



Name: chlorocyclohexane / chlorocyclohexane / 542-18-7

Legal entity owner: National Institute of Health Sciences / Tokyo / Japan

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Table of Contents

chlorocyclohexane	1
CORE	1
1 General information	1
1.1 Identification	1
Identification	1
OECD	2
D Health Effects	2
60 Acute toxicity: oral	2
Acute toxicity: oral.001	2
67 Repeated dose toxicity: oral	6
Repeated dose toxicity: oral.001	6
70 Genetic toxicity in vitro	13
Genetic toxicity in vitro.001	13
Genetic toxicity in vitro.002	17
73 Toxicity to reproduction	21
Toxicity to reproduction.001	21
References	28
A combined repeated-dose/reproductive-developmental toxicity study of chl orocyclohexane by oral administration in rats.	28
A combined repeated-dose/reproductive-developmental toxicity study of chl orocyclohexane by oral administration in rats.	29
chlorocyclohexane	30
chlorocyclohexane / 542-18-7 / 208-806-6	32
chlorocyclohexane / 542-18-7 / 208-806-6	33
chlorocyclohexane / 542-18-7 / 208-806-6	34
chlorocyclohexane / 542-18-7 / 208-806-6	35
In Vitro Chromosomal Aberration Test of Chlorocyclohexane on Cultured Chi nese Hamster Cells	36
National Institute of Health Sciences	37
Reverse Mutation Test of chlorocyclohexane on Bacteria.	39
Single Dose Oral Toxicity Test of Chlorocyclohexane in Rats	40

chlorocyclohexane

CORE

General information

Identification

SUBSTANCE: chlorocyclohexane

UUID: IUC5-cd10453c-72f2-45d4-a87e-fc2882f22d3b

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:41:51.000+09:00

Remarks:

Substance name
chlorocyclohexane

Legal entity
[National Institute of Health Sciences / Tokyo / Japan](#)

Identification of substance

Reference substance
[chlorocyclohexane / chlorocyclohexane / 542-18-7 / 208-806-6](#)

EC number	EC name
208-806-6	EC Inventory
CAS number	CAS name
542-18-7	
IUPAC name	
chlorocyclohexane	

Role in the supply chain

Manufacturer
false

Importer
false

Only representative
false

Downstream user
false

OECD

Health Effects

Acute toxicity: oral

ENDPOINT_STUDY_RECORD: Acute toxicity: oral.001

UUID: IUC5-6f52b438-c5b8-41b0-b587-ae524ef28122

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:42:38.000+09:00

Remarks:

Administrative data

Endpoint

acute toxicity: oral

Type of information

experimental study

Adequacy of study

key study

Robust study summary

false

Used for classification

false

Used for SDS

false

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

other: OECD Test Guideline study under GLP condition

Data source

Reference

[Single Dose Oral Toxicity Test of Chlorocyclohexane in Rats / MHLW \(Ministry of Health, Labour and Welfare\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to

Guideline

OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)

Deviations

no

GLP compliance

yes

Test type

standard acute method

Limit test

yes

Test material

Test material information

[chlorocyclohexane / 542-18-7 / 208-806-6](#)

Test animals

Species

rat

common species

Strain

Crj: CD(SD)

rat

Sex

female

Administration / exposure

Route of administration

oral: gavage

Vehicle

olive oil

Details on oral exposure

VEHICLE- Concentration in vehicle: 60 and 400 mg/ml. MAXIMUM DOSE VOLUME APPLIED: 5 ml/kg b.w.

Doses

300 mg/kg bw (first and second administration)

2000 mg/kg bw (third and fourth administration)

No. of animals per sex per dose

First and second administration (first purchase): 3 animals and 3 animals (animal ID No. 50101 – 50103 and 60101 - 60103) /female/300 mg/kg dose

Third and fourth administration (second purchase): 3 animals and 3 animals (animal ID No. 70101 – 70103 and 80101 - 80103) /female/2000 mg/kg dose

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations: Before dosing, Day 1 (day of administration):10, and 30 minutes and 1, 3, and 6 hrs after administration. After day 2: once a day
- Frequency of weighing: Days 1 (before administration), 4, 8 and 15
- Necropsy of survivors performed: Yes

Statistics

No

Results and discussion

Preliminary study

Dose levels were determined by the Globally Harmonized Classification System (GHS) (mg/kg b.w.). Mortality was not observed in each first and second administrations of 300 mg/kg groups and in each third and fourth administrations of 2000 mg/kg groups.

Effect levels

Key result

false

Sex

female

Dose descriptor

LD50

Effect level

> 2000

Mortality

No deaths were observed from first to fourth administration.

Clinical signs

No changes related to the test substance were observed from first to fourth administration.

Body weight

No changes related to the test substance were observed in first and second administrations of 300 mg/kg groups. Depression of body weight gains or decreases in body weight was observed in third and fourth administrations of 2000 mg/kg groups. However, their body weights were recovered at Day 8 and were equivalent to 300 mg/kg groups at Day 15.

Gross pathology

No changes related to the test substance were observed from first to fourth administration.

Other findings

- Organ weights: No data- Histopathology: No data- Potential target organs: Not identified- Other observations: No data

Applicant's summary and conclusion

Conclusions

The LD50 value was more than 2000 mg/kg bw for females.

GHS classification was classified in Category 5 (>2000 – 5000 mg/kg b.w.).

