



Name: OECD_SIDS / SUBSTANCE : 2,2'-[1,2-Ethanediy]bis(oxymethylene)]bis-oxirane /
2224-15-9 Tue, 29 Nov 2022, 15:19:07+0900 /

Legal entity owner: National Institute of Health Science

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Dossier header

Dossier submission type

Name

OECD SIDS

Version

core 7.0

Name (given by user)

Dossier subject

Dossier subject

[2,2'-\[1,2-Ethanediy\]bis\(oxymethylene\)\]bis-oxirane / 2224-15-9](#)

Public name

Submitting legal entity

[National Institute of Health Science](#)

Dossier creation date/time

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Used in category

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General information

Legal entity name

National Institute of Health Science

2,2'-[1,2-Ethanediy]bis(oxymethylene)]bis-oxirane

General information

Identification

Identification

SUBSTANCE: 2,2'-[1,2-Ethanediy]bis(oxymethylene)]bis-oxirane

UUID: a9230384-e00d-455b-9f00-e6a378a6b446

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Author:

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Remarks:

Substance name

2,2'-[1,2-Ethanediy]bis(oxymethylene)]bis-oxirane

Legal entity

[National Institute of Health Sciences / Kawasaki / Japan](#)

Identification of substance

Reference substance

[2,2'-\[1,2-Ethanediy\]bis\(oxymethylene\)\]bis-oxirane / 2224-15-9](#)

EC number

EC name

CAS number

CAS name

2224-15-9

IUPAC name

Role in the supply chain

Manufacturer

false

Importer

false

Only representative

false

Downstream user

false

Toxicological information

Repeated dose toxicity

Repeated dose toxicity: oral

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral. 001

UUID: c1195a3f-e3c2-49bc-93e1-55e2155c846d

Dossier UUID:

Author:

Date: 2022-11-29T15:13:13.559+09:00

Remarks:

Administrative data

Endpoint

short-term repeated dose 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

guideline study OECD Test Guideline study under GLP condition

Reliability 1

Cross-reference

Reason / purpose for cross-reference

reference to same study 7.8.1 Toxicity to reproduction: Toxicity to reproduction. 001

Related information

[OECD / Toxicity to reproduction / Toxicity to reproduction. 001 / 2,2,8-\[1,2-Ethanediybis\(oxymethylene\)\]bis-oxirane / 2224-15-9](#)

Data source

Reference

[Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of / Ministry of Health, Labour and Welfare \(MHLW\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to guideline

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

2,2'-[1,2-ethanediylbis(oxyethylene)]bis-oxirane

Specific details on test material used for the study

- Name of test material (as cited in study report): 2,2'-[1,2-ethanediylbis(oxyethylene)]bis-oxirane
- Analytical purity: contain 0.1% water
- Storage condition of test material: Room temperature, shading, airtightness
- Stability under test conditions: The stability of test material was identified by analysis of the remainder.

Test animals

Species

rat

common rodent species

Strain

other: CrI:CD(SD)

Sex

male/female

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

- Source: Charles River Japan, Inc., Hino Breeding Center.

- Age at study initiation: 10 weeks old

- Weight at study initiation:

Males (main study groups): 365-433 g, females (main study groups): 236-266 g, females in (mating study groups): 219-265 g

- Housing: Animals were individually housed in stainless steel suspension cage (240W × 380D × 200H mm), from gestation day 18 to lactation day 4, Dams were bred individually or with individual littermates in plastic cages (310W x 360D x 175H mm) and bedding.

- Diet: Solid feed (CRF-1: Oriental Yeast Co., Ltd.) was given ad libitum.

- Water: Tap water was given ad libitum.

- Acclimation period: Males (main study groups): 21 days, females (main study groups): 22 days, females (mating study groups): 21 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20-26°C (actual temperature: 22.5-24.7°C)
- Humidity (%): 40.0-70.0% (actual humidity: 41.3-58.4%)
- Air changes (per hr): 12
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 6:00~18:00)

Administration / exposure

Route of administration

oral: gavage

Vehicle

water for injection

Details on oral exposure

- Amount of vehicle (if gavage): 5 mL/kg
- Dosing volume: 5 mL/kg

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Concentrations of the test solutions using administration on day 1 were analyzed with GC. Analytical concentrations of the test solutions were all within the range of 92.2-106.4% of the nominal concentrations and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%).

Duration of treatment / exposure

Males: 28 days including 14 days pre-mating

Females (main study groups): 28 days

Females (mating groups): 42-46 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation

Frequency of treatment

Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
12.5	mg/kg bw/day (actual dose received)
Dose / conc.	
50	mg/kg bw/day (actual dose received)
Dose / conc.	
200	mg/kg bw/day (actual dose received)

No. of animals per sex per dose

- Main study groups:

Control- and high-dose groups: 12 males and 10 females per group (half of both sexes assigned as the treatment groups, and the remaining half assigned as the recovery groups)

Low- and middle-dose groups: 12 males and 5 females per group (half of males assigned as the treatment groups, and the remaining half assigned as the recovery groups)

- Mating groups:
12 females per dose

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: Based on the results of a 14-day preliminary study, the high dose was set to 200 mg/kg bw/day, and the intermediate dose and low dose were set to 50 mg/kg bw/day and 12.5 mg/kg bw/day, respectively.

[14-day preliminary study]

A 14-day repeated dose oral toxicity test (CrI:CD(SD) rats, doses: 0, 100, 200 or 500 mg/kg bw/day). At 500 mg/kg bw/day, all males died, and 4 females died or were moribund. At 200 mg/kg bw/day, transient salivation, decreased body weight and food consumption, and decreased eosinophil percentage were observed in males and females, and increased platelets, shortened PT and APTT, reddened gastric mucosa, and decreased absolute relative thymus weight were observed in males, and increased neutrophil percentage, and decreased ALT and total protein were observed in females. At 100 mg/kg bw/day, decreased body weight, increased platelets, shortened PT, and decreased absolute relative thymus weight were observed in males, and decreased eosinophil percentage, and decreased ALT and total protein were observed in females.

- Rationale for animal assignment (if not random): Body weight-balanced randomization

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes

- Time schedule:

Males and females (main study groups): 2 times/day (before administration, 1-183 minutes after administration) during the administration period. Once a day during the recovery period.

Females (mating groups): 2 times/day (before administration, 3-150 minutes after administration) during the administration period.

