Name: OECD_SIDS / SUBSTANCE : Terpineol / 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol / 8000-41-7 Fri, 29 Nov 2024, 09:44:21+0900 /

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Remarks:

Dossier header

Dossier submission type

Name
OECD SIDS

Version
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Name (given by user)

Dossier subject

Dossier subject
Terpineol / 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol / 8000-41-7

Public name

Submitting legal entity
National Institute of Health Sciences

Dossier creation date/time
Fri, 29 Nov 2024, 09:44:21+0900

Used in category

LEGAL_ENTITY: National Institute of Health Sciences

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General information

Legal entity name
National Institute of Health Sciences

Terpineol

General information

Identification

SUBSTANCE: Terpineol

UUID: 095eea6e-b243-414c-8db3-9a9f7e349bd8
Dossier UUID:
Author:
Date: 2023-01-12T15:48:54.000+09:00
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Substance name
Terpineol

Identification of substance

Reference substance
Terpineol / 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol / 8000-41-7 / 232-268-1

EC number	EC name
232-268-1	EC Inventory

CAS number	CAS name
8000-41-7	

IUPAC name
2-(4-methylcyclohex-3-en-1-yl)propan-2-ol

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:	16fad618-f670-455d-9c2e-319d46125351
Dossier UUID:	
Author:	
Date:	2023-02-24T16:24:37.000+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 / Terpineol / 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol / 8000-41-7

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/PDF8000-41-7d.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
Terpineol

Specific details on test material used for the study
- Name of test material (as cited in study report): Terpineol
- Analytical purity: 92.1% (α-Terpineol: 62.8%; β-Terpineol: 8.7% ; γ-Terpineol: 20.6%)
- 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 remai nder

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: 378 g (350-420 g), Female: 231 g (215-267 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: 22-24°C)

- Humidity (%): 50±20% (actual humidity: 39-65%)
- 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
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 HPLC. The results showed that the concentration of each suspension was 99.5 to 103.0% of the no minal concentration, and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%)

Duration of treatment / exposure
Males: 44 days including 14 days pre-mating
Females (mating group): 41-51 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation
Female (non-mating group): 44 days

Frequency of treatment
Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
100	mg/kg bw/day (actual dose received)
Dose / conc.	
300	mg/kg bw/day (actual dose received)
Dose / conc.	
1000	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex/dose (0, 100, 300 and 1000 mg/kg bw/day)

Non-mating group: 10 females/dose (0 and 1000 mg/kg bw/day)
Recovery group: 5 males/dose in the mating group and 3 females/dose in the non-mating groups (0 and 1000 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).
One male in the 1000 mg/kg bw/day group showed decreased body weight and food consumption, decreased fecal volume, decreased locomotor activity, and decreased respiratory rate, and died on Day 12. Autopsy revealed a small spleen and thymus, and dark red foci in the glandular stomach. In the surviving 1000 mg/kg bw/day group, increased liver weight, dark red foci in the glandular stomach, and increased urea nitrogen and total protein, increased kidney weight, and decreased spleen weight were observed in males and females. In the 500 mg/kg bw/day and 1000 mg/kg bw/day groups, 1 female each showed staggering gait, decreased muscle tone, and crawling gait only on Day 1.

- 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 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 (Gestation Days (GDs) 1, 7, 14 and 20 for mated females, Days 6 and 13 after mating for unmated females, and Lactation Day (LD) 4 for parturient females).

BODY WEIGHT: Yes
- Time schedule for examinations:
Males: Days 1, 8, 15, 22, 29, 36, 42 and 44 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 the day of necropsy.
Females in the non-mating group: Days 1, 8, 15, 22, 29, 36, 42 and 44 of administration and on the day of necropsy, and Days 1, 8 and 14 of recovery 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: Days 2, 8, 15, 30, 36, 42 and 44 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.
Females in the non-mating group: Days 2, 8, 15, 30, 36, 42 and 44 of administration, and Days 1, 8 and 14 of recovery

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: No

URINALYSIS: Yes
- Time schedule for collection of urine: On the final week of administration (Days 38 to 39 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, urine volume (20-hour volume), water intake (24-hour volume)

NEUROBEHAVIOURAL (FUNCTIONAL) EXAMINATION: Yes
- Time schedule for examinations:
Males: On the final week of administration (Day 37 of administration), and on the final week of recovery (Day 12 of recovery).
Females in the mating group: LD 4 (Day 41 to Day 43 of administration) after necropsy of F1 pups
Females in the non-mating group: On the final week of administration (Day 37 of administration), and on 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 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 site (Animal No. 4107, oviduct) (*).]
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).

