Name: OECD_SIDS / SUBSTANCE : Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate / diammonium hydrogen citrate / 3012-65-5 Fri, 16 Dec 2022, 14:40:50+0900 /

Legal entity owner: National Institute of Health Sciences

Printing date: 2022-12-16T14:40:50.467+09:00

Table of Contents

DOSSIER:

UUID: 0
Dossier UUID:
Author:
Date: 2022-12-16T14:40:50.254+09:00
Remarks:

Dossier header

Dossier submission type

Name
OECD SIDS

Version
core 7.0

Name (given by user)

Dossier subject

Dossier subject
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate / diammonium hydrogen citrate / 3012-65-5

Public name

Submitting legal entity
National Institute of Health Science

Dossier creation date/time
Fri, 16 Dec 2022, 14:40:50+0900

Used in category

LEGAL_ENTITY: National Institute of Health Science

UUID:	f51e7b54-9211-4863-90ce-fcf8a155d647
Dossier UUID:	
Author:	
Date:	2022-11-07T16:24:02.822+09:00
Remarks:	

General information

Legal entity name
National Institute of Health Science

Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

General information

Identification

Identification

SUBSTANCE: Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

UUID: IUC5-8627c376-bb47-4255-90c4-6639d07c0bb2
Dossier UUID:
Author:
Date: 2022-12-16T14:40:36.984+09:00
Remarks:

Substance name
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Legal entity
National Institute of Health Sciences / Kawasaki / Japan

Identification of substance

Reference substance
diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate / diammonium hydrogen citrate / 3012-65-5 / 221-146-3

EC number	EC name
221-146-3	EC Inventory
CAS number	**CAS name**
3012-65-5	
IUPAC name	
diammonium hydrogen citrate	

Role in the supply chain

Manufacturer
false

Importer
false

Only representative
false

Downstream user
false

Assessment approach (assessment entities)

FIXED_RECORD: Assessment approach

UUID:	b52b1221-3cd2-3919-b22b-e6acb33f56f3
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Author:	
Date:	2016-12-21T15:15:08.000+09:00
Remarks:	

Toxicological information

Acute Toxicity

Acute toxicity: oral

ENDPOINT_STUDY_RECORD: Acute toxicity: oral.001

UUID: IUC5-0469431d-735d-47de-8e42-89cac5c65922
Dossier UUID:
Author:
Date: 2022-12-16T14:35:59.541+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 diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate in Rats / MHLW / publication

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

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

Test material

Test material information
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Specific details on test material used for the study
- Name of test material (as cited in study report): diammonium hydrogen 2-hydroxypropane-1,2,3-trica rboxylate
- Analytical purity: 100.0%
- Lot/batch No.: 6803
- Stability under test conditions: The stability of test material was identified by analysis of the remainder.
- Storage condition of test material: At a cold place (temperature 2~7℃) in a refrigerator, with a stopper.

Test animals

Species
rat
common species

Strain
other: Crl:CD(SD)

Sex
female

Details on test animals or test system and environmental conditions
TEST ANIMALS
- Source :Charles River Japan Inc.
- Age at study initiation: 8-9 weeks old
- Weight at study initiation: Females, 207-224 g
- Fasting period before study: Approximately 16-18 hrs
- Housing:1/cage
- Diet (e.g. ad libitum): Ad libitum except fasting period for 16-18 hrs before administration to 4 hrs after administration
- Water (e.g. ad libitum):Ad libitum
- Acclimation period:6-16 days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22±3 ℃(actual temperature: 19-24℃)
- Humidity (%):50 ± 20% (actual humidity: 33-57%)
- Air changes (per hr): Approximately 10-15 times/hr
- Photoperiod (hrs dark / hrs light):12 hrs light / 12 hrs dark

Administration / exposure

Route of administration
oral: gavage

Vehicle
other: 0.5%CMC-Na

Details on oral exposure
- Amount of vehicle (if gavage):10 mL/kg bw

Doses
300, 2000 mg/kg bw

No. of animals per sex per dose
3 (1st step group), 3 (2nd step group), 3 (3rd step group) and 3 (4th step group)

Control animals
no

Details on study design
- Duration of observation period following administration: 14 days
- Frequency of observations: nearly successive observation (from time just to 1 hr after administration) and observation (at 2, 4 and 6 hr (only 3 & 4 steps) after administration) (day 0); twice a day (from day 1-day13) and once a day (day14)
- Frequency of weighing: just before administration (day 0), and 1,3,5,7,10 and 14 day after adminis tration
- Necropsy of survivors performed: yes

Results and discussion

Effect levels

Key result
false

Sex
female

Dose descriptor
LD50

Effect level

>	2000	mg/kg bw

Mortality
No deaths were observed in any group.

