Name: OECD_SIDS / SUBSTANCE : 1,4-Bis(isopropylamino)-9,10-anthracenedione / 14233-37-5 Tue, 29 Nov 2022, 14:56:31+0900 /

Legal entity owner: National Institute of Health Science

Printing date: 2022-11-29T14:56:31.744+09:00

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DOSSIER:

UUID: 0
Dossier UUID:
Author:
Date: 2022-11-29T14:56:31.592+09:00
Remarks:

Dossier header

Dossier submission type

Name
OECD SIDS

Version
core 7.0

Name (given by user)

Dossier subject

Dossier subject
1,4-Bis(isopropylamino)-9,10-anthracenedione / 14233-37-5

Public name

Submitting legal entity
National Institute of Health Science

Dossier creation date/time
Tue, 29 Nov 2022, 14:56:31+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

1,4-Bis(isopropylamino)-9,10-anthracenedione

General information

Identification

Identification

SUBSTANCE: 1,4-Bis(isopropylamino)-9,10-anthracenedione

UUID:	2a0b5c5e-11da-4464-bb85-dee4a7be72f1
Dossier UUID:	
Author:	
Date:	2022-11-29T14:48:32.584+09:00
Remarks:	

Substance name
1,4-Bis(isopropylamino)-9,10-anthracenedione

Legal entity
National Institute of Health Sciences / Kawasaki / Japan

Identification of substance

Reference substance
1,4-Bis(isopropylamino)anthraquinone / 14233-37-5

EC number	EC name
CAS number	**CAS name**
14233-37-5	

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: 5ded02e4-7dbc-4944-a357-e9a3a0abc2e4
Dossier UUID:
Author:
Date: 2022-11-29T14:45:24.742+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 / 1,4-Bis(isopropylamino)-9,10-anthracenedione / 14233-37-5

Data source

Reference

Combined repeat dose and reproductive/developmental toxicity screening test of 1,4-Bis(isopropylamin / Ministry of Health, Labour and Welfare (MHLW), Japan / study report

Data access
data published https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF14233-37-5d.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
1,4-Bis(isopropylamino)anthraquinone

Specific details on test material used for the study
- Name of test material (as cited in study report): 1,4-Bis(isopropylamino)anthraquinone
- Analytical purity: 100%
- Storage condition of test material: Cold and dark place (3 - 6°C)
- 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: Crl:CD(SD)

Sex
male/female

Details on test animals or test system and environmental conditions
TEST ANIMALS
- Source: Charles River Japan, Inc., Atsugi Breeding Center.
- Age at study initiation: 10 weeks old
- Weight at study initiation: Male: 416 g (385-475 g), Female: 253 g (230-279 g)
- Housing: Animals were individually housed in bracket-type metallic wire-mesh cages (254W × 350D × 170H mm), from gestation day 17 to lactation day 4, Dams were bred individually or with individual littermates in plastic cages (340W x 400D x 185H mm) and bedding.
- Diet: Solid feed (NMF: Oriental Yeast Co., ltd.) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation period: 19 days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23±3 (actual temperature: 22-26°C)

- Humidity (%): 50±20% (actual humidity: 33-50%)
- Air changes (per hr): 10-15
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 7:00~19:00)

Administration / exposure

Route of administration
oral: gavage

Vehicle
methylcellulose 0.5w/v%

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

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 group in week 1 and weeks 6 ad ministration were analyzed by absorptiometry. Results showed that the concentration of test suspensi ons in each concentration was 95.8 to 103.3% of the nominal concentration and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%)

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating
(P) Females: 41-46 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation
Female (non- mating, satellite group): 42 days

Frequency of treatment
Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
12	mg/kg bw/day (actual dose received)
Dose / conc.	
60	mg/kg bw/day (actual dose received)
Dose / conc.	
300	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex /dose (0, 12, 60, and 300 mg/kg bw/day). 5 males each in the 0 and 300 mg/kg bw/day were assigned to the recovery group.

Non-mating group (Satellite group): 10 females/dose (0 and 300 mg/kg bw/day), 5 females in each group were assigned to the recovery group.

Control animals
yes, concurrent vehicle

Details on study design
- Dose selection rationale: Based on the results of a 14-day preliminary study, the highest dose of 300 mg/kg bw/day was selected as an expected obvious toxic dose, and the lowest dose of 12 mg/kg bw/day was selected as an expected no toxic dose. The middle dose levels of 60 mg/kg bw/day were selected.
[14-day preliminary study]
A 14-day repeated dose oral toxicity test (Crl:CD(SD) rats, doses: 0, 100, 300 or 1000 mg/kg bw/day). At 100 mg/kg bw/day and above, bluish discoloration of skin, dark blue feces, purplish urine, bluish discoloration of whole body fat or organs, decreased food consumption and increased liver weight were observed. At 1,000 mg/kg bw/day, slight suppression of body weight gain in males and females, low urea nitrogen in males, high total cholesterol in females and high adrenal weight in females were observed.
- Rationale for animal assignment (if not random): Body weight-balanced randomization