- Oil red O stain and electron microscope examination
Since vacuoles were observed in the liver and adrenal glands of females, the adrenal glands and livers of the following animals were examined by Oil Red O staining and electron microscopy to determine the details.
Adrenal: 2 females in the control group (Animal Nos. 1103 and 1116) and 3 females in the 1000 mg/kg bw/day group (Animal Nos. 4102, 4104 and 4106)
Liver: 2 females in the control group (Animal Nos. 1103 and 1116) and 3 females in the 1000 mg/kg bw/day group (Animal Nos. 4103, 4105 and 4114)

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 and aerial righting reflex, Fisher's test was applied.

Results and discussion

Results of examinations

Clinical signs
effects observed, treatment-related

Description (incidence and severity)
CLINICAL SIGNS:
[At the dosing period]:
[General condition of living animals]:
In males, salivation and decreased feces were observed at 1000 mg/kg bw/day.
In non-mating females, salivation, decreased feces, emaciation, decrease in spontaneous movement, ataxia, and bradypnea were observed at 1000 mg/kg bw/day.
[General conditions of dead animals]:

In mating females, decreased feces, decrease in spontaneous movement, prone position/lateral positi on, emaciation, bradypnea, ataxia, and hypothermia were observed at 1000 mg/kg bw/day.
In non-mating females, salivation, decrease in spontaneous movement, emaciation, and ataxia were observed at 1000 mg/kg bw/day.
[At the recovery period]:
There were no effects related to the test substance in any groups.

DETAILED CLINICAL OBSERVATIONS:
[At the dosing period]:
In males, salivation was observed at 1000 mg/kg bw/day.
In mating females, ataxia was observed at 1000 mg/kg bw/day.
In non-mating females, salivation was observed at 1000 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 1000 mg/kg bw/day group, one female each died on day 14 of treatment and on day 3 of gestation, and one female each was euthanized on day 18 and day 21 of gestation due to worsening conditions.
In the non-mating females, at the 1000 mg/kg bw/day group, one female died on day 42 of treatment, and one female was euthanized due to deterioration of condition.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
[At the dosing period]:
A significant decrease in body weight gain was observed in males at 1000 mg/kg bw/day.
[At the recovery period]:
There were no changes related to the test substance in any groups.

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

Description (incidence and severity)
[At the dosing period]:
There were no changes related to the test substance in any groups.
[At the recovery period]:
In males, food consumption was significantly increased on days 1 and 14 at 1000 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 decrease in Hb was observed at 1000 mg/kg bw/day.
In non-mating females, significant decreases in Hb and Ht and increases in MCHC and fibrinogen were observed at 1000 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, a significant increase in γ-GTP was observed at 1000 mg/kg bw/day.
[At the end of recovery period]:
There were no changes related to the test substance in any groups.

Urinalysis findings
effects observed, treatment-related

Description (incidence and severity)
[At the dosing period]:
Significant increases in water intake and urine volume, a significant decrease in osmotic pressure were observed in males and non-mating females at 1000 mg/kg bw/day.
[At the recovery period]:
Significant increases in water intake, urine volume, potassium, and chloride, increasing tendency of s odium, a significant decrease in osmotic pressure were observed in males and non-mating females at 1000 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, a significant increase in relative liver weight and trend toward an increase in absolute liver weight, significant increase in relative and absolute kidney weights, significant decreases in relative and absolute testis and epididymis weights were observed 1000 mg/kg bw/day.
In mating females, a significant increase in relative liver weight and trend toward an increase in abso lute liver weight were observed at 300 mg/kg bw/day, and trend toward increases in absolute and rela tive liver weights were observed at 1000 mg/kg bw/day.
In non-mating females, significant increases in absolute and relative liver, kidney and adrenal weights were observed at 1000 mg/kg bw/day.
[At the end of recovery period]:
In males, a significant increase in relative liver weight and trend toward an increase in absolute li ver weight, significant increases in absolute and relative kidney weights, significant decreases in absolute and relative testis and epididymis weights were observed at 1000 mg/kg bw/day.
In non-mating females, a significant increase in relative liver weight was observed at 1000 mg/kg bw/ day.

