Name: OECD_SIDS / SUBSTANCE : cis-3-Hexenyl salicylate / hex-3-en-1-yl salicylate / 65405-77-8 Wed, 26 Nov 2025, 09:58:20+0900 /

Printing date: 2025-11-26T09:58:21.141+09:00

Table of Contents

DOSSIER:

UUID: 0
Dossier UUID:
Author:
Date: 2025-11-26T09:58:20.808+09:00
Remarks:

Dossier header

Dossier submission type

Name
OECD SIDS

Version
core 9.0

Name (given by user)

Dossier subject

Dossier subject
cis-3-Hexenyl salicylate / hex-3-en-1-yl salicylate / 65405-77-8

Public name

Submitting legal entity
National Institute of Health Sciences

Dossier creation date/time
Wed, 26 Nov 2025, 09:58:20+0900

Used in category

LEGAL_ENTITY: National Institute of Health Sciences

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

General information

Legal entity name
National Institute of Health Sciences

cis-3-Hexenyl salicylate

General information

Identification

SUBSTANCE: cis-3-Hexenyl salicylate

UUID: 0e68d149-c43c-42a5-bea4-1d43058286f4
Dossier UUID:
Author:
Date: 2025-02-26T15:36:34.655+09:00
Remarks:

Substance name
cis-3-Hexenyl salicylate

Identification of substance

Reference substance
(Z)-3-hexenyl salicylate / hex-3-en-1-yl salicylate / 65405-77-8 / 265-745-8

EC number	EC name
265-745-8	EC Inventory
CAS number	**CAS name**
65405-77-8	
IUPAC name	
hex-3-en-1-yl salicylate	

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:	d2680296-ca68-4ba3-89de-5ab1e57309e2
Dossier UUID:	
Author:	
Date:	2024-02-20T15:50:16.000+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

Study period: start date
2012-09-28

End date
2013-03-28

Reliability
1 (reliable without restriction)

Cross-reference

Reason / purpose for cross-reference
reference to same study

Related information
OECD / Toxicity to reproduction / Toxicity to reproduction. 001. / cis-3-Hexenyl salicylate / hex-3-en-1-yl salicylate / 65405-77-8

Data source

Reference

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

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
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
cis-3-Hexenyl salicylate

Specific details on test material used for the study
- Name of test material (as cited in study report): cis-3-Hexenyl salicylate
- Analytical purity: 98.86%
- Storage condition of test material: sealed, cool dark 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: 402 g (366-438 g), Female: 226 g (205-251 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 × 400D × 185H mm) and bedding.
- Diet: Solid feed (NMF: Oriental Yeast Co., ltd.) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation period: 19 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23±3 (actual temperature: 21-23°C)
- Humidity (%): 50±20% (actual humidity: 52-60%)

- 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 98.4 to 108.3% 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: 42 days including 14 days pre-mating
Females (mating group): 41-49 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.	
40	mg/kg bw/day (actual dose received)
Dose / conc.	
120	mg/kg bw/day (actual dose received)
Dose / conc.	
360	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex/dose (0, 40, 120 and 360 mg/kg bw/day)
Non-mating group: 10 females/dose (0 and 360 mg/kg bw/day)
Recovery group: 5 males/dose in the mating group and 5 females/dose in the non-mating groups (0 and 360 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 360 mg/kg, which is the intermediate dose between the intermediate and low doses of the preliminary st udy, and the intermediate and low doses were divided by a common ratio of 3, to 120 and 40 mg/kg respectively.