Examinations

Observations and examinations performed and frequency
CAGE SIDE OBSERVATIONS: Yes
- Time schedule: 3 times/day (before administration, immediately after administration and 1-3 hours after administration) during the administration period. Once a day during the recovery period.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule:
Males in the mating group and females in the non-mating (satellite) group: once before the start of administration, once every weekly during the administration and recovery periods.
Females in the mating group: once a week during the pre-mating period, on designated days during mating, gestation, and lactation (mated females: days 1, 7, 14 and 20 of pregnancy, parturient females: day 4 of lactation).

BODY WEIGHT: Yes
- Time schedule for examinations:
Males in the mating group and females in the non-mating (satellite) group: days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39 and 42 of administration and on the day of necropsy, and days 1, 4, 8, 11 and 14 of recovery and on the day of necropsy.
Females in the mating group: days 1, 4, 8, 11, and 15 of administration (uncopulated animals were weighed on days 18 of administration as well), days 0, 7, 14 and 20 of gestation, days 0 and 4 of lactation and the day of necropsy.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Males in the mating group and females in the non-mating (satellite) group: days 1, 4, 8, 11, 15, 32, 36, 39 and 42 of administration, and days 1, 4, 8, 11 and 14 of recovery
Females in the mating groups: days 1, 4, 8, 11 and 15 of administration, days 1, 4, 7, 11, 14, 17 and 20 of gestation and days 2 and 4 of lactation.

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: At the end of administration period, or at the end of recovery period in both sexes
- Anaesthetic used for blood collection: Isoflurane

- Animals fasted: Yes
- How many animals:
All animals/sex/group (Control and 300 mg/kg bw/day), 5 animals/sex/group (12 and 60 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, absolute number of each white blood cell, prothrombin time, activated partial thromboplastin time, fibrinogen.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: At the end of administration period, or at the end of recovery period in both sexes
- Animals fasted: Yes
- How many animals:
All animals/sex/group (Control and 300 mg/kg bw/day), 5 animals/sex/group (12 and 60 mg/kg bw/day)
- Parameters checked: ALP, total cholesterol, triglyceride, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, A/G ratio, AST (GOT), ALT (GPT), LDH, γ-GTP

BLOOD HORMONE: Yes
- Time schedule for collection of serum: Same as clinical chemistry
- Animals fasted: Yes
- How many animals:
All animals/sex/group (Control and 300 mg/kg bw/day), 5 animals/sex/group (12 and 60 mg/kg bw/day)
- Parameters checked: Triiodothyronine (T3), Thyroxin (T4), and thyroid stimulating hormone (THS) of thyroid hormone

URINALYSIS OF MALES: Yes
- Time schedule for collection of urine: final week of administration (days 36 to 37 of administration) and in the final week of recovery (days 8 to 9 of recovery)
- Metabolism cages used for collection of urine: Yes
A urine collector to collect four-hour urine samples under fasting but ad libitum drinking conditions, followed by collection of 20-hour urine samples under ad libitum feeding and drinking conditions.
- How many animals: 5 animals/group
- Parameters checked: pH, protein, ketones, glucose, occult blood, bilirubin, urobilinogen, color, sediment, urine volume (4-hour volume), osmotic pressure, urine volume (20-hour volume), water intake (24-hour volume).
NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations:
Males in the mating groups: final week of administration (day 40 of administration)
Females in the mating groups: lactation day 4 (day 41 to day 44 of administration) after necropsy of F1 pups
Males and females in the recovery groups: final week of administration (day 40 of administration) and in the final week of recovery (day 12 of recovery).
- Dose groups that were examined: All dose groups (5 animals/sex/group)
- Battery of functions tested:
1) Manipulative Test. Auditory response, approach response, touch response, tail pinch response, pupillary reflex, aerial righting reflex, landing foot splay
2) Measurement of Grip Strength. Following manipulative test, grip strength of forelimb and hind limb were measured by CPU gauge MODEL-RX-5 (AIKOH Engineering Co., Ltd.).
3) Measurement of Motor Activity. Following measurement of grip strength, motor activity was measured by a motor activity sensor for experimental animals NS-AS01 (Neuro Science, Inc.). The measurement was conducted for 1 hour, and measured values at 10-minute intervals and from 0 to 60 minutes were collected.