Gross pathological findings
effects observed, treatment-related

Description (incidence and severity)
[At the end of dosing period]:
In males, white foci in the kidneys and small foci in the testes were observed at 1000 mg/kg bw/day.
[At the end of recovery period]:
In males, small of testes were observed at 1000 mg/kg bw/day.

Neuropathological findings
not examined

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

Description (incidence and severity)
[At the end of dosing period]:
Adrenal:
In mating females, vacuolation of cortical cell was observed at 300 mg/kg bw/day and above.
In non-mating females, vacuolation of cortical cell was observed at 1000 mg/kg bw/day.
The vacuole of the cortical cell was the swelling of the mitochondrion in the electron microscopic ex amination. Some vacuoles were positive for oil red O, and some vacuoles containing neutral fat were considered to be present. In the case with strong swelling, the crista disappeared.
Testis:
In males, atrophy of seminiferous tubular, multinucleated giant cell, vacuolation of seminiferous tubular were observed at 1000 mg/kg bw/day.
Epididymis：
In males, hypospermia, and cell debris in lumen were observed at 1000 mg/kg bw/day.
Kidney:
In males, regeneration of cortex tubular, and eosinophilic body of tubular cell were observed at 300 mg/kg bw/day and above, dilatation of tubular, vacuolation of distal tuble/collecting duct, single cell necrosis of papillary duct, cell infiltration of cortex, and regeneration of papillary collecting duct were observed at 1000 mg/kg bw/day.
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.
In mating females, vacuolation of proximal tubular was observed at 300 mg/kg bw/day. vacuolation of distal tuble/collecting duct, necrosis of papillary, and cell infiltration of papillary were observed at 1000 mg/kg bw/day.
In non-mating females, dilatation of tubular, vacuolation of distal tuble/collecting duct, regeneration of cortex tubular, single cell necrosis of papillary duct, cell infiltration of cortex, necrosis of papillary and cell infiltration of papillary were observed at 1000 mg/kg bw/day.
Liver:
In males, hypertrophy of centrilobular hepatocyte was observed at 1000 mg/kg bw/day.
In mating females, vacuolation of hepatocyte was observed at 1000 mg/kg bw/day.
In non-mating females, vacuolation of hepatocyte was observed at 1000 mg/kg bw/day.
The vacuoles of the hepatocytes were not stained with Oil Red O, and electron microscopy showed enl arged mitochondria. In the case with strong swelling, the crista disappeared.
Urinary bladder
In males, vacuolation of umbrella cell was observed at 300 mg/kg bw/day and above, atrophy of um brella cell, hypertrophy/hyperplasia of transitional epithelial were observed at 1000 mg/kg bw/day.
In mating females, vacuolation of umbrella cell was observed at 300 mg/kg bw/day, atrophy of umbrella cell, and hypertrophy/hyperplasia of transitional epithelial were observed at 1000 mg/kg bw/day.
In non-mating females, atrophy of umbrella cell and hypertrophy/hyperplasia of transitional epithelial were observed at 1000 mg/kg bw/day.
Pancreas
In mating females, decreased zymogen granule was observed at 300 mg/kg bw/day.
In non-mating females, decreased zymogen granule was observed at 1000 mg/kg bw/day.
[At the end of recovery period]:
Testis:

In males, atrophy of seminiferous tubular, multinucleated giant cell, and vacuolation of seminiferous tubular were observed at 1000 mg/kg bw/day.
Epididymis：
In males, hypospermia, and cell debris in lumen were observed at 1000 mg/kg bw/day.
Kidney:
In males, necrosis of papillary, and regeneration of cortex tubular were observed at 1000 mg/kg bw/day, respectively.
In non-mating females, necrosis of papillary was observed at 1000 mg/kg bw/day

Histopathological findings: neoplastic
not examined

Effect levels

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male

Basis for effect level
histopathology: non-neoplastic
Regeneration of cortex tubular and eosinophilic body of tubular cell in the kidney, vacuolation of umbrella cell in the urinary bladder were observed in males at 300 mg/kg bw/day.