Clinical signs
other: At 2000 mg/kg bw, mucus feces were observed.
No changes related to the test substance were observed in any group.

Gross pathology
No changes related to the test substance were observed in any group.

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/PDF3012 -65 -5a.pdf

Applicant's summary and conclusion

Executive summary

The acute oral LD50 of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate was > 2,000 mg/kg bw in female rats based on a study conducted according to OECD TG 423. No deaths were observed at 2,000 mg/kg bw. This substance caused mucoid stools at 2,000 mg/kg bw.

Repeated dose toxicity

Repeated dose toxicity: oral

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.001

UUID: IUC5-55581c53-51f6-4e2f-a181-994678536d94
Dossier UUID:
Author:
Date: 2022-12-16T14:37:06.491+09:00
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: GLP guideline study

Cross-reference

Reason / purpose for cross-reference
reference to same study

Remarks
7.8.1 Reproductive/developmental toxicity.001

Data source

Reference

Combined repeat dose and reproductive/developmental toxicity screening test of diammonium hydrogen 2 / 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
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Specific details on test material used for the study
- Name of test material (as cited in study report): diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate
- Purity: 100.0%
- Impurities (identity and concentrations):
- Lot/batch No.: 6803
- Stability under test conditions: Stable
- Storage condition of test material: Refrigeration
- Dosing solution storage condition: Room temperature
- Other: The dosing solution was used within 10 days of preparation.

Test animals

Species
rat
common rodent species

Strain
other: Crl: CD(SD)

Sex
male/female

Details on test animals or test system and environmental conditions
TEST ANIMALS
- Source: Charles River Laboratories Japan, Inc. Atsugi
- Age at study initiation: 10 weeks
- Weight at study initiation: Males: 371-439 g; Females: 219-273 g
- Housing: Steel wire-mesh cage (250 mm x 350 mm x 200 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 15 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21-26
- Humidity (%): 42-64
- Air changes: 10-15 times / hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
water

Details on oral exposure
PREPARATION OF DOSING SOLUTIONS:
VEHICLE
- Amount of vehicle (if gavage): 5 mL/kg bw
- Lot/batch no. (if required): 6F74

Details on analytical verification of doses or concentrations
Test suspensions at each concentration to be used for males in week 1 and week 6 of administration were analyzed by the HPLC method at Bozo Research Center Inc. Results showed that the concentration of the test article in each suspension was 93.3 to 100.0% of the nominal concentration and both values were within the acceptable range (concentration: percentage of the nominal concentration, 100 ± 10%; C.V.: 10% or below)

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating and mating periods, and thereafter 14 days (P) Females: 41-47 days including 14 days pre-mating, mating and gestation periods, and the days until day 4 of lactation; satellite animals: 42 days.

Frequency of treatment
Once/day, 7days/week

Doses / concentrations

Remarks
Doses / Concentrations:
0 (vehicle), 100, 300, and 1000 mg/kg bw/day
Basis:
actual ingested

No. of animals per sex per dose
12 animals/sex/dose as a main dose group,
5 males and 5 females at 0 and 1000 mg/kg bw/day as a satellite group (without mating)

Control animals
yes, concurrent vehicle

Details on study design
- Dose selection rationale: Doses in this test were set based on the results of the following study: 14-day repeated dose oral toxicity test (doses: 100, 300, and 1000 mg/kg bw/day). In the 14-day repeated dose oral toxicity test, abnormalities were observed in animals in the 1000 mg/kg bw/day group, such as an increase in relative kidney weight. No effects were observed at 300 mg/kg bw/day. On th

e basis of these effects, a dose level of 1000 mg/kg was selected as the maximum dose expecting to induce the toxic changes, and then dose levels of 300 and 100 mg/kg bw/day were selected as a middle dose and a minimum dose levels, respectively, in accordance with a common ratio of approximately 3.
- Rationale for animal assignment (if not random): Body weight-balanced randomization
- Post-exposure recovery period in satellite groups: 14 days