Sacrifice and pathology
GROSS PATHOLOGY: Yes

ORGAN WEIGHT: Yes [brain, pituitary, thyroids (including parathyroids), adrenal gland, thymus, spleen, heart, liver, kidney, testis, epididymis, prostate, seminal vesicles, ovary, uterus]
HISTOPATHOLOGY: Yes, [cerebrum, cerebellum (including pons), sciatic nerve, spinal cord (thoracic), eyeball, optic nerve, Harderian gland, pituitary, thyroid, parathyroid, adrenal glands, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, thoracic aorta, trachea, lung (including bronchial), tongue, larynx, esophagus, stomach, duodenum, jejunum, ileum (including payer's patch), cecum, colon, rectum, submandibular gland, sublingual gland, liver, pancreas, kidney, bladder, testis, epididymis, ovary, uterus, vagina, prostate, seminal vesicles (including coagulating gland), mammary gland (inguinal region), skin (inguinal region), sternum and femur (including bone marrows), femoral s keletal muscle, gross abnormalities site and Individual identification site (pinna with ear tag)]
Underlined organs and tissues are fixed and stored only.

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 be tween two groups in the recovery test, homogeneity of variance was analyzed by F-test. Then, if homo genous, student's t-test was applied. If not homogenous, Aspin-Welch's t-test was used.
Regarding clinical observation (except for frequency of urination, defecation, rearing and grooming) and sensory reactivity, Steel test was applied. Regarding auditory response, approach response, touch response, tail pinch response, pupillary reflex, aerial righting reflex, Fisher's 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]:
Blue skin, dark blue stools, and purple urine were observed in males at 60 mg/kg bw/day and above, and in females at 12 mg/kg bw/day and above, which were considered to reflect the color of the test substance, independent of adverse reactions.
[At the recovery period]:
These findings diminished during the recovery period.

DETAILED CLINICAL OBSERVATIONS:
There were no changes related to the test substance in any groups at the administration and recovery periods.

Mortality
no mortality observed

Body weight and weight changes
no effects observed

Food consumption and compound intake (if feeding study)
effects observed, treatment-related

Description (incidence and severity)
[At the end of administration period]:

Decrease in food consumption were observed in mating females at 12 mg/kg bw/day and above.
[At the end of 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)
not examined

Ophthalmological findings
not examined

Haematological findings
effects observed, treatment-related

Description (incidence and severity)
[At the end of administration period]:
Decreases in white blood cell count, neutrophil count, eosinophil count and monocyte count were observed in mating females at 60 mg/kg bw/day and above.
[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]:
Increase in ALT was observed in non-mating females at 300 mg/kg bw/day.
[At the end of recovery period]:
There were no changes related to the test substance in any groups.

BLOOD HORMONE:
[At the end of administration period]:
Increases in T3 and T4 were observed in mating females at 60 mg/kg bw/day and above.
[At the end of recovery period]:
There were no changes related to the test substance in any groups.

Urinalysis findings
effects observed, non-treatment-related

Description (incidence and severity)
[At the administration period]:
Light purple to deep purple urine was observed in the treated groups, which were considered to reflect the color of the test substance independent of adverse effects.
[At the recovery period]:
Light purple urine was observed in males and females at 300 mg/kg bw/day.

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]:
Increases in absolute and relative liver weights were observed in males at 60 mg/kg bw/day and increase in relative liver weight in males at 300 mg/kg bw/day. Increases in absolute and relative liver and adrenal weights, decreases in absolute and relative spleen weights, and decreases in absolute ovary weight were observed in non-mating females at 300 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
effects observed, non-treatment-related

Description (incidence and severity)
[At the end of administration period]:
In the treated groups, skin, adipose tissue, and organs of the whole body were blue, which were consi dered to reflect the color of the test substance independent of adverse effects.
[At the end of recovery period]:
In the treated groups, skin and adipose tissue were blue, which were considered to reflect the color of the test substance independent of adverse effects.

Neuropathological findings
not examined

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

Description (incidence and severity)
[At the end of administration period]:
Liver: Centrilobular hypertrophy of hepatocyte was observed in males and mating females at 60 mg/kg bw/day and above, and non-mating females at 300 mg/kg bw/day.
Adrenal gland: Hypertrophy of cortical cell was observed in non-mating females at 300 mg/kg bw/day.
Thymus: Atrophy was observed in mating females at 300 mg/kg bw/day.
[At the end of recovery period]:
There were no changes related to the test substance in any groups.

Histopathological findings: neoplastic
not examined

Effect levels

Key result
false

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male

Basis for effect level
histopathology: non-neoplastic
At 60 mg/kg bw/day, centrilobular hypertrophy of hepatocyte was observed in males.
organ weights and organ / body weight ratios
At 60 mg/kg bw/day, increase in liver weight was observed in males.

Key result
false

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
clinical biochemistry
At 60 mg/kg bw/day, increases in T3 and T4 were observed in mating females.
haematology
At 60 mg/kg bw/day, decreases in white blood cell count, neutrophil count, eosinophil count and monocyte count were observed in mating females.
histopathology: non-neoplastic
At 60 mg/kg bw/day, centrilobular hypertrophy of hepatocytes was observed in females. The centrilobular hypertrophy of hepatocyte was observed in mating females at 60 mg/kg bw/day and above, and non-mating females at 300 mg/kg bw/day. (The non-mating females received only 2 doses of 0 and 300 mg/kg bw/day.)