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule:

Males and females (main study groups): on day of grouping, on days 7, 14, 21 and 27 of administration period.

Females (mating groups): on day of grouping, on days 7 and 14 of administration period, on days 1, 8 and 15 of gestation period, on day 4 of lactation period.

BODY WEIGHT: Yes

- Time schedule for examinations:

Males and females (main study groups):

Twice a week (On days 1, 4, 8, 11, 15, 18, 22, 25, 28 and 29 of administration period, on days 1, 4, 8, 11, 14 and 15 of recovery period).

Females (mating groups): Twice a week (On days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 43, 46, 50 and 53 of administration period, on days 0, 7, 14 and 20 of gestation period, on days 0, 4 and 5 of lactation period).

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

- Food consumption: Yes

- Time schedule for examinations:

Males and females (main study groups):

Twice a week (Males: On days 2, 5, 9 and 12 of administration period, on days 2, 5, 9 and 12 of recovery period; Females: On days 2, 5, 9, 12, 16, 19, 23 and 26 of administration period, on days 2, 5, 9 and 12 of recovery period).

Females (mating groups): Twice a week (On days 2, 5, 9 and 12 of administration period, on days 2, 9, 16 and 20 of gestation period, on days 2 of lactation period).

WATER INTAKE

- Time schedule for examinations:

Males and females (main study groups):

Twice a week (Males: On days 2, 5, 9 and 12 of administration period, on days 2, 5, 9 and 12 of recovery period; Females: On days 2, 5, 9, 12, 16, 19, 23 and 26 of administration period, on days 2, 5, 9 and 12 of recovery period).

Females (mating groups): Twice a week (On days 2, 5, 9 and 12 of administration period, on days 2, 9, 16 and 20 of gestation period, on days 2 of lactation period).

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes

- Time schedule for collection of blood:

Males and females (main study groups): At the end of administration period, or at the end of recovery period in both sexes

- Anaesthetic used for blood collection: Pentobarbital sodium

- Animals fasted: Yes

- How many animals:

At the end of administration period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

At the end of recovery period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0 and 200 mg/kg bw/day)

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

CLINICAL BIOCHEMISTRY: Yes

- Time schedule for collection of blood:

Males and females (main study groups): At the end of administration period, or at the end of recovery period in both sexes

- Animals fasted: Yes

- How many animals:

At the end of administration period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

At the end of recovery period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0 and 200 mg/kg bw/day)

- Parameters checked: ALP, total cholesterol, triglyceride, total bilirubin, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, A/G ratio, AST, ALT, γ -GT

BLOOD HORMONE: Yes

- Time schedule for collection of serum:

Males and females (main study groups): At the end of administration period in both sexes

- Animals fasted: Yes

- How many animals:

6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

- Parameters checked: Triiodothyronine (T3), Thyroxin (T4), and thyroid stimulating hormone (TSH)

URINALYSIS: Yes

- Time schedule for collection of urine:

Males and females (main study groups): Before the end of the administration period (day 23 of administration period) and before the end of recovery (day 12 of recovery period).

- Metabolism cages used for collection of urine: Yes

A urine collector to collect fresh urine samples under fasting but ad libitum drinking conditions, followed by collection of 24-hour urine samples under ad libitum feeding and drinking conditions.

- How many animals:

At the end of administration period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

At the end of recovery period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0 and 200 mg/kg bw/day)

- Parameters checked:

Fresh urine: Color, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, sediment

24-urine: Specific gravity, urine volume (24-hour volume),

NEUROBEHAVIOURAL EXAMINATION: Yes

- Time schedule for examinations:

Males and females (main study groups): Final week of administration (Manipulative test and measurement of grip strength: Day 27 of administration, measurement of motor activity: Day 26 of administration)

- Dose groups that were examined: Autopsy animals after the end of the administration period

- Battery of functions tested:

1) Manipulative Test. Pupillary reflex, approaching behavior, response to touch, auditory reflex, pain reflex

2) Measurement of Grip Strength. Following manipulative test, grip strength of forelimb and hind limb were measured by CPU gauge (San Diego Instruments Inc.).

3) Measurement of Spontaneous Motor Activity. Spontaneous motor activity (Ambulatory and vertical counts) was measured by Activity Monitor (MED Associates Inc.).

The measurements were collected at 10-minute intervals from 1 hour to 2 hours after administration.

Sacrifice and pathology

GROSS PATHOLOGY: Yes

ORGAN WEIGHT: Yes [main study groups: brain, pituitary, salivary glands, thyroids, adrenal gland, thymus, spleen, heart, liver, kidney, testes, epididymides, ventral prostate, seminal vesicles, ovaries, uterus; females in mating group: ovary, uterus]

HISTOPATHOLOGY: Yes, [main study groups: heart, lung, trachea, liver, pancreas, sublingual gland, submandibular gland, esophagus, stomach, duodenum, jejunum, ileum (including Peyer's patch), cecum, colon, rectum, thymus, spleen, mandibular lymph nodes, mesenteric lymph nodes, kidney, urinary bladder, testis, epididymis, ventral prostate, seminal vesicles (including coagulating gland), ovaries, uterus, vagina, pituitary, adrenal glands, thyroid (including parathyroid), cerebrum, cerebellum, pons, spinal cord, sciatic nerve, eye ball, Harderian gland, sternum and femur (including bone marrows), muscle (rectus femoris), mammary gland; females in mating group: ovaries, uterus, and vagina]

Statistics

For quantitative data, homogeneity of variance was tested using Bartlett method first. If the variance was homogenous, statistical difference between each treatment group and the control group was analyzed using Dunnett method. If not homogenous, statistical difference between each treatment group and the control group was tested using Steel method. For comparison of quantitative data between two groups in the recovery test, homogeneity of variance was analyzed by F-test. Then, if homogenous, student's t-test was applied. If not homogenous, Aspin-Welch's t-test was used.

For histopathological findings, statistical analysis was carried out in combination with Steel-test and Cochran-Armitage trend test.

Regarding clinical observation (except for frequency of urination, defecation, rearing and grooming) and sensory reactivity, Steel test was applied.

Results and discussion

Results of examinations

Clinical signs

effects observed, non-treatment-related

Description (incidence and severity)

CLINICAL SIGNS:

[At the administration period]:

Transient salivation was observed in males and females at 200 mg/kg bw/day. This was considered to be due to the irritant properties of the test substance.

