Name: OECD_SIDS / SUBSTANCE : 2-tert-Butylcyclohexane-1-yl=acetate / 2-tert-butylcyclohexyl acetate / 88-41-5 Fri, 29 Nov 2024, 09:35:03+0900 /

Printing date: 2024-11-29T09:35:05.023+09:00

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DOSSIER:

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Author:
Date: 2024-11-29T09:35:03.515+09:00
Remarks:

Dossier header

Dossier submission type

Name
OECD SIDS

Version
core 9.0

Name (given by user)

Dossier subject

Dossier subject
2-tert-Butylcyclohexane-1-yl=acetate / 2-tert-butylcyclohexyl acetate / 88-41-5

Public name

Submitting legal entity
National Institute of Health Sciences

Dossier creation date/time
Fri, 29 Nov 2024, 09:35:03+0900

Used in category

LEGAL_ENTITY: National Institute of Health Sciences

UUID:	71368d76-19ad-4a2e-bc26-6c8ef515e6e3
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Author:	
Date:	2024-05-29T16:58:20.759+09:00
Remarks:	

General information

Legal entity name
National Institute of Health Sciences

2-tert-Butylcyclohexane-1-yl=acetate

General information

Identification

SUBSTANCE: 2-tert-Butylcyclohexane-1-yl=acetate

UUID: d00ff986-5395-4ffc-a302-329dc086ddb0
Dossier UUID:
Author:
Date: 2023-01-12T14:45:32.000+09:00
Remarks:

Substance name
2-tert-Butylcyclohexane-1-yl=acetate

Identification of substance

Reference substance
2-tert-butylcyclohexyl acetate / 2-tert-butylcyclohexyl acetate / 88-41-5 / 201-828-7

EC number	EC name
201-828-7	EC Inventory
CAS number	**CAS name**
88-41-5	

IUPAC name
2-tert-butylcyclohexyl acetate

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:	7b12bcb1-799c-49af-9d03-c821ae34cf6f
Dossier UUID:	
Author:	
Date:	2023-01-31T10:03:20.000+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
Reliability 1

Cross-reference

Reason / purpose for cross-reference
reference to same study

Related information
OECD / Toxicity to reproduction / Toxicity to reproduction. 001 / 2-tert-Butylcyclohexane-1-yl=acetate / 2-tert-butylcyclohexyl acetate / 88-41-5

Data source

Reference

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

Data access
data published https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF88-41-5d.pdf

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
other: Guideline for Combined Repeated Dose Study with the Reproduction /Developmental Toxicity Screening Test in Mammalian Species (Chemical Substances Control Law of Japan)

GLP compliance
yes

Limit test
no

Test material

Test material information
2-tert-Butylcyclohexan-1-yl acetate

Specific details on test material used for the study
- Name of test material (as cited in study report): 2-tert-Butylcyclohexan-1-yl acetate
- Analytical purity: 99.4%
- Storage condition of test material: sealed, cool place (actual temperature: 2 - 8°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: 391 g (353-465 g), Female: 251 g (226-280 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: 20 days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23±3 (actual temperature: 22-23°C)
- Humidity (%): 50±20% (actual humidity: 41-61%)

- Air changes (per hr): 12-17
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 7:00-19:00)

Administration / exposure

Route of administration
oral: gavage

Vehicle
corn oil

Details on oral exposure
- Amount of vehicle (if gavage): 5 mL/kg
- Dosing volume: 5 mL/kg

Analytical verification of doses or concentrations
yes

Details on analytical verification of doses or concentrations
The concentrations of each suspension used at weeks 1 and 6 of administration were analyzed by GC. The results showed that the concentration of each suspension was 101.0 to 106.0% of the nominal concentration, and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%)

Duration of treatment / exposure
Males: 42 days including 14 days pre-mating
Females (mating group): 41-46 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation
Female (non-mating 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.	
50	mg/kg bw/day (actual dose received)
Dose / conc.	
150	mg/kg bw/day (actual dose received)
Dose / conc.	
500	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex/dose (0, 50, 150, and 500 mg/kg bw/day)
Non-mating group: 10 females/dose (0 and 500 mg/kg bw/day)

Recovery group: 5 males/dose in the mating group and 5 females/dose in the non-mating groups (0 and 500 mg/kg bw/day)

Control animals
yes, concurrent vehicle

Details on study design
- Dose selection rationale: Based on the results of a 14-day preliminary study, the high dose was 1000 mg/kg bw/day, which was expected to cause clear signs of toxicity, and the intermediate dose and low dose were set to 300 mg/kg bw/day and 100 mg/kg bw/day, respectively.

[14-day preliminary study]
A 14-day repeated dose oral toxicity test (Crl: CD (SD) rats, doses: 0, 250, 500, and 1000 mg/kg bw/day).
In the 1000 mg/kg group, all females died, and males showed decreased fecal volume and salivation, in the early phase of treatment, decreased body weight and food consumption, suppressed weight gain, high total protein, and high absolute and relative weights of liver and kidney. In the 500 mg/kg group, tonic convulsion, salivation, or decreased fecal volume were observed in females, low food consumption was observed in males and females in the early phase of treatment, suppressed weight gain was observed in males during the treatment period, suppressed weight gain was observed in females in the early phase of treatment, high relative weight of the liver and high absolute and relative weight of the kidney were observed in males, low hemoglobin content, high absolute and relative weight of the liver, adrenal glands and high relative weights of the thyroid gland were observed in females. In the 250 mg/kg group, females showed low food consumption in the early phase of treatment and males showed high relative weight of the liver.
- 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. Twice a day during the recovery period.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule:
Males in the mating group and females in the non-mating group: Once before the start of administration, once a week 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 (Gestation Days (GDs) 1, 7, 14 and 20 for mated females, and Lactation Day (LD) 4 for parturient females).