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/PDF14233-37-5d.pdf

Applicant's summary and conclusion

Conclusions
Based on the effects of 1,4-bis(isopropylamino)anthraquinone on the liver, the no observed adverse e ffect levels (NOAELs) for repeated oral dosing were determined to be 12 mg/kg bw/day in male and female rats.

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, 60 and 300 mg/kg bw/day, Males were dosed for 42 days including 14-days pre-mating and mating periods. Females were dosed during the periods of pre-mating, mating, gestation and days until day 4 of lactation (41 to 46 days).

No animals died in any of the treatment groups, and there were no treatment-related effects on clinical signs, detailed clinical observations, body weight, functional tests, grip strength, motor activity, or

urinalysis. The following findings were observed in examination at the end of administration period. In the haematological examination, decreases in white blood cell count, neutrophil count, eosinophil count and monocyte count were observed in mating females at 60 mg/kg bw/day and above. In the clinical chemistry, increases in T3 and T4 were observed in mating females at 60 mg/kg bw/day and above. An increase in ALT was observed in non-mating females at 300 mg/kg bw /day. In the organ weights, increases in liver weight were observed in males at 60 mg/kg bw/day and above. Increases in liver and adrenal weights, decreases in spleen and ovary weights were observed in non-mated females at 300 mg/kg bw/day. In the histopathological examination, centrilobular hypertrophy of hepatocyte was observed in males and mating females at 60 mg/kg bw/day and above, and non-mating females at 300 mg/kg bw/day. Hypertrophy of cortical cell of adrenal gland was observed in non-mating females at 300 mg/kg bw/day. Atrophy of thymus was observed in mating females at 300 mg/kg bw/day. At the end of recovery period, all changes observed in haematological examination, clinical chemistry, organ weight and histopathological examination were disappeared or reduced.

Based on the above results, NOAELs for repeated dose toxicity of 1,4-bis(isopropylamino)anthraquinone were determined to be 12 mg/kg bw/ day in male and female rats.

Genetic toxicity

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

UUID: 1c509d79-867b-4e07-aa01-3ab79523f867
Dossier UUID:
Author:
Date: 2022-11-14T15:04:14.000+09:00
Remarks:

Administrative data

Endpoint
in vitro gene mutation study in bacteria

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
Reliability 1

Data source

Reference

Reverse Mutation Test of 1,4-Bis(isopropylamino)anthraquinone 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
1,4-Bis(isopropylamino)anthraquinone

Specific details on test material used for the study
Purity 99.73%

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:
2.44, 4.88, 9.77, 19.5, 39.1, 78.1 µg/plate (TA100 strain)
9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate (TA1535, TA98, TA1537 strains)
313, 625, 1250, 2500, 5000 µg/plate (WP2uvrA strain)
+S9 mix:
313, 625, 1250, 2500, 5000 µg/plate (TA100, TA1535, TA1537 strains)

156, 313, 625, 1250, 2500, 5000 µg/plate (TA98 strain)
313, 625, 1250, 2500, 5000 µg/plate (WP2uvrA strain)

Maximum concentration was established based on the result of the preliminary test at concentration up to 5000 ug/plate. In this test, the growth inhibition was observed at 78.1 µg/plate and above for S. typhimurium TA100 without S9 mix, at 313 µg/plate and above for S. typhimurium TA1535, TA98, TA 1537 without S9 mix.

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

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
sodium azide
SAZ
benzo(a)pyrene
furylfuramide
AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide
other: ICR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine 2HCl, 2AA: 2-aminoanthracene

Remarks
-S9 mix: AF-2: (TA 100, TA98 and WP2 uvrA), SAZ: (TA1535), ICR-191: (TA1537).
+S9 mix: 2AA: (TA1535 and WP2 uvrA), benzo(a)pyrene (TA100, TA98 and TA1537)

Details on test system and experimental conditions
METHOD OF APPLICATION: Preincubation
DURATION- Preincubation period: 20 min at 37°C
- Exposure duration: 48 or 48.5 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
false

Species / strain
S. typhimurium TA 1535
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 313 µg/plate

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
with

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 / strain
S. typhimurium TA 1537
bacteria

Metabolic activation
without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 156 µg/plate and above

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

Genotoxicity
positive

Cytotoxicity / choice of top concentrations
cytotoxicity +S9 mix: 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
S. typhimurium TA 98
bacteria

Metabolic activation
without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 313 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
S. typhimurium TA 100
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 78.1 µg/plate and above

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
with and without

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

Additional information on results
The maximum specific activity of mutation was 750 revertants/mg, which was observed in plates of Salmonella typhimurium TA98 treated with the test article at 156 µg/plate without metabolic a ctivation.