Repeated dose toxicity: oral

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.001

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Dossier UUID:

Author: SuperUser

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Remarks:

Administrative data

Endpoint

short-term repeated dose toxicity: oral combined repeated dose and reproduction / developmental screening

Type of information

experimental study

Adequacy of study

key study

Robust study summary

false

Used for classification

false

Used for SDS

false

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

other: OECD Test Guideline study under GLP condition

Cross-reference

Reason / purpose

reference to same study

Remarks

7.8.1 Toxicity to reproduction: Toxicity to reproduction.001

Data source

Reference

[A combined repeated-dose/reproductive-developmental toxicity study of chlorocyclohexane by oral adm... / MHLW \(Ministry of Health, Labour and Welfare\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to

Guideline

OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)

Deviations

no

GLP compliance

yes

Limit test

no

Test material

Test material information

[chlorocyclohexane / 542-18-7 / 208-806-6](#)

Test animals

Species

rat

common rodent species

Strain

Crj: CD(SD)

rat

Sex

male/female

Details on test animals and environmental conditions**TEST ANIMALS**

- Source: Atsugi Breeding Center, Charles River Laboratories Japan, Inc.
 - Age at study initiation: 9 weeks of age
 - Weight at study initiation: 325-386 g for males and 193-231 g for females
 - Housing: bracket-type metallic wire-mesh cages/males and females excluding gestation and lactation periods (W 195 × D 325 × H 180 mm), and polycarbonate cage during gestation and lactation periods/females (W 265 × D 426 × H 200 mm)
 - Water (e.g. ad libitum): ad libitum
 - Acclimation period: 5 days
- ENVIRONMENTAL CONDITIONS**
- Temperature (°C): 21.4 to 23.7°C
 - Humidity (%): 48.8 to 65.0%
 - Air changes (per hr): 6 to 20 times per hour
 - Photoperiod (hrs dark / hrs light): 12-hour lighting per day

Administration / exposure

Route of administration

oral: gavage

Vehicle

olive oil

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS: Test substance was dissolved in olive oil for injection.
VEHICLE

- Justification for use and choice of vehicle: No data
- Amount of vehicle (if gavage): 5 ml/kg bw
- Lot/batch no. (if required): No data
- Dosing volume: 5 mL/kg
- Stability (test solutions): For 8 days
- Storage condition of test solution: Stored in a dark place at room temperature

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Test suspensions at each concentration of initial and final preparations were analyzed by the GC method at Mitsubishi Safety Institute Ltd. Results showed that the concentration of the test article in each suspension was 95.0 to 106.2% of the nominal concentration and both values were within the acceptable range (concentration: percentage of the nominal concentration, 100 ± 10%)

Duration of treatment / exposure

(P) Males: 42 days including 14 days pre-mating and mating periods

(P) Females: Days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation

Frequency of treatment

Daily: 7 times / week

Doses / concentrations

Remarks

Doses / Concentrations:

0 (vehicle), 10, 60 and 300 mg/kg bw/day

Basis:

actual ingested

No. of animals per sex per dose

12 females/dose (0, 10, 60, 300 mg/kg), 7, 12, 12, 7 males of 0, 10, 60, 300 mg/kg, respectively, 5 males and 5 females at 0 and 300 mg/kg bw/day (recovery group)

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: A preliminary study was conducted to determine the doses to be employed. Three males and three female SD rats receiving 0, 30, 100, 300, and 1000 mg/kg groups of the substance were administered for 14 days. As a result, death or moribundity was observed in all males and females receiving 1000 mg/kg groups. Salivation and increases in absolute and relative adrenal weights were observed in females receiving 300 mg/kg group. No clear changes related to the test substance were observed in males receiving 300 mg/kg group. Therefore, the high dose was set at 300 mg/kg/day, and the middle and low dose were set at 60 and 10 mg/kg/day using common ratio 5. Vehicle control groups were set using olive oil only.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes

- Time schedule:

Males and females: once before the start of administration, during the administration period, and during the recovery period

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule:

Males: once before the start of administration, once every week until Week 6 during the administration

Females: once before the start of administration, once every week until Week 6 during the administration and once during the lactation period.

No observation performed during the recovery period because no observed clinical changes during the administration period.

BODY WEIGHT: Yes

- Time schedule for examinations:

Males in the main and recovery groups were weighed on Day 1, 8, 15, 22, 29, 36, 42, and 43 of administration, and males of recovery groups were weighed on Day 50 and 56. Female satellite groups were weighed same frequencies to male recovery groups. Females in the main groups were weighed on Day 1, 8 and 15 of administration and copulated females were weighed on Day 0, 7, 14 and 20 of gestation, and days 0 and 4 of lactation.

FOOD CONSUMPTION : Yes

- Food consumption (g/day/rat) for each animal determined from the difference of the of the previous day's feeding amount: Yes

Measurement of food consumption was conducted on all animals at the following frequencies:

Males in the main and recovery groups on Day 1-8, 8-15, 15-22, 22-29, 29-36, 36-38, 43-50, and 50-52. Female satellite groups on Day 1-8, 8-15, 15-22, 22-29, 29-36, 36-42, 43-50, and 50-56. Females in the main group on Day 1-8, 8-15, 22-29, 29-36, 36-38, 43-50, and 50-52.

FOOD INTAKE: No

HAEMATOLOGY: Yes

- Time schedule for collection of blood: On the day after the final day of administration and on the final day of the recovery period

- Anaesthetic used for blood collection: Yes

- Animals fasted: Yes

- How many animals: 5 animals/sex/group

- Parameters examined red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, platelet count, white blood cell count, differential white blood cell count, prothrombin time, activated partial thromboplastin time

CLINICAL CHEMISTRY: Yes

- Time schedule for collection of blood: On the day after the final day of administration and on the ending day of the recovery period

- Animals fasted: Yes

- How many animals: 5 animals/sex/group

- Parameters checked: ASAT (GOT), ALAT (GPT), γ -GT, ALP, total bilirubin, blood urea nitrogen, creatinine, glucose, total cholesterol, triglyceride, total protein, albumin, A/G ratio, calcium, inorganic phosphorus, sodium, potassium, chloride

URINALYSIS: Yes

- Time schedule for collection of urine: final week of administration (Day 38 of administration) in males

- Metabolism cages used for collection of urine: Yes

- Animals fasted: Yes

- How many animals: 5 animals/males/group

- Parameters checked: pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen

BLOOD HORMONE: No

NEUROBEHAVIOURAL EXAMINATION: Yes

- Battery of functions tested:

1) Open field observation: Aerial righting reaction, arousal, urination, defecation, posture and body position, breathing, co-ordination movement, gait, tremor, clonic convulsion, tonic convulsion, stereotypy, and bizarre behavior.