Examinations

Observations and examinations performed and frequency
CAGE SIDE OBSERVATIONS: Yes
- Time schedule:
Males and females: once before the start of administration, 3 times/day during the administration period, and once during the recovery period

DETAILED CLINICAL OBSERVATIONS: Yes
The functional observational battery testing (FOB) was performed on all animals. Among the measures in the FOB, detailed clinical observations were made before the initiation of dosing. Thereafter, in males of the main groups, detailed clinical observations were made once a week. Also in females of the main groups, detailed clinical observations were made once a week in pre-mating and mating periods thereafter, and then those were made on days 1,7,14 and 20 of gestation, and on day 4 of lactation. For the satellite group, detailed clinical observations were made once a week in dosing and recovery periods.
Sensory motor reflexes, forelimb and hindlimb grip strengths, and motor activity were measured on week 6 of administration period (main/recovery group animals) and week 2 of recovery period (recovery group animals).

BODY WEIGHT: Yes
- Time schedule for examinations: Males (main) & males and females (recovery group): Days 1, 4, 8, 11, 15, 22, 25, 29, 32, 36, 39, 42, and the day of necropsy (after ca. 16h-fasting) in dosing period
Males and females (recovery group): Days 1, 4, 8, 11, 14, and the day of necropsy (after ca. 16h-fasting) in recovery period
Females (main group): Twice a week during the precopulation period (days 1, 4, 8, 11, and 15); gestation days 0, 4, 7, 11, 14, 17, and 20; lactation days 0 and 4; and the day of necropsy (after ca. 16 h-fasting)
FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Males (main) & males and females (recovery group): Days 1, 4, 8, 11, 15, 32, 36, and 39 in dosing period
Males and females (recovery group): Days 1, 4, 8, 11, and 14 in recovery period
Females (main group): Days 1, 4, 8, 11, and 15; gestation days 1, 4, 7, 11, 14, 17, and 20; lactation days 2 and 4

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: Blood was collected on the day of necropsy
- Anaesthetic used for blood collection: Yes (ether)
- Animals fasted: Yes, 16-20h
- How many animals: 5 sex/dose/group
- Parameters checked in table were examined.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: Same as hematology
- Animals fasted: Same as hematology
- How many animals: Same as hematology
- Parameters checked in table were examined.

URINALYSIS: Yes (males only)
- Time schedule for collection of urine: Day 37-38 in dosing period, day 11-12 in recovery period
- Metabolism cages used for collection of urine: No data
- Animals fasted: fasting and only water at libitum (4h-urine), no fasting (20h-urine)

Sacrifice and pathology
SACRIFICE:
Male animals: Rats were euthanized by exsanguination under ether anesthesia on the day after the l ast administration.
Maternal animals: Rats were euthanized by exsanguination under ether anesthesia on day 4 of lactati on.

GROSS PATHOLOGY, Yes: whole organs and tissues

ORGAN WEIGHTS, Yes: Brain, thyroids(including parathyroids), thymus, heart, liver, spleen, kidneys, adrenals, testes, epididymis

HISTOPATHOLOGY, Yes: Cerebrum, cerebellum, pituitary gland, spinal cord (thoracic), sciatic nerve, thyroid, parathyroid, adrenal glands, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, lung (including the bronchi), stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, kidney, bladder, testis, epididymis, ovary, uterus, seminal vesicles, sternum and femur (including bone marrows), macroscopic lesions.

Other examinations
Organ weight: Brian, thyroids (including parathyroids), thymus, heart, liver, spleen, kidneys, adrenals, testes, epididymis

Statistics
The data were analyzed for homogeneity of variance by the Bartlett test. If variances were homogeneous, data was analyzed by the Dunnett test, whereas heterogeneous data was analyzed by the Dunnett type mean rank test ($p<0.05$, two-sided).
In the recovery test, these values of two groups were analyzed by F test. If variances were homogene ous, data was analyzed by the Student t-test, whereas heterogeneous data was analyzed by the Aspin-Welch t-test ($p<0.05$, two-sided).