Any other information on results incl. tables

Figures and Tables (in Japanese) are available in the following full report of the study.
https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF14233-37-5e.pdf

Please also see the attached files (Tables in English)

Applicant's summary and conclusion

Conclusions
Interpretation of results (migrated information): positive with 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), 1,4-bis(isopropylamino)anthraquinone was positive for TA98 and TA 1537 with metabolic activation. The maximum specific activity of mutation was 750 revertants/mg/plate, which was observed in plates of Salmonella typhimurium TA98 treated with the test article at 156 µg/plate without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

UUID: 27e78527-2620-4760-bb8d-1ce5b7fa4523
Dossier UUID:
Author:
Date: 2021-03-08T16:43:54.000+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 1,4-Bis(isopropylamino)anthraquinone Cultured Chinese Ham / 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
1,4-Bis(isopropylamino)anthraquinone

Specific details on test material used for the study
Purity: 99.73%

Method

Species / strain

Species / strain / cell type
Chinese hamster lung (CHL/IU)
mammalian cell line

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): 25.8, 51.6, 103, 206, 413, 825, 1650, 3300 ug/mL
+S9 mix (short-term treatment): 25.8, 51.6, 103, 206, 413, 825, 1650, 3300 ug/mL
-S9 mix (continuous treatment, 24hr): 25.8, 51.6, 103, 206, 413, 825, 1650, 3300 ug/mL
-S9 mix (continuous treatment, 48hr): 25.8, 51.6, 103, 206, 413, 825, 1650, 3300 ug/mL
Main study
-S9 (short-term treatment): 825, 1650, 3300 ug/mL
+S9 (short-term treatment): 825, 1650, 3300 ug/mL

Vehicle / solvent
- Vehicle(s)/solvent(s) used: 0.5%CNC Na

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
cyclophosphamide
+S9
mitomycin C
-S9

Details on test system and experimental conditions
METHOD OF APPLICATION: Exposure duration: [short-term treatment]:6 hrs + 18 hr
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(±): 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

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

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/PDF14233-37-5f.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), 1,4-Bis(isopropylamino)anthraquinone induced chromosome numerical aberrations but did not induce structural chromosomal aberrations under the conditions of this study.

Toxicity to reproduction

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction. 001

UUID: 32dedd3b-9b7d-4733-83b9-32b7011bf119
Dossier UUID:
Author:
Date: 2022-11-29T14:48:32.584+09:00
Remarks:

Administrative data

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

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 7.5.1 Repeated dose toxicity: oral: Repeated dose toxicity: oral. 001

Related information
OECD / Repeated dose toxicity: oral / Repeated dose toxicity: oral. 001 / 1,4-Bis(isopropylamino)-9,10-anthracenedione / 14233-37-5

Data source

Reference

Combined repeat dose and reproductive/developmental toxicity screening test of 1,4-Bis(isopropylamin / Ministry of Health, Labour and Welfare (MHLW), Japan / study report

Data access
data published https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF14233-37-5d.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
1,4-Bis(isopropylamino)anthraquinone

Specific details on test material used for the study
- Name of test material (as cited in study report): 1,4-Bis(isopropylamino)anthraquinone
- Analytical purity: 100%

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 Japan, Inc., Atsugi Breeding Center.
- Age at study initiation: 10 weeks old
- Weight at study initiation: Male: 416 g (385-475 g), Female: 253 g (230-279 g)
- Housing: Animals were individually housed in bracket-type metallic wire-mesh cages (254W × 350D × 170H mm), from gestation day 17 to lactation day 4, Dams were bred individually or with individual littermates in plastic cages (340W x 400D x 185H mm) and bedding.
- Diet: Solid feed (NMF: Oriental Yeast Co., ltd.) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation period: 19 days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23±3 (actual temperature: 22-26°C)
- Humidity (%): 50±20% (actual humidity: 33-50%)
- Air changes (per hr): 10-15
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 7:00~19:00)

Administration / exposure

Route of administration
oral: gavage

Vehicle
other: 0.5w/v% methylcellulose

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

Details on mating procedure
- M/F ratio per cage:1/1
- Length of cohabitation: up to 5 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 group in week 1 and weeks 6 ad ministration were analyzed by absorptiometry. Results showed that the concentration of test suspensi ons in each concentration was 95.8 to 103.3% of the nominal concentration and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%)

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating
(P) Females: 41-46 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation
Female (non-mating, satellite group): 42 days

Frequency of treatment
Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
12	mg/kg bw/day (actual dose received)
Dose / conc.	
60	mg/kg bw/day (actual dose received)
Dose / conc.	
300	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex /dose (0, 12, 60, and 300 mg/kg bw/day). 5 males each in the 0 and 300 mg/kg bw/day were assigned to the recovery group.

