/kg b.w./day for 48 days from 14 days prior to mating in males and for 42-53 days from 14 days prior to mating to day 4 of lactation throughout the mating and pregnancy period in females [MHLW, Japan: 2002].

Regarding the reproductive ability of parent animals, no effects were detected on the estrus cycle, copulation index, fertility index, gestation length, numbers of corpora lutea and implantations, implantation index, gestation index, delivery index, parturition or maternal behavior. And regarding the developmental parameters, no effects were detected on viability, body weight, general appearance or autopsy findings of offspring. The NOAEL for reproduction/developmental toxicity was considered to be 500 mg/kg b.w./day.

Studies in Humans

There is no information available on humans.

Conclusion

In an OECD combined study, there were no effects of this chemical on reproduction/developmental parameters. The NOAEL for reproduction/developmental toxicity was considered to be 500 mg/kg b.w./day.

3.2 Initial Assessment for Human Health

An oxidation of cyclohexene at the allylic position has been shown by *in vitro* studies but there is no detailed information *in vivo* regarding absorption, metabolism and excretion.

The oral acute toxicity of cyclohexene is low: the LD_{50} in rats is 1,000-2,000 mg/kg [OECD TG 401]. Some clinical signs including hypoactivity were observed. Dermal acute toxicity is negligible: the LD_{50} in guinea pigs is > 16,220 mg/kg. Acute inhalation toxicity is very low: exposure of rats to 21,388 mg/m^3 produced no deaths. There is no reliable information on eye and skin irritation and sensitization.

According to a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test [OECD TG 422], SD rats received gavage doses of 0, 50, 150 and 500 mg/kg b.w./day for 48 days in males and for 42-53 days from 14 days before mating to day 4 of lactation throughout the mating and pregnancy period in females. Salivation was observed for about 5 minutes after dosing in 3 of 12 males and 2 of 12 females at 150 mg/kg b.w./day and up to 6 hours after dosing in all of 12 males and 12 females at 500 mg/kg b.w./day. Blood chemical examination showed a decrease in triglyceride in males at 150 and 500 mg/kg b.w./day, and increases in total bilirubin in males at 500 mg/kg b.w./day and in total bile acid in both sexes at 150 mg/kg b.w./day and more. In males of the 500 mg/kg b.w./day group, there was an increase in the relative kidney weight. On histopathological examinations, no dose-related changes were observed. Therefore, the NOAEL for repeated dose toxicity was considered to be 50 mg/kg b.w./day for both sexes.

In a reverse gene mutation assay [OECD TG 471], this chemical was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 urvA with and without exogenous metabolic activation. In a chromosomal aberration test [OECD TG 473], structural chromosomal aberrations and polyploidy were not induced with and without exogenous metabolic activation in cultured Chinese hamster lung (CHL/IU) cells.

There is no data available on the carcinogenicity.

Regarding the effects on the reproductive/developmental parameters, in the above-mentioned combined study [OECD TG 422], no effects of this chemical were observed on mating, pregnancy, delivery, lactation of parent animals and viability, body weight, general appearance and the autopsy finding of offspring. The NOAEL for reproductive/developmental toxicity was considered to be 500 mg/kg b.w./day.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Two algal growth inhibition test results are available. A test with *Selenastrum capricornutum* was conducted according to OECD TG 201with a final concentration of carrier of 0.003 mg/L polyoxyethylene sorbitan fatty acid ester (HCO-xx) and 0.1 ml/L acetone in each test solution. EC50 values were not determined as the growth inhibition rate was about 20 % (calculated by the

biomass or growth rate method) at the highest concentration tested (MOE Japan, unpublished data, 2000). Furthermore, a test with *Chlorella pyrenoidosa* according to GLP was reported. This test was conducted according to a different test guideline with a 48 h exposure and without analytical monitoring (Canton & Wegman, 1983). In this test the EbC50 was determined to be 3.5 mg/L but the value obtained without analytical monitoring may be not reliable because the concentration could not be kept due to the high volatility of the substance.

One reliable toxicity test with *Daphnia magna* is available. The 48 h EC50 was 2.1 mg/L (MOE Japan, 2000; soft water and closed system).

Acute toxicity tests with two different species were performed. A 96 h LC50 of 5.8 mg/L was determined with *Oryzias latipes* (MOE Japan, 2000) and a 96 h LC50 of 12.4 mg/L with guppy, *Poecilia reticulata* (Canton & Wegman, 1983). In the latter study, an EC50 of 4.7 mg/L for paralysis effects of cyclohexene was also reported.

Toxicity test results with other species, *Chilomonas* sp and *Uronema parduzci* (both were protozoa, Bringmann et al., 1980 and Bringmann &Kuhn, 1980) were reported. However these test results were judged invalid or not assignable as less information was available.

Chronic Toxicity Test Results

There are chronic toxicity test results with cyclohexene available. In algae, a 72 h NOErC of 0.67 mg/L and a NOEbC 1.8 mg/L in *Selenastrum capricornutum* were reported. And a 48 h NOEbC of 0.22 mg/L in *Chlorella* was reported however this value was regarded to be of low reliability due to lack of analytical monitoring.

In daphnids, a 21 d EC50 of 1.0 mg/L and a 21 d NOEC of 0.53 mg/L for reproduction of *Daphnia magna* were reported (MOE Japan, 2000). This test was conducted using a carrier as in the acute test.

4.2 Terrestrial Effects

There is no available information.

4.3 Initial Assessment for the Environment

Cyclohexene is not readily biodegradable and its bioconcentration factor is less than 45. In the air, this chemical is expected to be photodegraded ($T_{1/2}$= 1.4 hours) by ozone. Hydrolysis is not expected to occur. A generic level III fugacity model shows that if Cyclohexene is released to one of the compartments of air, water and soil, it has a tendency to remain in the original compartment.

Regarding acute toxicity test results to aquatic organisms, for daphnids an EC50 of 2.1- 5.3 mg/L and for fish an LC50 of 5.8 mg/L (*Oryzias latipes*) and a LC 50 of 12.4 mg/L (*Poecilia reticulata*) have been reported.

Three chronic toxicity test results from two trophic level species were available, a NOErC of 0.67 mg/L and a NOEbC of 1.8 mg/L in algae and a NOEC of 0.53 mg/L for reproduction of daphnids are available.

A predicted no effect concentration (PNEC) of 0.0053 mg/L for the aquatic organisms was calculated from the NOEC for *Daphnia magna* on reproduction using an assessment factor of 100, because only two chronic data (daphnids and algae) are available.

5 RECOMMENDATIONS

Human Health: The chemical is a currently of low priority for further work.

Environment: The chemical is a candidate for further work.

There is no information available on the production volume or use as a solvent, and since the substance shows chronic toxicity to aquatic organisms, an environmental exposure assessment is recommended.

6 REFERENCES

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NTIS (National Technical Information Service): OTS0555329

NTIS (National Technical Information Service): OTS0556686

I U C L I D

D A T A S E T

Existing Chemical	:	ID: 110-83-8
CAS No.	:	110-83-8
EINECS Name	:	cyclohexene
EINECS No.	:	203-807-8
Molecular Formula	:	C6H10

Producer Related Part

Company	:	National Institute of Health & Sciences
Creation date	:	24.12.0002

Substance Related Part

Company	:	National Institute of Health & Sciences
Creation date	:	24.12.0002
Memo	:	
Printing date	:	08.01.2003
Revision date	:	24.12.0002
Date of last Update	:	24.12.2002
Number of Pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD AND COMPANY INFORMATION

Type : sponsor country
Name : Chemicals Evaluation and Research Institute (CERI)
Partner :
Date :
Street : 1-4-25 Koraku, Bunkyo-ku
Town : 112-0004 Tokyo
Country : Japan
Phone : 03-5804-6135
Telefax : 03-5804-6139
Telex :
Cedex :
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
Flag : Critical study for SIDS endpoint
24.07.2002

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : organic
Physical status : liquid
Purity : % w/w
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
24.07.2002

1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

1,2,3,4-Tetrahydrobenzene
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
Flag : Critical study for SIDS endpoint
24.07.2002

Benzenetetrahydride
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
24.07.2002

Cyclohexene
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
24.07.2002

Hexanaphthylene

Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
24.07.2002

Tetrahydrobenzene
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
24.07.2002

1.3 IMPURITIES

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

Type : industrial
Category : Chemical industry: used in synthesis
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
Flag : Critical study for SIDS endpoint
24.07.2002

Type : industrial
Category : other: specific solvent
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
29.07.2002

1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value	:	= -103.5 ° C
Sublimation	:	
Method	:	other: unknown
Year	:	
GLP	:	no data
Test substance	:	no data
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	Melting point is -103.5 degree C.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

31.10.2002 (29)

2.2 BOILING POINT

Value	:	= 83 ° C at 1013 hPa
Decomposition	:	
Method	:	other: unknown
Year	:	
GLP	:	no data
Test substance	:	no data
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	Boiling point is 83 degree C.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

31.10.2002 (29)

2.3 DENSITY

Type	:	density
Value	:	= .81 g/cm3 at 20° C
Method	:	other: unknown
Year	:	1999
GLP	:	no data
Test substance	:	
Source	:	Wako Pure Chemical Industries, Ltd. Chemicals Evaluation and Research Institute (CERI) Tokyo
Test substance	:	Wako Pure Chemical Industries, Ltd. Purity 99.8%
Conclusion	:	Density is 0.810 g/cm3.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

01.11.2002

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= 119 hPa at 25° C
Decomposition	:	
Method		other (measured)
Year	:	

GLP	:	no data
Test substance	:	no data
Source	:	SRC PhysProp Database Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion		Vapour pressure is 210 hPa.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

31.10.2002 (7)

2.5 PARTITION COEFFICIENT

Log pow	:	= 2.99 at 25° C
Method		OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year	:	2001
GLP	:	yes
Test substance	:	
Result	:	The results at each conditions were as follows; Condition 1: 2.96, 3.03 Condition 2: 2.99, 3.00 Condition 3: 2.94, 3.02. The pH was 6.3 - 6.9. The logPow was averaged using all data.
Source	:	Chemicals Evaluation and Research Institute, Japan Chemicals Evaluation and Research Institute (CERI) Tokyo
Test substance	:	Wako Pure Chemical Industries, Ltd. Purity 99.8%
Conclusion	:	LogPow is 2.99.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

31.10.2002 (16)