[14-day preliminary study]
A 14-day repeated dose oral toxicity test in Crl: CD(SD) rats, doses: 0, 250, 500, and 1000 mg/kg bw/ day). In the 1000 mg/kg bw/day group, all males and females died from 3 to 6 days of treatment. Clinical signs of the animals included decreased motor activity, decreased feces, pale auricle and limbs, decreased respiration rate and decreased body weight with low food consumption. Necropsy revealed small thymus and spleen, dark red focus in the glandular stomach or the forestomach, eleva ted lesion of the forestomach, dark red contents in the small intestine, white focus in the epididymis, and pale auricle and limbs. In the 500 mg/kg dose group, one female showed decreased motor activ ity, decreased feces, and pale pinna and limbs on day 7 and died on day 8. Necropsy revealed dark re d contents in the small intestine, and pale pinna and limbs. In surviving cases, transient weight loss or suppression of body weight gain with low food consumption was observed in the early phase of t reatment, but there were no abnormalities of the clinical symptoms. Increased reticulocyte percentag e, AST and liver weight in males and females, increased ALT, and decreased glucose and the thymus weight in males, and increased the heart weight in females were observed. Necropsy revealed dark red focus in the lung in males. In the 250 mg/kg dose group, transient low food consumption in males on day 4, increased AST in males and females, and dark red focus in the lung in males were observed.

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

Examinations

Observations and examinations performed and frequency
CAGE SIDE OBSERVATIONS: Yes
- Time schedule: 3 times/day (before administration, immediately after administration and 1-3 hours after administration) during the administration period. 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 admi nistration, 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, Day 6 after the start of 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 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 and 15 of administration (and Day 22 for unmated females), 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 and 42 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 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.

Females in the non-mating group: Days 2, 8, 15, 30, 36 and 42 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.
* Neutrophil, eosinophil, basophil, lymphocyte, monocyte and large unstained cells.

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 10 to 11 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: On the final week of administration (Day 37 of administration), and on the final week of recovery (Day 9 of recovery).
Females in the mating group: LD 4 (Day 41 to Day 45 of administration)
Females in the non-mating group: On the final week of administration (Day 37 of administration), and on the final week of recovery (Day 9 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 (including bone marrows)*, femur (including bone marrows), femoral skeletal muscle, and Individual identification site (pinna with ear tag)*]
Asterisked organs and tissues are fixed and stored only.

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)
In dead animals, soiled fur and pale skin were observed in one mating female at 360 mg/kg bw/day.

Mortality
mortality observed, treatment-related

Description (incidence)
In mating females, at the 360 mg/kg bw/day group, two females died on day 23 of gestation and one female died on day 1 of lactation.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
[At the dosing period]:
In mating females, significantly decreased body weight on day 20 of gestation and body weight gain during gestation and lactation period were observed at 360 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)
effects observed, treatment-related

Description (incidence and severity)
[At the dosing period]:
In mating females, food consumption was significantly decreased on lactation day 4 at 360 mg/kg bw/day.

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

Food efficiency
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 red blood cell count, significant increases in mean corpuscular volume, mean corpuscular hemoglobin and reticulocyte percentage, a significant prolongation of activated partial thromboplastin time and trend towards prolonged prothrombin time were observed at 360 mg/kg bw/day.
In mating females, a significant decrease in platelet count was observed at 360 mg/kg bw/day.

[At the end of recovery period]:
In males, a significant decrease in red blood cell count, significant increases in mean corpuscular volume and mean corpuscular hemoglobin were observed at 360 mg/kg bw/day.

Clinical biochemistry findings
effects observed, treatment-related

Description (incidence and severity)
[At the end of dosing period]:
In males, significant increases in AST, total bile acid, phospholipids, creatinine, inorganic phosphorus, albumin and A/G ratio were observed at 360 mg/kg bw/day.
In mating females, significant increases in creatinine and A/G ratio were observed at 360 mg/kg bw/day.
In non-mating females, significant increases in AST, ALT, triglyceride, inorganic phosphorus and A/G ratio, and significant decreases in glucose, potassium and chloride were observed at 360 mg/kg bw/day.

[At the end of recovery period]:
In males, a significant increase in creatinine was observed at 360 mg/kg bw/day.

Endocrine findings
not examined

Urinalysis findings
effects observed, treatment-related

Description (incidence and severity)
[At the dosing period]:
In males, significant increases in water intake and urine volume, and a significant decrease in osmotic pressure were observed at 360 mg/kg bw/day.

[At the recovery period]:

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

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 mating females, a significant decrease in absolute and relative weight of the pituitary was observed at 360 mg/kg bw/day.
In non-mating females, a significant increase in absolute and relative weight of the liver was observed at 360 mg/kg bw/day.