2) Manipulative Test: Approach response, touch response, auditory response, tail pinch response, and aerial righting reflex

3) Measurement of Grip Strength: Grip strength of forelimb and hind limb

4) Measurement of Motor Activity. Motor activity: 10-minute intervals from 0 to 60 minutes

Sacrifice and pathology

GROSS PATHOLOGY AND ORGAN WEIGHTS : Brain, heart, liver, kidneys, adrenals, thymus, spleen, testes, and epididymis.

HISTOPATHOLOGY: Cerebrum, pituitary, thymus, lymph nodes (including mesenteric and mandibular lymph nodes), trachea, lung, stomach, intestinal tract (duodenum, jejunum, ileum, cecum, colon, rectum), thyroids, parathyroid, heart, liver, spleen, kidneys, adrenals, urinary bladder, testes, epididymis, seminal vesicles (including the coagulating gland), prostate (ventral lobe), ovaries, uterus, vagina, bone marrow (one side femur), Sciatic nerve (one side femur), spinal cord, and gross abnormal sites.

Statistics

The data were analyzed for homogeneity of variance by the Bartlett test. If variances were homogeneous, data was analyzed by one-way analysis of variance, whereas heterogenous data was analyzed by Kruskal-Wallis ranking test. When a significant difference was observed, Dunnett's multiple comparison test was conducted between control and treated groups. If not homogenous, analysis was performed using the Kruskal-Wallis ranking test. Qualitative value as the pathological findings was analyzed by Wilcoxon test and Fisher's exact test. Urinalyses data were analyzed by Kruskal-Wallis and Dunnett's type mean rank test. Statistical significance was set at < 5% by two-sided.

Results and discussion

Results of examinations

Clinical signs

effects observed, treatment-related

Mortality

mortality observed, treatment-related

Body weight and weight changes

effects observed, treatment-related

Food consumption and compound intake (if feeding study)

no effects observed

Food efficiency

not examined

Haematological findings

no effects observed

Description (incidence and severity)

Significant increase in reticulocyte counts was observed in males receiving 300 mg/kg bw/day, but it was not considered to be toxicological effects.

Clinical biochemistry findings

effects observed, treatment-related

Description (incidence and severity)

Significant decrease in inorganic phosphorus concentration was observed in females receiving 300 mg/kg bw/day, but it was not considered to be toxicological effects.

Urinalysis findings

no effects observed

Behaviour (functional findings)

no effects observed

Organ weight findings including organ / body weight ratios

effects observed, treatment-related

Gross pathological findings

effects observed, treatment-related

Histopathological findings: non-neoplastic

effects observed, treatment-related

Histopathological findings: neoplastic

not specified

Details on results

CLINICAL SIGNS AND MORTALITY: No animal died in any group. Salivation was observed in males and females receiving 300 mg/kg from Day 9 to the end of dosing. No abnormal changes were observed in both sexes with recovery animals during the recovery period.

DETAILED CLINICAL OBSERVATIONS, MANIPULATIVE TEST, GRIP STRENGTH TEST AND LOCOMOTOR ACTIVITY MEASUREMENT: There were no changes related to the test substance in any group during the dosing.

BODY WEIGHT: Depression of body weight gains was observed in males and females receiving 300 mg/kg from Day 8 to 42.

ORGAN WEIGHTS: A significant increase in relative kidney weight was observed in males receiving 300 mg/kg bw/day at the end of both administration and recovery periods. **HISTOPATHOLOGY:** Hyaline droplet of proximal tubular epithelium in kidneys was observed in all males receiving 300 mg/kg. Minimal hyperplasia of mucosal epithelium in urinary bladder was observed in males receiving 60 mg/kg and males and females receiving 300 mg/kg at the end of the administration. This pathological change was found in females receiving 300 mg/kg after the recovery period. Minimal cell infiltration was present in one male and one female receiving 300 mg/kg at the end of administration and recovery periods.

Effect levels**Dose descriptor**

NOAEL

Effect level

10

mg/kg bw/day (actual dose received)

Sex

male

Basis for effect level

other: Effects of lesions in the hyperplasia of mucosal epithelium of urinary bladder in males receiving 60 mg/kg .

Dose descriptor

NOAEL

Effect level

60

mg/kg bw/day (actual dose received)

Sex

female

Basis for effect level

other: Effects of lesions in the hyperplasia of mucosal epithelium of urinary bladder in females receiving 300 mg/kg.

Target system / organ toxicity

Key result

false

Critical effects observed

not specified

Any other information on results incl. tables

Figures and Tables (in English) are available in the following full report of the study.

http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF542-18-7d.pdf

Applicant's summary and conclusion

Conclusions

Based on the effects of chlorocyclohexane on the urinary bladder, the no observed adverse effect level (NOAEL) for repeated oral dosing was determined to be 10 mg/kg bw/day in male rats and 60 mg/kg bw/day in female rats.

Executive summary

A combined repeated oral dose toxicity study and reproduction/developmental toxicity screening test was performed according to the OECD TG 422. Male and female rats (12 animals/sex/dose) were administered chlorocyclohexane at 0, 10, 60, and 300 mg/kg bw/day. Males were dosed for 42 days, including a 14 day pre-mating period and subsequent mating period. Females were dosed for up to 55 days, including 14 day pre-mating, mating, and gestation periods, and the time until lactation day 4. Five out of 12 males dosed at 0 and 300 mg/kg bw/day were treated as a recovery group. In addition, 5 females/dose 0 and 300 mg/kg bw/day groups were dosed for 42 days without mating and examined after the recovery period. At 300 mg/kg bw/day, increased salivation and decreased body weight gain were observed in both sexes. Absolute and relative kidney weights increased and hyaline droplet formation in the proximal tubular epithelium increased in males administered 300 mg/kg bw/day. Hyperplasia of the urinary bladder mucosal epithelium was observed in males administered 60 and 300 mg/kg bw/day and in females administered 300 mg/kg bw/day. Among these changes, increased relative kidney weight in males and hyperplasia of the urinary bladder mucosal epithelium in females persisted after the recovery period. Based on these effects in the kidney and urinary bladder, the NOAELs for repeated dose toxicity were determined to be 10 mg/kg bw/day and 60 mg/kg bw/day in male and female rats, respectively.