Results and discussion

Results of examinations

Clinical signs
no effects observed

Mortality
no mortality observed

Body weight and weight changes
no effects observed

Food consumption and compound intake (if feeding study)
no effects observed

Food efficiency
not examined

Water consumption and compound intake (if drinking water study)
not examined

Ophthalmological findings
not examined

Haematological findings
no effects observed

Clinical biochemistry findings
effects observed, treatment-related

Urinalysis findings
effects observed, treatment-related

Behaviour (functional findings)
no effects observed

Organ weight findings including organ / body weight ratios
no effects observed

Gross pathological findings
no effects observed

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

Histopathological findings: neoplastic
not examined

Details on results
CLINICAL CHEMISTRY
High value of glucose was observed at 1000 mg/kg bw/day at the end of dosing.

URINALYSIS (male only)
At week 6 of administration in the main group, an increased trend of acidification and a decreased incidence of crystallization of phosphate were observed dose-dependently.

HISTOPATHOLOGY: NON-NEOPLASTIC
[At the end of dosing] and [At the end of recovery period]
Stomach: Minimal or mild hyperplasia of squamous cells in the limiting ridge were observed in males and females at 1000 mg/kg bw/day.

Any other changes with statistically significant in the tables were considered to be incidental due to temporary, dose-independent, or within the normal ranges of physiological variability.

Effect levels

Key result
false

Dose descriptor
LOAEL

Effect level

300	mg/kg bw/day (actual dose received)

Based on
test mat.

Sex
male/female

Basis for effect level
other: effects on the stomach

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/PDF3012 -65 -5d.pdf

Applicant's summary and conclusion

Conclusions
Based on the effects on the stomach in males and females at 1000 mg/kg bw/day, the NOAEL for repeated oral dosing was determined to be 300 mg/kg bw/day in male and female rats.

Executive summary

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed according to OECD TG 422. Male and female rats (12 animals/sex/dose) were administered diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate at 0, 100, 300, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14-day pre-mating and mating periods. Females were dosed for 41–47 days, including a 14-day pre-mating, mating, and gestation periods and the time until day 4 of lactation. Five animals/sex/dose administered 0 and 1,000 mg/kg bw/day were treated as the recovery group and examined after a 14-day recovery period. After the administration period, squamous cell hyperplasia of the boundary edge in the stomach was observed at 1,000 mg/kg bw/day in both sexes. This change resolved after the recovery period. On the basis of the observed stomach changes, NOAEL for repeated-dose toxicity was determined to be 300 mg/kg bw/day in male and female rats.

Genetic toxicity

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

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Dossier UUID:
Author:
Date: 2022-12-16T14:38:16.038+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 diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate on Bacteria. / MHLW, Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals

Qualifier
according to guideline

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

GLP compliance
yes

Type of assay
bacterial reverse mutation assay
in vitro gene mutation study in bacteria

Test material

Test material information
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Specific details on test material used for the study
- Name of test material (as cited in study report): diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate
- Analytical purity: 100.0%
- Lot/batch No.: 6803
- Storage condition of test material: in a hermetically sealed and light-resistant container at cool (2-8 ℃) place
- Stability under test conditions: The stability of test material was identified by analysis of the remainder.

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

Test concentrations with justification for top dose
-S9 mix and + S9 mix: 313, 625, 1250, 2500, 5000 µg/plate (all strains)

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
other: -S9 mix: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF2:TA100, TA98, WP2 uvrA), sodium a zide (SA:TA1535) and 9-aminoacridine hydrochloride (9AA:TA1537). +S9 mix: 2-aminoanthracene (2AA:all strains).

Details on test system and experimental conditions
RANGE-FINDING/SCREENING STUDIES:
Concentration: 5-5000 µg/plate
Cytotoxic conc.: No.
Precipitate: No.

METHOD OF APPLICATION: Preincubation
DURATION
- Preincubation period: 20 min at 37 °C
- Exposure duration:48-49 hrs
NUMBER OF PLATES: 3

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-r elated 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 and TA 100
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

Key result
false

Species / strain
E. coli WP2 uvr A
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

Additional information on results
Contamination with any other bacterias was not found.