Non-mating group (Satellite group): 10 females/dose (0 and 300 mg/kg bw/day), 5 females in each group were assigned to the recovery group.

Control animals
yes, concurrent vehicle

Details on study design
- Dose selection rationale: Based on the results of a 14-day preliminary study, the highest dose of 300 mg/kg bw/day was selected as an expected obvious toxic dose, and the lowest dose of 12 mg/kg bw/day was selected as an expected no toxic dose. The middle dose levels of 60 mg/kg bw/day were selected.
[14-day preliminary study]
A 14-day repeated dose oral toxicity test (Crl:CD(SD) rats, doses: 0, 100, 300 or 1000 mg/kg bw/day). At 100 mg/kg bw/day and above, bluish discoloration of skin, dark blue feces, purplish urine, bluish discoloration of whole body fat or organs, decreased food consumption and increased liver weight were observed. At 1,000 mg/kg bw/day slight suppression of body weight gain was also observed in males and females, low urea nitrogen in males, high total cholesterol in females and high adrenal wei ght 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: 3 times/day (before administration, immediately after administration and 1-3 hours a fter administration) during the administration period. Once a day during the recovery period.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule:
Males in the mating group and females in the non-mating (satellite) group: once before the start of ad ministration, once every weekly during the administration and recovery periods.
Females in the mating group: once a week during the pre-mating period, on designated days during mating, gestation, and lactation (mated females: days 1, 7, 14 and 20 of pregnancy, parturient females: day 4 of lactation).

BODY WEIGHT: Yes
- Time schedule for examinations:
Males in the mating group and females in the non-mating (satellite) group: days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39 and 42 of administration and on the day of necropsy, and days 1, 4, 8, 11 and 14 of recovery and on the day of necropsy.
Females in the mating group: days 1, 4, 8, 11, and 15 of administration (uncopulated animals were wei ghed on days 18 of administration as well), days 0, 7, 14 and 20 of gestation, days 0 and 4 of lactation and the day of necropsy.
FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Males in the mating group and females in the non-mating (satellite) group: days 1, 4, 8, 11, 15, 32, 36, 39 and 42 of administration, and days 1, 4, 8, 11 and 14 of recovery
Females in the mating groups: days 1, 4, 8, 11 and 15 of administration, days 1, 4, 7, 11, 14, 17 and 20 of gestation and days 2 and 4 of lactation.

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: At the end of administration period, or at the end of recovery period in both sexes
- Anaesthetic used for blood collection: Isoflurane

- Animals fasted: Yes
- How many animals:
All animals/sex/group (Control and 300 mg/kg bw/day), 5 animals/sex/group (12 and 60 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, absolute number of each white blood cell, prothrombin time, activated partial thromboplastin time, fibrinogen.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: At the end of administration period, or at the end of recovery period in both sexes
- Animals fasted: Yes
- How many animals:
All animals/sex/group (Control and 300 mg/kg bw/day), 5 animals/sex/group (12 and 60 mg/kg bw/day)
- Parameters checked: ALP, total cholesterol, triglyceride, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, A/G ratio, AST (GOT), ALT (GPT), LDH, γ-GTP

BLOOD HORMONE: Yes
- Time schedule for collection of serum: Same as clinical chemistry
- Animals fasted: Yes
- How many animals:
All animals/sex/group (Control and 300 mg/kg bw/day), 5 animals/sex/group (12 and 60 mg/kg bw/day)
- Parameters checked: Triiodothyronine (T3), Thyroxin (T4), and thyroid stimulating hormone (THS) of thyroid hormone

URINALYSIS OF MALES: Yes
- Time schedule for collection of urine: final week of administration (days 36 to 37 of administration) and in the final week of recovery (days 8 to 9 of recovery)
- Metabolism cages used for collection of urine: Yes
A urine collector to collect four-hour urine samples under fasting but ad libitum drinking conditions, followed by collection of 20-hour urine samples under ad libitum feeding and drinking conditions.
- How many animals: 5 animals/group
- Parameters checked: pH, protein, ketones, glucose, occult blood, bilirubin, urobilinogen, color, sediment, urine volume (4-hour volume), osmotic pressure, urine volume (20-hour volume), water intake (24-hour volume).

NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations:
Males in the mating groups: final week of administration (day 40 of administration)
Females in the mating groups: lactation day 4 (day 41 to day 44 of administration) after necropsy of F1 pups
Males and females in the recovery groups: final week of administration (day 40 of administration) and in the final week of recovery (day 12 of recovery).
- Dose groups that were examined: All dose groups (5 animals/sex/group)
- Battery of functions tested:
1) Manipulative Test. Auditory response, approach response, touch response, tail pinch response, pupillary reflex, aerial righting reflex, landing foot splay
2) Measurement of Grip Strength. Following manipulative test, grip strength of forelimb and hind limb were measured by CPU gauge MODEL-RX-5 (AIKOH Engineering Co., Ltd.).
3) Measurement of Motor Activity. Following measurement of grip strength, motor activity was measured by a motor activity sensor for experimental animals NS-AS01 (Neuro Science, Inc.). The measurement was conducted for 1 hour, and measured values at 10-minute intervals and from 0 to 60 minutes were collected.