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

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Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:42:56.000+09:00

Remarks:

Administrative data

Endpoint

in vitro gene mutation study in bacteria Type of genotoxicity: gene mutation

Type of information

experimental study

Adequacy of study

key study

Robust study summary

false

Used for classification

false

Used for SDS

false

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

other: OECD Test Guideline study under GLP condition

Data source

Reference

[Reverse Mutation Test of chlorocyclohexane on Bacteria. / MHLW \(Ministry of Health, Labour and Welfare\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to

Guideline

OECD Guideline 471 (Bacterial Reverse Mutation Assay)
in vitro gene mutation study in bacteria

Deviations

no

Qualifier

according to

Guideline

JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals

Deviations

no

GLP compliance

yes

Type of assay

bacterial reverse mutation assay

in vitro gene mutation study in bacteria

Test material

Test material information

[chlorocyclohexane / 542-18-7 / 208-806-6](#)

Method

Species / strain

Species / strain

S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2
bacteria

Metabolic activation

with and without

Metabolic activation system

SD male rat liver, induced by phenobarbital and 5,6-benzoflavone

Test concentrations with justification for top dose

-S9 mix: 2.44, 4.88, 9.77, 19.5, 39.1, 78.1, 156 µg/plate (TA100, TA1535 strains),

9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate (TA98, TA1537, WP2uvrA/pKM101 strains)

+S9 mix: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate (TA100, TA1535, TA98, TA1537 strains),

9.77, 19.5, 39.1, 78.1, 156, 313, 625 µg/plate (WP2uvrA/pKM101 strain)

Vehicle

- Vehicle(s)/solvent(s) used: DMSO

Controls

Negative controls

no

Solvent controls

yes

True negative controls

no

Positive controls

yes

Positive control substance

other: -S9 mix: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA 100, TA98 and WP2 uvrA/pKM101), sodium azide (TA1535) and 9-aminoacridine hydrochloride (TA1537). +S9 mix: 2-aminoanthracene (all strains)

Details on test system and conditions

METHOD OF APPLICATION: Preincubation DURATION- Preincubation period: 20 min at 37°C - Exposure duration:48 hrs NUMBER OF PLATES: 3 NUMBER OF REPLICATIONS: 1 DETERMINATION OF CYTOTOXICITY- Method: other: growth inhibition

Evaluation criteria

In any strain(s) tested with or without S9 mix, when the mean number of revertant colonies per plate increased twice more than that of the negative control and when the increase was shown to be dose-related and reproducible, the chemical was judged mutagenic.

Statistics

No

Results and discussion

Test results

Key result

false

Species / strain

S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2 bacteria

Metabolic activation

with and without

Genotoxicity

negative

Cytotoxicity

yes

Vehicle controls valid

yes

Negative controls valid

not examined

Positive controls valid

yes

Remarks on result

other: all strains/cell types tested Migrated from field 'Test system'.

Additional information on results

RANGE-FINDING/SCREENING STUDIES: Concentration: 1.22, 4.88, 19.5, 78.1, 313, 1250, 5000 µg/plate with and without S9 mix
Cytotoxic conc.: -S9 at 78.1µg/plate and higher, +S9 at 313 µg/plate and higher

Any other information on results incl. tables

Figures and Tables (in Japanese) are available in the following full report of the study.

http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF542-18-7e.pdf

Overall remarks, attachments

Overall remarks

No increase in revertant colonies was observed in the test with either the non-activation method (-S9) or activation (+S9) method. Reverse mutation assays using microorganisms (*Salmonella typhimurium*, *Escherichia coli*) were conducted to assess the potential of chlorocyclohexane to induce gene mutations. Chlorocyclohexane did not induce gene mutations in the bacteria under the conditions of this study. The positive control showed expected results.

Applicant's summary and conclusion

Conclusions

Chlorocyclohexane did not induce gene mutations in the in vitro bacteria test.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

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Remarks:

Administrative data

Endpoint

in vitro cytogenicity / chromosome aberration study in mammalian cells Type of genotoxicity: chromosome aberration

Type of information

experimental study

Adequacy of study

key study

Robust study summary

false

Used for classification

false

Used for SDS

false

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

other: OECD Test Guideline study under GLP condition

Data source

Reference

[In Vitro Chromosomal Aberration Test of Chlorocyclohexane on Cultured Chinese Hamster Cells / MHLW \(Ministry of Health, Labour and Welfare\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to

Guideline

OECD Guideline 473 (In Vitro Mammalian Chromosome Aberration Test)
in vitro cytogenicity / chromosome aberration study in mammalian cells

Deviations

no

Qualifier

according to

Guideline

JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals

Deviations

no

GLP compliance

yes

Type of assay

in vitro mammalian chromosome aberration test
chromosome aberration

Test material**Test material information**

[chlorocyclohexane / 542-18-7 / 208-806-6](#)

Method**Target gene**

Chromosome

Species / strain**Species / strain**

other: Chinese hamster lung(CHL/IU) cells

Metabolic activation

with and without

Metabolic activation system

SD male rat liver, induced by phenobarbital and 5,6-benzoflavone

Test concentrations with justification for top dose

-S9 mix (short term treatment): 100, 150, 200, 250, 300, 350 ug/mL

+S9 mix (short term treatment): 200, 300, 400, 450, 500 ug/mL

Continuous treatment: 150, 175, 200, 225, 275 ug/mL

Vehicle

- Vehicle(s)/solvent(s) used: DMSO

Controls**Negative controls**

no

Solvent controls

yes

True negative controls

no

Positive controls

yes

Positive control substance

other: [continuous treatment -S9]: mitomycin C; [continuous treatment +S9]: Benzo[a]pyrene

Details on test system and conditions

METHOD OF APPLICATION: Exposure duration: [continuous treatment]: 24 hrs [short-term treatment]: 6 hrs + 18 hr

SPINDLE INHIBITOR: Colcemid

STAIN: Giemsa stain for 20 min.