[At the recovery period]:

There were no changes related to the test substance in any groups.

DETAILED CLINICAL OBSERVATIONS:

[At the administration period]:

Transient salivation was observed in males and females at 200 mg/kg bw/day. This was considered to be due to the irritant properties of the test substance.

Mortality

no mortality observed

Body weight and weight changes

effects observed, treatment-related

Description (incidence and severity)

[At the administration period]:

Decreases in body weights were observed throughout the administration period in males at 50 mg/kg /day and above, in late the administration period in females (main study and mating groups) at 200 mg/kg bw/day.

[At the recovery period]:

Decrease in body weight was observed throughout the recovery period in males at 200 mg/kg/day.

Food consumption and compound intake (if feeding study)

effects observed, treatment-related

Description (incidence and severity)

[At the administration period]:

Decreases in food consumption were observed throughout the administration period in males at 200 mg/kg bw/day and early in the administration period in females (main study and mating groups) at 200 mg/kg bw/day.

[At the recovery period]:

There were no changes related to the test substance in any groups.

Food efficiency

not examined

Water consumption and compound intake (if drinking water study)

effects observed, treatment-related

Description (incidence and severity)

[At the administration period]:

Increase in water consumption was observed late in the administration period in females (main study groups) at 200 mg/kg bw/day.

[At the recovery period]:

Increase in water consumption was observed early in the recovery period in females (main study groups) at 200 mg/kg bw/day.

Ophthalmological findings

not examined

Haematological findings

effects observed, treatment-related

Description (incidence and severity)

[At the end of administration period]:

Increases in platelet count and lymphocyte percentage, and decrease in neutrophil percentage were observed in females (main study groups) at 50 mg/kg bw/day and above. Increases in reticulocyte percentage and PT were observed in females (main study groups) at 200 mg/kg bw/day.

[At the end of recovery period]:

There were no changes related to the test substance in any groups.

Clinical biochemistry findings

effects observed, treatment-related

Description (incidence and severity)

Including blood hormones (T3, T4, TSH)

CLINICAL BIOCHEMISTRY:

[At the end of administration period]:

Decrease in triglyceride was observed in males at 50 mg/kg bw/day and above.

[At the end of recovery period]:

There were no changes related to the test substance in any groups.

BLOOD HORMONES:

There were no changes related to the test substance in any groups at the end of administration and recovery periods.

Urinalysis findings

no effects observed

Behaviour (functional findings)

no effects observed

Immunological findings

not examined

Organ weight findings including organ / body weight ratios

effects observed, treatment-related

Description (incidence and severity)

[At the end of administration period]:

Decreases in absolute and relative ovaries weights were observed in females (mating groups) at 12.5 mg/kg bw/day and 50 mg/kg bw/day. Decreases in absolute and relative uterus weights observed in females (mating groups) at 50 mg/kg bw/day.

[At the end of recovery period]:

There were no changes related to the test substance in any groups.

Gross pathological findings

no effects observed

Neuropathological findings

not examined

Histopathological findings: non-neoplastic

effects observed, treatment-related

Description (incidence and severity)

[At the end of administration period]:

Stomach: Chronic ulcer of the glandular stomach was observed in males and females (main study groups) at 50 mg/kg bw/day and above. Squamous epithelium hyperplasia of the forestomach was observed in males and females (main study groups) at 200 mg/kg bw/day.

[At the end of recovery period]:

Stomach: Scar of the glandular stomach was observed in males at 50 mg/kg bw/day and above. Squamous epithelium hyperplasia of the forestomach was observed in males and females (main study groups) at 200 mg/kg bw/day.

Histopathological findings: neoplastic

not examined

Effect levels

Key result false	
Dose descriptor NOAEL	
Effect level	
12.5	mg/kg bw/day (actual dose received)
Based on test mat.	
Sex male	
Basis for effect level body weight and weight gain At 50 mg/kg bw/day, decrease in body weight was observed in males. clinical biochemistry At 50 mg/kg bw/day, decrease in triglyceride was observed in males. histopathology: non-neoplastic At 50 mg/kg bw/day, chronic ulcer of the glandular stomach was observed in males.	
Key result false	
Dose descriptor NOAEL (non-mating females)	
Effect level	
12.5	mg/kg bw/day (actual dose received)
Based on test mat.	
Sex female	

Basis for effect level

haematology

At 50 mg/kg bw/day, Increases in platelet count and lymphocyte percentage, and decrease in neutrophil percentage were observed in females (main study groups).

histopathology: non-neoplastic

At 50 mg/kg bw/day, chronic ulcer of the glandular stomach was observed in females (main study groups).

Key result

false

Dose descriptor

NOAEL (maternal toxicity)

Effect level

< 12.5 mg/kg bw/day (actual dose received)

Based on

test mat.

Sex

female

Basis for effect level

organ weights and organ / body weight ratios

At 12.5 mg/kg bw/day, decreases in absolute and relative ovaries weights were observed in females (mating study groups).

Any other information on results incl. tables

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

https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9d.pdf

Applicant's summary and conclusion**Conclusions**

The NOAELs for repeated-dose toxicity were determined to be 12.5 mg/kg bw/day for males and females (non-mating groups), and the NOAEL for maternal toxicity was determined to be less than 12.5 mg/kg bw/day.

Executive summary

In the combined repeated dose and reproductive/developmental screening test (OECD TG422), SD rats were treated orally with the test substance at the doses of 0, 12.5, 50 and 200 mg/kg bw/day. Males (12 animals/dose: 6 animals were treated as a recovery group) were dosed for 28 days including a 14 day pre-mating period. Females (12 animals/dose) were dosed for 42-46 days including 14 day pre-mating, mating, and gestation periods and days until day 4 of lactation. In addition, as the main study group of females, 5 or 10 females/group was dosed for 28 days without mating (5 females at 0 and 200 mg/kg bw/day were treated as recovery groups).