BODY WEIGHT: Yes
- Time schedule for examinations:
Males in the mating group and females in the non-mating group: Days 1, 8, 15, 22, 29, 36 and 42 of administration and on the day of necropsy, and Days 1, 8 and 14 of recovery and on the day of necropsy.
Females in the mating group: Days 1, 8, 15 and 22 of administration, GDs 0, 7, 14 and 20, LDs 0 and 4 and on the day of necropsy.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Measurement of food consumption was conducted on all animals at the following frequencies:
Males in the mating group and females in the non-mating group: Days 2, 8, 15, 30, 36 and 42 of administration, and Days 1, 8 and 14 of recovery
Females in the mating group: Days 2, 8 and 15 of administration, GDs 1, 7, 14 and 20, LDs 2 and 4.

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: 5 animals/sex/group
- 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: 5 animals/sex/group
- Parameters checked: ALP, total bile acid, 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 blood: At the end of administration period, or at the end of recovery period in both sexes
- Animals fasted: Yes
- How many animals: 5 animals/sex/group
- Parameters checked: T3, T4, TSH

URINALYSIS: Yes
- Time schedule for collection of urine: On the final week of administration (Days 36 to 37 of administration) and on 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, sodium, potassium, chloride, urine volume (20-hour volume), water intake (24-hour volume)

NEUROBEHAVIOURAL (FUNCTIONAL) EXAMINATION: Yes
- Time schedule for examinations:
Males in the mating group and females in the non-mating group: On the final week of administration (Day 38 of administration), and on the final week of recovery (Day 10 of recovery).
Females in the mating group: LD 4 (Day 41 to Day 44 of administration).
- 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 was 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 (including coagulating gland), 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), skin (inguinal region)*, mammary gland (inguinal region)*, sternum* and femur (including bone marrows), femoral skeletal muscle, and Individual identification site (pinna with ear tag)*, and gross abnormalities sites* (Animal Nos. 1118, 1122 and 4119: stomach, Animal No. 4120: kidney).]
Asterisked organs and tissues are fixed and stored only.

Other:
Special stain and electron microscope examination
- Anti-α2 μ-globulin antibody and PAS-stain
As eosinophilic bodies were observed in renal tubular epithelial cells in the high-dose group of males, immunohistochemically stained specimens with anti-α2 μ-globulin antibody and PAS-stained specimens were prepared and examined microscopically in representative kidneys (Animal Nos. 4001 and 4002).

Statistics
For quantitative data, the homogeneity of variances was first tested using the Bartlett method. If the variance was homogeneous, statistical differences between the treatment and control groups were analyzed using the Dunnett method. If not homogeneous, the steel method was used to test for statistical differences between each treatment group and the control group. For comparison of quantitative data between the two groups in the recovery study, homogeneity of variance was analyzed by the F-test. Then, if homogeneous, the Student's t-test was applied. If not, the Aspin-Welch t-test was used.
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, treatment-related

Description (incidence and severity)
[At the dosing period]:
[General condition of living and dead animals]:
In males and females, salivation and/or clonic convulsion were observed at 500 mg/kg bw/day.
[At the recovery period]:
There were no effects related to the test substance in any groups.

Mortality
mortality observed, treatment-related

Description (incidence)
In mating females, at the 500 mg/kg bw/day group, one female each died on day 10 of treatment and on day 12, 13 or 17 of gestation. In the non-mating females, at the 500 mg/kg bw/day group, two females died on day 15 of treatment, and one female died on day 29 of treatment.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
[At the dosing period]:
In mating females, body weight gain was significantly decreased during gestation at 500 mg/kg bw/day.
[At the recovery period]:
In males, body weight gain was significantly increased at 500 mg/kg bw/day.

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

Description (incidence and severity)
[At the dosing period]:
In males, food consumption was significantly decreased on day 2 of treatment at 500 mg/kg bw/day.
In mating females, food consumption was significantly decreased on days 2 and 8 of treatment at 500 mg/kg bw/day.
In non-mating females, food consumption was significantly decreased on day 2 of treatment at 500 mg/kg bw/day.
[At the recovery period]:
In males and non-mating females, food consumption was significantly increased on day 8 at 500 mg/kg bw/day.

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 dosing period]:
In males, a significant prolongation of prothrombin time and a significant increase in fibrinogen were observed at 500 mg/kg bw/day.
In non-mating females, a significant decrease in red blood cell count was observed at 500 mg/kg bw/day.
[At the end of recovery period]:
There were no changes related to the test substance in any groups.

Clinical biochemistry findings
effects observed, treatment-related

Description (incidence and severity)
[At the end of dosing period]:
In males, significant decreases in total bile acid and glucose were observed at 500 mg/kg bw/day.
In non-mating females, a significant decrease in total bile acid was observed at 500 mg/kg bw/day.

[At the end of recovery period]:
There were no changes related to the test substance in any groups.