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. 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 all P male parental generations: testis weight, epididymis weight, histopatho logical 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 isoflurane anesthesia.
SACRIFICE: Males in the mating groups and female in the non-mating (satellite) groups: On next day after the last administration (Day 43), Maternal animals: on Day 4 of lactation, and Males and females in the recovery groups: on Day 14 of recovery.

GROSS PATHOLOGY: Yes

ORGAN WEIGHT: Yes [brain, pituitary, thyroids (including parathyroids), adrenal gland, thymus, spleen, heart, liver, kidney, testis, epididymis, prostate, seminal vesicles, ovary, uterus]

HISTOPATHOLOGY: Yes, [cerebrum, cerebellum (including pons), sciatic nerve, spinal cord (thoracic), eyeball, optic nerve, Harderian gland, pituitary, thyroid, parathyroid, adrenal glands, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, thoracic aorta, trachea, lung (including bronchial), tongue, larynx, esophagus, stomach, duodenum, jejunum, ileum (including payer's patch), cecum, colon, rectum, submandibular gland, sublingual gland, liver, pancreas, kidney, bladder, testis, epididymis, ovary, uterus, vagina, prostate, seminal vesicles (including coagulating gland), mammary gland (inguinal region), skin (inguinal region), sternum and femur (including bone marrows), femoral skeletal muscle, gross abnormalities site and Individual identification site (pinna with ear tag)]
Underlined organs and tissues are fixed and stored only.

Postmortem examinations (offspring)
SACRIFICE
- The F1 offsprings were euthanized on PND4 by exsanguination under isoflurane anesthesia.
GROSS NECROPSY：Yes
- Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.
HISTOPATHOLOGY / ORGAN WEIGTHS
- 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 be tween two groups in the recovery test, homogeneity of variance was analyzed by F-test. Then, if homo genous, student's t-test was applied. If not homogenous, Aspin-Welch's t-test was used.
Regarding clinical observation (except for frequency of urination, defecation, rearing and grooming) and sensory reactivity, Steel test was applied. Regarding implantation index, stillborn index, live birth index, viability index and external abnormalities, Steel test was applied. Regarding copulation index,

insemination index, fertility index, and delivery index, auditory response, approach response, touch response, tail pinch response, pupillary reflex, aerial righting reflex, Fisher's test was applied.

Reproductive indices
Each parameter was determined by the following equations:
Copulation index (%) = (No. of copulated animals / No. of mated animals) × 100
Fertility index (%) = (No. of pregnant females / No. of copulated females) × 100
Insemination index (%) = (No. of males which impregnated females / No. of copulated males) × 100
Gestation length (days) = No. of days from pregnancy day 0 to parturition day
Delivery index (%) = (No. of females which delivered liveborns / No. of pregnant females) × 100
Implantation index (%) = (No. of implantation sites / No. of corpora lutea) × 100
Stillborn index (%) = (No. of stillborn / No of liveborns and stillborns) × 100
Live birth index (%) = (No. of liveborn / No. of implantation sites) × 100
External abnormalities (%) = (No. of pups with external abnormalities / No. of liveborns) × 100
Sex ratio = No. of liveborns males / No. of liveborns
Sex ratio of live pups on day 4= No. of live males on day 4/ No. of live pups on day 4

Offspring viability indices
Viability index on postnatal day 4 (%) = (No. of live pups on day 4 / No. of liveborns on day 0) × 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
no effects observed

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)
not examined

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 Repeated dose toxicity.001

Urinalysis findings
effects observed, non-treatment-related

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

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
effects observed, non-treatment-related

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

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 function / performance: Decreased nursing behavior was observed in dams at the 12 mg/kg bw/day and above. Death of all nursing infants in almost all dams were observed at 300 mg/kg bw/day.

Effect levels (P0)

Key result
false

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male

Basis for effect level
other: No effects on reproduction

Key result
true

Dose descriptor
NOAEL

Effect level

< 12 mg/kg bw/day (actual dose received)

Based on
test mat.

Sex
female

Basis for effect level
food consumption and compound intake
Decreased food consumption was observed in dams during lactation at the 12 mg/kg bw/day.
reproductive performance
Decreased nursing behavior was observed in dams at the 12 mg/kg bw/day.