2.6.1 WATER SOLUBILITY

Value	:	= 250 mg/l at 25 ° C
Qualitative	:	moderately soluble (100-1000 mg/L)
Pka	:	at 25 ° C
PH	:	at and ° C
Method	:	OECD Guide-line 105 "Water Solubility"
Year	:	1999
GLP	:	no
Test substance	:	
Source	:	Chemicals Evaluation and Research Institute, Japan Chemicals Evaluation and Research Institute (CERI) Tokyo
Test substance	:	Wako Pure Chemical Industries, Ltd. Purity 99.8%
Conclusion	:	This chemical is moderately soluble in water (250mg/L).
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

31.10.2002 (16)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value	:	= -12 ° C
Type	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Source	:	Material safety data sheet of Aldrich chemical Co.,Inc Chemicals Evaluation and Research Institute (CERI) Tokyo
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

31.10.2002

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

Memo	:	Viscosity : 0.625mPaS at 25 degree C
Source	:	CRC Handbook of Chemistry and Physics. 75th ed. Chemicals Evaluation and Research Institute (CERI) Tokyo
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

31.10.2002

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	O3
Conc. of sens.	:	700000000000 molecule/cm3
Rate constant	:	= .0000000000000002 cm3/(molecule*sec)
Degradation	:	= 50 % after .8 hour(s)
Deg. Product	:	
Method	:	other (calculated)
Year	:	2002
GLP	:	
Test substance	:	
Remark	:	The reaction rate constant with OH was estimated by SRC AOPWIN. The half-life time (2.086 hours) was calculated based on the calculated rate constant (61.5E-12 cm3/mol-sec) and OH concentration in atmosphere of 1.5E6 OH/cm3. The reaction rate constant with ozone was estimated by SRC AOPWIN. The half-life time (1.375 hours) was calculated based on the calculated rate constant (20E-17 cm3/mol-sec) and ozone concentration in atmosphere of 7E11 mol/cm3. The half-life time with ozone and hydroxyl radical is 0.83 hours.
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	The half-life time with ozone and hydroxyl radical in air is 0.83 hours.
Flag	:	Critical study for SIDS endpoint

01.11.2002

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at degree C
t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Degradation	:	< 10 % after 5 day at pH and 50 degree C
Deg. Product	:	no
Method	:	OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year	:	2000
GLP	:	no
Test substance	:	
Source	:	Chemicals Evaluation and Research Institute, Japan Chemicals Evaluation and Research Institute (CERI) Tokyo
Test substance	:	Wako Pure Chemical Industries, Ltd. Purity 99.8 %
Conclusion	:	This chemical is stable at pH 4, 7 and 9 at 50 degree C for five days.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

31.10.2002 (17)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type :
Media :
Air (level I) :
Water (level I) :
Soil (level I) :
Biota (level II / III) :
Soil (level II / III) :
Method :
Year : 2002
Method : Parameters used in calculation of distribution by Mackay level III fugacity model are as follows;
Melting point: 83 degree C
Vapour pressure: 11900 Pa
Water solbility: 250 mg/L
LogPow: 2.99

As this chemical is not readily biodegradable, we assume the following half-life times;
Half-life time in air: 0.83 hours
Half-life times in water: 240000 hours
Half-life times in soil: 240000 hours
Half-life times in sediment: 720000 hours

Result :

Compartment	Release: 100 % to air	100 % to water	100 % to soil
Air	99.7 %	0.6 %	0.9 %
Water	0.1 %	94.2 %	0.2 %
Soil	0.2 %	0.0 %	98.9 %
Sediment	0.0 %	5.2 %	0.0 %

Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion : If Cyclohexene is released to one of the compartments of air, water and soil, it has a tendency to remain in the original compartment.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
31.10.2002

3.3.2 DISTRIBUTION

Type :
Media : Water-air
Year : 1975
Method : Henry's Law Constant
Result : The reported Henry's Law Constant of cyclohexene is 4.55 E-2 at m-m3/mol
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
Remarks : Reliable secondary data source
Reliability : (2) valid with restriction

Flag : Critical study for SIDS endpoint
22.12.2003 (30)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge
Concentration	:	100mg/l related to Test substance related to
Contact time	:	28 day
Degradation	:	= 0 % after 28 day
Result	:	under test conditions no biodegradation observed
Control substance	:	Aniline
Kinetic	:	**%** **%**
Deg. Product	:	no
Method	:	OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year	:	1999
GLP	:	yes
Test substance	:	other TS
Remark	:	The concentration of activated sludge is 30mg/L. The concentration of control substance (aniline) is 100mg/L.
Result	:	The biodegradations of this chemical were as follows; 0, 0, 0 % by BOD after 28 days 0, 1, 0 % by GC after 28 days.
Source	:	Chemicals Evaluation and Research Institute, Japan Chemicals Evaluation and Research Institute (CERI) Tokyo
Test substance	:	Wako Pure Chemical Industries, Ltd. Purity 99.8%
Conclusion	:	This chemical is not readily biodegradable.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

31.10.2002 (17)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	28 day at 25 degree C
Concentration	:	100µg/l
BCF	:	= 12 - 38
Elimination	:	no
Method	:	OECD Guide-line 305 E "Bioaccumulation: Flow-through Fish Test"
Year	:	2001
GLP	:	yes
Test substance	:	other TS
Result	:	The bioconcentration of the test substance was 12 - 38 at 100 ug/L of test concentration. The bioconcentration of the test substance was 23 - 45 at 10 ug/L of test concentration.
Source	:	Chemicals Evaluation and Research Institute, Japan

Chemicals Evaluation and Research Institute (CERI) Tokyo

Test substance : Wako Pure Chemical Industries, Ltd.
Purity 99.8 %

Conclusion : The bioconcentration potential is low.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

25.07.2002 (17)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC0	:	= 4
LC50	:	= 5.8
LC100	:	= 17
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	2000
GLP	:	yes
Test substance	:	other TS:Kanto Kagaku, Lot. No.; 204D2060, Purity >= 99 %
Method	:	-Test Organisms: a) Supplier: Test organisms were obtained from Takizawa Yougyo-jo (Private Fish Farm, Japan) and had been reproduced in the testing laboratory for four years. b) Size (length and weight): 2.0 cm (1.8-2.0 cm) in length; 0.11 g (0.091 - 0.13 g) in weight c) Age: Not described d) Any pretreatment: Test organisms were acclimated for at least 7 days before testing, any groups showing > 5 % mortality were not used for testing. During acclimination, test fishes were fed with TETRAMINE equivalent to 3% of weight. These test organisms were not fed for 24 hours before the test started. -Test substance: Cyclohexene a) Empirical Formula: C6H10 b) Molecular Weight: 82.15g/mol c) Purity: >=99 % -Test Conditions: a) Dilution Water Source: Dilution water was prepared from tap water (Tama city, Tokyo). The tap water was dechlorinated and treated by activated carbone. After that Residual Chlorine was removed from the water. Before using the dilution water, aeration was fully carried out. b) Dilution Water Chemistry: pH: = 7.9 Total hardness (as CaCO3): = 76 mg/L c) Exposure Vessel Type: 4 L test solution in a 5 L Glass Tank d) Nominal Concentrations: control, solvent control, 3.2, 4.2, 5.6, 7.5, 10, 13 and 18 mg/L e) Vehicle/Solvent and Concentrations: 100mL of Solvent was prepared by dissolving 3g polyoxyethylene sorbitan fattyacid ester in acetone and it was made 100mL. The final concentrations of polyoxyethylene sorbitan fatty acid ester and acetone were at maximum of 3ug/L and 100 uL/L respectively in test solution and in solvent control. f) Number of Replicates: 1 g) Fish per Replicates: 10 h) Renewal Rate of Test Water: Every 24 hours i) Water Temperature: 24+/-1ºC j) Light Condition: 16:8 hours, light-darkness cycle k) Feeding: None l) Aeration : Not described -Analytical Procedure: Thetested concentrations were measured at the start and 24th hour (before exchange of test solution). -Statistical Method:

a) Data Analysis: Probit method for LC50
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Time-weighted mean

Result : - Measured Concentrations : The test concentrations were measured at 0 h and 24 h (before exchange of test solution).
For some of them, the deviation from the nominal were not less than +/- 20%.

Nominal Conc. mg/L	Measured Conc., mg/L 0 Hour Fresh	24 Hours Old	Mean* mg/L	Percent of Nominal 0 Hour Fresh	24Hours Old
Control	<0.10	<0.10	---	---	---
Solvent Control	<0.10	<0.10	---	---	---
3.2	3.31	1.48	2.27	104	46
4.2	3.91	1.88	2.77	93	45
5.6	5.61	2.76	4.02	100	49
7.5	7.99	4.02	5.78	107	54
10	10.3	5.69	7.77	103	57
13	13.7	8.19	10.7	105	63
18	18.5	14.9	16.6	103	83

*: Mean measured concentration (Time-weighted Mean)
Fresh: Start of renewal period
Old: End of renewal period

- Water chemistry (pH and DO) and temperature in test: Water chmistry and temperature were measured for old and renewal solution with control and each concentration at the start of test and every 24 hours.
pH: 7.3 - 8.2
DO: 5.4 - 9.0 mg/L
Water Temperature: 23.9 - 24.3ºC

-Effect Data(mortality):
LC50 (96hr) = 5.8mg/L (mc)
LC0 (96hr) = 4mg/L (mc)
LC100 (96hr) = 17mg/L (mc)
mc: based on measured concentration (time weighted mean)

- Cumulative Mortality: None of test organisms were killed during exposure period at control, solvent control, 3.2, 4.2 and 5.6 mg/L, however all test organisms were killed at 18mg/L on and after 24hours.

Measured Conc. -mg/L	Cumulative Number of Dead (PercentMortality) 24hr	48hr	72hr	96hr
Control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
2.3	0 (0)	0 (0)	0 (0)	0 (0)
2.8	0 (0)	0 (0)	0 (0)	0 (0)
4.0	0 (0)	0 (0)	0 (0)	0 (0)
5.8	7 (70)	7 (70)	7 (70)	7 (70)
7.8	9 (90)	9 (90)	9 (90)	9 (90)
10.7	9 (90)	9 (90)	9 (90)	9 (90)
16.6	---	---	---	---

---: All fishes were dead at this observation time.

-Other Effect:Toxicological symptom was observed at 5.8mg/L (24 hour) and higher concentration.

Nominal Conc. mg/L	Symptoms 24hr	48hr	72hr	96hr
Control	n	n	n	n
Solvent Control	n	n	n	n
2.3	n	n	n	n
2.8	n	n	n	n
4.0	n	n	n	n
5.8	le	le	n	n
7.8	le	le	n	n
10.7	le	le	n	n
16.6	---	---	---	---

n: No abnormalities are detected
le: Lethargy
---: All fishes were dead at this observation time.

- Calculation of toxicity values: The calculation oftoxicity values was the mean measured concentrations. The reason is that some of the error ranges of measured concentration were not less than +/-20% of nominalconcentration.