Endocrine findings
no effects observed

Urinalysis findings
effects observed, treatment-related

Description (incidence and severity)
[At the dosing period]:

In males, in association with increasing tendencies of water intake and urine volume, a decrease in osmotic pressure, increasing tendencies of sodium and chloride, and a significant increase in chloride were observed at 500 mg/kg bw/day.
In non-mated females, in association with increasing tendencies of water intake and urine volume, a significant decrease in osmotic pressure was observed at 500 mg/kg bw/day.
[At the recovery period]:
In males, in association with increasing tendencies of water intake and urine volume, a decrease in osmotic pressure and a significant increase in chloride were observed at 500 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 dosing period]:
In males, significant increases in absolute and relative weight of liver* and relative weight of kidney were observed at 50 mg/kg bw/day and above, and a significant increase in absolute weight of kidney was observed at 500 mg/kg bw/day. (*Note: the absolute weight of liver at 500 mg/kg bw/day was increased, but not significant)
In mating females, significant increases in relative weight of liver, adrenal and kidney were observed at 150 and 500 mg/kg bw/day, and significant increases in absolute weight of liver and adrenal and a significant decrease in absolute and relative weight of thymus were observed at 500 mg/kg bw/day.
In non-mating females, significant increases in absolute and relative weight of thyroid* and liver, a significant decrease in relative weight of thymus and a decrease in absolute weight of thymus were observed at 500 mg/kg bw/day. (*Note: the absolute weight of thyroid at 500 mg/kg bw/day was increased, but not significant)
[At the end of recovery period]:
In males, a significant increase in absolute and relative weight of kidney was observed at 500 mg/kg bw/day.

Gross pathological findings
effects observed, treatment-related

Description (incidence and severity)
[At the end of dosing period]:
In males, large kidneys were observed at 150 and 500 mg/kg bw/day, and large livers were observed at 150 mg/kg bw/day.
[At the end of recovery period]:
There were no changes related to the test substance in any groups.

Neuropathological findings
not examined

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

Description (incidence and severity)
[At the end of dosing period]:
Kidney:
In males, regeneration of tubular was observed at 150 mg/kg bw/day and above, and dilatation of tubular, granular cast and cell infiltration of interstitial were observed at 500 mg/kg bw/day.
Eosinophilic bodies of tubular epithelial cells observed at 50 mg/kg bw/day and above were considered to be derived from α2μ globulin since they were positive in the immunohistochemical stained specimen of α2μ globulin and negative in the PAS stain.

Liver:
Hypertrophy of centrilobular hepatocyte was observed at 150 and 500 mg/kg bw/day in males and mating females and at 500 mg/kg bw/day in non-mating females.

Thyroid:
Hypertrophy of follicular epithelial cells were observed at 150 and 500 mg/kg bw/day in males and at 500 mg/kg bw/day in mating and non-mating females.

Adrenal:
In mating and non-mating females, vacuolation of cortical cell was observed at 500 mg/kg bw/day.

[At the end of recovery period]:
Kidney:
In males, regeneration of tubular and granular cast did not show any obvious differences between the end of dosing period and the end of recovery period. The dilatation of tubular, cell infiltration of interstitial and eosinophilic bodies of tubular epithelial cells disappeared or diminished in degrees and frequencies.

Liver:
Hypertrophy of centrilobular hepatocyte was observed at 500 mg/kg bw/day in males and non-mating females, but the degree and frequency were diminished.

Thyroid:
In non-mating females, hypertrophy of follicular epithelial cells were observed at 500 mg/kg bw/day, but the degree and frequency were diminished.

Adrenal:
In mating and non-mating females, vacuolation of cortical cell was observed at 500 mg/kg bw/day.

Histopathological findings: neoplastic
not examined

Effect levels

Key result
true

Dose descriptor
NOAEL

Effect level

< 50 mg/kg bw/day (actual dose received)

Based on
test mat.

Sex
male

Basis for effect level
organ weights and organ / body weight ratios
significant increases in absolute and relative weight of liver and relative weight of kidney were observed in males at 50 mg/kg bw/day.

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
histopathology: non-neoplastic
Hypertrophy of centrilobular hepatocyte was observed in mating females at 150 mg/kg bw/day.
organ weights and organ / body weight ratios
Significant increases in relative weights of liver, kidney and adrenal were observed in mating femal es at 150 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/PDF88-41-5d.pdf

Applicant's summary and conclusion

Conclusions
The NOAEL for repeated dose toxicity in this study was determined to be less than 50 mg/kg bw/day for males and 50 mg/kg bw/day for females.

Executive summary

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed according to a Japanese guideline (similar to OECD TG 422). Male and female rats (12 animals/sex/dose) were administered 2-tert-butylcyclohexan-1-yl acetate mixed isomers by gavage at 0 (vehicle: corn oil), 50, 150, and 500 mg/kg bw/day. Males were administered for 42 days, including a 14-day premating period and subsequent mating period, whereas females in the mating group were administered for 41–46 days, including the 14-day premating, mating, and gestation periods, and until lactation day 4. Five males at the 0 and 500 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period. Ten additional females were administered at 0 and 500 mg/kg bw/day as a satellite group. These females were administered for 42 days without mating, and five females at 0 and 500 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period.

At 500 mg/kg bw/day, 4 females in the mating group, and 3 females in the non-mating group died.

In the clinical signs, salivation and/or clonic convulsion were observed in males and females at 500 mg/kg bw/day.

In the body weight, a significant decrease in body weight gain during gestation was observed in mating females at 500 mg/kg bw/day.

In the food consumption, significant decrease in the early stage of treatment was observed in males and females at 500 mg/kg bw/day.

In the urinalysis, in association with increasing tendencies of water intake and urine volume, a decrease in osmotic pressure, increasing tendencies of sodium and chloride, and a significant increase in chloride were observed in males at 500 mg/kg bw/day.

In the haematology, a significant prolongation of prothrombin time and a significant increase in fibrinogen were observed in males, and a significant decrease in red blood cell count was observed in non-mating females at 500 mg/kg bw/day.