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

Any other information on results incl. tables

Field content is not in a valid XML format and thus ignored!

Applicant's summary and conclusion

Conclusions
Interpretation of results (migrated information):
negative

Executive summary

In a bacterial reverse mutation assay using S. typhimurium TA100, TA1535, TA98, and TA1537 and E. coli WP2uvrA (OECD TG 471), diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate was negative with or without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

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Dossier UUID:	
Author:	
Date:	2022-12-16T14:39:15.804+09:00
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 diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate on / MHLW, Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

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

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
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Specific details on test material used for the study
- Name of test material (as cited in study report): diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate
- Analytical purity: 100.0%
- Lot/batch No.: 6803
- Storage condition of test material: in a hermetically sealed and light-resistant container at cool (2-8 ℃) place
- Stability under test conditions: The stability of test material was identified by analysis of the remainder.

Method

Target gene
Chromosome

Species / strain

Species / strain / cell type
other: Chinese hamster lung(CHL/IU) cells

Metabolic activation
with and without

Metabolic activation system
rat liver, induced by phenobarbital and 5,6-benzoflavone

Test concentrations with justification for top dose
-S9 mix (short-term treatment): 0, 565, 1130, 2260 ug/mL
+S9 mix (short-term treatment): 0, 565, 1130, 2260 ug/mL
-S9 mix (continuous treatment, 24 h): 0, 283, 656, 1130, 1695, 2260 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
benzo(a)pyrene
mitomycin C

Remarks
mitomycin C (without S9 mix), benzo[a]pyrene (with S9 mix)

Details on test system and experimental conditions
METHOD OF APPLICATION: Exposure duration: [continuous treatment]: 24 hrs [short-term treat ment]:6 hrs + 18 hr
SPINDLE INHIBITOR: Colcemid

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 were employed.
Appearance incidence of cells with chromosomal aberrations: Negative (-): < 5%; equivocal (±): 5-10%; positive (+): > 10%.
Finally, the substance is positive when the incidence is considered to be dose-related and repro ducible.

Statistics
not used.

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 / choice of top concentrations
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

Key result
false

Species / strain
other: Chinese hamster lung (CHL/IU) cells

Metabolic activation
without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity 50% cell growth inhibition: 2260 ug/mL (24h continuous)

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

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/PDF3012 -65 -5f.pdf

Applicant's summary and conclusion

Executive summary

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

Toxicity to reproduction

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Reproductive/developmental toxicity.001

UUID: IUC5-c1ff5a74-f962-4d95-a7cd-a59a30a70cb5
Dossier UUID:
Author:
Date: 2022-12-16T14:40:21.935+09:00
Remarks:

Administrative data

Endpoint
screening for reproductive / developmental toxicity based on test type (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 for cross-reference
reference to same study

Remarks
7.5.1 Repeated dose toxicity: oral.001

Data source

Reference

Combined repeat dose and reproductive/developmental toxicity screening test of diammonium hydrogen 2 / 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
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Specific details on test material used for the study
- Name of test material (as cited in study report): diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate
- Purity: 100.0%
- Impurities (identity and concentrations):
- Lot/batch No.: 6803
- Stability under test conditions: Stable
- Storage condition of test material: Refrigeration
- Dosing solution storage condition: Room temperature
- Other: The dosing solution was used within 10 days of preparation.

Test animals

Species
rat

Strain
other: Crl:CD(SD)

Sex
male/female

Details on test animals or test system and environmental conditions
TEST ANIMALS
- Source: Charles River Laboratories Japan, Inc. Atsugi
- Age at study initiation: 10 weeks
- Weight at study initiation: Males: 371-439 g; Females: 219-273 g
- Housing: Steel wire-mesh cage (250 mm x 350 mm x 200 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 15 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 21-26
- Humidity (%): 42-64
- Air changes: 10-15 times / hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
water

Details on exposure
PREPARATION OF DOSING SOLUTIONS:
VEHICLE
- Amount of vehicle (if gavage): 5 mL/kg bw
- Lot/batch no. (if required): 6F74

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
Test suspensions at each concentration to be used for males in week 1 and week 6 of administration were analyzed by the HPLC method at Bozo Research Center Inc. Results showed that the concentration of the test article in each suspension was 93.3 to 100.0% of the nominal concentration and both values were within the acceptable range (concentration: percentage of the nominal concentration, 100 ± 10%; C.V.: 10% or below)

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating and mating periods, and thereafter 14 days
(P) Females: 41-47 days including 14 days pre-mating, mating and gestation periods, and the days until day 4 of lactation; satellite animals: 42 days.