NUMBER OF REPLICATIONS: 2

NUMBER OF CELLS EVALUATED: 200 cells / dose

DETERMINATION OF CYTOTOXICITY

- Method: relative total growth

Evaluation criteria

For the evaluation of the frequencies of structural aberrations and of polyploidy induced, the following criteria, which are usually used for chromosomal aberration testing with CHL, were employed. Appearance incidence of cell with chromosomal aberrations: Negative(-): less than 5% Equivocal(±): 5% or more, less than 10% Positive(+): 10% or more

Statistics

No analyses

Any other information on materials and methods incl. tables

Figures and Tables (in Japanese) are available in the following full report of the study.

http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF542-18-7f.pdf

Results and discussion

Test results

Key result

false

Species / strain

other: Chinese hamster lung (CHL/IU) cells

Metabolic activation

with and without

Genotoxicity

negative

Cytotoxicity

no

Vehicle controls valid

yes

Negative controls valid

not examined

Positive controls valid

yes

Remarks on result

other: strain/cell type: Chinese hamster lung (CHL/IU) cells Migrated from field 'Test system'.

Applicant's summary and conclusion

Conclusions

Interpretation of results (migrated information):
negative

Chlorocyclohexane did not induce chromosomal aberrations in cultured cells.

Executive summary

An in vitro chromosomal aberration test using CHL/IU cells (OECD TG 473) was negative with or without metabolic activation.

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction.001

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Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:43:08.000+09:00

Remarks:

Administrative data

Endpoint

one-generation reproductive toxicity based on generations indicated in Effect levels (migrated information)

Type of information

experimental study

Adequacy of study

key study

Robust study summary

false

Used for classification

false

Used for SDS

false

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

other: OECD Test Guideline study under GLP condition

Cross-reference

Reason / purpose

reference to same study

Remarks

7.5. Repeated dose toxicity: oral: Repeated dose toxicity: oral.001

Data source

Reference

[A combined repeated-dose/reproductive-developmental toxicity study of chlorocyclohexane by oral admi... / MHLW \(Ministry of Health, Labour and Welfare\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to

Guideline

OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)

Deviations

no

GLP compliance

yes

Limit test

no

Test material

Test material information

[chlorocyclohexane / 542-18-7 / 208-806-6](#)

Test animals

Species

rat

Strain

Crj: CD(SD)

rat

Sex

male/female

Details on test animals and environmental conditions**TEST ANIMALS**

- Source: Atsugi Breeding Center, Charles River Laboratories Japan, Inc.
- Age at study initiation: 9 weeks of age
- Weight at study initiation: 325-386 g for males and 193-231 g for females
- Housing: bracket-type metallic wire-mesh cages/males and females excluding gestation and lactation periods (W 195 × D 325 × H 180 mm), and polycarbonate cage during gestation and lactation periods/females (W 265 × D 426 × H 200 mm)
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum excluding collected fresh urine
- Acclimation period: 8 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 21.4 to 23.7°C
- Humidity (%): 48.8 to 65.0%
- Air changes (per hr): 9 to 20 times per hour
- Photoperiod (hrs dark / hrs light): 12-hour lighting per day

Administration / exposure

Route of administration

oral: gavage

Vehicle

olive oil

Details on exposure

PREPARATION OF DOSING SOLUTIONS: Test substance was dissolved in olive oil for injection.
VEHICLE

- Justification for use and choice of vehicle: No data
- Amount of vehicle (if gavage): 5 ml/kg bw
- Lot/batch no. (if required): No data
- Dosing volume: 5 mL/kg
- Stability (test solutions): At least 7 days
- Storage condition of test solution: Stored in a refrigerator

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Test suspensions at each concentration of initial and final preparations were analyzed by the GC method at Mitsubishi Safety Institute Ltd. Results showed that the concentration of the test article in each suspension was 95.0 to 106.2% of the nominal concentration and both values were within the acceptable range (concentration: percentage of the nominal concentration, $100 \pm 10\%$)

Duration of treatment / exposure

(P) Males: 42 days including 14 days pre-mating and mating periods (P) Females: Days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation

Frequency of treatment

Daily: 7 times / week

Doses / concentrations

Remarks

Doses / Concentrations:

0 (vehicle), 10, 60 and 300 mg/kg bw/day

Basis:

actual ingested

No. of animals per sex per dose

12 females/dose (0, 10, 60, 300 mg/kg), 7, 12, 12, 7 males of 0, 10, 60, 300 mg/kg, respectively, 5 males and 5 females at 0 and 300 mg/kg bw/day (recovery group)

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: A preliminary study was conducted to determine the doses to be employed. Three males and three female SD rats were receiving 0, 30, 100, 300, and 1000 mg/kg groups of the substance were administered for 14 days. As a result, death or dying was observed in all males and females receiving 1000 mg/kg groups. Salivation and increases in absolute and relative adrenal weights were observed in females receiving 300 mg/kg group. No clear changes related to the test substance were observed in males receiving 300 mg/kg group. Therefore, the high dose was set at 300 mg/kg/day, and the middle and low dose were set at 60 and 10 mg/kg/day using common ratio 5. Vehicle control groups were set using olive oil only.

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes

- Time schedule:

Males and females: once before the start of administration, two times/day during the administration period, and once during the recovery period

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule:

Males: once before the start of administration, during the administration and recovery periods

Females: once before the start of administration, days 1, 7, 14 and 20 of gestation, and day 4 of lactation

BODY WEIGHT: Yes

- Time schedule for examinations:

Males in the main and recovery groups were weighed on Day 1, 8, 15, 22, 29, 36, 42, and 43 of administration, and males of recovery groups were weighed on Day 50 and 56. Female satellite groups were weighed same frequencies to male recovery groups. Females in the main groups were weighed on Day 1, 8 and 15 of administration and copulated females were weighed on Day 0, 7, 14 and 20 of gestation, and days 0 and 4 of lactation.

FOOD CONSUMPTION: Yes

- Food consumption (g/day/rat) for each animal determined from the difference of the of the previous day's feeding amount: Yes

Measurement of food consumption was conducted on all animals at the following frequencies:

Males in the main and recovery groups were measured on Day 1-8, 8-15, 15-22, 22-29, 29-36, 36-38, 43-50, and 50-52. Female satellite groups were measured on Day 1-8, 8-15, 15-22, 22-29, 29-36, 36-42, 43-50, and 50-56. Main females were measured same frequencies to body weighted days. It is not measured during the mating.