In the clinical chemistry, significant decreases in total bile acid and glucose were observed in males at 500 mg/kg bw/day. A significant decrease in total bile acid was observed in non-mating females at 500 mg/kg bw/day.

In organ weights, weights of the liver, kidney, adrenal gland and thymus were affected by the administration of the test substance. A significant increase in liver weight was observed in males at 50 mg/kg bw/day and above, in mating females at 150 and 500 mg/kg bw/day, and in non-mating females at 500 mg/kg bw/day. A significant increase in kidney weight was observed in males at 50 mg/kg bw/day and above and in mating females at 150 and 500 mg/kg bw/day. A significant increase in adrenal gland weights was observed in mating females at 150 and 500 mg/g bw/day. Significant increases in thymus and thyroid weights were observed in mating and non-mating females at 500 mg/g bw/day.

In the gross pathology, in males, large kidneys were observed at 150 and 500 mg/kg bw/day, and large livers were observed at 150 mg/kg bw/day.

In the histopathological examination, treatment-related lesions were observed in the liver and thyroid in males and females, kidney in males, and adrenal in females. As lesion of the liver, hypertrophy of centrilobular hepatocyte was observed at 150 and 500 mg/kg bw/day in males and mating females and at 500 mg/kg bw/day in non-mating females. As lesion of the thyroid, hypertrophy of follicular epithelial cell was observed in males at 150 and 500 mg/kg bw/day and in mating and non-mating females at 500 mg/kg bw/day. As lesion of the kidney, in males, regeneration of tubular was observed at 150 and 500 mg/kg bw/day, dilatation of tubular, granular cast and cell infiltration of interstitial were observed at 500 mg/kg bw/day and eosinophilic bodies of tubular epithelial cells were observed at 50 mg/kg bw/day and above. The eosinophilic bodies of tubular epithelial cells were positive for α2μ-globulin and negative by PAS staining, suggesting that the substance was derived from α2μ-globulin.

As lesion of adrenal glands, vacuolation of cortical cell was observed in mating and non-mating females at 500 mg/kg bw/day.

In the recovery study, food consumption was significantly increased in males and non-mating females at 500 mg/kg bw/day. A decrease in osmotic pressure and a significant increase in chloride associated with increasing tendencies of water intake and urine volume and a significant increase in absolute and relative weight of kidney were observed in males at 500 mg/kg bw/day. However, other changes were reversible, decreasing or disappearing.

Based on these results, the NOAEL for repeated dose toxicity under the conditions of this study were determined to be less than 50 mg/kg bw/day for males, because the increased weight of livers and kidney at 50 mg/kg bw/day, and 50 mg/kg bw/day for females, because hypertrophy of centrilobular hepatocyte and increased weights of liver, kidney and adrenal were observed in mating females at 150 mg/kg bw/day.

Genetic toxicity

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

UUID:	f341dfc5-8e38-4774-8c78-4ace51c505d1
Dossier UUID:	
Author:	
Date:	2023-01-31T10:02:24.000+09:00
Remarks:	

Administrative data

Endpoint
in vitro gene mutation study in bacteria

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

Reverse Mutation Test of 2-tert-Butylcyclohexan-1-yl acetate on Bacteria. / Ministry of Health, Labour and Welfare (MHLW), Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

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

Deviations
no

Qualifier
according to guideline

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

Deviations
no

GLP compliance
yes

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

Test material

Test material information
2-tert-Butylcyclohexan-1-yl acetate

Specific details on test material used for the study
-Name of test material (as cited in study report): 2-tert-Butylcyclohexan-1-yl acetate
- Analytical purity: 99.4%
- Storage condition of test material: Seald and refrigerated (actual temperature: 2 - 8°C)
- Stability under test conditions: The stability of test material was identified by analysis of the remai
nder

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

Justification for deviation from the high dose level
-S9 mix:
2.44, 4.88, 9.77, 19.5, 39.1, 78.1 µg/plate (TA100, TA1535, TA98, TA1537 strains)

313, 625, 1250, 2500, 5000 µg/plate (WP2uvrA strain)
+S9 mix:
2.44, 4.88, 9.77, 19.5, 39.1, 78.1µg/plate (TA100, TA98, TA1537 strains)
0.61, 1.22, 2.44, 4.88, 9.77, 19.5, 39.1, 78.1 µg/plate (TA1535 strain)
39.1, 78.1, 156, 313, 625, 1250 µg/plate (WP2uvrA strain)
Maximum concentration was established based on the result of the preliminary test at concentration up to 5000 ug/plate. In the preliminary test, the growth inhibition was observed at 19.5 µg/plate and above for S. typhimurium TA1535 with S9, at 78.1 µg/plate and above for all TA strains without S9 mix and TA100, TA98 and TA1537 strains with S9, and at 1250 µg/plate and above for E. coli WP2uvrA strains with 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
other: -S9 mix: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), sodium azide (SAZ) and 2-methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine 2HCl (ICR-191) ;
+S9 mix: 2-aminoanthracene (2AA), benzo[a]pyrene (B[a]P)

Details on test system and experimental conditions
METHOD OF APPLICATION: Preincubation
DURATION- Preincubation period: 20 min at 37°C
- Exposure duration: 48.5 or 48 hrs
NUMBER OF PLATES: 3
NUMBER OF REPLICATIONS: 2
DETERMINATION OF CYTOTOXICITY
- Method: other: growth inhibition