Source : Ministry of Environment, Japan (2000)
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
19.12.2002 (22)

Type : semistatic
Species : Poecilia reticulata (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
LC0 : = 3.1
LC50 : = 12.4
Method : other:NEN6501, 6502, 6504 and 6506 (1980) Dutch Standardization Organization
Year : 1982
GLP : yes
Test substance : other TS: Fluka, Purity>99.5%
Method : -Test Organisms: The 3 - 4 weeks old fishes were used for test.

-Test substance: Cyclohexene
a) Empirical Formula: C6H10
b) Molecular Weight: 82.15g/mol
c) Purity: >99.5 %

-Test Conditions:
a) Dilution Water Chemistry: Total hardness (ºDH): = 11.7
NaHCO3 = 100 mg/L
CaCl2·H2O = 200mg/L
KHCO3 = 20mg/L
MgSO4·7H2O = 180mg/L

b) Test Volume: 3 L test solution per group
c) Nominal Concentrations: the lowest concentration =4.1mg/L, the highest concentration = 20mg/L, factor=1.8
d) Fish per Group: 10
e) Renewal Rate of Test Water: once per 2days
f) Water Temperature: 23+/-2ºC
g) Light Condition: 14:10 hours, light-darkness cycle

-Analytical Procedure: At 0 hour and 48th hour, samples were taken from the lowest and the highest concentrations.

- Water chemistry (pH and DO) and temperature in test: The pH and Oxygen content of the test solutions were checked at 0 hour and 48th hour.

-

Result : - Measured Concentrations : At 0 hour and 48th hour, samples were taken from the lowest and the highest concentrations.

Nominal Conc. mg/L	Measured Conc., mg/L		Percent of Nominal	
	0 Hour Fresh	48 Hours Old	0 Hour Fresh	48 Hours Old
4.1	3.5	2.7	85.4	65.9
20	17	14	85.0	70.0

Fresh: Start of renewal period
Old: End of renewal period

-Effect Data(mortality, paralysis):
LC50 (96hr) = 12.4mg/L
LC0 (96hr) = 3.1mg/L
EC50 (96hr) = 4.7mg/L

Source : Canton, J. H. and Wegman, R. C. C.(1983)
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
19.12.2002 (5)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : semistatic
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : yes
EC0 : = 1.5
EC50 : = 2.1
Method : OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year : 2000
GLP : yes
Test substance : other TS:Kanto Kagaku, Lot. No.; 204D2060, Purity >= 99 %
Method : - Test Organisms:
a) Age: < 24 hours old
b) Supplier/Source: Test organisms were obtained from National Institute for Environmental Studies (JAPAN) and had been reproduced in the testing

laboratory for three years.
c) Any pretreatment: Parental daphnids were acclimated for at least 14 days on test condition before testing, any groups showing high mortality were not used for testing.
During acclimination, test daphnids were fed with Chlorella vulgaris, 0.1 - 0.2 mg carbon/day/individual.

-Test substance: Cyclohexene
a) Empirical Formula: C6H10
b) Molecular Weight: 82.15g/mol
c) Purity: >=99 %

-Test Conditions:
a) Dilution Water Source: Dilution water was prepared from tap water. The tap water was dechlorinated. After that Residual Chlorine was removed from the water. Before using the dilution water, aeration was fully carried out.
b) Dilution Water Chemistry: pH: = 8.0
Total hardness (as CaCO3): = 81 mg/L
c) Exposure Vessel Type: 250 mL test solution in a 270 mLGlass Beaker with glass cap (closed system)
d) Nominal Concentrations: control, solvent control, 1.0,1.5, 2.2, 3.2, 4.6, 6.8 and 10 mg/L
e) Vehicle/Solvent and Concentrations: 100mL of Solvent was prepared by dissolving 3g polyoxyethylene sorbitan fatty acid ester in acetone and it was made 100mL. At the maximum,100uL/L solvent could be be contained in the test solutions. 100uL/L solvent was contained in solvent control.
f) Number of Replicates: 4
g) Individuals per Replicates: 5
h) Renewal Rate of Test Water: Every 24 hours
i) Water Temperature: 20+/-1ºC
j) Light Condition: 16:8 hours, light-darkness cycle
k) Feeding: None- Analytical Procedure: Test concentrations were measured at the start and 24th hour.

- Statistical Method:
a) Data Analysis: Binominal method for EC50
b) Method of Calculating Mean Measured Concentrations: Time-Weighted Mean

-

Result **:** - Measured Concentrations : The test concentrations were measured at the start and 24th hour (before exchange of test solution). For some of them, the deviation from the nominal were not less than +/-20%

Nominal Conc. mg/L	Measured Conc., mg/L 0 Hour Fresh	24 Hour Old	Mean* mg/L	Percent of Nominal 0 Hour Fresh	24 Hour Old
Control	<0.05	<0.05	---	---	---
Solvent Control	<0.05	<0.05	---	---	---
1.0	1.04	0.779	0.914	104	80
1.5	1.80	1.22	1.49	120	81
2.2	2.52	1.45	1.94	115	66
3.2	3.14	1.89	2.46	98	59
4.6	4.77	2.57	3.56	104	56

6.8	7.35	4.22	5.64	108	62
10	9.87	5.41	7.42	99	54

Fresh: freshly prepared test solution
Old: test solution after 24 hours exposure
*: Mean measured concentration (Time-weighted Mean)

- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for control and each concentration at the start and 24th hour.
pH: 7.8 - 8.1
DO: 8.8 - 9.1 mg/L
Water Temperature: 19.6 - 20.8ºC

-Effect Data:
EC0 (48hr) = 1.5 mg/L (mc)
EC50 (48hr) = 2.1 mg/L (mc)
mc: based on measured concentration

-Mortality or Immobility: No test organism was Immobilized at control, solvent control, 1.0 and 1.5mg/L. The lowest concentration that the test organisms were affected was 1.9mg/L after 48 hours. All test organisms were affected at 3.6, 5.6and 7.4mg/L on and after 24th hour.

Measured Conc. mg/L	Cumulative Number of Dead or Immobilized Daphnids (Percent Mortality or Immobility) 24 Hour	48 Hour
Control	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)
0.91	0 (0)	0 (0)
1.5	0 (0)	0 (0)
1.9	0 (0)	3 (15)
2.5	10 (50)	20 (100)
3.6	20 (100)	20 (100)
5.6	20 (100)	20 (100)
7.4	20 (100)	20 (100)

- Calculation of toxic values: Based on the mean measured concentrations.

Source : Ministry of Environment, Japan (2000)
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
19.12.2002 (22)

Type : semistatic
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : yes
EC0 : = 3.8
EC50 : = 5.3
Method : other:NEN6501, 6502, 6504 and 6506 (1980) Dutch Standardization Organization
Year : 1983
GLP : yes

Test substance	:	other TS: Fluka, Purity>99.5%
Method	:	- Test Organisms: The 24 hours old daphnids were used for test. -Test substance: Cyclohexene a) Empirical Formula: C6H10 b) Molecular Weight: 82.15g/mol c) Purity: >99.5 % -Test Conditions: a) Dilution Water Chemistry: Total hardness (ºDH): = 11.7 NaHCO3 = 100 mg/L CaCl2·H2O = 200mg/L KHCO3 = 20mg/L MgSO4·7H2O = 180mg/L b) Test Volume: 1 L test solution per group c) Nominal Concentrations: factor=1.8 d) Individuals per Group: 25 e) Water Temperature: 19+/-1ºC f) Light Condition: 12:12 hours, light-darkness cycle-Analytical Procedure: At 0 hour and 48th hour, samples were taken form the lowest and the highest concentrations. - Water chemistry (pH and DO) and temperature in test: The pH and Oxygen content of the test solutions were checked at 0 hour and 48th hour. -
Result	:	-Effect Data: EC50 (48hr) = 5.3 mg/L LC50 (48hr) = 9.4 mg/L EC0(48hr) = 3.8 mg/L
Source	:	Canton, J. H. and Wegman, R. C. C. National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
Reliability	:	(4) not assignable - Remark: Details on test condition and results are not available. - Remark: Details on test condition and results are not available.

20.12.2002 (6)

Type	:	
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
EC0	:	= 563
EC50	:	= 720
EC100	:	= 750
Method	:	
Year	:	1982
GLP	:	
Test substance	:	other TS
Method	:	- Test Organisms: The 24 hours old daphnids (IRCHA strain) were used for test. -Test Conditions: a) Dilution Water: For reasons of national and international standardization, artificial fresh water according to DIN (Deutsches Institut fuer Normung) was used. This water was aerated to oxygen saturation level and the pH was measured (8.0 +/- 0.2). b) Test Volume: 20 mL test solution in a 50 mL beaker

c) Water Temperature: approximately 20ºC
d) Number of Replicates: 2

- Effect of end point: When the test period (24 hr) was over, the test organisms that could swim were counted.-

Water chemistry (pH and DO) and temperature in test: At the end of the test period pH values and dissolved oxygen concentration were measured in the test and control vesseles. The dissolved oxygen concentration had not dropped below 2mg/L at the end of the test period.

Source : National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki

Reliability : (4) not assignable -
Remark: Details of the test were not available and test was a 24-h exposure.

Remark -:Details of the test were not available and test was a 24-h exposure.
20.12.2002 (3)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : yes
NOEC : = .67
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 2000
GLP : yes
Test substance : other TS:Kanto Kagaku, Lot. No.; 204D2060, Purity >= 99 %
Method : - Test Organisms:
a) Supplier/Source: Obtained from American Type Culture Collection and reproduced in aseptic culture for 6 months.
b) Method of Cultivation: Sterile
c) Stain Number: ATCC22662

-Test substance: Cyclohexene
a) Empirical Formula: C6H10
b) Molecular Weight: 82.15g/mol
c) Purity: >=99 %

- Test Conditions:

a) Medium: OECD medium
b) Exposure Vessel Type: 100 mL Medium in a 500mL Erlenmeyer Flask with glass cap (closed system)
c) Nominal Concentrations: control, solvent control, 1.8, 3.2, 5.6, 10 and 18 mg/L
d)Vehicle/Solvent and Concentrations: 100mL of Solvent was prepared by dissolving 3g polyoxyethylene sorbitan fatty acid ester in acetone and it was made 100mL The final concentration of polyoxyethylene sorbitan fatty acid ester and acetone were at maximum 3ug/L and 100 uL/L respectively in test solution and in solvent control.
e) Stock Solutions Preparations and Stability: Not described.

f) Number of Replicates: 3
g) Initial Cell Number: 10,000 cells/mL
h) Water Temperature: 23+/-2ºC
i) Light Condition: 4,000 - 5,000 lux, continuously
j)Shaking: 100 rpm

- Analytical Procedure: Test concentrations were measured at the start and the 72nd hour using GC-MS with detection limit of 0.05 mg/l.