FOOD INTAKE: No

COMPOUND INTAKE: No

FOOD EFFICIENCY: No

WATER CONSUMPTION: No

Estrous cyclicity (parental animals)

Vaginal smears were collected from all females in the main groups and microscopically examined every day from the day after the start of administration until the day copulation was confirmed.

Mean estrous cycle (day) and abnormal estrous cycle animals (not 4 to 6 day in estrous cycle) were examined by dams.

Sperm parameters (parental animals)

Parameters examined in P male parental generations: testes weight, epididymides weight

Litter observations

PARAMETERS EXAMINED: The following parameters were examined in F1 offspring: Number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, and weight gain.

Postmortem examinations (parental animals)

SACRIFICE: The F1 pups were euthanized on PND 4 by exsanguination pentobarbital sodium anesthesia, intraperitoneally.

GROSS NECROPSY: Yes

Statistics

Parametric data such as grip strength, motor activity, body weight and gain, food consumption, urine volume, specific gravity, Hematology, blood biochemistry, and absolute and relative organ weights were analyzed by Bartlett's test for homogeneity of distribution. When homogeneity was recognized, one-way analysis of variance was performed. When a significant difference was observed, Dunnett's multiple comparison test was conducted for comparison between control and treated groups. If not homogenous, analysis was performed using the Kruskal-Wallis ranking test. In consequence, if not homogenous, Dunnett's type mean rank sum test was conducted to compare to control and individual treatment groups. Qualitative value as the pathological findings was analyzed by Wilcoxon test and Fisher's exact test. Urinalyses data were analyzed by Kruskal-Wallis and Dunnett's type mean rank test. Reproductive incidences of estrous cycle, fertility index, copulation index, delivery index, sex ratio, and external abnormalities were analysed by Fisher's exact test. Significance level was set at 0.05 compared with the control group and among the groups.

Reproductive indices

1) Each parameter was determined by the following equations:

Mean estrus cycle, incidence of females with irregular estrus cycle, mating periods,

Copulation index (%) = (No. of copulated animals/No. of co-housed animals) × 100

Fertility index (%) = (No. of pregnant females/No. of copulated females) × 100

Gestation length, number of corpora lutea, number of implantation sites, total number of offspring,

Implantation index (%) = (No. of implantation sites/No. of corpora lutea) × 100

Delivery index (%) = (No. of females delivered liveborn pups/No. of pregnant females) × 100

Gestation index (%) = (No. of pregnant animals delivered live offspring/number of pregnant animals) × 100

Offspring viability indices

Total number of offspring at birth, number of live offspring at birth,

Number of live pups on day 0 of lactation Birth index (%) = (Number of live pups on day 0/Number of implantation sites) × 100

Viability index = (Number of live pups on day 4 after birth/Number of live pups born) × 100

External examination of offspring, necropsy finding

Pups weight on day 0 of lactation

Sex ratio on day 0 of lactation

Number of live pups on day 4 of lactation

Pups weight on day 4 of lactation

Sex ratio on day 4 of lactation

Results and discussion

Results: P0 (first parental animals)

General toxicity (P0)

Clinical signs

effects observed, treatment-related

Body weight and weight changes

effects observed, treatment-related

Food consumption and compound intake (if feeding study)

effects observed, treatment-related

Organ weight findings including organ / body weight ratios

effects observed, treatment-related

Gross pathological findings

effects observed, treatment-related

Histopathological findings: non-neoplastic

effects observed, treatment-related

Other effects

no effects observed

Reproductive function / performance (P0)

Reproductive function: estrous cycle

no effects observed

Reproductive function: sperm measures

not examined

Reproductive performance

no effects observed

Details on results (P0)

1) Estrous Cycle

There were no animals showing abnormal estrous cycles, and there were no significant differences in the average length of the estrous cycle between the control group and any treatment group.

2) Results of Mating

There were no significant differences in the incidence of females with irregular estrus cycle, mating period with the number of estrus and day of conceiving, copulation index, and fertility index between the control group and any treatment groups.

3) Delivery Data and Delivery

There were no significant differences in the gestation length, number of corpora lutea, number of implantation sites, implantation index, and delivery index between the control group and any treatment groups.

GROSS PATHOLOGY

See 7.5.1 Repeated dose toxicity: oral

HISTOPATHOLOGY

See 7.5.1 Repeated dose toxicity: oral

Effect levels (P0)

Dose descriptor	
NOAEL	
Effect level	
300	mg/kg bw/day
Sex	
male/female	
Basis for effect level	
other: No reproductive effects up to highest dose tested	

Results: F1 generation

General toxicity (F1)

Clinical signs

no effects observed

Mortality / viability

no mortality observed

Body weight and weight changes

no effects observed

Sexual maturation

no effects observed

Organ weight findings including organ / body weight ratios

not specified

Gross pathological findings

no effects observed

Histopathological findings

not specified

Effect levels (F1)

Dose descriptor

NOAEL

Generation

F1

Effect level

300

mg/kg bw/day

Sex

male/female

Basis for effect level

other: No developmental effects up to highest dose tested

Overall reproductive toxicity

Key result

false

Reproductive effects observed

not specified

Any other information on results incl. tables

Figures and Tables (in English) are available in the following full report of the study.

http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF542-18-7d.pdf

Applicant's summary and conclusion

Conclusions

The NOAEL for rat reproductive and developmental toxicity was determined to be 300 mg/kg bw/day.

Executive summary

A combined repeated oral dose toxicity study and reproduction/developmental toxicity screening test was performed according to the OECD TG 422. Male and female rats (12 animals/sex/dose) were administered chlorocyclohexane at 0, 10, 60, and 300 mg/kg bw/day. Males were dosed for 42 days, including a 14 day pre-mating period and subsequent mating period. Females were dosed for up to 55 days, including 14 day pre-mating, mating, and gestation periods, and the time until lactation day 4. Five out of 12 males dosed at 0 and 300 mg/kg bw/day were treated as a recovery group. In addition, 5 females/dose 0 and 300 mg/kg bw/day groups were dosed for 42 days without mating and examined after the recovery period. There were no effects on reproductive and developmental parameters at 300 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of chlorocyclohexane was determined to be 300 mg/kg bw/day, the highest dose tested.