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
histopathology: non-neoplastic
Vacuolation of cortical cell in the adrenal, vacuolation of proximal tubular in the kidney, vacuolation of umbrella cell in the urinary bladder, and decreased zymogen granule in the pancreas were observed in mating females at 300 mg/kg bw/day.
organ weights and organ / body weight ratios

A significant increase in relative liver weight and trend toward an increase in absolute liver weight were observed in mating females at 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/PDF8000-41-7d.pdf

Applicant's summary and conclusion

Conclusions
The NOAEL for repeated dose toxicity in this study was determined to be 100 mg/kg bw/day for males and 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 terpineols mixed isomers by gavage at 0 (vehicle: corn oil), 100, 300, and 1000 mg/kg bw/day. Males were administered for 44 days, including a 14-day premating period and subsequent mating period, whereas females in the mating group were administered for 41–51 days, including the 14-day premating, mating, and gestation periods, and until lactation day 4. Five males at the 0 and 1000 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 1,000 mg/kg bw/day as a satellite group. These females were administered for 44 days without mating, and five females at 0 and 1000 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period.

At 1000 mg/kg bw/day, 4 females in the mating group, and 2 females in the non-mating group died or were euthanized due to deteriorating conditions.

In the clinical signs, salivation and decreased feces were observed in males and females at 1000 mg/kg bw/day, and emaciation, decrease in spontaneous movement, ataxia, and bradypnea were observed in females at 1000 mg/kg bw/day.

In the detailed clinical observation, salivation was observed in males and females at 1000 mg/kg bw/day, and staggering gait was observed on day 1 of gestation in mating females at 1000 mg/kg bw/day.

In the body weight, decrease in body weight gain was observed in males at 1000 mg/kg bw/day.

In the food consumption, there were no changes related to the test substance in any groups.

In the urinalysis, increase in water intake and urine volume, decrease in osmotic pressure were observed in males and females at 1000 mg/kg bw/day.

In the haematology, decrease in Hb was observed in males and females at 1000 mg/kg bw/day, decrease in Ht, increases in MCHC and fibrinogen were observed in females at 1000 mg/kg bw/day.

In the clinical chemistry, increase in γ-GTP was observed in males at 1000 mg/kg bw/day.

In organ weights, increase in liver weight was observed in females at 300 mg/kg bw/day or more in males at 1000 mg/kg bw/day. Increase in kidney weight was observed in males and females at 1000 mg/kg bw/day. Decreases in testis and epididymis weights were observed in males at 1000 mg/kg bw/day. Increase in adrenal weight was observed in females at 1000 mg/kg bw/day.

In the histopathological examination, treatment-related lesions were observed in the liver, kidneys, adrenal glands and bladder in males and females, testis and epididymis in males, and pancreas in

females. Liver lesions included hypertrophy of centrilobular hepatocyte (males), and vacuolation of hepatocyte due to mitochondrial swelling (females) at 1000 mg/kg bw/day. Kidney lesions such as vacuolization, regeneration and dilation of tubule, papillary necrosis and cell infiltration of the papillary were observed in males and females at 300 mg/kg bw/day and above. Eosinophilic body of tubular cell due to α2μ-globulin was observed in males at 300 mg/kg bw/day and above. As adrenal glands lesion, vacuolation of cortical cell due to mitochondrial swelling was observed in female at 300 mg/kg bw/day and above. As bladder lesion, vacuolation of umbrella cell was observed in males and females at 300 mg/kg bw/day and above, and atrophy of umbrella cell and hypertrophy/hyperplasia of transitional epithelial were observed in males and females at 1000 mg/kg bw/day. As testicular lesion, atrophy of seminiferous tubular and multinucleated giant cell, vacuolation of seminiferous tubular was observed in males at 1000 mg/kg bw/day. As epididymal lesions, hypospermia, cell debris in lumen were observed in males at 1000 mg/kg bw/day. As pancreatic lesions, decreased zymogen granule was observed in females at 300 mg/kg bw/day and above.

In the recovery study, an increase in urine volume accompanied by an increase in water consumption, a decrease in urine osmolality and an increase in electrolyte excretion were observed in males and females at 1000 mg/kg bw/day, and renal papillary necrosis continued to be observed in females, indicating insufficient recovery. Renal papillary necrosis was not observed in males at the end of treatment but was observed at the end of the recovery period.