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Mortality / viability
mortality observed, treatment-related

Description (incidence and severity)
Decrease in viability index on PND 4 was observed at 60 mg/kg bw/day and above.
viability index on PND 4:
0, 12, 60, 300 mg/kg bw/day: 85.8% (+/-30.6), 87.4% (+/-27.8), 29.8% (+/-25.4), 0.6% (+/-2.0)

All pups died during the lactation period in 1/10, 1/12, 2/12, 11/12 litters at the 0, 12, 60 and 300 mg/kg bw/day.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
The body weight of pups on PND 0 was lower at 60 mg/kg bw/day and above, and the body weight of pups on PND 4 was lower at 12 mg/kg bw/day and above.

Gross pathological findings
effects observed, non-treatment-related

Description (incidence and severity)
Bluish discoloration of organs/tissues or gastrointestinal contents was observed. These were conside red to reflect the color of the test substance.

Details on results (F1)

In the offspring, death of all nursing infants in almost all dams were observed at 300 mg/kg bw/day, decreased viability index on PND 4 was observed at 60 mg/kg bw/day and above, decreased body weights on PND 4 were observed at 12 mg/kg/day and above.

Effect levels (F1)

Key result
true

Dose descriptor
NOAEL

Generation
F1

Effect level

< 12 mg/kg bw/day (actual dose received)

Based on
test mat.

Sex
male/female

Basis for effect level
body weight and weight gain
In the offspring, decreased body weights on PND 4 were observed at 12 mg/kg/day and above.

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/PDF14233-37-5d.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, decreases in nursing behavior were observed in dams at 12 mg/kg bw/day and above, and deaths of all nursing infants were observed in almost all dams at 300 mg/kg bw/day. Also, food consumption at doses of 12 mg/kg bw/day and above during lactation were significantly lower. In offspring, a decrease in body weight was observed at 60 mg/kg bw/day on postnatal day (PND) 0 and at 12 mg/kg bw/day or more on PND 4, and a decrease in viability index on PND 4 was observed at 60 mg/kg bw/day or more.
The NOAEL for the reproductive/developmental toxicity was determined to be less than 12 mg/kg bw/day based on decreased nursing behavior in dams during lactation, decreased body weight in offspring at the 12 mg/kg bw/day dose, which was the lowest dose tested.

References

Reference Substances

REFERENCE_SUBSTANCE: 1,4-Bis(isopropylamino)anthraquinone

UUID: 6a24b2e9-eb9e-4f35-9ee6-32197dfe3c12
Dossier UUID:
Author:
Date: 2020-12-29T14:25:33.000+09:00
Remarks:

Reference substance name
1,4-Bis(isopropylamino)anthraquinone

Inventory

CAS number
14233-37-5

Molecular and structural information

Molecular formula
C20H22N2O2

Molecular weight

322.4

Test Materials

TEST_MATERIAL_INFORMATION: 1,4-Bis(isopropylamino)anthraquinone

UUID: a128e41f-ac9d-4fe1-b4f8-ca2042de1b42
Dossier UUID:
Author:
Date: 2020-12-29T14:26:29.000+09:00
Remarks:

Name
1,4-Bis(isopropylamino)anthraquinone

Composition

Composition

Reference substance
1,4-Bis(isopropylamino)anthraquinone / 14233-37-5

EC number	EC name
CAS number	CAS name
14233-37-5	

IUPAC name

Concentration

100

Literatures

LITERATURE: Combined repeat dose and reproductive/ developmental toxicity screening test of 1,4-Bis(isopropylamino)anthraquinone by oral administration in rats

UUID: 37839122-27b9-4487-944a-14d4e960cee6
Dossier UUID:
Author:
Date: 2020-12-29T14:17:59.000+09:00
Remarks:

General information

Reference Type
study report

Title
Combined repeat dose and reproductive/developmental toxicity screening test of 1,4-Bis(iso propylamino)anthraquinone by oral administration in rats

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

Year
2012

Bibliographic source
available in the web of Japan Existing Chemical Data Base (JECDB) at https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF14233-37-5d.pdf

Testing facility
BoZo Research Center

Report number
R-1068

LITERATURE: In Vitro Chromosomal Aberration Test of on 1,4-Bis(isopropylamino)anthraquinone Cultured Chinese Hamster Cells.

UUID: 8c138619-d7fd-4829-99bc-88d75ed25201
Dossier UUID:
Author:
Date: 2021-03-08T15:44:08.000+09:00
Remarks:

General information

Reference Type
study report

Title
In Vitro Chromosomal Aberration Test of on 1,4-Bis(isopropylamino)anthraquinone Cultured Chinese Hamster Cells.

Author
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Year
2011

Bibliographic source
Japan Existing Chemical Data Base (JECDB)
https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF14233-37-5f.pdf

Testing facility
Bozo Research Center Inc.

Report number
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LITERATURE: Reverse Mutation Test of 1,4-Bis(isopropylamino)anthraquinone on Bacteria.

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