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

Statistics
no

Results and discussion

Test results

Key result
true

Species / strain
S. typhimurium TA 1535
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 39.1 µg/plate and above
+S9 mix: 19.5 µg/plate

Vehicle controls validity
valid

Positive controls validity
valid

Key result
true

Species / strain
S. typhimurium TA 1537
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 39.1 µg/plate and above
+S9 mix: 39.1 µg/plate and above

Vehicle controls validity
valid

Positive controls validity
valid

Key result
true

Species / strain
S. typhimurium TA 98
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 39.1 µg/plate and above
+S9 mix: 39.1 µg/plate and above

Vehicle controls validity
valid

Positive controls validity
valid

Key result
true

Species / strain
S. typhimurium TA 100
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity -S9 mix: 39.1 µg/plate and above
+S9 mix: 39.1 µg/plate and above

Vehicle controls validity
valid

Positive controls validity
valid

Key result
true

Species / strain
E. coli WP2 uvr A
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity +S9 mix: 625 µg/plate and above

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 Japanese) are available in the following full report of the study.

https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF88-41-5e.pdf

Please also see the attached files (Tables in English)

Overall remarks, attachments

Attachments

Attached (sanitised) documents for publication
88-41-5_Ames Tables.xlsx / 49.538 KB (application/vnd.openxmlformats-officedocument.spreadsheetml.sheet)

Applicant's summary and conclusion

Conclusions
Interpretation of results (migrated information): negative
In a bacterial reverse mutation assay using Salmonella typhimurium TA100, TA1535, TA98, and TA1537, and Escherichia coli WP2uvrA (OECD TG 471), 2-tert-butylcyclohexan-1-yl acetate was negative with or without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

UUID:	7ace2550-f43b-42ad-b3b7-74cb1647564f
Dossier UUID:	
Author:	
Date:	2023-01-12T14:45:32.000+09:00
Remarks:	

Administrative data

Endpoint
in vitro 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
Reliability 1

Data source

Reference

In Vitro Chromosomal Aberration Test of 2-tert-Butylcyclohexan-1-yl acetate on Cultured Chinese Hams / 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 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
genetic toxicity in vitro, other

Deviations
no

GLP compliance
yes

Type of assay
other: in vitro mammalian chromosome aberration test

Test material

Test material information
2-tert-Butylcyclohexan-1-yl acetate

Specific details on test material used for the study
-Name of test material (as cited in study report): o-tert-Butylcyclohexyl acetate, mixture of isomers
- Analytical purity: 99.4%
- Storage condition of test material: Seald and refrigerated (actual temperature: 1.7 - 8.0°C)
- Stability under test conditions: The stability of test material was identified by analysis of the remain
der

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

Justification for deviation from the high dose level
Cell growth inhibition study
-S9 mix (short-term treatment): 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000 ug/mL
+S9 mix (short-term treatment): 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000 ug/mL
-S9 mix (continuous treatment, 24hr): 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000 ug/mL
-S9 mix (continuous treatment, 48hr): 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000 ug/mL

Main study
-S9 mix (short-term treatment): 20, 40, 60, 80, 100 ug/mL
+S9 mix (short-term treatment): 27.7, 41.5, 62.2, 93.3, 140 ug/mL
-S9 mix (continuous treatment, 24hr): 20, 40, 60, 80, 100 ug/mL
-S9 mix (continuous treatment, 48hr): 20, 40, 60, 80, 100 ug/mL

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
cyclophosphamide
+S9
mitomycin C
-S9

Details on test system and experimental conditions
METHOD OF APPLICATION:
Exposure duration:
- [short-term treatment]: 6 hrs + 18 hrs
- [continuous treatment]: 24, 48 hrs
SPINDLE INHIBITOR: Colcemid
STAIN: Giemsa stain (2 v/v%) for 15 min.
NUMBER OF REPLICATIONS: 2
NUMBER OF CELLS EVALUATED: 100 + 100 cells /concentration
DETERMINATION OF CYTOTOXICITY
- Method: relative total growth

Evaluation criteria
For the evaluation of the frequencies of structural aberrations and of polyploidy induced, the following criteria were employed. Appearance incidence of cell with chromosomal aberrations: Negative (-): less than 5%, Equivocal(±): more than 5% and less than 10%, Positive(+): 10% and above

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
negative

Cytotoxicity / choice of top concentrations
cytotoxicity

Vehicle controls validity
valid

Positive controls validity
valid

Additional information on results
RANGE-FINDING/SCREENING STUDIES (if applicable):
50% cell growth inhibition (IC50): 115 ug/mL (short-term treatment, +S9 mix), 81 ug/mL (short-term tr
eatment, -S9 mix), 70 ug/mL (continuous treatment, 24hr), 80 ug/mL (continuous treatment, 48hr)

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/PDF*88-41-5*f.pdf*

Applicant's summary and conclusion

Conclusions
Interpretation of results (migrated information): negative with or without metabolic activation
In an in vitro chromosomal aberration test using CHL/IU cells (OECD TG 473), 2-tert-butylcyclo
hexan-1-yl acetate was negative with or without metabolic activation

Toxicity to reproduction

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction. 001

UUID:	e0fcbafa-cdca-44a0-ac22-20e1f5183a5e
Dossier UUID:	
Author:	
Date:	2023-01-31T10:04:12.000+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
Reliability 1

Cross-reference

Reason / purpose for cross-reference
reference to same study

Related information
OECD / Repeated dose toxicity: oral / Repeated dose toxicity: oral. 001 / 2-tert-Butylcyclohexane-1-yl=acetate / 2-tert-butylcyclohexyl acetate / 88-41-5