Decreases in body weights were observed during the administration period in males at 50 mg/kg bw/day and above and in females (main study and mating groups) at 200 mg/kg bw/day. Decreases in food consumption were observed during the administration period in males and females (main study and mating groups) at 200 mg/kg bw/day. An increase in water consumption was observed during the

administration period and early recovery periods in females (main study group) at 200 mg/kg bw/day. The following findings were observed in the examination at the end of the administration period. In the haematological examination, increases in platelet count and lymphocyte percentage, and decreases in neutrophil percentage were observed in females (main study groups) at 50 mg/kg bw/day and above. Increases in reticulocyte percentage and PT were observed in females (main study groups) at 200 mg/kg bw/day. In the clinical chemistry, decreases in triglyceride were observed in males at 50 mg/kg bw/day and above. In the organ weights, decreases in absolute and relative ovaries weights were observed in females (mating groups) at 12.5 mg/kg bw/day and 50 mg/kg bw/day. Decreases in absolute and relative uterus weights were observed in females (mating groups) at 50 mg/kg bw/day. In the histopathological examination, chronic ulcer of the glandular stomach was observed in males and females (main study groups) at 50 mg/kg bw/day and above. Squamous epithelium hyperplasia of the forestomach was observed in males and females (main study groups) at 200 mg/kg bw/day. At the end of the recovery period, all changes observed in haematological examination, clinical chemistry, organ weight and histopathological examination were disappeared or reduced

Based on the above results, the NOAELs for repeated dose toxicity of 2,2'-[1,2-ethanediylbis(oxyethylene)]bis-oxirane were determined to be 12.5 mg/kg bw/day in male and female (non-mating groups), and NOAEL for maternal toxicity was determined to be less than 12.5 mg/kg bw/day.

Genetic toxicity

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

UUID: 1066351b-57a7-49e8-9ed4-e7477de31a7f

Dossier UUID:

Author:

Date: 2022-11-07T15:03:48.000+09:00

Remarks:

Administrative data

Endpoint

in vitro gene mutation study in bacteria

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

guideline study under GLP condition

Reliability 1

Data source

Reference

[Reverse Mutation Test of 2,2'-\[1,2-ethanediylbis\(oxymethylene\)\]bis-oxirane on Bacteria. / Ministry of Health, Labour and Welfare \(MHLW\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to guideline

Guideline

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

Deviations

no

Qualifier

according to guideline

Guideline

JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals
genetic toxicity in vitro, other

Deviations

no

GLP compliance

yes

Type of assay

bacterial reverse mutation assay
in vitro gene mutation study in bacteria

Test material**Test material information**

[2,2'-\[1,2-ethanediylbis\(oxymethylene\)\]bis-oxirane](#)

Specific details on test material used for the study

Purity: contain 0.1% water

Method**Species / strain****Species / strain / cell type**

S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
bacteria

Species / strain / cell type

E. coli WP2 uvr A
bacteria

Metabolic activation

with and without

Metabolic activation system

S9 mix: SD male rat liver, induced by phenobarbital and 5,6-benzoflavone

Test concentrations with justification for top dose

-S9 mix:

9.77, 19.5, 39.1, 78.1, 156.3, 312.5, 625, 1250, 2500, 5000 µg/plate (TA 1535 and TA 100 strains)

78.1, 156.3, 312.5, 625, 1250, 2500, 5000 µg/plate (WP2uvrA strain)

312.5, 625, 1250, 2500, 5000 µg/plate (TA 98 and TA 1537 strains)

+S9 mix:

39.1, 78.1, 156.3, 312.5, 625, 1250, 2500, 5000 µg/plate (TA 1535 and TA 100 strains)
78.1, 156.3, 312.5, 625, 1250, 2500, 5000 µg/plate (WP2uvrA strain)
312.5, 625, 1250, 2500, 5000 µg/plate (TA 98 and TA 1537 strains)

Maximum concentration was established based on the result of the preliminary test at concentration up to 5000 µg/plate. In this test, the growth inhibition was observed at 5000 µg/plate for WP2uvrA strain with or without S9 mix.

Vehicle / solvent

- Vehicle(s)/solvent(s) used: water for injection

Controls

Untreated negative controls

no

Negative solvent / vehicle controls

yes

True negative controls

no

Positive controls

yes

Positive control substance

9-aminoacridine

9-aminoacridine hydrochloride (9AA): -S9 mix: (TA1537)

sodium azide

NaN₃: -S9 mix: (TA1535)

furylfuramide

2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2): -S9 mix: (TA100, TA98, WP2 uvrA)

other: 2-aminoanthracene (2AA)

+S9 mix: (TA1535, TA100, TA98, TA1537 and WP2 uvrA)

Details on test system and experimental conditions

METHOD OF APPLICATION: Preincubation

DURATION- Preincubation period: 20 min at 37°C

- Exposure duration: ca.48 hrs

NUMBER OF PLATES: 3

NUMBER OF REPLICATIONS: 2

DETERMINATION OF CYTOTOXICITY

- Method: other: growth inhibition

Evaluation criteria

A chemical was judged to be mutagenic when the mean number of revertant colonies per plate increased more than twice that of the negative control and when the dose-related and reproducible increase was observed.

Statistics

no

Results and discussion

Test results**Key result**

true

Species / strainS. typhimurium TA 1535
bacteria**Metabolic activation**

with and without

Genotoxicity

positive

Cytotoxicity / choice of top concentrations

no cytotoxicity

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Key result

false

Species / strainS. typhimurium TA 1537
bacteria**Metabolic activation**

with

Genotoxicity

negative

Cytotoxicity / choice of top concentrations

no cytotoxicity

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Key result

true

Species / strain

S. typhimurium TA 1537
bacteria

Metabolic activation

without

Genotoxicity

positive

Cytotoxicity / choice of top concentrations

no cytotoxicity

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Key result

true

Species / strain

S. typhimurium TA 98
bacteria

Metabolic activation

with and without

Genotoxicity

positive

Cytotoxicity / choice of top concentrations

no cytotoxicity

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Key result

true

Species / strain

S. typhimurium TA 100
bacteria

Metabolic activation

with and without

Genotoxicity

positive

Cytotoxicity / choice of top concentrations

no cytotoxicity

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Key result

true

Species / strain

E. coli WP2 uvr A
bacteria

Metabolic activation

with

Genotoxicity

positive

Cytotoxicity / choice of top concentrations

cytotoxicity 5000 µg/plate

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Key result

false

Species / strain

E. coli WP2 uvr A
bacteria

Metabolic activation

without

Genotoxicity

negative

Cytotoxicity / choice of top concentrations

cytotoxicity 2500 µg/plate or more

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Additional information on results

The maximum specific activity of mutation was 2151.1 revertants/mg/plate, which was observed in plates of Salmonella typhimurium TA100 treated with the test article at 78.1 µg/plate without metabolic activation.