The no-observed-adverse-effect level (NOAEL) for repeated-dose toxicity was determined to be 100 mg/kg bw/day for both males and females because effects on the kidneys and bladder in males and females and the liver, adrenal glands and pancreas in females were observed at 300 mg/kg bw/day and above.

Genetic toxicity

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

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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 terpineol 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
Terpineol

Specific details on test material used for the study
- Name of test material (as cited in study report): Terpineol
- Analytical purity: 92.1% (α-Terpineol: 62.8%; β-Terpineol: 8.7% ; γ-Terpineol: 20.6%)
- Storage condition of test material: sealed, cool place (actual temperature: 1.7 - 7.0°C)
- Stability under test conditions: The stability of test material was identified by analysis of the r emainder

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:
39.1, 78.1, 156, 313, 625, 1250 µg/plate (All strains)
+S9 mix:
39.1, 78.1, 156, 313, 625, 1250 µg/plate (TA100, WP2uvrA strains)

9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate (TA1535, TA98, TA1537 strains)

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 313 µg/plate and above for S. typhimurium TA98, TA 1535 and TA1537 strains with S9 mix, and at 1250 µg/plate and above for S. typhimurium TA100 and E. coli WP2uvrA strains with S9 mix, and for all strains 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
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 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 i ncrease 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: 625 µg/plate and above
+S9 mix: 313 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: 625 µg/plate and above
+S9 mix: 313 µg/plate

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: 625 µg/plate and above
+S9 mix: 313 µg/plate

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: 625 µg/plate and above
+S9 mix: 625 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: 1250 µg/plate;
+S9 mix: 1250 µg/plate

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/PDF8000-41-7e.pdf

Please also see the attached files (Tables in English)

Overall remarks, attachments

Attachments

Attached (sanitised) documents for publication
8000-41-7_Ames Tables.xlsx / 42.616 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), terpineol was negative with or without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

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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 terpineol on Cultured Chinese Hamster Cells. / 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
Terpineol

Specific details on test material used for the study
- Name of test material (as cited in study report): Terpineol
- Analytical purity: 92.1% (α-Terpineol: 62.8%; β-Terpineol: 8.7% ; γ-Terpineol: 20.6%)
- Storage condition of test material: sealed, cool place (actual temperature: 1.7 - 7.0°C)
- Stability under test conditions: The stability of test material was identified by analysis of the r emainder

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): 12.5, 25.0, 50.0, 100, 200, 400, 800, 1600 ug/mL
+S9 mix (short-term treatment): 12.5, 25.0, 50.0, 100, 200, 400, 800, 1600 ug/mL
-S9 mix (continuous treatment, 24hr): 12.5, 25.0, 50.0, 100, 200, 400, 800, 1600 ug/mL
-S9 mix (continuous treatment, 48hr): 12.5, 25.0, 50.0, 100, 200, 400, 800, 1600 ug/mL

Main study
-S9 mix (short-term treatment): 100, 200, 300, 400 ug/mL
+S9 mix (short-term treatment): 100, 200, 300, 400 ug/mL
-S9 mix (continuous treatment, 24hr): 100, 200, 300, 400 ug/mL
-S9 mix (continuous treatment, 48hr): 100, 200, 300, 400 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 Short-term treatment (+/-S9 mix): no cytotoxicity; Continuous treatment (24hr/48hr): cytotoxicity

Vehicle controls validity
valid

Positive controls validity
valid

Additional information on results
RANGE-FINDING/SCREENING STUDIES (if applicable):
50% cell growth inhibition (IC50): 449 ug/mL (short-term treatment, +S9 mix), 295 ug/mL (short-term treatment, -S9 mix), 276 ug/mL (continuous treatment, 24hr), 242 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/PDF8000-41-7f.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), terpineol was negative with or without metabolic activation

Toxicity to reproduction

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction. 001

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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 / Terpineol / 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol / 8000-41-7

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/PDF8000-41-7d.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
Terpineol

Specific details on test material used for the study
- Name of test material (as cited in study report): Terpineol
- Analytical purity: 92.1% (α-Terpineol: 62.8%; β-Terpineol: 8.7% ; γ-Terpineol: 20.6%)
- 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 remai nder