Data source

Reference

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

Data access
data published https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF88-41-5d.pdf

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
other: Guideline for Combined Repeated Dose Toxicity Study with the Reproduction /Developmental Toxicity Screening Test in Mammalian Species (Chemical Substances Control Law of Japan)

GLP compliance
yes

Limit test
no

Test material

Test material information
2-tert-Butylcyclohexan-1-yl acetate

Specific details on test material used for the study
- Name of test material (as cited in study report): 2-tert-Butylcyclohexan-1-yl acetate
- Analytical purity: 99.4%
- Storage condition of test material: sealed, cool place (actual temperature: 2 - 8°C)
- Stability under test conditions: The stability of test material was identified by analysis of the remainder

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: 391 g (353-465 g), Female: 251 g (226-280 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: 20 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23±3 (actual temperature: 22-23°C)
- Humidity (%): 50±20% (actual humidity: 41-61%)
- Air changes (per hr): 12-17
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 7:00-19:00)

Administration / exposure

Route of administration
oral: gavage

Vehicle
corn oil

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

Details on mating procedure
- M/F ratio per cage:1/1
- Length of cohabitation: up to 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
The concentrations of each suspension used at weeks 1 and 6 of administration were analyzed by GC. The results showed that the concentration of each suspension was 101.0 to 106.0% of the nominal concentration, and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%)

Duration of treatment / exposure
Males: 42 days including 14 days pre-mating
Females (mating group): 41-46 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation
Female (non-mating 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.	
50	mg/kg bw/day (actual dose received)
Dose / conc.	
150	mg/kg bw/day (actual dose received)
Dose / conc.	
500	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex/dose (0, 50, 150, and 500 mg/kg bw/day)
Non-mating group: 10 females/dose (0 and 500 mg/kg bw/day)

Recovery group: 5 males/dose in the mating group and 5 females/dose in the non-mating groups (0 and 500 mg/kg bw/day)

Control animals
yes, concurrent vehicle

Details on study design
- Dose selection rationale: Based on the results of a 14-day preliminary study, the high dose was 500 mg/kg bw/day, which was expected to cause clear signs of toxicity, and the intermediate dose and l ow dose were set to 150 mg/kg bw/day and 50 mg/kg bw/day, respectively.
[14-day preliminary study]
A 14-day repeated dose oral toxicity test (Crl: CD (SD) rats, doses: 0, 250, 500, and 1000 mg/kg bw/d ay).
In the 1000 mg/kg group, all females died, and males showed decreased fecal volume and salivation, in the early phase of treatment, decreased body weight and food consumption, suppressed weight gain, high total protein, and high absolute and relative weights of liver and kidney. In the 500 mg/kg group, tonic convulsion, salivation, or decreased fecal volume were observed in females, low food consumption was observed in males and females in the early phase of treatment, suppressed weight gain was observed in males during the treatment period, suppressed weight gain was observed in females in the early phase of treatment, high relative weight of the liver and high absolute and relative weight of the kidney were observed in males, low hemoglobin content, high absolute and relative weight of the liver, adrenal glands and high relative weights of the thyroid gland were observed in females. In the 250 mg/kg group, females showed low food consumption in the early phase of treat ment and males showed high relative weight of the liver.

- 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 after admi nistration) during the administration period. Twice a day during the recovery period.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule:
Males in the mating group and females in the non-mating group: Once before the start of administr ation, once a week 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 (Gestation Days (GDs) 1, 7, 14 and 20 for mated females, and L actation Day (LD) 4 for parturient females).

BODY WEIGHT: Yes
- Time schedule for examinations:
Males in the mating group and females in the non-mating group: Days 1, 8, 15, 22, 29, 36 and 42 of a dministration and on the day of necropsy, and Days 1, 8 and 14 of recovery and on the day of necrops y.
Females in the mating group: Days 1, 8, 15 and 22 of administration, GDs 0, 7, 14 and 20, LDs 0 and 4 and on the day of necropsy.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Measurement of food consumption was conducted on all animals at the following frequencies:
Males in the mating group and females in the non-mating group: Days 2, 8, 15, 30, 36 and 42 of admi nistration, and Days 1, 8 and 14 of recovery
Females in the mating group: Days 2, 8 and 15 of administration, GDs 1, 7, 14 and 20, LDs 2 and 4.

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: 5 animals/sex/group
- 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: 5 animals/sex/group
- Parameters checked: ALP, total bile acid, 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 blood: At the end of administration period, or at the end of recovery period in both sexes
- Animals fasted: Yes
- How many animals: 5 animals/sex/group
- Parameters checked: T3, T4, TSH

URINALYSIS: Yes
- Time schedule for collection of urine: On the final week of administration (Days 36 to 37 of administration) and on 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, sodium, potassium, chloride, urine volume (20-hour volume), water intake (24-hour volume)

NEUROBEHAVIOURAL (FUNCTIONAL) EXAMINATION: Yes
- Time schedule for examinations:
Males in the mating group and females in the non-mating group: On the final week of administration (Day 38 of administration), and on the final week of recovery (Day 10 of recovery).
Females in the mating group: LD 4 (Day 41 to Day 44 of administration).
- 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 was 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: weights and histopathological examinations for testis, epididymis, prostate and seminal vesicles (including coagulating gland).

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 main groups and females in non-mating groups: On Day 43 (next day after the last administration), Maternal animals: on Day 5 of lactation, and Males and females recovery groups: on Day 15 of recovery.