- Statistical Method:
a) Data Analysis: regression analysis for EC50, and Dunnett multiple comparison for NOEC,
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): geometric mean

Remark : Some deviations were present in this study. These are
1) Dispersant was used as a solvent although the concentration of test substance was not greater than it's solubility.
2) Light intensity was shown as a unit of lux instead of E/m2/s
3) Exponential growth had not kept throughout the test period
4) EC50 was extrapolated using limited data without value near EC5

Result : - Measured Concentrations : The tested concentrations were measured at the start and the 72nd hour.

- All the error ranges of measured concentration were not less than +/- 20% of nominal concentration. Especially the concentrations of 72 hours after were low. Those were 4 - 8% of nominal.

Nominal conc. mg/L	Measured Conc., mg/L		Percent of nominal		Mean*
	0 Hour	72 Hour	0 Hour	72 Hour	
Control	<0.05	<0.05	---	---	---
Solvent Control	<0.05	<0.05	---	---	---
1.8	2.02	0.0686	112	4	0.37
3.2	3.37	0.133	105	4	0.67
5.6	6.72	0.458	120	8	1.8
10	10.0	0.484	100	5	2.2
18	17.2	0.741	96	4	3.6

*Geometric Mean

- Water chemistry (pH) in test: pH was measured for control and each concentration at the start and end of test
pH: 8.0 - 10.6

-Effect Data:biomass
Area Method
EbC50(0-72hr) not available
NOEC (0-72hr) = 1.8 mg/L (mc)

Rate Method
ErC50(24-48hr) = not available
NOEC (24-48hr) not available
ErC50(0-72hr) not available
NOEC (0-72hr) = 0.67 mg/L (mc)
mc: mean measured concentration

- Percent Growth Inhibition of Selenastrum capricornutum

Measured	Area under the growth curves (Average)

Conc. mg/L	Area A (0-72hr)	Inhibition (%)* IA (0-72hr)
Solvent Control	23,176,000	---
0.37	26,960,000	-16.3
0.67	21,976,000	5.2
1.8	20,328,000	12.3
2.2	19,504,000	15.8*
3.6	18,312,000	21.0*

Mean Measured Conc. mg/L	Growth rates and percent inhibition (Average) Rate (24-48hr)	Inhibition(%) (24-48hr)	Rate (0-72hr)	Inhibition(%) (0-72hr)
Solvent Control	0.069538	---	0.044231	---
0.37	0.072891	-4.82	0.043983	0.56
0.67	0.063976	8.00	0.039025	11.8
1.8	0.068153	1.99	0.038331	13.3**
2.2	0.061519	11.53	0.038339	13.3*
3.6	0.059582	14.32	0.036012	18.5**

*: alpha = 0.05 (significant difference)
**: alpha = 0.01 (significant difference)Inhibition(%) was calculated by comparing result in the solvent control

- Growth Curves: Log phase during the test period

- Growth Curves: Until 48 hrs, logarithmic growth was occurred, but growth rate during 48-72 was smaller than that of first 48 hr slightly at all concentrations.

- Calculation of toxicity value: Did not calculate for EC50s because the highest inhibition rate was below 50 %: Inhibition rates by a area method and a rate method were calculated 21% and 18.5% at the highest concentration of 3.6 mg/L respectively.

-For N(L)OECs, measured relative concentrations to those in solvent control were confirmed their homogeneity of variances by Bartlett's test and then Dunnett's multiple comparison test were carried out. The results were shown in the above tables as indicated by asterisks.

-

Source : Ministry of Environment, Japan (2000)
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki

Reliability : (2) valid with restrictions
-
Remark:
There are some deviation from OECD TG 201 and guidance document 23 as below.

1) The test substance were removed during test period up to 96% however the test was conducted by closed system. One of the reason for that was due to use vessels which had a too large headspace. This substance was expected as a volatile and it should be used smaller vessels to prevent to loss of the substance.

2) Any EC50s could not be deriven. At the highest concentration the

inhibition rate was showed only 18.5 % based on rate method calculation.

Flag : Critical study for SIDS endpoint
20.12.2002 (23)

Species : Chlorella pyrenoidosa (Algae)
Endpoint : biomass
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
NOEC : = .22
EC50 : = 3.8
Method : other: NEN6501, 6502, 6504 and 6506 (1980) Dutch Standardization Organization
Year : 2000
GLP : yes
Test substance : other TS: Fluka, Purity>99.5%
Method : -Test substance: Cyclohexene
a) Empirical Formula: C6H10
b) Molecular Weight: 82.15g/mol
c) Purity: >99.5 %

- Test Conditions:
a) Compounds of standard media: Total hardness (ºDH): = 30.2

$CaCl_2 \cdot H_2O$ = 35mg/L
$MgSO_4 \cdot 7H_2O$ = 75mg/L
K_2HPO_4 = 52mg/L
Citric acid = 6mg/L
Na_2CO_3 = 500mg/L
$Na_2CO_3 \cdot H_2O$ = 54mg/L
Ferricitrate = 6mg/L
NH_4NO_3 = 330mg/L

b) Test Volume: 300mL test solution per group
c) Nominal Concentrations: factor=1.8
d) Algae per Group: 10,000 cells/L
e) Water Temperature: 24+/-1ºC
f) Light Condition: 5,000 lux, continuously

- Analytical Procedure: No analyses were performed.

- Water chemistry (pH and DO) and temperature in test: The pH and Oxygen content of the test solutions were checked at 0 hour and 48th hour.

Result : -Effect Data:Area Method (biomass)
EbC50(48hr) =3.5 mg/L (nc)
NOEC (48hr) = 0.22 mg/L (nc)
nc: based on nominal concentration

Source : Canton, J. H. And Wegman, R. C. C.
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
Reliability : (4) not assignable
Remark - Test substance concentration was no measured.
20.12.2002 (6)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	Chilomonas sp. (Protozoa)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
Method	:	
Year	:	1980
GLP	:	
Test substance	:	
Method	:	- Test Conditions: a) Exposure Vessel: 300mL Erlenmeyer Flask with metal cap b) Number of Replicates: 2 c) pH of test solution: adjusted to 6.9 d) Water Temperature: 20ºC -Effect of end point: If cell counts in the test cultures are 5% below the average of the counts in reference cultures free of toxic influence, this may be considered as an initial inhibition of protozoan cell multiplication by pollutant and thus serve for determining the toxicity threshold for that particular pollutant.
Result	:	-Effect Data: The threshold toxicity concentration for initial influence of the cyclohexene at the exposure period for 48th hour was >160 mg/L.
Source	:	National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
Reliability	:	(4) not assignable
Remark		- Insufficient information on test condition

20.12.2002 (4)

Type	:	aquatic
Species	:	Uronema parduzci (Protozoa)
Exposure period	:	20 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
Method	:	
Year	:	1980
GLP	:	
Test substance	:	
Method	:	- Test Conditions: a) Exposure Vessel: 300mL Erlenmeyer Flask with metal cap b) Number of Replicates: 2 c) pH of test solution: adjusted to 6.9 d) Water Temperature: 20ºC -Effect of end point: If cell counts in the test culturesare 5% below the average of the counts in reference cultures free of toxic influence, this may be considered as an initial inhibition of protozoan cell multiplication by pollutant and thus serve for determining the toxicity threshold for that particular pollutant.
Result	:	-Effect Data: The threshold toxicity concentration for initial influence of the cyclohexene at the exposure period for 20th hour was >50 mg/L.

-

Source : National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki

Reliability : (4) not assignable

Remark - Insufficient information on test condition

20.12.2002 (2)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day
Unit	:	mg/l
Analytical monitoring	:	yes
NOEC	:	= .53
LCEC	:	= .74
EC50	:	= 1
Method	:	other: OECD Guide-line 211
Year	:	2000
GLP	:	yes
Test substance	:	other TS:Kanto Kagaku, Lot. No.; 204D2060, Purity >= 99 %
Method	:	-Test Organisms: a) Age: < 24 hours old b) Supplier/Source: Test organisms were obtained from National Institute for Environmental Studies (JAPAN) and had been reproduced in the testing laboratory for 2 years and 6 months. c) Any pretreatment: Parental daphnids were acclimated for at least 21 days on test conditions before testing, any groups showing high mortality were not used for testing. -Test substance: Cyclohexene a) Empirical Formula: C6H10 b) Molecular Weight: 82.15g/mol c) Purity: >=99 % - Test Conditions: a) Dilution Water Source: Dilution water was prepared from tap water. The tap water was dechlorinated. After that Residual Chlorine was removed from the water. Before using the dilution water, aeration was fully carried out. b) Dilution Water Chemistry: pH: = 8.0 Total hardness (as CaCO3): = 81 mg/L c) Exposure Vessel Type: 80 mL test solution in a heat-resistance glass jar with glass screw cap d) Nominal Concentrations: control, solvent control, 1.0, 1.5, 2.2, 3.2, 4.6, 6.8 and 10 mg/L e) Vehicle/Solvent and Concentrations: 100mL of Solvent was prepared by dissolving 3g polyoxyethylene sorbitan fatty acid ester in acetone and it was made 100mL. In all concentrations except contol, 100uL/L of solvent solution was added. The final concetration of polyoxyethylene sorbitan fatty acid ester was 3ug/L

f) Stock Solutions Preparations and Stability: prepared with the test substance using the solvent solution (3 g polyoxyethylene sorbitan fatty acid ester in per 100 ml acetone) as the dilutant. Stability was not checked.
g) Number of Replicates: 10
h) Individuals per Replicates: 10
i) Renewal Rate of Test Water: Every 48 hours
j) Water Temperature: 20+/-1oC
k) Light Condition: 16:8 hours, light-darkness
l) Feeding: 0.1 - 0.2 mg carbon/day/individual (Chlorella vulgaris: Green Algae)

- Analytical Procedure: The test concentrations were measured for both renewal and old test solution at the start of test and 2nd, 6th 8th, 14th and 16th day.

- Statistical Method:
a) Data Analysis:
t-test for NOEC and LOEC
Doudoroff method for EC50
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Time-weighted Mean

-

Remark : NOEC was determined based on the cumulative number of alive juveniles produced per adult alive for 21 days.

Result : - Effect: reproduction- Measured Concentrations : The test concentrations were measured for both renewal and old test solution at the start of test and 2nd, 6th 8th, 14th and 16th day. For some of them, the deviation from the nominal concentration was not less than +/- 20%.