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: 378 g (350-420 g), Female: 231 g (215-267 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: 22-24°C)
- Humidity (%): 50±20% (actual humidity: 39-65%)
- 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
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 HPLC. The results showed that the concentration of each suspension was 99.5 to 103.0% of the no minal concentration, and both values were within the acceptable range (concentration: percentage of nominal concentration, 100±10%)

Duration of treatment / exposure
Males: 44 days including 14 days pre-mating
Females (mating group): 41-51 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation
Female (non-mating group): 44 days

Frequency of treatment
Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
100	mg/kg bw/day (actual dose received)
Dose / conc.	
300	mg/kg bw/day (actual dose received)
Dose / conc.	
1000	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex/dose (0, 100, 300, and 1000 mg/kg bw/day)

Non-mating group: 10 females/dose (0 and 1000 mg/kg bw/day)
Recovery group: 5 males/dose in the mating group and 3 females/dose in the non-mating groups (0 and 1000 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).
One male in the 1000 mg/kg bw/day group showed decreased body weight and food consumption, decreased fecal volume, decreased locomotor activity, and decreased respiratory rate, and died on Day 12. Autopsy revealed a small spleen and thymus, and dark red foci in the glandular stomach. In the surviving 1000 mg/kg bw/day group, increased liver weight, dark red foci in the glandular stomach, and increased urea nitrogen and total protein, increased kidney weight, and decreased spleen weight were observed in males and females. In the 500 mg/kg bw/day and 1000 mg/kg bw/day groups, 1 female each showed staggering gait, decreased muscle tone, and crawling gait only on Day 1.

- 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 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 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 (Gestation Days (GDs) 1, 7, 14 and 20 for mated females, Days 6 and 13 after mating for unmated females, and Lactation Day (LD) 4 for parturient females).

BODY WEIGHT: Yes
- Time schedule for examinations:
Males: Days 1, 8, 15, 22, 29, 36, 42 and 44 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 the day of necropsy.
Females in the non-mating group: Days 1, 8, 15, 22, 29, 36, 42 and 44 of administration and on the day of necropsy, and Days 1, 8 and 14 of recovery 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: Days 2, 8, 15, 30, 36, 42 and 44 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.
Females in the non-mating group: Days 2, 8, 15, 30, 36, 42 and 44 of administration, and Days 1, 8 and 14 of recovery

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: No

URINALYSIS: Yes
- Time schedule for collection of urine: On the final week of administration (Days 38 to 39 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, urine volume (20-hour volume), water intake (24-hour volume)

NEUROBEHAVIOURAL (FUNCTIONAL) EXAMINATION: Yes
- Time schedule for examinations:
Males: On the final week of administration (Day 37 of administration), and on the final week of recovery (Day 12 of recovery).
Females in the mating group: LD 4 (Day 41 to Day 43 of administration) after necropsy of F1 pups
Females in the non-mating group: On the final week of administration (Day 37 of administration), and on 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 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: 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 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, sp leen, submandibular lymph nodes, mesenteric lymph nodes, heart, thoracic aorta (*), trachea, lung (i ncluding bronchial), tongue (*), larynx (*), esophagus (*), stomach, duodenum, jejunum, ileum (inclu ding payer's patch), cecum, colon, rectum, submandibular gland, sublingual gland, liver, pancreas, kidney, bladder, testis, epididymis, ovary, uterus, vagina, prostate, seminal vesicles (including coa gulating 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 site (Animal No. 4107, oviduct) (*).]
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).
- Oil red O stain and electron microscope examination
Since vacuoles were observed in the liver and adrenal glands of females, the adrenal glands and livers of the following animals were examined by Oil Red O staining and electron microscopy to determine the details.
Adrenal: 2 females in the control group (Animal Nos. 1103 and 1116) and 3 females in the 1000 mg/kg bw/day group (Animal Nos. 4102, 4104 and 4106)
Liver: 2 females in the control group (Animal Nos. 1103 and 1116) and 3 females in the 1000 mg/kg bw/day group (Animal Nos. 4103, 4105 and 4114)

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, 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 implantation index, delivery index, live birth index, stillborn index, viability index and external abnormalities, Steel test was applied. Regarding 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 and 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)
no effects observed

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 7.5.1 Repeated dose toxicity. 001

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
effects observed, treatment-related

Description (incidence and severity)
Significant decreases in relative and absolute testis and epididymis weights were observed in males at 1000 mg/kg bw/day.
Atrophy of seminiferous tubular, multinucleated giant cell, vacuolation of seminiferous tubular of the testis, hypospermia, and cell debris in lumen of the epididymis were observed in males at 1000 mg/kg bw/day.