Any other information on results incl. tables

Figures and Tables (in English) are available in the following full report of the study.
https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9e.pdf

Applicant's summary and conclusion

Conclusions

Positive with or without metabolic activation

Executive summary

In a bacterial reverse mutation assay using Salmonella typhimurium TA100, TA1535, TA98, and TA 1537, and Escherichia coli WP2uvrA (OECD TG 471), 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane was positive for TA100, TA1535 and TA98 with or without metabolic activation, positive for Escherichia coli WP2uvrA/pKM101 with metabolic activation, positive for TA1537 without metabolic activation. The maximum specific activity of mutation was 2151.1 revertants/mg/plate, which was observed in plates of Salmonella typhimurium TA100 treated with the test article at 78.1 µg/plate without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

UUID: 193b622d-2af8-4946-818b-3a1f18c337b8

Dossier UUID:

Author:

Date: 2022-11-07T15:04:52.000+09:00

Remarks:

Administrative data

Endpoint

in vitro cytogenicity / chromosome aberration study in mammalian cells

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

guideline study under GLP condition

Reliability 1

Data source

Reference

[In Vitro Chromosomal Aberration Test of on 2,2'-\[1,2-ethanediylbis\(oxymethylene\)\]bis-oxirane Culture / Ministry of Health, Labour and Welfare \(MHLW\), Japan / study report](#)

Data access

data published

Materials and methods

Test guideline

Qualifier

according to guideline

Guideline

OECD Guideline 473 (In Vitro Mammalian Chromosomal Aberration Test)

in vitro cytogenicity / chromosomal aberration study in mammalian cells (from 26 September 2014)

Deviations

no

Qualifier

according to guideline

Guideline

JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals
genetic toxicity in vitro, other

Deviations

no

GLP compliance

yes

Type of assay

in vitro mammalian chromosome aberration test
in vitro cytogenicity / chromosome aberration study in mammalian cells

Test material**Test material information**

[2,2'-\[1,2-ethanediylbis\(oxymethylene\)\]bis-oxirane](#)

Specific details on test material used for the study

Purity: contain 0.1% water

Method**Species / strain****Species / strain / cell type**

Chinese hamster lung (CHL/IU)
mammalian cell line

Cytokinesis block (if used)

colcemid

Metabolic activation

with and without

Metabolic activation system

S9 mix: SD male rat liver, induced by phenobarbital and 5,6-benzoflavone

Test concentrations with justification for top dose

Cell growth inhibition study

-S9 mix (short-term treatment): 14.1, 28.1, 56.3, 112.5, 225, 450, 900, 1800 ug/mL

+S9 mix (short-term treatment): 14.1, 28.1, 56.3, 112.5, 225, 450, 900, 1800 ug/mL

-S9 mix (continuous treatment, 24hr): 14.1, 28.1, 56.3, 112.5, 225, 450, 900, 1800 ug/mL

Main study

-S9 (short-term treatment): 62.5, 125, 250, 500 ug/mL

+S9 (short-term treatment): 125, 250, 500, 1000 ug/mL

-S9 mix (continuous treatment, 24hr): 31.3, 62.5, 125, 250 ug/mL

Vehicle / solvent

- Vehicle(s)/solvent(s) used: water for injection

Controls

Untreated negative controls

no

Negative solvent / vehicle controls

yes

True negative controls

no

Positive controls

yes

Positive control substance

N-dimethylnitrosamine

+S9

mitomycin C

-S9

Details on test system and experimental conditions

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

SPINDLE INHIBITOR: Colcemid

STAIN: Giemsa stain (2 v/v%) for 15 min.

NUMBER OF REPLICATIONS: 2

NUMBER OF CELLS EVALUATED: 100 + 100 cells /concentration

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 were employed. Appearance incidence of cell with chromosomal aberrations: Negative (-): less than 5%, Equivocal(\pm): 5% or more and less than 10%, Positive(+): 10% or more

Statistics

no

Results and discussion

Test results**Key result**

true

Species / strain

Chinese hamster lung (CHL/IU)

mammalian cell line

Metabolic activation

with and without

Genotoxicity

positive structural aberration, D20: 0.051 mg/mL, TR: 1300

Cytotoxicity / choice of top concentrations

cytotoxicity

Vehicle controls validity

valid

Untreated negative controls validity

not examined

True negative controls validity

not examined

Positive controls validity

valid

Additional information on results

RANGE-FINDING/SCREENING STUDIES (if applicable):

50% cell growth inhibition (IC50): 650 ug/mL (short-term treatment, +S9 mix), 270 ug/mL (short-term treatment, -S9 mix), 150 ug/mL (continuous treatment)

Any other information on results incl. tables _____

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

https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9f.pdf

Applicant's summary and conclusion _____**Conclusions**

Positive with or without metabolic activation

Executive summary

In an in vitro chromosomal aberration test using CHL/IU cells (OECD TG 473), 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane induced structural chromosomal aberrations but did not induce chromosome numerical aberrations under the conditions of this study.

Toxicity to reproduction

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction. 001

UUID: ff758fd1-dff2-4058-87b4-6afdc80229cf

Dossier UUID:

Author:

Date: 2022-11-29T14:38:14.704+09:00

Remarks:

Administrative data

Endpoint

reproductive toxicity, other A combined repeated dose/reproductive developmental toxicity study

Type of information

experimental study

Robust study summary

false

Used for classification

false

Used for SDS

false

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

guideline study OECD Test Guideline study under GLP condition

Reliability 1

Cross-reference

Reason / purpose for cross-reference

reference to same study 7.5.1 Repeated dose toxicity: oral: Repeated dose toxicity: oral. 001

Related information

[OECD / Repeated dose toxicity: oral / Repeated dose toxicity: oral. 001 / 2,2,4,4-tetrahydro-2H-pyridine-3,5-dione / 2224-15-9](#)

Data source

Reference

[Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of / Ministry of Health, Labour and Welfare \(MHLW\), Japan / study report](#)

Data access

data published https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9d.pdf

Materials and methods

Test guideline

Qualifier

according to guideline

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

[2,2'-\[1,2-ethanediylbis\(oxymethylene\)\]bis-oxirane](#)

Specific details on test material used for the study

- Name of test material (as cited in study report): 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane
- Analytical purity: contain 0.1% water
- Storage condition of test material: Room temperature, shading, airtightness
- Stability under test conditions: The stability of test material was identified by analysis of the remainder.