Nominal Conc. mg/L	Measured Conc., mg/L							
Date	0	2	6	8	14	16	TWM*	% of
	Fresh	Old	Fresh	Old	Fresh	Old	mg/L	Nominal
Control	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	---	---
Solvent Control	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	---	---
1.0	1.15	0.05	1.08	<0.05	1.15	<0.05	0.27	27
1.5	1.69	0.08	1.58	0.06	1.67	0.13	0.53	35
2.2	2.49	0.13	2.27	0.08	2.31	0.13	0.74	34
3.2	3.40	0.22	3.55	0.18	3.54	0.21	1.17	37
4.6	4.90	0.38	4.67	0.31	5.42	0.37	1.75	38
6.8	8.03	0.52	6.83	1.27	---	---	3.02	44
10	11.5	0.77	---	---	---	---	3.97	40

Fresh: Start of renewal period
Old: End of renewal period
*: Time-weighted mean of measured concentration during 21days
---: No measured was made because all daphnids were dead at this time.

- Measured Concentration as a Percentage of Nominal

Nominal Concentration mg/L	Measured Concentration as a Percentage of Nominal					
Date	0	2	6	8	14	16
	Fresh	Old	Fresh	Old	Fresh	Old
1.0	115	5	108	<1	112	<1

1.5	113	5	105	4	111	9
2.2	113	6	103	4	105	6
3.2	106	7	111	6	111	7
4.6	107	8	102	7	118	8
6.8	118	8	100	19	---	---
10	115	8	---	---	---	---

Fresh: Start of renewal period
Old: End of renewal period
---: No measured was made because all daphnids were dead at this time.

-
- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for control and each concentration at the start of test and before and after renewal of the test solutions.
pH: 7.7 - 8.8
DO: 8.4 - 10.2 mg/L
Water Temperature: 19.6 - 20.8ºC

- Total hardness: 80 - 82 mg/L

-Effect Data:
EC50 (21day) = 1.0 mg/L (mc)
NOEC (21day) = 0.53 mg/L (mc)
LOEC (21day) = 0.74 mg/L (mc)
mc: based on measured concentration (Time weighted mean)

- Cumulative Number of Died Parental Daphnids: No test organism was killed at control solvent control, 1.0, 1.5 and 2.2 mg/L. The lowest concentration that test organisms were dead was at 3.2 mg/L after 4days. All test organisms were dead at 6.8 mg/L after 9days and at 10 mg/L after 4days.

Nominal Conc. (mg/L)	Cumulative Number of Dead Parental Daphnids (days) 1	2	3	4	5	6	7	8	9	10
Control	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0
2.2	0	0	0	0	0	0	0	0	0	0
3.2	0	0	0	1	1	1	1	1	1	1
4.6	0	0	0	2	5	6	6	6	6	6
6.8	0	0	0	4	5	5	7	9	10	10
10	0	0	7	10	10	10	10	10	10	10

Nominal Conc. (mg/L)	Cumulative Number of Dead Parental Daphnids (days) 11	12	13	14	15	16	17	18	19	20	21
Control	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0	0
2.2	0	0	0	0	0	0	0	0	0	0	0
3.2	1	1	1	1	1	1	1	1	1	2	2
4.6	6	8	8	9	9	9	9	9	9	9	9
6.8	10	10	10	10	10	10	10	10	10	10	10

10	10	10	10	10	10	10	10	10	10	10	10

-Effect Data(reproduction):Juveniels were first producted on the 8th day control and all concentrations. At 4.6 mg/L, no juveniele was producted.

Nominal Conc. mg/L	Mean Cumulative Numbers of Juvenieles Produced per Adult (days)							
	0 --- 6	7	8	9	10	11	12	13
Control	0 --- 0	1.9	6.1	7.3	18.9	25.4	29.4	50.3
Solvent Control	0 --- 0	5.6	10.7	12.3	29.3	31.4	34.0	62.9
1.0	0 --- 0	2.3	6.2	9.3	18.7	26.0	32.2	49.9
1.5	0 --- 0	1.1	6.7	8.4	14.1	26.8	30.2	44.4
2.2	0 --- 0	0.0	0.5	1.4	11.9	13.9	19.8	30.8
3.2	0 --- 0	0.0	0.0	0.0	1.5	2.0	3.5	6.8
4.6	0 --- 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.8	0 --- 0	0.0	D	D	D	D	D	D
10	0 --- 0	D	D	D	D	D	D	D

Nominal Conc. mg/L	Mean Cumulative Numbers of Juvenieles Produced per Adult (days)							
	14	15	16	17	18	19	20	21
Control	58.9	62.1	82.8	91.7	94.7	110.3	123.1	127.2
Solvent Control	65.9	69.7	99.5	102.9	106.8	137.0	140.5	144.3
1.0	60.4	65.6	84.0	94.4	101.8	120.2	131.1	138.4
1.5	61.8	64.8	78.3	95.9	98.6	112.7	129.8	133.2
2.2	34.7	44.7	67.3	70.8	83.3	105.3	109.8	119.7
3.2	8.4	11.9	18.8	20.5	25.6	31.4	37.3	43.3
4.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.8	D	D	D	D	D	D	D	D
10	D	D	D	D	D	D	D	D

D: The parental daphnids were dead during a 21-day testing period

-Cumulative numbers of juveniles produced per adult alive for 21days

Vessel No.	Solv Cont.	Nominal Conc., mg/L (Measured Conc.1) , mg/L) 2) 1.0 (0.27)	1.5 (0.53)	2.2 (0.74)	3.2 (1.17)	4.6 (1.75)	6.8 (3.02)	10 (3.97)
1	146	129	149	144	47	D	D	D
2	146	145	131	104	27	D	D	D
3	151	144	131	93	55	D	D	D
4	161	154	130	118	54	D	D	D
5	152	124	134	109	D	D	D	D
6	140	135	139	148	47	0	D	D
7	128	133	108	136	D	D	D	D
8	143	135	128	105	14	D	D	D
9	127	147	140	102	55	D	D	D
10	149	138	142	138	47	D	D	D
Mean	144.3	138.4	133.2	119.3	43.3	0.0	---	---

S. D. 10.5 9.1 11.0 20.0 14.9 --- --- ---

Inhibiton
rate(%) --- 4.1 7.7* 17.0** 70.0** 100 100 100 100

---

1): Time-wighted mean measured concentration
2): Solvent control; Inhibition rate was calculated versus solvent control.
*: significant (alpha = 0.05)
**: significant at alpha = 0.01 level
D: Were not included for calculation because the parental daphnids died.

- Calculation of toxicity values: The calculation of toxicity values was the mean measured concentrations. The reason is that some of the error ranges of measured concentration were not less than +/-20% of nominal concentration.

Source : Ministry of Environment, Japan (2000)
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
19.12.2002 (23)

Species : Daphnia magna (Crustacea)
Endpoint : reproduction rate
Exposure period : 15 day
Unit : mg/l
Analytical monitoring : yes
NOEC : = 2.4
LCEC : = 2.9
EC50 : = 4
Method : other: NEN6501, 6502, 6504 and 6506 (1980) Dutch Standardization Organization
Year : 1982
GLP :
Test substance : other TS: Fluka, Purity>99.5%
Method : - Test Organisms: The 24 hours old daphnids were used fortest.

-Test substance: Cyclohexene
a) Empirical Formula: C6H10
b) Molecular Weight: 82.15g/mol
c) Purity: >99.5 %

-Test Conditions:
a) Dilution Water Chemistry: Total hardness (ºDH): = 11.7
NaHCO3 = 100 mg/L
CaCl2·H2O = 200mg/L
KHCO3 = 20mg/L
MgSO4·7H2O = 180mg/L
b) Test Volume: 1 L test solution per group
c) Renewal Rate of Test Water: Once per 2 - 3 days
d) Nominal Concentrations: ratio=1.8
e) Individuals per Group: 15
f) Water Temperature: 19+/-1ºC
g) Light Condition: 12:12 hours, light-darkness cycle

-Analytical Procedure: At 0 hour and 48th hour, samples were taken from the lowest and the highest concentrations for analysis to get an impression about the actual concentrations during test.

- Water chemistry (pH and DO) and temperature in test: The pH and Oxygen content of the test solutions were checked at 0 hour and 48th hour.

Result : -Effect Data:
EC50 (15days) = 2.9 mg/L
LC50 (15days) = 4.0 mg/L
NOEC (15days) = 2.4 mg/L

Source : National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki
National Institute of Environmental Studies, Environment Agency Tsukuba-Ibaraki

Reliability : (4) not assignable

Remark - Method has a deviation from an aditional standard, and the detail was not available to assess reliability.

20.12.2002 (6)

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Crj: CD(SD)
Sex	:	male/female
Number of animals	:	5
Vehicle	:	other:corn oil
Method	:	OECD Guide-line 401 "Acute Oral Toxicity"
Year	:	2002
GLP	:	yes
Test substance	:	other TS
Remark	:	Doses were 0, 500, 1000, and 2000mg/kgbw for both sexes.
Result	:	LD50 value was greater than 1,000 mg/kg bw. Each 3 of 5 animals of male and female rats at 2,000 mg/kg bw showed abnormal gait, adoption of a prone position, salivation, piloerection and tremor, and then died within 3 days after dosing. Hypoactivity was observed in all male and female rats given the test substance. Lacrimation was also observed in both sexes just after dosing at 1,000 mg/kg bw and more. Necropsy of the dead animals revealed pulmonary congestion. Mortality: Dose(mg/kgbw) 0 500 1000 2000 No.of animals 5 5 5 5 No.of death Male 0 0 0 3 Female 0 0 0 3
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa
Test substance	:	Purity:98.6%
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

17.07.2002 (18)

Type	:	LD50
Species	:	mouse
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Value	:	> 3.2 ml/kg bw
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Result	:	Toxic effects: Altered sleep time including change in righting reflex, somnolence (general depressed activity) and ataxia.
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (26)

Type	:	LD50
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	other
Value	:	= 2.4 ml/kg bw
Method	:	other
Year	:	

GLP	:	no data
Test substance	:	no data
Result	:	Toxic effects: Somnolence (general depressed activity), tremor and ataxia
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (25)

Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

03.06.2002

5.1.2 ACUTE INHALATION TOXICITY

Type	:	other:Lethal concentration
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Exposure time	:	4 hour(s)
Value	:	> 6370 ppm
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Result	:	Toxic effects: Tremor and ataxia.
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (27)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Species	:	guinea pig
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Value	:	> 20 ml/kg bw
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Result	:	Details of toxic effects were not reported other than lethal dose value.
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (28)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LC50
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data

Route of admin.	:	other:no data
Exposure time	:	
Value	:	= 2000 mg/kg bw
Method	:	
Year	:	1985
GLP	:	no data
Test substance	:	no data
Result	:	Details of toxic effect were not reported other than lethal dose value.
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (10)