Frequency of treatment
Once/day, 7days/week

Doses / concentrations

Remarks
Doses / Concentrations:
0 (vehicle), 100, 300, and 1000 mg/kg bw/day
Basis:
actual ingested

No. of animals per sex per dose
12 animals/sex/dose (main dose group), 5 males and 5 females at 0 and 1000 mg/kg bw/day as a satellite group (without mating).

Control animals
yes, concurrent vehicle

Examinations

Parental animals: Observations and examinations
CAGE SIDE OBSERVATIONS: Yes
- Time schedule:
Males and females: once before the start of administration, 3 times/day during the administration period, and once during the recovery period

DETAILED CLINICAL OBSERVATIONS: Yes
The functional observational battery testing (FOB) was performed on all animals. Among the measures in the FOB, detailed clinical observations were made before the initiation of dosing. Thereafter, in males of the main groups, detailed clinical observations were made once a week. Also in females of the main groups, detailed clinical observations were made once a week in pre-mating and mating periods thereafter, and then those were made on days 1,7,14 and 20 of gestation, and on day 4 of lactation. For the satellite group, detailed clinical observations were made once a week in dosing and recovery periods.
Sensory motor reflexes, forelimb and hindlimb grip strengths, and motor activity were measured on week 6 of administration period (main/recovery group animals) and week 2 of recovery period (recovery group animals).

BODY WEIGHT: Yes
- Time schedule for examinations: Males (main) & males and females (recovery group): Days 1, 4, 8, 11, 15, 22, 25, 29, 32, 36, 39, 42, and the day of necropsy (after ca. 16h-fasting) in dosing period
Males and females (recovery group): Days 1, 4, 8, 11, 14, and the day of necropsy (after ca. 16h-fasting) in recovery period
Females (main group): Twice a week during the precopulation period (days 1, 4, 8, 11, and 15); gestation days 0, 4, 7, 11, 14, 17, and 20; lactation days 0 and 4; and the day of necropsy (after ca. 16 h-fasting)
FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Males (main) & males and females (recovery group): Days 1, 4, 8, 11, 15, 32, 36, and 39 in dosing period
Males and females (recovery group): Days 1, 4, 8, 11, and 14 in recovery period
Females (main group): Days 1, 4, 8, 11, and 15; gestation days 1, 4, 7, 11, 14, 17, and 20; lactation days 2 and 4

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: Blood was collected on the day of necropsy
- Anaesthetic used for blood collection: Yes (ether)
- Animals fasted: Yes, 16-20h
- How many animals: 5 sex/dose/group
- Parameters checked in table were examined.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: Same as hematology
- Animals fasted: Same as hematology
- How many animals: Same as hematology
- Parameters checked in table were examined.

URINALYSIS: Yes (males only)
- Time schedule for collection of urine: Day 37-38 in dosing period, day 11-12 in recovery period
- Metabolism cages used for collection of urine: No data
- Animals fasted: fasting and only water at libitum (4h-urine), no fasting (20h-urine)

Oestrous 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. During the pre-mating administration period, vaginal smear pictures were classified as proestrus, estrus, metestrus or diestrus and examined for the frequency of estrus and interval between estruses (estrous cycle). During the mating period, vaginal smears were examined for the presence of sperm.

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

Postmortem examinations (parental animals)
SACRIFICE:
Male animals: Rats were euthanized by exsanguination under ether anesthesia on the day after the l ast administration.
Maternal animals: Rats were euthanized by exsanguination under ether anesthesia on day 4 of lactati on.

GROSS PATHOLOGY, Yes: whole organs and tissues

ORGAN WEIGHTS, Yes: Brain, thyroids(including parathyroids), thymus, heart, liver, spleen, kidneys, adrenals, testes, epididymis

HISTOPATHOLOGY, Yes: Cerebrum, cerebellum, pituitary gland, spinal cord (thoracic), sciatic nerve, thyroid, parathyroid, adrenal glands, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, lung (including the bronchi), stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, kidney, bladder, testis, epididymis, ovary, uterus, seminal vesicles, sternum and femur (including bone marrows), macroscopic lesions.