Test animals

Species

rat

Strain

other: CrI:CD(SD)

Sex

male/female

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

- Source: Charles River Japan, Inc., Hino Breeding Center.
- Age at study initiation: 10 weeks old
- Weight at study initiation:
Males (main study groups): 365-433 g, females (main study groups): 236-266 g, females in (mating study groups): 219-265 g
- Housing: Animals were individually housed in stainless steel suspension cage (240W × 380D × 200H mm), from gestation day 18 to lactation day 4, Dams were bred individually or with individual littermates in plastic cages (310W x 360D x 175H mm) and bedding.
- Diet: Solid feed (CRF-1: Oriental Yeast Co., Ltd.) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation period: Males (main study groups): 21 days, females (main study groups): 22 days, females (mating study groups): 21 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20-26°C (actual temperature: 22.5-24.7°C)
- Humidity (%): 40.0-70.0% (actual humidity: 41.3-58.4%)
- Air changes (per hr): 12

- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 6:00~18:00)

Administration / exposure

Route of administration

oral: gavage

Vehicle

water for injection

Details on exposure

- Amount of vehicle (if gavage): 5 mL/kg
- Dosing volume: 5 mL/kg

Details on mating procedure

- M/F ratio per cage: 1/1
- Length of cohabitation: up to 14 days
- Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0 of pregnancy

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Concentrations of the test solutions using administration on day 1 were analyzed with GC. Analytical concentrations of the test solutions were all within the range of 92.2-106.4% of the nominal concentrations and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%).

Duration of treatment / exposure

Males: 28 days including 14 days pre-mating

Females (main study groups): 28 days

Females (mating groups): 42-46 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation

Frequency of treatment

Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
12.5	mg/kg bw/day (actual dose received)
Dose / conc.	
50	mg/kg bw/day (actual dose received)
Dose / conc.	
200	mg/kg bw/day (actual dose received)

No. of animals per sex per dose

- Main study groups:

Control- and high-dose groups: 12 males and 10 females per group (half of both sexes assigned as the treatment groups, and the remaining half assigned as the recovery groups)

Low- and middle-dose groups: 12 males and 5 females per group (half of males assigned as the treatment groups, and the remaining half assigned as the recovery groups)

- Mating groups:
12 females per dose

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: Based on the results of a 14-day preliminary study, the high dose was set to 200 mg/kg bw/day, and the intermediate dose and low dose were set to 50 mg/kg bw/day and 12.5 mg/kg bw/day, respectively.

[14-day preliminary study]

A 14-day repeated dose oral toxicity test (CrI:CD(SD) rats, doses: 0, 100, 200 or 500 mg/kg bw/day). At 500 mg/kg bw/day, all males died, and 4 females died or were moribund. At 200 mg/kg bw/day, transient salivation, decreased body weight and food consumption, and decreased eosinophil percentage were observed in males and females, and increased platelets, shortened PT and APTT, reddened gastric mucosa, and decreased absolute relative thymus weight were observed in males, and increased neutrophil percentage, and decreased ALT and total protein were observed in females. At 100 mg/kg bw/day, decreased body weight, increased platelets, shortened PT, and decreased absolute relative thymus weight were observed in males, and decreased eosinophil percentage, and decreased ALT and total protein were observed in females.

- Rationale for animal assignment (if not random): Body weight-balanced randomization

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes

- Time schedule:

Males and females (main study groups): 2 times/day (before administration, 1-183 minutes after administration) during the administration period. Once a day during the recovery period.

Females (mating groups): 2 times/day (before administration, 3-150 minutes after administration) during the administration period.

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule:

Males and females (main study groups): on day of grouping, on days 7, 14, 21 and 27 of administration period.

Females (mating groups): on day of grouping, on days 7 and 14 of administration period, on days 1, 8 and 15 of gestation period, on day 4 of lactation period.

BODY WEIGHT: Yes

- Time schedule for examinations:

Males and females (main study groups):

Twice a week (On days 1, 4, 8, 11, 15, 18, 22, 25, 28 and 29 of administration period, on days 1, 4, 8, 11, 14 and 15 of recovery period).

Females (mating groups): Twice a week (On days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 43, 46, 50 and 53 of administration period, on days 0, 7, 14 and 20 of gestation period, on days 0, 4 and 5 of lactation period).

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

- Food consumption: Yes

- Time schedule for examinations:

Males and females (main study groups):

Twice a week (Males: On days 2, 5, 9 and 12 of administration period, on days 2, 5, 9 and 12 of recovery period; Females: On days 2, 5, 9, 12, 16, 19, 23 and 26 of administration period, on days 2, 5, 9 and 12 of recovery period).

Females (mating groups): Twice a week (On days 2, 5, 9 and 12 of administration period, on days 2, 9, 16 and 20 of gestation period, on days 2 of lactation period).

WATER INTAKE

- Time schedule for examinations:

Males and females (main study groups):

Twice a week (Males: On days 2, 5, 9 and 12 of administration period, on days 2, 5, 9 and 12 of recovery period; Females: On days 2, 5, 9, 12, 16, 19, 23 and 26 of administration period, on days 2, 5, 9 and 12 of recovery period).

Females (mating groups): Twice a week (On days 2, 5, 9 and 12 of administration period, on days 2, 9, 16 and 20 of gestation period, on days 2 of lactation period).

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes

- Time schedule for collection of blood:

Males and females (main study groups): At the end of administration period, or at the end of recovery period in both sexes

- Anaesthetic used for blood collection: Pentobarbital sodium

- Animals fasted: Yes

- How many animals:

At the end of administration period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

At the end of recovery period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0 and 200 mg/kg bw/day)

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

CLINICAL BIOCHEMISTRY: Yes

- Time schedule for collection of blood:

Males and females (main study groups): At the end of administration period, or at the end of recovery period in both sexes

- Animals fasted: Yes

- How many animals:

At the end of administration period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

At the end of recovery period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0 and 200 mg/kg bw/day)

- Parameters checked: ALP, total cholesterol, triglyceride, total bilirubin, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, A/G ratio, AST, ALT, γ -GT

BLOOD HORMONE: Yes

- Time schedule for collection of serum:

Males and females (main study groups): At the end of administration period in both sexes

- Animals fasted: Yes

- How many animals:

6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

- Parameters checked: Triiodothyronine (T3), Thyroxin (T4), and thyroid stimulating hormone (TSH)

URINALYSIS: Yes

- Time schedule for collection of urine:

Males and females (main study groups): Before the end of the administration period (day 23 of administration period) and before the end of recovery (day 12 of recovery period).