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	other:Crj:CD(SD)IGS
Route of admin.	:	gavage
Exposure period	:	Males:48 days Females:42-53 days from 14 days before mating to day 4 of lactation
Frequency of treatment	:	Once a day
Post obs. period	:	None
Doses	:	50, 150, 500 mg/kgbw
Control group	:	yes, concurrent vehicle
NOAEL	:	= 50 mg/kg bw
Method	:	OECD combined study TG422
Year	:	2002
GLP	:	yes
Test substance	:	other TS
Remark	:	This study was conducted to examine both repeated dose toxicity and reproductive/developmental toxicity as an OECD screening combined study (Test guideline: 422). Study design: Vehicle: Corn oil Clinical observation performed and frequency: General condition was observed once a day, body weights were determined at days 1(before dosing),8,15,22,29,36,43 and 49 of treatment for males and at days 1, 8 and 15 of treatment and at days 0,7,14,and 20 of gestation period and at days 0 and 4 of lactation period and at autopsy for females, food consumption was determined at days 1,8,15,22,29,36,43 and 48 of treatment for males and at days 1,8 and 15 of treatment and at days 0, 7,14 and 20 of gestation period and at days 0 and 4 of lactation for females, but food consumption were not determined during mating period for males and females. For 5 males per group, urinalysis was carried out at 43-48 days of administration period. For all males and all females after childbirth, hematology and biochemistry were carried out at time of necropsy after 49

days for males and at 5 days after delivery for females. Organs examined at necropsy.
Organ weight: Brain, liver, kidney, spleen, adrenal, thymus, testis and epididymis
Microscopic examination: Brain, pituitary, thymus, thyroid, parathyroid, adrenal, spleen, heart, thoracic aorta, tongue, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, larynx, trachea, lung, kidney, urinary bladder, testis, epididymis, prostate, seminal vesicle, ovary, uterus, vagina, eye, harderian gland, mammary gland, skin, sternum, femur, spinal cord, skeletal muscle, mesentery lymph node, mandibular lymph node, submandibular gland, sublingual gland, parotid gland, ischiadic nerve, bone marrow,
Statistical methods: Dunnett's test for continuous data and Steel test for quantal data

Result : Mortality: There was no mortality related to the test substance treatment.
Clinical signs: Salivation was apparent in three animals of 150 mg/kg bw group and in twelve animals of 500 mg/kg bw group for males and in two animals of 150 mg/kg bw group and twelve animals of 500 mg/kg bw group for females. Although the grades of salivation were not reported, the sign was observed for about 5 minutes after dosing at 150 mg/kg bw , and for 30 minutes to 5 hours after dosing at 500 mg/kg bw during treatment period. In addition, lacrimation was observed in two animals of 500 mg/kg bw group for males and in one animal each of 150 and 500 mg/kg bw groups for females. The onsets and grades of lacrimation were not reported.
Body weight: No statistically significant changes for males and females.
Food consumption: No effects for males and females.
Urinalysis: No statistically significant changes.
Hematology: No effects for males and females
Blood biochemistry: Males: Decreases in triglyceride in 150 and 500 mg/kg bw groups, increases in total bilirubin in 500 mg/kg bw group, and total bile acid in 150 and 500 mg/kg bw.

Dose (mg/kg bw)		0	50	150	500
No.of animals		12	11	12	12
Triglyceride (mg/dL)	Mean	39.2	6.8	27.7	22.5
	SD	22.4	18.8	16.7	7.7
T.bilirubin (mg/dL)	Mean	0.03	0.04	0.05	0.05*
	SD	0.01	0.01	0.01	0.01
T.bile acid (umol/L)	Mean	18.8	20.8	39.9*	32.6
	SD	15.0	16.6	21.0	25.5

Note:*,P<0.05

Females: Increase in total bile acid in 50, 150, and 500 mg/kg bw.

Dose (mg/kg bw)		0	50	150	500
No. of animals		10	10	10	10
T.bile acid (umol/L)	Mean	19.3	49.2*	31.2	82.2*
	SD	8.6	28.8	19.7	81.1

Note:*,p<0.05

Necropsy and histopathology: No adverse effects for males and females
Organ weights: Males: Increase in a relative kidney weight in 500 mg/kg bw group.

Dose (mg/kg bw)		0	50	150	500
No.of animals		12	11	12	12
Kidney					
Absolute (g)	Mean	3.21	3.09	3.20	3.31
	SD	0.33	0.27	0.27	0.27
Relative (g%)	Mean	0.652	0.619	0.667	0.705*

SD 0.057 0.031 0.059 0.053
Note:*, p<0.05
Females: No statistically significant changes.

Histopathology: No changes related to test substance.

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa
Test substance : Purity:98.6%
Conclusion : Increase in total bile acid noted in females of 50 mg/kg bw was not considered as an adverse effect because of no accompanying changes. Therefore, based on salivation observed at 150 mg/kg bw, the NOAEL for repeated dose toxicity was considered to be 50 mg/kg bw/day.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.07.2002 (19)

Species : rat
Sex : male
Strain : no data
Route of admin. : inhalation
Exposure period : 6 months
Frequency of treatment : 6 hrs/day, 5 days /week
Post obs. period : none
Doses : 75, 150, 300, 600 ppm
Control group : yes, concurrent vehicle
Method : other
Year : 1976
GLP : no data
Test substance : no data
Remark : Animal: 20 rats per group.
Cyclohexene levels were determined by continuous monitoring of the chambers, using an automatic sampling system connected to a Carlo Erba gas chromatograph. Humidity, temperature, and pressure were also monitored inside and outside the chambers. Body weight of all animals was recorded weekly. Hematological profile (WBC, RBC, PI, Hb, Ht, differential count) was obtained before, during, and after exposure. Biochemical profile (glucose, BUN, cholesterol, SGOT, SGPT, LDH, alkaline phosphatase, electrolytes, etc.) and gross pathology of the hemopoietic organs were carried out after 6 months of exposure.
Result : Significant increase in body weight($p<0.05$) was observed in the rats exposed 600 ppm, and significant increase in alkaline phosphatase($P<0.01$-0.02) was observed in all groups of rats exposed to cyclohexene.
Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa
12.07.2002 (11)

Species : guinea pig
Sex : male
Strain : no data
Route of admin. : inhalation
Exposure period : 6 months
Frequency of treatment : 6 hrs/day, 5 days/week
Post obs. period : none
Doses : 75, 150, 300, 600 ppm
Control group : yes, concurrent vehicle
Method : other
Year : 1976
GLP : no data

Test substance	:	no data
Remark	:	Animal: 10 guinea pigs per group. Cyclohexene levels were determined by continuous monitering of the chambers, using an automatic sampling system connected to a Carlo Erba gas chromatograph. Humidity, temperature, and pressure were also monitored inside and outside the chambers. Body weight of all animals was recorded weekly. Hematological profile (WBC, RBC, PI, Hb, Ht, differential count) was obtained before, during, and after exposure. Biochemical profile (glucose, BUN, cholesterol, SGOT, SGPT, LDH, alkaline phosphatase, electrolytes, etc.) and gross pathology of the hemopoietic organs were carried out after 6 months of exposure.
Result	:	No significant changes were observed in all groups of guinea pigs exposed to cyclohexene.
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (11)

Species	:	rabbit
Sex	:	male
Strain	:	no data
Route of admin.	:	inhalation
Exposure period	:	6 months
Frequency of treatment	:	6 hrs/day, 5 days/week
Post obs. period	:	none
Doses	:	75, 150, 300, 600 ppm
Control group	:	yes, concurrent vehicle
Method	:	other
Year	:	1976
GLP	:	no data
Test substance	:	no data
Remark	:	Animal: 6 rabbits per group. Cyclohexene levels were determined by continuous monitoring of the chambers, using an automatic sampling system connected to a Carlo Erba gas chromatograph. Humidity, temperature, and pressure were also monitored inside and outside the chambers. Body weight of all animals was recorded weekly. Hematological profile (WBC, RBC, PI, Hb, Ht, differential count) was obtained before, during, and after exposure. Biochemical profile (glucose, BUN, cholesterol, SGOT, SGPT, LDH, alkaline phosphatase, electrolytes, etc.) and gross pathology of the hemopoietic organs were carried out after 6 months of exposure.
Result	:	No significant changes were observed in all groups of rabbits exposed to cyclohexene.
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (11)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Test spicies/strain: Salmonella typhimurium TA100, TA1535, TA98, TA1537, Escherichia coli WP2 uvrA
Concentration	:	See "Remark"
Cycotoxic conc.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other:Chemical Substance Control Law of Japan and OECD Test Guideline 471
Year	:	2002

GLP	:	yes
Test substance	:	other TS
Remark	:	Procedures: Pre-incubation method Solvent: Ethanol Dosage of each strain with or without S9 -S9 mix: 0, 19.5, 39.1, 78.1, 156, 313, 625, 1250 ug/plate (TA100, TA1535, TA98, TA1537); 0, 78.1, 156, 313, 625, 1250, 2500, 5000 ug/plate (WP2 uvrA) +S9 mix: 0, 19.5, 39.1, 78.1, 156, 313, 625, 1250 ug/plate (all strain) *Maximum concentration was established based on the result of the preliminary test up to 5000 ug/plate. In this test, the growth inhibition was observed at 1250 ug/plate and more with and without S9 mix in Salmonella typhimurium TA100, TA98, TA1535, TA1537 and with S9 mix in Escherichia coli WP2 uvrA. Positive control: without S9 mix: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2 uvrA), Sodium azide (TA 1535), 2-methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acriine 2HCl with S9 mix: Benzo[a]pyrene (TA100, TA98), 2-aminoanthracene (TA1535, WP2 uvrA, TA1537) S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone Plates/test: 3
Result	:	There were no precipitations in any test concentration. Cytotoxic concentration: Growth inhibition was observed at 625 ug/plate or more with or without S9 mix in Salmonella typhimurium TA100, TA1535, TA98, TA1537, and at 1250 ug/plate or more with S9 in Escherichia coli WP2 uvrA. Genotoxic effects: With metabolic activation: negative Without metabolic activation: negative
Source	:	Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa
Test substance	:	Purity:98.63%
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

17.07.2002 (20)