Reproductive performance
effects observed, treatment-related

Description (incidence and severity)
At 1000 mg/kg bw/day, decreased insemination index (2/11: 18%) and decreased fertility index (2/10: 20%) were observed, which were considered to be due to testicular toxicity.
All pups died on day 2 of lactation in 1 dam (Animal No. 3103, 4112) at 300 and 1000 mg/kg bw/day, respectively.
Histopathological examination of these dams revealed the following findings, suggesting that all pup deaths were associated with poor nursing due to poor general condition of the dams.
Animal No.3103: vacuolation of cortical cell in the adrenal, vacuolation of proximal tubular in the kidney, lesions considered secondary to deterioration of general condition (atrophy of acinar cells in the submandibular gland, atrophy of thymus)
Animal No. 4112: vacuolation of cortical cell in the adrenal, vacuolation of distal tuble/collecting duct in the kidney, atrophy of umbrella cell, single cell necrosis of transitional epithelial, hypertrophy/hyperplasia of transitional epithelial in the urinary bladder, lesions considered secondary to deterioration of general condition (atrophy of spleen, atrophy of thymus, atrophy of the colonic mucosa, atrophy of mucosal of colon, atrophy of acinar cells in the submandibular gland)

Effect levels (P0)

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/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

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

Based on
test mat.

Sex
male

Basis for effect level
reproductive function (sperm measures)
Significant decreases in relative and absolute testis and epididymis weights were observed in males at 1000 mg/kg bw/day.
Atrophy of seminiferous tubular, multinucleated giant cell, vacuolation of seminiferous tubular of the testis, hypospermia, and cell debris in lumen of the epididymis were observed in males at 1000 mg/kg bw/day.
reproductive performance
Decreased insemination index were observed at 1000 mg/kg bw/day.
Decreased fertility index were observed at 1000 mg/kg bw/day.

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
reproductive performance
Poor nursing was observed in dams at 300 mg/kg bw/day and above.

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Mortality / viability
mortality observed, treatment-related

Description (incidence and severity)
Decrease tendency of viability index on postnatal day 4 was observed at 300 mg/kg bw/day and above. This was because all pups died due to poor nursing in 1 dam at 300 and 1000 mg/kg bw/day, respectively.

Body weight and weight changes
no effects observed

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

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

Based on
test mat.

Sex
male/female

Basis for effect level
other:
No significant effect was observed at 300 mg/kg bw/day. The effects on pups could not be clearly defined at 1000 mg/kg bw/day because the fertility index was as low as 2/10(20%) and one of the dams showed poor nursing due to deteriorating conditions.

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/PDF8000-41-7d.pdf

Applicant's summary and conclusion

Conclusions
In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test described above, decreased insemination index and decreased fertility index were observed at 1000 mg/kg bw/day, possibly due to testicular toxicity, and poor nursing was observed in the dams at 300 mg/kg bw/day and above. As for effects on pups, the fertility index was as low as 2/10 (20%) in the 1000 mg/kg bw/day, and 1 of the dams had all infant deaths due to poor maternal condition, so no effects on pups could be confirmed at this dose. However, no effects on pups were observed in the 300 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of terpineols mixed isomers was regarded as 300 mg/kg bw/day for males, 100 mg/kg bw/day for females and 300 mg/kg bw/day for pups.