- Metabolism cages used for collection of urine: Yes

A urine collector to collect fresh urine samples under fasting but ad libitum drinking conditions, followed by collection of 24-hour urine samples under ad libitum feeding and drinking conditions.

- How many animals:

At the end of administration period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0, 12.5, 50, 200 mg/kg bw/day)

At the end of recovery period: 6 males/dose (0, 12.5, 50, 200 mg/kg bw/day), 5 females/dose (0 and 200 mg/kg bw/day)

- Parameters checked:

Fresh urine: Color, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, sediment

24-urine: Specific gravity, urine volume (24-hour volume),

NEUROBEHAVIOURAL EXAMINATION: Yes

- Time schedule for examinations:

Males and females (main study groups): Final week of administration (Manipulative test and measurement of grip strength: Day 27 of administration, measurement of motor activity: Day 26 of administration)

- Dose groups that were examined: Autopsy animals after the end of the administration period

- Battery of functions tested:

1) Manipulative Test. Pupillary reflex, approaching behavior, response to touch, auditory reflex, pain reflex

2) Measurement of Grip Strength. Following manipulative test, grip strength of forelimb and hind limb were measured by CPU gauge (San Diego Instruments Inc.).

3) Measurement of Spontaneous Motor Activity. Spontaneous motor activity (Ambulatory and vertical counts) was measured by Activity Monitor (MED Associates Inc.).

The measurements were collected at 10-minute intervals from 1 hour to 2 hours after administration.

Oestrous cyclicity (parental animals)

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

Sperm parameters (parental animals)

Parameters examined in all P male parental generations: testis, epididymis and seminal vesicle weight, histopathological examinations for testes, epididymides, seminal vesicle and ventral prostate.

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.

GROSS EXAMINATION OF DEAD PUPS: Yes, for external and internal abnormalities.

Postmortem examinations (parental animals)

METHOD OF SACRIFICED: All animals were sacrificed by exsanguination under pentobarbital sodium anesthesia.

SACRIFICE: Males and females (main study groups): On next day after the last administration,

Maternal animals: on Day 5 of lactation, and males and females recovery group: on Day 14 of recovery

.

GROSS PATHOLOGY: Yes

ORGAN WEIGHT: Yes [main study groups: brain, pituitary, salivary glands, thyroids, adrenal gland, thymus, spleen, heart, liver, kidney, testes, epididymides, ventral prostate, seminal vesicles, ovaries, uterus; females in mating group: ovary, uterus]

HISTOPATHOLOGY: Yes, [main study groups: heart, lung, trachea, liver, pancreas, sublingual gland, submandibular gland, esophagus, stomach, duodenum, jejunum, ileum (including Peyer's patch),

cecum, colon, rectum, thymus, spleen, mandibular lymph nodes, mesenteric lymph nodes, kidney, urinary bladder, testis, epididymis, ventral prostate, seminal vesicles (including coagulating gland), ovaries, uterus, vagina, pituitary, adrenal glands, thyroid (including parathyroid), cerebrum, cerebellum, pons, spinal cord, sciatic nerve, eye ball, Harderian gland, sternum and femur (including bone marrow), muscle (rectus femoris), mammary gland; females in mating group: ovaries, uterus, vagina]

Postmortem examinations (offspring)

SACRIFICE

- The F1 offsprings were euthanized on PND4 by exsanguination under 20% Isoflurane anesthesia.

GROSS NECROPSY: Yes

- Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

HISTOPATHOLOGY / ORGAN WEIGHTS

- Not examined.

Statistics

For quantitative data, homogeneity of variance was tested using Bartlett method first. If the variance was homogenous, statistical difference between each treatment group and the control group was analyzed using Dunnett method. If not homogenous, statistical difference between each treatment group and the control group was tested using Steel method. For comparison of quantitative data between two groups in the recovery test, homogeneity of variance was analyzed by F-test. Then, if homogenous, student's t-test was applied. If not homogenous, Aspin-Welch's t-test was used.

For histopathological findings, statistical analysis was carried out in combination with Steel-test and Cochran-Armitage trend test. Regarding clinical observation (except for frequency of urination, defecation, rearing and grooming) and sensory reactivity, Steel test was applied. Regarding implantation index, delivery index, birth index, live birth index, viability index, sex ratio and external abnormalities, Steel test was performed between administration groups and control groups. Regarding copulation, fertility index, and gestation index, Fisher's test was applied.

Reproductive indices

Each parameter was determined by the following equations:

Copulation index (%) = (No. of pairs with successful copulation / No. of pairs) × 100

Fertility index (%) = (No. of pregnant females / No. of pairs with successful copulation) × 100

Gestation index (%) = (No. of dams having live pups / No. of pregnant dams) × 100

Length of gestation (days)

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

Delivery index (%) = (No. of pups born / No. of implantation scars) × 100

Birth index (%) = (No. of live pups born / No. of implantation scars) × 100

Live birth index (%) = (No. of live pups born / No. of pups born) × 100

Sex ratio on Day 4 of lactation = No. of male pups / No. of female pups

External abnormalities (%) = (No. of pups with external abnormalities / No. of live pups) × 100

Offspring viability indices

Viability index (%) = (No. of live pups on Day 4 of lactation / No. of live pups born) × 100

Results and discussion

Results: P0 (first parental generation)

General toxicity (P0)

Clinical signs

effects observed, non-treatment-related

Description (incidence and severity)

See 7.5.1 Repeated dose toxicity.001

Mortality

no mortality observed

Body weight and weight changes

effects observed, treatment-related

Description (incidence and severity)

See 7.5.1 Repeated dose toxicity.001

Food consumption and compound intake (if feeding study)

effects observed, treatment-related

Description (incidence and severity)

See 7.5.1 Repeated dose toxicity.001

Food efficiency

not examined

Water consumption and compound intake (if drinking water study)

effects observed, treatment-related

Description (incidence and severity)