Type	:	Chromosomal aberration test
System of testing	:	Type of cell used: Chinese hamster lung(CHL/IU) cell
Concentration	:	0, 100, 150, 200, 250, 300, 350, 400 ug/mL
Cycotoxic conc.	:	400 ug/mL
Metabolic activation	:	with and without
Result	:	negative
Method	:	other:Chemical Substances Control Law of Japan and OECD Test Guideline 473
Year	:	2002
GLP	:	yes
Test substance	:	other TS
Remark	:	Solvent: Ethanol S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone Plates/test: 2 The maximum concentration was established, based on the growth inhibition test. In this test, 50% growth inhibition was observed between 250 and 300 ug/mL for short-term treatment and continuous treatment with or without S9.
Result	:	No increase in chromosomal aberrations was observed after short-term or continuous treatment with or without S9 mix. Cell toxicity was observed at 400 ug/mL after continuous treatments for 24 and 48 hrs. Genetic effects:

		Clastogenicity			Polyploidy		
		+	?	-	+	?	-
	Without metabolic activation	[ ]	[ ]	[*]	[ ]	[ ]	[*]
	With metabolic activation	[ ]	[ ]	[*]	[ ]	[ ]	[*]

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa
Test substance : Purity: 98.63%
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.07.2002 (21)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENITY

5.8 TOXICITY TO REPRODUCTION

Type : other
Species : rat
Sex : male/female
Strain : other:Crj:CD(SD)IGS
Route of admin. : gavage
Exposure period : Males:48 days, females:42-53 days from 14 days before mating to day 4 of lactation
Frequency of treatment : once a day
Premating exposure period
Male : 14 days
Female : 14 days
Duration of test : Males: 49 days, females: from 14 days before day 5 of lactation
Doses : 50, 150, 500 mg/kgbw
Control group : yes, concurrent vehicle
NOAEL Parental : = 500 mg/kg bw
NOAEL F1 Offspr. : = 500 - mg/kg bw
Method : other:OECD Test guideline 422
Year : 2002
GLP : yes
Test substance : other TS
Remark : This study was conducted to examine both repeated dose toxicity and reproductive/developmental toxicity as an OECD screening combined study (Test guideline: 422).
Study design:
Vehicle: Corn oil
Clinical observation performed and frequency: General condition was observed once a day, body weights were determined at days 1(before dosing),8,15,22,29,36,43 and 49 of treatment for males and at days 1, 8 and 15 of treatment and at days 0,7,14,and 20 of gestation period and at days 0 and 4 of lactation period and at autopsy for females, food consumption was determined at days 1,8,15,22,29,36,43 and 48 of treatment for males and at days 1,8 and 15 of treatment and at days 0, 7,14 and 20 of gestation period and at days 0 and 4 of lactation for females. but food consumption were not determined during mating period for males and females.
For 5 males per group, urinalysis was carried out at 43-48 days of administration period. For all males and all females after childbirth per

group, hematology and biochemistry were carried out at time of necropsy after 49 days for males and at 5 days after delivery for females. Organs examined at necropsy.
Organ weight: Brain, liver, kidney, spleen, adrenal, thymus, testis and epididymis
Microscopic examination: Brain, pituitary, thymus, thyroid, parathyroid, adrenal, spleen, heart, thoracic aorta, tongue, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, larynx, trachea, lung, kidney, urinary bladder, testis, epididymis, prostate, seminal vesicle, ovary, uterus, vagina, eye, harderian gland, mammary gland, skin, sternum, femur, spinal cord, skeletal muscle, mesentery lymph node, mandibular lymph node, submandibular gland, sublingual gland, parotid gland, ischiadic nerve, bone marrow.
Reproductive and developmental parameters: No.of pairs with successful copulation, No. of pregnant females, copulation index (No. of pairs with successful copulation/No. of pairs mated x 100), fertility index (No. of pregnant animals/No.of animals with successful copulation x 100), estrous cycle, No. of dams delivered live pups, duration of gestation, No. of total corpora lutea, No. of total implants, No. of total pups born, No. of total live pups born, sex ratio, No. of total dead pups, No. of total cannibalism, gestation index (No. of females with live pups/No. of pregnant females x 100), implantation index (No. of implants/No.of corpora lutea x 100), delivery index (No. of pups born/No. of implants x 100), live birth index (No. of live pups born/No. of pups born x 100), and viability index on day 4 (No. of live pups on day 4 after birth/No. of live pups born x 100). Statistical methods: Dunnett's test for continuous data and Steel test for quantal data.

Result : Mortality: There was no mortality related to the test substance treatment.
Clinical signs: Salivation was apparent in three animals of 150 mg/kg bw group and in twelve animals of 500 mg/kg bw group for males and in two animals of 150 mg/kg bw group and twelve animals of 500 mg/kg bw group for females. Although the grades of salivation were not reported, the sign was observed for about 5 minutes after dosing at 150 mg/kg bw , and for 30 minutes to 5 hours after dosing at 500 mg/kg bw during treatment period. In addition, lacrimation was observed in two animals of 500 mg/kg bw group for males and in one animal each of 150 and 500 mg/kg bw groups for females. The onsets and grades of lacrimation were not reported.
Body weight: No statistically significant changes for males and females.
Food consumption: No effects for males and females.
Urinalysis: No statistically significant changes.
Hematology: No effects for males and females
Blood biochemistry:
Males: Decreases in triglyceride in 150 and 500 mg/kg bw groups, increases in total bilirubin in 500 mg/kg bw group, and total bile acid in 150 and 500 mg/kg bw.
Females: Increase in total bile acid in 50, 150, and 500mg/kg bw.
Necropsy and histopathology: No adverse effects for males and females.
Organ weights:
Males: Increase in a relative kidney weight in 500 mg/kg bw group.
Females: No statistically changes.

Histopathology: No changes related to test substance.
Reproductive and developmental parameters: No effects observed on reproductive performance in males and females given each dose, and developmental performance of the newborns.

Dose(mg/kg bw)	0	50	150	500
No. of pairs mated	12	12	12	12
No. of pairs copulated	12	11	12	12
No. of pregnant females	11	10	10	10
Copulation index (%)	100.0	91.7	100.0	100.0

Fertility index (%)		91.7	90.9	83.3	83.3
No. of dams observed		11	10	10	10
No. of dams delivered live pups		11	10	10	10
Duration of gestation:	Mean	22.5	22.2	22.3	22.5
	SD	0.5	0.4	0.5	0.5
No. of total corpora lutea:	Mean	19.2	17.4	18.4	20.1
	SD	2.6	3.3	3.2	3.8
No. of total implants:	Mean	13.7	14.4	14.3	14.3
	SD	3.0	1.6	1.5	1.6
No. of total pups born:	Mean	12.8	13.4	13.5	12.5
	SD	3.5	1.6	2.1	2.2
Sex ratio:	Mean	0.80	1.32	1.14	0.81
	SD	0.23	0.68	1.60	0.56
No. of total live pups on day 4					
Male:	Mean	5.5	6.9	6.7	5.0
	SD	2.2	1.9	2.4	2.0
Female:	Mean	6.8	6.2	6.7	7.2
	SD	2.8	1.9	2.4	2.0
No. of total dead pups:	Mean	0.3	0.1	0.1	0.0
	SD	0.6	0.3	0.3	0.0
Gestation index (%):		100.0	100.0	100.0	100.0
Implantation index (%):	Mean	73.2	84.1	79.0	72.9
	SD	20.3	9.7	10.6	13.1
Delivery index (%):	Mean	93.2	93.8	97.3	90.0
	SD	11.9	6.1	4.7	10.3
Live birth index (%):	Mean	98.0	99.3	96.9	97.0
	SD	4.7	2.3	9.7	9.5
Viability index day 4					
Male:	Mean	90.9	95.3	100.0	96.7
	SD	30.2	10.0	0.0	10.5
Female:	Mean	88.6	100.0	98.3	98.3
	SD	29.8	0.0	5.3	5.3

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa
Test substance : Purity:98.6%
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.07.2002 (19)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

See 5.8.

5.10 OTHER RELEVANT INFORMATION

Type : Toxicokinetics
Remark : Hydroxylation of cyclohexene at the allylic position has been shown to occur in hepatic microsomes and 9000 g supernatant fractions of male Holtzman rats and male New Zealand White rabbits (incubation time was 10 to 60 min). After 10-min incubation of 9000 g supernatant fractions from rats with 40 mM cyclohexene, 2-cyclohexen-1-ol at 0.93 ± 0.10 umol/g liver, cyclohexene oxide at 0.06 ± 0.02 umol/g liver and trans-cyclohexanediol at 0.89 ± 0.05 umol/g liver was produced in preparations from rats. Pretreatment of rats with phenobarbital induced cyclohexene oxidation by more than 3.5 times (2-cyclohexen-1-ol at 5.14 ± 0.64 umol/g liver, trans-cyclohexanediol at 3.08 ± 0.24 umol/g liver and cyclohexene oxide 4.47 ± 0.75 umol/g liver). A small amount of 2-cyclohexen-1-one (0.03 ± 0.01 umol/g liver) was also formed in preparations from

phenobarbital-pretreated rats . The formation of the product, 2-cyclohexen-1-ol, requires the presence of a NADPH-generating system, is inhibited by CO, metyrapone, and SKF 525-A.
In in vivo study, two male Holtzman rats (300 g) were each given 0.1 mL (0.07 mmol) of cyclohexene, followed by 1 mL of water, by stomach tube. Urine was collected at room temperature for 24 hr, after which it was filtered. To 5 mL samples of filtered urine were added 0.8 mL of 1 M acetate buffer, pH 5.0, and 2.5 mL of a preparation containing 2500 Fisherman units of beta-glucuronidase. After incubation overnight at 37 ・C, the mixtures were extracted with ether and assayed by gas chromatography. One week later, control 24-hr urine samples from the same rats were collected. Part of the urine was allowed to stand at room temperature for an additional 18 hr, and another, 5-mL aliquot, to which 1 mg of 2-cyclohexen-1-ol had been added, was incubated for 18 hr at 37 ・C. Ether extracts of the samples were then examined by gas chromatography.The urine of the two rats contained no detectable material hydrolyzable to 2-cyclohexen-1-ol by beta-glucuronidase. These rats, however, excreted 636 and 750 nmol of 2-cyclohexen-1-one in 24 hr. Chromatography of extracts of control urine revealed no peak corresponding in retention time to this ketone, and allowing control urine mixed with 2-cyclohexen-1-ol to stand at room temperature for 18 hr did not result in any formation of ketone. The presence of the ketone and absence of the alcohol was therefore probably not an artifact caused by the oxidation of one to the other in the voided urine. Although only 0.1 % of the oral dose of cyclohexene was excreted as the ketone, it is not known how much of the dose was absorbed nor how much was excreted by the pulmonary route.