Postmortem examinations (offspring)
GROSS NECROPSY
- Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

Statistics
The data were analyzed for homogeneity of variance by the Bartlett test. If variances were homogeneous, data was analyzed by the Dunnett test, whereas heterogeneous data was analyzed by the Dunnett type mean rank test ($p<0.05$, two-sided).
In the recovery test, these values of two groups were analyzed by F test. If variances were homogene ous, data was analyzed by the Student t-test, whereas heterogeneous data was analyzed by the Aspin-Welch t-test ($p<0.05$, two-sided).

Reproductive indices
Each parameter was determined by the following equations:
Copulation index (%) = (No. of copulated animals/No. of co-housed animals) × 100
Fertility index (%) = (No. of pregnant females/No. of copulated females) × 100
Insemination index (%) = (No. of pregnant females/No. of copulated males) × 100
Duration of gestation (days) = day 0 of lactation – day 0 of gestation
Delivery index (%) = (No. of females delivered liveborn pups/No. of pregnant females) × 100
Implantation index (%) = (No. of implantation sites/No. of corpora lutea) × 100
Stillborn index (%) = (No. of stillborn pups/Total No. of pups born) × 100
Liveborn index (%) = (No. of liveborn pups/Total No. of pups born) × 100
External abnormalities (%) = (No. of pups with external abnormalities/No. of liveborn pups) × 100

Sex ratio = No. of liveborn male pups/(No. of liveborn male pups + No. of liveborn female pups)

Offspring viability indices
Viability index (%) = (No. of surviving pus on day 4 after birth/No. of liveborn pups on day 0 after birth) × 100

Results and discussion

Results: P0 (first parental generation)

General toxicity (P0)

Clinical signs
no effects observed

Body weight and weight changes
no effects observed

Food consumption and compound intake (if feeding study)
no effects observed

Organ weight findings including organ / body weight ratios
no effects observed

Description (incidence and severity)
on reproductive organs

Gross pathological findings
no effects observed

Description (incidence and severity)
on reproductive organs

Histopathological findings: non-neoplastic
no effects observed

Description (incidence and severity)
on reproductive organs

Reproductive function / performance (P0)

Reproductive function: oestrous cycle
no effects observed

Reproductive function: sperm measures
not examined

Reproductive performance
no effects observed

Description (incidence and severity)
on reproductive organs

Effect levels (P0)

Key result
false

Dose descriptor
NOAEL

Effect level

300 mg/kg bw/day (actual dose received)

Sex
male/female

Basis for effect level
other: Effects of stomach (see repeated dose toxicity)

Key result
false

Dose descriptor
NOAEL

Effect level

1000 mg/kg bw/day (actual dose received)

Sex
male/female

Basis for effect level
other: no effects on reproduction

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
not examined

Organ weight findings including organ / body weight ratios
not examined

Gross pathological findings
no effects observed

Histopathological findings
not examined

Effect levels (F1)

Key result
false

Dose descriptor
NOAEL

Generation
F1

Effect level

1000	mg/kg bw/day (actual dose received)

Sex
male/female

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/PDF3012 -65 -5d.pdf

Applicant's summary and conclusion

Conclusions
NOAEL for rat reproductive/developmental toxicity was determined to be 1000 mg/kg bw/day.

Executive summary

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (0, 100, 300, and 1,000 mg/kg bw/day) (OECD TG 422), no effects of this substance on reproductive and developmental parameters were observed at 1,000 mg/kg bw/day. NOAEL for the rat reproductive/developmental toxicity of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate was regarded as 1,000 mg/kg bw/day, the highest dose tested.