See 7.5.1 Repeated dose toxicity.001

Ophthalmological findings

not examined

Haematological findings

effects observed, treatment-related

Description (incidence and severity)

See 7.5.1 Repeated dose toxicity.001

Clinical biochemistry findings

effects observed, treatment-related

Description (incidence and severity)

See 7.5.1 Repeated dose toxicity.001

Urinalysis findings

no effects observed

Behaviour (functional findings)

no effects observed

Immunological findings

not examined

Organ weight findings including organ / body weight ratios

effects observed, treatment-related

Description (incidence and severity)

See 7.5.1 Repeated dose toxicity.001

Gross pathological findings

no effects observed

Neuropathological findings

not examined

Histopathological findings: non-neoplastic
effects observed, treatment-related

Description (incidence and severity)
See 7.5.1 Repeated dose toxicity.001

Histopathological findings: neoplastic
not examined

Reproductive function / performance (P0)

Reproductive function: oestrous cycle
no effects observed

Reproductive function: sperm measures
no effects observed

Reproductive performance
effects observed, treatment-related

Details on results (P0)

General toxicity: See 7.5.1 Repeated dose toxicity.001

Reproductive performance: An increased non-pregnant females, decreased fertility index were observed at 50 mg/kg bw/day and above. Decreases in number of corpora lutea, number of implantations, implantation index, and birth index, prolonged gestation length were observed at 50 mg/kg bw/day.

Effect levels (P0)

Key result

false

Dose descriptor

NOAEL

Effect level

12.5

mg/kg bw/day (actual dose received)

Based on

test mat.

Sex

male/female

Basis for effect level

reproductive performance

At 50 mg/kg bw/day, decreases in number of corpora lutea, number of implantations, implantation index, and birth index, prolonged gestation length were observed.

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Mortality / viability

mortality observed, treatment-related

Body weight and weight changes

effects observed, treatment-related

Gross pathological findings

no effects observed

Effect levels (F1)

Key result

false

Dose descriptor

NOAEL

Generation

F1

Effect level

12.5

mg/kg bw/day (actual dose received)

Based on

test mat.

Sex

male/female

Basis for effect level

other: At 50 mg/kg bw/day, decreases in pups born, live pups born, live pups on Day 4 of lactation, viability index, increases in body weights at Day0 and Day4 of lactation were observed.

Any other information on results incl. tables

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

https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9d.pdf

Applicant's summary and conclusion

Conclusions

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, reproductive effects in parent animals and effects on offspring were observed at 50 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of 2,2'-[1,2-ethanediylbis(oxyethylene)]bis-oxirane was regarded as 12.5 mg/kg bw/day.

References

Reference Substances

REFERENCE_SUBSTANCE: 2,2'-[1,2-Ethanediy]bis(oxymethylene)]bis-oxirane

UUID: e03f3fed-a3ac-4bd4-b972-e670071cb252

Dossier UUID:

Author:

Date: 2022-11-07T15:00:24.000+09:00

Remarks:

Reference substance name

2,2'-[1,2-Ethanediy]bis(oxymethylene)]bis-oxirane

Inventory

CAS number

2224-15-9

Synonyms

Synonyms

Identifier

other: Ethylene glycol diglycidyl ether

Molecular and structural information

Molecular formula

C₈H₁₄O₄

Molecular weight

174.19

Test Materials

TEST_MATERIAL_INFORMATION: 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane

UUID: 931e20ce-f068-4902-9747-41c723b5f5e0

Dossier UUID:

Author:

Date: 2022-11-07T15:02:41.000+09:00

Remarks:

Name

2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane

Composition

Composition

Reference substance

2,2'-[1,2-Ethanediylbis(oxymethylene)]bis-oxirane / 2224-15-9

EC number

EC name

CAS number

CAS name

2224-15-9

IUPAC name

Literatures

LITERATURE: Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane by oral administration in rats

UUID: bd9f6f04-067d-4005-9e92-e1af12f2c22b

Dossier UUID:

Author:

Date: 2022-11-07T15:00:55.000+09:00

Remarks:

General information

Reference Type
study report

Title
Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane by oral administration in rats

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

Year
2013

Bibliographic source
available in the web of Japan Existing Chemical Data Base (JECDB) https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9d.pdf

Testing facility
Nihon Bioresearch Inc.

Report number
100130

LITERATURE: In Vitro Chromosomal Aberration Test of on 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane Cultured Chinese Hamster Cells.

UUID: a091c3ef-9a83-4112-8765-688872d9dd16

Dossier UUID:

Author:

Date: 2022-11-07T15:04:18.000+09:00

Remarks:

General information

Reference Type

study report

Title

In Vitro Chromosomal Aberration Test of on 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane Cultured Chinese Hamster Cells.

Author

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

Year

2011

Bibliographic source

Japan Existing Chemical Data Base (JECDB)

https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9f.pdf

Testing facility

Nihon Bioresearch Inc.

Report number

970530

LITERATURE: Reverse Mutation Test of 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane on Bacteria.

UUID: 475e1779-7bd5-4491-96f7-4ef097fb790f

Dossier UUID:

Author:

Date: 2022-11-07T15:02:24.000+09:00

Remarks:

General information

Reference Type

study report

Title

Reverse Mutation Test of 2,2'-[1,2-ethanediylbis(oxymethylene)]bis-oxirane on Bacteria.

Author

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

Year

2011

Bibliographic source

Japan Existing Chemical Data Base (JECDB)

https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF2224-15-9e.pdf

Testing facility

Nihon Bioresearch Inc.

Report number

900830

Legal Entities

LEGAL_ENTITY: National Institute of Health Sciences

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

Dossier UUID:

Author:

Date: 2022-11-07T15:49:29.000+09:00

Remarks:

General information

Legal entity name

National Institute of Health Sciences

Remarks

Disclaimer: The contents in this document were created based on the MHLW (Ministry of Health, Labour and Welfare) peer reviewed study reports (in Japanese) in JECDB (Japan Existing Chemical Database) at http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp. Authorship is in the Division of Risk Assessment, the National Institute of Health Sciences, and the contents do not reflect any official MHLW opinions or any other regulatory policies.

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Region / State

Kanagawa

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Identifiers

Other IT system identifiers

IT system

LEO

ID

10767

IT system

IUCLID4

ID

16558402024DIV750