ORGAN WEIGHT: Yes [brain, pituitary, thyroids (including parathyroids), adrenal gland, thymus, spleen, heart, liver, kidney, testis, epididymis, prostate, seminal vesicles (including coagulating gland), 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), skin (inguinal region)*, mammary gland (inguinal region)*, sternum* and femur (including bone marrows), femoral skeletal muscle, and Individual identification site (pinna with ear tag)*, and gross abnorma lities sites* (Animal Nos. 1118, 1122 and 4119: stomach, Animal No. 4120: kidney).]
Asterisked organs and tissues are fixed and stored only.

Other:
Special stain and electron microscope examination
- Anti-α2 μ-globulin antibody and PAS-stain
As eosinophilic bodies were observed in renal tubular epithelial cells in the high-dose group of male s, immunohistochemically stained specimens with anti-α2 μ-globulin antibody and PAS-stained specimens were prepared and examined microscopically in representative kidneys (Animal Nos. 4001 and 4002).

Postmortem examinations (offspring)
SACRIFICE
- The F1 offsprings were fixed on day 4 by immersion in Bouin's solution under isoflurane anesthesia and stored.
GROSS NECROPSY
- Not examined.
HISTOPATHOLOGY / ORGAN WEIGTHS
- Not examined.

Statistics
For quantitative data, the homogeneity of variances was first tested using the Bartlett method. If the variance was homogeneous, statistical differences between the treatment and control groups were a nalyzed using the Dunnett method. If not homogeneous, the steel method was used to test for statisti cal differences between each treatment group and the control group. For comparison of quantitative data between the two groups in the recovery study, homogeneity of variance was analyzed by the F-test. Then, if homogeneous, the Student's t-test was applied. If not, the Aspin-Welch t-test was used. R

egarding implantation index, delivery index, live birth index, stillborn index, external abnormalities and viability index on PND4 and, Steel test was applied. Regarding the index of animals with abnormal estrous cycle, copulation index, insemination index, fertility index, and gestation 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:
Index of animals with abnormal estrous cycle (%) = No. of animals with abnormal estrous cycle / No. of animals examined) × 100
Copulation index (%) = (No. of copulated animals / No. of mated animals) × 100
Insemination index (%) = (No. of males which impregnated females / No. of copulated males) × 100
Fertility index (%) = (No. of pregnant females / No. of copulated females) × 100
Gestation index (%) = (No. of females which delivered liveborns / No. of pregnant females) × 100
Gestation length (days) = No. of days from pregnancy day 0 to parturition day
Implantation index (%) = (No. of implantation sites / No. of corpora lutea) × 100
Delivery index (%) = (No. of delivered pups / No. of implantation sites) × 100
Stillborn index (%) = (No. of stillborn / No. of delivered pups) × 100
External abnormalities (%) = (No. of delivered pups with external abnormalities / No. of delivered pups) × 100
Live birth index (%) = (No. of liveborn / No. of delivered pups) × 100
Sex ratio of delivered pups = No. of delivered males / No. of delivered pups
Sex ratio of liveborns = 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) × 100

Results and discussion

Results: P0 (first parental generation)

General toxicity (P0)

Clinical signs
effects observed, treatment-related

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

Mortality
mortality observed, treatment-related

Description (incidence)
See 7.5.1 Repeated dose toxicity. 001

Body weight and weight changes
effects observed, treatment-related

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

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

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

Food efficiency
not examined

Ophthalmological findings
not examined

Haematological findings
effects observed, treatment-related

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

Clinical biochemistry findings
effects observed, treatment-related

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

Endocrine findings
no effects observed

Urinalysis findings
effects observed, 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, 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
no effects observed

Details on results (P0)

General toxicity:
See 7.5.1 Repeated dose toxicity.001

Reproductive function / performance:
No effects were observed in the 500 mg/kg dose group of males and females.
No effects were observed on reproductive functions such as copulation, insemination, and fertility in males and females, and pregnancy maintenance, parturition, and nursing behavior in dams.

Effect levels (P0)

Key result
true

Dose descriptor
NOAEL

Effect level

< 50 mg/kg bw/day (actual dose received)

Based on
test mat.

Sex
male

Basis for effect level
organ weights and organ / body weight ratios
See 7.5.1 Repeated dose toxicity. 001

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
organ weights and organ / body weight ratios
See 7.5.1 Repeated dose toxicity. 001
histopathology: non-neoplastic
See 7.5.1 Repeated dose toxicity. 001

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
reproductive performance
No effects observed.

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Mortality / viability
no mortality observed

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
Not significant but dose-dependent decreases in body weights on postnatal day 4 and body weight gain during postnatal days 0-4 were observed in pups at 500 mg/kg bw/day, and the body weight gain is more than 17% less than the control group.

Gross pathological findings
no effects observed

Details on results (F1)

No effects observed.

Effect levels (F1)

Key result
true

Dose descriptor
NOAEL

Generation
F1

Effect level

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

Based on
test mat.

Sex
not specified

Basis for effect level
body weight and weight gain
In the 500 mg/kg bw/day, the trend in declining weight gain was observed on postnatal days 0-4.

Overall reproductive toxicity

Key result
false

Reproductive effects observed
no

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/PDF88-41-5d.pdf

Applicant's summary and conclusion

Conclusions
In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity scree ning test described above, no effects were observed in the dams. However, the trend in declining wei ght gain during postnatal days 0-4 was observed in pups at 500 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of 2-tert-Butylcyclohexan-1-yl acetate was regarded as 500 mg/ kg bw/day for males and females and 150 mg/kg bw/day for pups.