References

LITERATURE: A combined repeated-dose/ reproductive-developmental toxicity study of chlorocyclohexane by oral administration in rats.

UUID: 830ba0b0-26d5-37a9-8ef7-84c4a85199b0

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:42:46.000+09:00

Remarks:

General information

Reference Type

study report

Title

A combined repeated-dose/reproductive-developmental toxicity study of chlorocyclohexane by oral administration in rats.

Author

MHLW (Ministry of Health, Labour and Welfare), Japan

Year

2007

Bibliographic source

Japan Existing Chemical Data Base (JECDB)

Testing facility

Mitsubishi Safety Institute Ltd.

Study no.

B041794

Report Date

2007-01-23

LITERATURE: A combined repeated-dose/ reproductive-developmental toxicity study of chlorocyclohexane by oral administration in rats.

UUID: be12d00b-9f91-3613-a630-e0d51b1f4304

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:43:08.000+09:00

Remarks:

General information

Reference Type

study report

Title

A combined repeated-dose/reproductive-developmental toxicity study of chlorocyclohexane by oral administration in rats.

Author

MHLW (Ministry of Health, Labour and Welfare), Japan

Year

2007

Bibliographic source

Japan Existing Chemical Data Base (JECDB)

Testing facility

Mitsubishi Safety Institute Ltd.

Study no.

B041794

Report Date

2007-01-23

REFERENCE_SUBSTANCE: chlorocyclohexane

UUID: ECB5-9483ca56-6e33-4ec9-a0f4-545b621d4c14

Dossier UUID:

Author: SuperUser

Date: 2007-05-10T18:00:00.000+09:00

Remarks:

General information

Reference substance name

chlorocyclohexane

Inventory

Inventory name

chlorocyclohexane

Inventory

EC

Inventory number

208-806-6

CAS number

542-18-7

Molecular formula

C₆H₁₁Cl

Description

Reference substance information

IUPAC name

chlorocyclohexane

Synonyms

Identity
chlorocyclohexane
Identity
Cyclohexane, chloro-
Identity
Cyclohexane, chloro-

CAS information

CAS number

542-18-7

Related substances

Group / category information

DSL Category: Organics

Molecular and structural information

Molecular formula

C₆H₁₁Cl

Molecular weight

118.6045

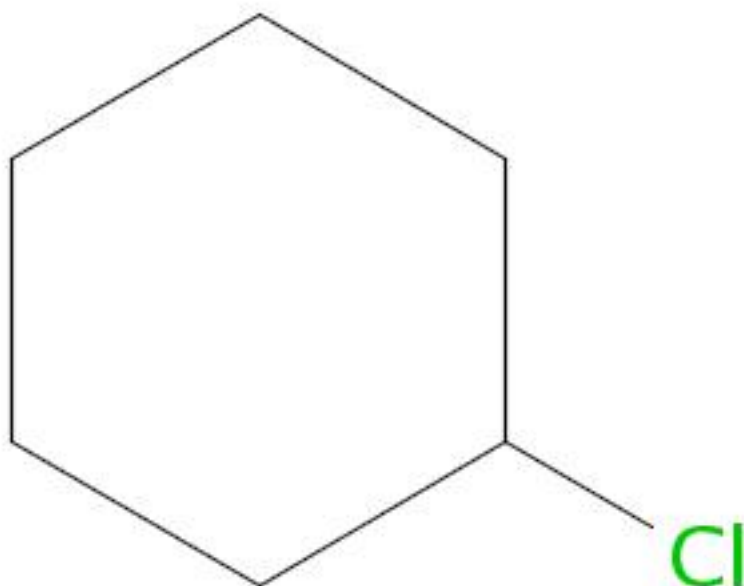
SMILES notation

ClC1CCCCC1

InChI

InChI=1/C6H11Cl/c7-6-4-2-1-3-5-6/h6H,1-5H2

Structural formula



TEST_MATERIAL_INFORMATION: chlorocyclohexane / 542-18-7 / 208-806-6

UUID: 12489c5c-655c-3e0c-b6cf-5f492c38624a

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:42:38.000+09:00

Remarks:

Name

chlorocyclohexane / 542-18-7 / 208-806-6

Composition

Type

Constituent

Reference substance

chlorocyclohexane / chlorocyclohexane / 542-18-7 / 208-806-6

EC number

208-806-6

EC name

EC Inventory

CAS number

542-18-7

CAS name

IUPAC name

chlorocyclohexane

Other characteristics

Details on test material

- Name of test material (as cited in study report): Chlorocyclohexane
- CAS No.: 542-18-7
- Molecular weight: 118.61
- Lot No.: 5C1114
- Purity: 99.7%
- Supplier: Junsei Chemical Co., Ltd.
- Vapor pressure: 6.73 mmHg (25°C)
- Vapor density : 4.12 (air = 1)
- Boiling point : 142°C
- Melting point: -44°C
- Flash point: °C
- Specific gravity: 1.004 (20/4°C)
- Solubility: Insoluble in water and miscible by organic solvents as the alcohol and ether et al.
- Odor: Characteristic odor
- Physical state: Slightly pale yellow from colorless liquid
- Storage condition of test material: Dark and sealed in refrigerator (2.8 – 8.4°C)

TEST_MATERIAL_INFORMATION: chlorocyclohexane / 542-18-7 / 208-806-6

UUID: 9dd9b486-9f57-3868-8e7d-75c58cc0f5e6

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:42:46.000+09:00

Remarks:

Name

chlorocyclohexane / 542-18-7 / 208-806-6

Composition

Type

Constituent

Reference substance

chlorocyclohexane / chlorocyclohexane / 542-18-7 / 208-806-6

EC number

208-806-6

EC name

EC Inventory

CAS number

542-18-7

CAS name

IUPAC name

chlorocyclohexane

Other characteristics

Details on test material

- Name of test material (as cited in study report): Chlorocyclohexane
- CAS No.: 542-18-7
- Molecular weight: 118.60
- Lot No.: 5C1114
- Purity: 99.7%
- Supplier: Junsei Chemical Co., Ltd.
- Vapor pressure: 6.73 mmHg (25°C)
- Vapor density : 4.12 (air = 1)
- Boiling point : 142°C
- Melting point: -44°C
- Flash point: 29°C
- Specific gravity: 1.004 (20/4°C)
- Solubility: Insoluble in water and miscible with organic solvents such as alcohol and ether etc.
- Odor: Peculiar odor
- Physical state: Colorless liquid to slightly pale yellow
- Storage condition of test material: Dark and sealed in refrigerator (2.8 – 8.4°C)