References

Reference Substances

REFERENCE_SUBSTANCE: diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

UUID: ECB5-399f7091-4bb7-447c-b011-267f474d9c96
Dossier UUID:
Author:
Date: 2016-12-21T15:14:55.000+09:00
Remarks:

Reference substance name
diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

IUPAC name
diammonium hydrogen citrate

Inventory

Inventory number

Inventory name
diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Inventory
EC Inventory

Inventory number
221-146-3

CAS number
3012-65-5

Molecular formula
C6H8O7.2H3N

Description

CAS number
3012-65-5

Synonyms

Synonyms

Identity
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, diammonium salt

Identity
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, diammonium salt

Identity
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, diammonium salt

Molecular and structural information

Molecular formula
C6H8O7.2H3N

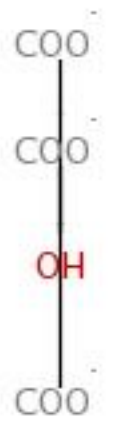
Molecular weight

225.1772

SMILES notation
[NH4+].[NH4+].OC(CC(=O)[O-])(CC(=O)[O-])C(=O)[O-]

InChI
InChI=1/C6H8O7.2H3N/c7-3(8)1-6(13,5(11)12)2-4(9)10;;/h13H,1-2H2,(H,7,8)(H,9,10)(H,11,12);2*1H3/p-1

Structural formula



Related substances

Group / category information
DSL Category: Organics

Test Materials

TEST_MATERIAL_INFORMATION: Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

UUID: 80817e13-2fe0-3773-a70c-2e2ef08eef5c
Dossier UUID:
Author:
Date: 2022-12-12T11:00:40.265+09:00
Remarks:

Name
Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

Composition

Composition

Type
Constituent

Reference substance
diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate / diammonium hydrogen citrate / 3012-65-5 / 221-146-3

EC number	EC name
221-146-3	EC Inventory
CAS number	**CAS name**
3012-65-5	
IUPAC name	
diammonium hydrogen citrate	

Literatures

LITERATURE: Combined repeat dose and reproductive/ developmental toxicity screening test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate by oral administration in rats

UUID: 5871efdc-ac0e-3345-bf1f-4eaf58d59637
Dossier UUID:
Author:
Date: 2022-12-12T11:12:26.146+09:00
Remarks:

General information

Reference Type
study report

Title
Combined repeat dose and reproductive/developmental toxicity screening test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate by oral administration in rats

Author
MHLW, Japan

Year
2009

Bibliographic source
available in the web of Japan Existing Chemical Data Base (JECDB) at http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp

Testing facility
BoZo Research Center

LITERATURE: In Vitro Chromosomal Aberration Test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate on Cultured Chinese Hamster Cells.

UUID: ed9f5c39-923d-3218-a027-aa04dad90a22
Dossier UUID:
Author:
Date: 2017-02-15T15:49:29.000+09:00
Remarks:

General information

Reference Type
study report

Title
In Vitro Chromosomal Aberration Test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarb oxylate on Cultured Chinese Hamster Cells.

Author
MHLW, Japan

Year
2011

Bibliographic source
Japan Existing Chemical Data Base (JECDB)

Testing facility
Safety Research Institute for Chemical Compounds Co., Ltd.

LITERATURE: Reverse Mutation Test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate on Bacteria.

UUID: 435d0925-d6a4-3611-87a0-697e7ce9d1bb
Dossier UUID:
Author:
Date: 2017-02-15T15:48:17.000+09:00
Remarks:

General information

Reference Type
study report

Title
Reverse Mutation Test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate on Bacteria.

Author
MHLW, Japan

Year
2011

Bibliographic source
Japan Existing Chemical Data Base (JECDB)

Testing facility
Safety Research Institute for Chemical Compounds Co., Ltd.

LITERATURE: Single Dose Oral Toxicity Test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate in Rats

UUID: 711f2de5-2173-37ae-b376-f1c8b65aca3e
Dossier UUID:
Author:
Date: 2017-02-15T15:51:02.000+09:00
Remarks:

General information

Reference Type
publication

Title
Single Dose Oral Toxicity Test of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate in Rats

Author
MHLW

Year
2011

Bibliographic source
available in the web of Japan Existing Chemical Data Base (JECDB) at http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp

Testing facility
Safety Research Institute for Chemical Compounds Co., Ltd.

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 o fficial MHLW opinions or any other regulatory policies.

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210-9501

Town
Kawasaki

Region / State
Kanagawa

Country
Japan
JP

Identifiers

Other IT system identifiers

IT system LEO **ID** 10767
IT system IUCLID4

ID
16558402024DIV750