References

Reference Substances

REFERENCE_SUBSTANCE: 2-tert-butylcyclohexyl acetate

UUID: ECB5-2c44f264-f67a-4c87-b06e-7abc236dbcd6
Dossier UUID:
Author:
Date: 2023-01-13T10:33:03.000+09:00
Remarks:

Reference substance name
2-tert-butylcyclohexyl acetate

IUPAC name
2-tert-butylcyclohexyl acetate

Inventory

Inventory number

Inventory name
2-tert-butylcyclohexyl acetate

Inventory
EC Inventory

Inventory number
201-828-7

CAS number
88-41-5

Molecular formula
C12H22O2

Description

CAS number
88-41-5

Synonyms

Synonyms

Identity
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate

Identity
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate

Molecular and structural information

Molecular formula
C12H22O2

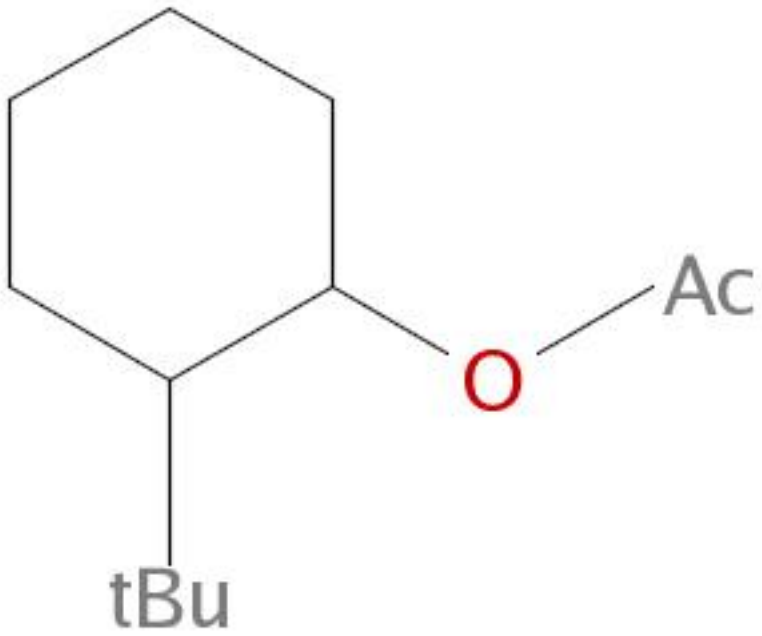
Molecular weight

198.3019

SMILES notation
CC(=O)OC1CCCCC1C(C)(C)C

InChI
InChI=1/C12H22O2/c1-9(13)14-11-8-6-5-7-10(11)12(2,3)4/h10-11H,5-8H2,1-4H3

Structural formula



Related substances

Group / category information
DSL Category: Organics

Test Materials

TEST_MATERIAL_INFORMATION: 2-tert-Butylcyclohexan-1-yl acetate

UUID: f5810082-4aef-425f-a703-d34bdf8454a4
Dossier UUID:
Author:
Date: 2023-01-13T10:33:05.000+09:00
Remarks:

Name
2-tert-Butylcyclohexan-1-yl acetate

Composition

Composition

Type
Constituent

Reference substance
2-tert-butylcyclohexyl acetate / 2-tert-butylcyclohexyl acetate / 88-41-5 / 201-828-7

EC number	EC name
201-828-7	EC Inventory
CAS number	**CAS name**
88-41-5	

IUPAC name
2-tert-butylcyclohexyl acetate

Concentration

99.35	% (v/v)

Literatures

LITERATURE: Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of 2-tert-butylcyclohexan-1-yl acetate by oral administration in rats

UUID: df79ea87-e9da-4b5f-87b1-ade65291a016
Dossier UUID:
Author:
Date: 2023-01-16T18:02:45.000+09:00
Remarks:

General information

Reference Type
study report

Title
Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of 2-tert-butylcyclohexan-1-yl acetate by oral administration in rats

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

Year
2013

Bibliographic source
available in the web of Japan Existing Chemical Data Base (JECDB) https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF88-41-5d.pdf

Testing facility
BoZo Research Center

Report date
2013-09-25

Report number
R-1105

LITERATURE: In Vitro Chromosomal Aberration Test of 2-tert-Butylcyclohexan-1-yl acetate on Cultured Chinese Hamster Cells.

UUID: 0a1efee3-2ccf-40c7-8f1e-2f701a80df60
Dossier UUID:
Author:
Date: 2023-01-12T14:34:50.000+09:00
Remarks:

General information

Reference Type
study report

Title
In Vitro Chromosomal Aberration Test of 2-tert-Butylcyclohexan-1-yl acetate on Cultured Chinese Hamster Cells.

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

Year
2013

Bibliographic source
Japan Existing Chemical Data Base (JECDB) https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF88-41-5f.pdf

Testing facility
Bozo Research Center Inc.

Report date
2013-03-22

Report number
T-G060

LITERATURE: Reverse Mutation Test of 2-tert-Butylcyclohexan-1-yl acetate on Bacteria.

UUID: ba31f751-58c0-44db-80b7-8c25cb8f2b63
Dossier UUID:
Author:
Date: 2023-01-12T13:38:11.000+09:00
Remarks:

General information

Reference Type
study report

Title
Reverse Mutation Test of 2-tert-Butylcyclohexan-1-yl acetate on Bacteria.

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

Year
2013

Bibliographic source
Japan Existing Chemical Data Base (JECDB) https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF88-41-5e.pdf

Testing facility
Bozo Research Center Inc.

Report date
2013-03-22

Report number
T-1112