TEST_MATERIAL_INFORMATION: chlorocycl ohexane / 542-18-7 / 208-806-6

UUID: 72271859-2295-3aba-b05b-c54f5fa53646

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:43:02.000+09:00

Remarks:

Name

chlorocyclohexane / 542-18-7 / 208-806-6

Composition

Type

Constituent

Reference substance

chlorocyclohexane / chlorocyclohexane / 542-18-7 / 208-806-6

EC number

208-806-6

EC name

EC Inventory

CAS number

542-18-7

CAS name

IUPAC name

chlorocyclohexane

Other characteristics

Details on test material

- Name of test material (as cited in study report): Chlorocyclohexane
- CAS No.: 542-18-7
- Molecular weight: 118.61
- Lot No.: 5C1114
- Purity: 99.7%
- Supplier: Junsei Chemical Co., Ltd.
- Vapor pressure: 6.73 mmHg (25°C)
- Vapor density : 4.12 (air = 1)
- Boiling point : 142°C
- Melting point: -44°C
- Flash point: °C
- Specific gravity: 1.004 (20/4°C)
- Solubility: Insoluble in water and miscible by organic solvents as the alcohol and ether et al.
- Odor: Characteristic odor
- Physical state: Slightly pale yellow from colorless liquid
- Storage condition of test material: Dark and sealed in refrigerator (2.8 - 8.4°C)

TEST_MATERIAL_INFORMATION: chlorocycl ohexane / 542-18-7 / 208-806-6

UUID: 514a0d9e-6dac-3812-a0de-aadb2c6ec9cf

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:43:08.000+09:00

Remarks:

Name

chlorocyclohexane / 542-18-7 / 208-806-6

Composition

Type

Constituent

Reference substance

chlorocyclohexane / chlorocyclohexane / 542-18-7 / 208-806-6

EC number

208-806-6

EC name

EC Inventory

CAS number

542-18-7

CAS name

IUPAC name

chlorocyclohexane

Other characteristics

Details on test material

- Name of test material (as cited in study report): Chlorocyclohexane
- CAS No.: 542-18-7
- Molecular weight: 118.61
- Lot No.: 5C1114
- Purity: 99.7%
- Supplier: Junsei Chemical Co., Ltd.
- Vapor pressure: 6.73 mmHg (25°C)
- Vapor density : 4.12 (air = 1)
- Boiling point : 142°C
- Melting point: -44°C
- Flash point: 29°C
- Specific gravity: 1.004 (20/4°C)
- Solubility: Insoluble in water and miscible with organic solvents such as the alcohol and ether etc.
- Odor: Peculiar odor
- Physical state: Slightly pale yellow from colorless liquid
- Storage condition of test material: Dark and sealed in refrigerator (2.8 - 8.4°C)

LITERATURE: In Vitro Chromosomal Aberration Test of Chlorocyclohexane on Cultured Chinese Hamster Cells

UUID: 9d974c31-5cd8-3cff-a6ab-0ee04becc208

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:43:02.000+09:00

Remarks:

General information

Reference Type

study report

Title

In Vitro Chromosomal Aberration Test of Chlorocyclohexane on Cultured Chinese Hamster Cells

Author

MHLW (Ministry of Health, Labour and Welfare), Japan

Year

2006

Bibliographic source

Japan Existing Chemical Data Base (JECDB)

Testing facility

Mitsubishi Safety Institute Ltd.

Study no.

B041796

Report Date

2016-09-15

LEGAL_ENTITY: National Institute of Health Sciences

UUID: IUC4-b036ff75-0f3c-323b-b200-ed5f46cf5101

Dossier UUID:

Author: SuperUser

Date: 2011-06-23T11:55:01.000+09:00

Remarks:

General information

Legal entity name

National Institute of Health Sciences

Identifiers

Other IT system identifiers

IT system

LEO

ID

10767

IT system

IUCLID4

ID

16558402024DIV750

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Address 2

Setagaya-ku

Postal code

158-8501

Town

Tokyo

Country

Japan

Contact persons

Person

Hirose, Akihiko; National Institute of Health Sciences

Last name

Hirose

First name

Akihiko

Organisation

National Institute of Health Sciences

Department

Division of Risk Assessment

Title

Dr.

Address 1

1-18-1 Kamiyoga

Address 2

Setagaya-ku

Postal code

158-8501

Town

Tokyo

Country

Japan

LITERATURE: Reverse Mutation Test of chlorocyclohexane on Bacteria.

UUID: 04ba63b5-4aaf-38de-a757-3593a38ec717

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:42:56.000+09:00

Remarks:

General information

Reference Type

study report

Title

Reverse Mutation Test of chlorocyclohexane on Bacteria.

Author

MHLW (Ministry of Health, Labour and Welfare), Japan

Year

2006

Bibliographic source

Japan Existing Chemical Data Base (JECDB)

Testing facility

Mitsubishi Safety Institute Ltd.

Study no.

B041795

Report Date

2006-09-14

LITERATURE: Single Dose Oral Toxicity Test of Chlorocyclohexane in Rats

UUID: 5eb44718-fca3-3d6a-ba1f-2cc869f878f5

Dossier UUID:

Author: SuperUser

Date: 2017-01-04T16:42:38.000+09:00

Remarks:

General information

Reference Type

study report

Title

Single Dose Oral Toxicity Test of Chlorocyclohexane in Rats

Author

MHLW (Ministry of Health, Labour and Welfare), Japan

Year

2007

Bibliographic source

Japan Existing Chemical Data Base (JECDB)

Testing facility

Mitsubishi Safety Institute Ltd.

Study no.

B041793

Report Date

2007-01-23