References

Reference Substances

REFERENCE_SUBSTANCE: Terpineol

UUID: ECB5-93f9fde6-aca7-4cb2-b29b-9048014c0dbd
Dossier UUID:
Author:
Date: 2007-05-10T18:00:00.000+09:00
Remarks:

Reference substance name
Terpineol

IUPAC name
2-(4-methylcyclohex-3-en-1-yl)propan-2-ol

Inventory

Inventory number

Inventory name
Terpineol

Inventory
EC Inventory

Inventory number
232-268-1

CAS number
8000-41-7

Molecular formula
C10H18O

Description

CAS number
8000-41-7

Synonyms

Synonyms

Identity
Terpineol

Identity
Terpineol

Molecular and structural information

Molecular formula
C10H18O

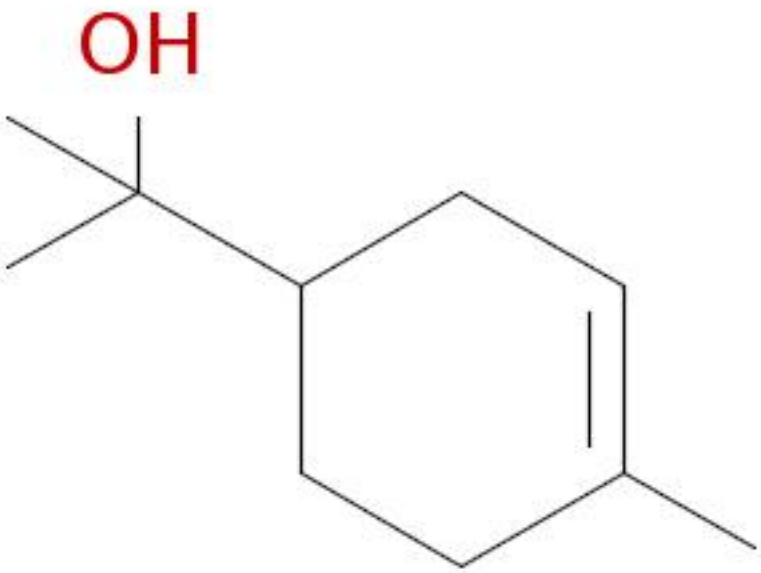
Molecular weight

154.2493

SMILES notation
CC1=CCC(CC1)C(C)(C)O

InChI
InChI=1/C10H18O/c1-8-4-6-9(7-5-8)10(2,3)11/h4,9,11H,5-7H2,1-3H3

Structural formula



Related substances

Group / category information
DSL Category: Organics

Test Materials

TEST_MATERIAL_INFORMATION: Terpineol

UUID: d156fb54-33c0-4c55-9804-bab98ce18177
Dossier UUID:
Author:
Date: 2023-01-13T11:03:36.000+09:00
Remarks:

Name
Terpineol

Composition

Composition

Type
Constituent

Reference substance
Terpineol / 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol / 8000-41-7 / 232-268-1

EC number	EC name
232-268-1	EC Inventory
CAS number	**CAS name**
8000-41-7	

IUPAC name
2-(4-methylcyclohex-3-en-1-yl)propan-2-ol

Concentration

92.1 % (v/v)

Remarks
α-Terpineol: 62.8%; β-Terpineol: 8.7% ; γ-Terpineol: 20.6%

Literatures

LITERATURE: Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of terpineol by oral administration in rats

UUID: 7b2aa043-eddc-4102-8476-62bde9edf463
Dossier UUID:
Author:
Date: 2023-02-27T09:29:22.000+09:00
Remarks:

General information

Reference Type
study report

Title
Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of terpineol 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/PDF8000-41-7d.pdf

Testing facility
Bozo Research Center

Report date
2013-09-10

Report number
R-1104

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

UUID: 6a93fca3-bf96-4fa5-9b10-2fb4e7d5d6ce
Dossier UUID:
Author:
Date: 2023-01-12T11:57:17.000+09:00
Remarks:

General information

Reference Type
study report

Title
In Vitro Chromosomal Aberration Test of terpineol 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/PDF8000-41-7f.pdf

Testing facility
Bozo Research Center Inc.

Report date
2013-03-22

Report number
T-G059

LITERATURE: Reverse Mutation Test of terpineol on Bacteria.

UUID: c1e98b4e-b395-41bf-a496-6223253843ee
Dossier UUID:
Author:
Date: 2023-02-27T09:30:56.000+09:00
Remarks:

General information

Reference Type
study report

Title
Reverse Mutation Test of terpineol 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/PDF8000-41-7e.pdf

Testing facility
Bozo Research Center Inc.

Report date
2013-03-22

Report number
T-1111