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

26.07.2002 (14)

Type : Toxicokinetics
Remark : Male F344/N rats (200-300 g, 8-12 weeks old) were exposed nose-only to the gaseous cyclohexene at 600 ppm (2,015 mg/m3) or cyclohexene oxide at 30 ppm (120 mg/m3). Blood samples were collected at various times during the 60-minute exposure. For hepatic cytochrome p-450 analyses, tissue samples were taken after 20 or 360 minutes of exposure, or after 20 or 60 minutes of exposure for the epoxide.
RESULT:
During exposure of rats to 600 ppm cyclohexene, blood concentrations of cyclohexene oxide were below detection limits. During this exposure, however, cyclohexene blood levels increased to 2 ug/g blood. When rats were exposed to 30 ppm cyclohexene oxide, blood concentrations of the epoxide increased during the first 25 minute of exposure and then leveled off at up to 20 ug/g blood. No significant effect was observed on the p-450 concentration.

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

23.07.2002 (15)

Type : Metabolism
Remark : The oxidation of cyclohexene was studied in vitro using hepatic microsomes isolated from male Newzealand white rabbits (1.3-2.0 kg bw) pretreated for 3 days with sodium-phenobarbital, 15 mg/kg/day i.p.. Microsomes were incubated with 20 mM cyclohexene for up to 20 minutes. The reaction mixtures were analyzed by gas chromatography and thin layer chromatography.

RESULTS: Peaks were observed corresponding in retention time to those given by authentic samples of cyclohexene oxide (about 1.3 minutes);

there were in areas which were devoid of peaks in chromatograms of blank experiments in which cyclohexene was added at the end of the incubation, immediately before extraction. Chromatograms of blank extracts to which cyclohexene oxide had been added were very similar to those of experimental extracts. As for the time course of appearance and disappearance of cyclohexene oxide, the amount recovered reached a maximum after about 10 minutes and then declined until the level was no longer detectable.
No peaks with the retention times of cyclohexene oxide were observed when diols were injected in the gas chromatograph; under the conditions used, the retention times of trans-cyclohexanediol were 9.0 minutes. No peaks, or only very slight peaks, corresponding to cyclohexene oxide could be found in experiments in which the NADPH-generating system was absent. When isolated microsomes, free of cytosol, were used in place of 9000 x g supernatant fraction, and NADPH was added instead of NADP and glucose 6-phosphate, cyclohexene oxide products could be demonstrated by gas chromatography.
As results of thin-layer chromatography of picrate derivatives of the oxidation products of cyclohexene, a spot was found corresponding in Rf to the derivatives of cyclohexene oxide. Neither cyclohexene itself nor its glycol yielded any picrate derivative visible either with or without ammonia treatment, at concentrations over 100 times that which afforded detectable spot in case of the epoxide.
In an attempt to obtain a greater yield of an epoxide in intermediate by inhibiting its hydration with a different epoxide, the epoxidation of cyclohexene was studied in the presence of styrene oxide. The presence of a low concentration of styrene oxide allowed the recovery of about 3 times as much cyclohexene oxide as was recovered in its absence. Higher concentrations of styrene oxide, however, inhibited the cyclohexene-epoxidizing system.

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

17.07.2002 (12)

Type : Metabolism
Remark : The conversion of cyclohexene to trans diols in liver microsomes was studied. Liver of male Holtzmann rats (100-150 g bw) or Newzealand White rabbits (1.5-2.5 kg bw) pretreated with phenobarbital (rats, 75 mg/kg/day; rabbits, 15 mg/kg/day) i.p. for 3 days were homogenized in 4 vol. of 0.1M tris buffer, pH 7.5. The supernatant fraction derived from centrifugation for 15 min. at 9000 g was used immediately, or was lyophilized and stored at -15 degree C. Microsomes were prepared from, the 9000 g supernatant fraction by centrifugation at 105,00 g for 1 hr, followed by resuspension in tris buffer and recentrifugation. Cyclohexene were introduced as 10 per cent solutions in absolute ethanol (0.2 mL). Incubation time was 1 hr at 37 degree C, and30 min. in those described in Table 1. The oxidation products were identified by gas or thin-layer chromatography.

Table 1 Cyclohexene oxidation in rabbit liver preparations

Preparation	Nucleotide	Diol produced$
9000 g Supernatant fraction(freshly prepared)	NADPH	3.23
9000 g Supernatant fraction(freshly prepared	NADP+G6P	3.14
9000 g Supernatant fraction(lyophilized)	NADP+G6P	1.42
Microsomes	NADPH	1.19
Microsomes	NADH	0.41
Microsomes	NADP	0.04

Microsomes

Note:
Concentration when used: NADH or NADPH, 0.3 mM; ADP, 0.06 mM; G6p, 5 mM. $: Amounts were estimated from gas chromatographic peak heights.

RESULTS:
As gas chromatographic evidence for the production of trans-1,2-cyclohexanediol by oxidation of cyclohexene in reconstituted lyophilized 9000 g supernatant fraction of rat liver, a peak identical in retention time to that produced upon injection of a reference sample of trans-1,2-cyclohexanediol was found in the experimental chromatogram, in an area devoid of peaks in a corresponding chromatogram of an extract of a similar reaction mixture in which cyclohexene was not added until the end of the incubation, immediately preceding extraction. No peak was found corresponding in reaction time to cis-1,2-cyclohexanediol.Oxidation of cyclohexene to the trans-diol was also demonstrated in rabbit liver preparations. As shown in Table 1, the diol was formed in freshly prepared 9000 g supernatant fraction of rabbit liver as well as in the reconstituted lyophilized preparation produced from the same fraction. The lyophilized preparation contained, in this case, 45 per cent of the activity of the freshly prepared supernatant fraction. Activity was also found in the microsomes prepared from the fresh supernatant fraction. About one-third of the activity was exhibited when NADH was substituted equimolarly for NADPH. Very little activity was found when NADP was substituted for NADPH or when no pyridine nucleotide was present. In no case was any peak corresponding to cis-1,2-cyclohexanediol found in gas chromatogram. Further evidence of the nature of the diol products of oxidation of the cyclohexene was afforded by thin-layer chromatography, in which one metabolite spot was found, corresponding in Rf value to the trans-1,2-cyclohexanediol.The product of cyclohexene oxidation was chromatographed both as the free diol and as its benzoyl ester. In no case did a spot appear with the Rf of a cis-cyclohexanediol.

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

23.07.2002 (13)

Type : Cytotoxicity
Remark : The effect of cyclohexene on oncogenic cell transformation with 3-methylcholanthrene in C3H 10T1/2 CL8 mouse embryofibroblast was examined. Cyclohexene enhanced cell transformation by inhibiting epoxide-hydratase activity, allowing increased concentrations of arene oxides to accumulate in cells.
Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

24.07.2002 (24)

Type : other:Eye irritation of photo-oxidizing cyclohexene-nitrogen oxide mixture in air
Remark : The broad objective of this research was to investigate the existence of such a synergistic effect on eye irritation, using aerosols produced by the addition of trace amount of sulfur dioxide to photo-oxidizing cyclohexene-nitrogen oxide mixtures in air.
Subjects, who were males between the ages of 21 and 31, underwent one 5 minute eye exposure at chamber ports and then subjectively evaluated eye irritant effects on a scale from 0(no irritation) to 3(very severe irritation) and the time to initial eye irritation. Subjects were exposed to photo-oxidizing cyclohexene-nitrogen oxide mixture (cyclohexene at 0.05 ppm and nitrogen dioxide at 0.18 to 0.19 ppm with or without sulfur-dioxide at

0.05 ppm) (measured concentration before irradiation: cyclohexene: 1.0 ppm, nitrogen dioxide: 0.44 ppm, sulfur-dioxide: 0.05 ppm).

The effect of adding sulfur dioxide to photo-oxidizing mixtures on eye irritation was found to be barely significant. The trace concentration of cyclohexene could produce significant amounts of eye irritants other than formaldehyde, which is a major irritant obtained from most of the olefins.

Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (9)

Type : other:Inhibition of tumour induction

Remark : In the previous study carried out by the author (Berenblum 1929), it was shown that the induction of warts in mice by the repeated application of tar could be almost completely prevented by the addition to the tar of 0.1 percent. mustard gas. The same effect was obtained when the tar and a 0.1 percent. mustard gas in acetone were applied separately to the same area of skin at alternate intervals of 3 and 4 days. It was therefore suggested that the inhibition was due to some direct effect of the mustard gas on the animal, preventing the latter from responding to the carcinogenic tar, and not to an inactivation of the tar itself. Cyclohexene, which is not chemically related to the mustard gas but is known to be a skin irritant, was dissolved at 50 percent. concentration in dry acetone freshly before application, thereby avoiding any risk of decomposition. The tar was applied to a small area of skin in the region of the shoulder blades; the acetone solution including the test substance was applied by touching the skin with the end of a thick-walled capillary tube containing a small quantity of the appropriate solution. The survival rate and the time of appearance of warts were determined for 19 weeks. In control group using 50 mice, acetone was applied in place of the solution of the test substance. Preliminary tests were undertaken on small groups of mice to determine the degree of irritation produced by the test substance in different concentration. Each dilution was applied 3 times at weekly intervals to the skin of 4 mice, and a week after the last application the skins were examined both macroscopically and microscopically. In the group treated with tar and 50 percent. cyclohexene, a slight but definite inhibition was obtained followed by 7th degree of irritant action. There was a tendency for some of the established warts to disappear.

Group:	Control	50 percent. cyclohexene
No. of animals	50	50
Survivors after 15 weeks	45	40
Total number of animals with warts after 15 weeks	18	11
Time taken for 50 percent. of survivors to develop tumors	16 weeks	>19 weeks
Inhibition of tumor induction	-	++*
Irritant action	-	7**

Note: *: A delay of 4-6 weeks in reaching the 50 percent. level can be accepted as definite evidence of inhibition.
**: Marked thickening of the skin with loss of hair but little or no evidence of ulceration.

Source : Research Institute for Animal Science in Biochemistry and Toxicology

Sagamihara Kanagawa

12.07.2002 (1)

Type : other:Ovarian Toxicity
Remark : Twenty-eight day old female B6C3F1 mice were administered cyclohexene 7.5mmol/kgbw ip daily for 30 days. Following day 30, mice were killed by CO2 inhalation on the first day of diestrus of their cycle. Ovaries were removed, weighed, fixed in Bouin's solution for 24 hours, and transferred to 70 % ethanol. Serial sections were prepared and stained with hematoxylin and eosin. Oocytes contained in small and growing pre-antral follicles were counted microscopically. No alteration of small ovarian follicle counts occurred following treatment with cyclohexene that contain only a single unsaturated site. Cyclohexene, which is converted to monoepoxides both in vitro and in vivo, was not ovotoxic in mice
Purity: 95-99%
Source : Research Institute for Animal Science in Biochemistry and Toxicology Sagamihara Kanagawa

12.07.2002 (8)

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