[At the end of recovery period]:
In non-mating females, a significant increase in absolute and relative weight of the adrenal gland was observed at 360 mg/kg bw/day.

Gross pathological findings
effects observed, treatment-related

Description (incidence and severity)
[Dead animals]:
Pale discoloration of skin, hemorrhagic smudge fur from perineal to abdominal area, dark red focus in the glandular stomach, dark red discoloration of the uterus, pale discoloration of the kidney were obs erved at 360 mg/kg bw/day in mating females.

[At the end of dosing period]:
Dark red focus in the glandular stomach was observed at 360 mg/kg bw/day in males, mating fe males and non-mating females.
[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)
[Dead animals]:
Kidney: acute tubular necrosis was observed at 360 mg/kg bw/day in mating females.

Liver: centrilobular necrosis or vacuolation of hepatocyte were observed at 360 mg/kg bw/day in mati ng females.

Femur: increased trabecular bone was observed at 360 mg/kg bw/day in mating females.

Glandular stomach: erosion was observed at 360 mg/kg bw/day in mating females.

[At the end of dosing period]:
Femur:
Increased trabecular bone was observed at 360 mg/kg bw/day in males, mating females and non-mating females.

Glandular stomach:
Erosion was observed at 360 mg/kg bw/day in males, mating females and non-mating females.

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

Histopathological findings: neoplastic
not examined

Effect levels

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male

Basis for effect level
histopathology: non-neoplastic
Increased trabecular bone in femur and erosion in glandular stomach at 360 mg/kg bw/day.

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
histopathology: non-neoplastic
Increased trabecular bone in femur and erosion in glandular stomach in mating/non-mating females at 360 mg/kg bw/day.
mortality
Three mating females died on gestation and lactation period at the 360 mg/kg bw/day group.
organ weights and organ / body weight ratios
Significant increases in absolute and relative weights of liver were observed in non-mating females at 360 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/PDF65405-77-8d.pdf

Applicant's summary and conclusion

Conclusions
The NOAEL for repeated dose toxicity in this study was determined to be 120 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 cis-3-hexenyl salicylate by gavage at 0 (vehicle: corn oil), 40, 120, and 360 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 41–49 days, including the 14-day premating, mating, and gestation periods, and until lactation day 4. Five males at the 0 and 360 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 360 mg/kg bw/day as a satellite group. These females were administered for 42 days without mating, and five females at 0 and 360 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period.

At 360 mg/kg bw/day, 3 females in the mating group died.

In the body weights, a significant decrease in body weight and body weight gain during gestation and lactation were observed in mating females at 360 mg/kg bw/day.

In the food consumptions, significant decrease during lactation was observed in mating females at 360 mg/kg bw/day.

In the urinalysis, significant increases in water intake and urine volume, and a significant decrease in osmotic pressure were observed in males at 360 mg/kg bw/day.

In the haematology results, a significant decrease in red blood cell count, significant increases in mean corpuscular volume, mean corpuscular hemoglobin and reticulocyte percentage, a significant prolongation of activated partial thromboplastin time and trend towards prolonged prothrombin time were observed in males, and a significant decrease in platelet count was observed in mating females at 360 mg/kg bw/day.

In the clinical chemistry results, significant increases in AST, total bile acid, phospholipids, creatinine, inorganic phosphorus, albumin and A/G ratio were observed in males, significant increases in creatinine and A/G ratio were observed in mating females, and significant increases in AST, ALT, triglyceride, inorganic phosphorus and A/G ratio, and significant decreases in glucose, potassium and chloride were observed in non-mating females at 360 mg/kg bw/day.

In the organ weights, significant decreases in absolute and relative weights of the pituitary were observed in mating females, and significant increases in absolute and relative weights of the liver were observed in non-mating females at 360 mg/kg bw/day.

In the gross pathology, dark red focus in the glandular stomach was observed in males, mating females and non-mating females at 360 mg/kg bw/day.

In the histopathological examination, increased trabecular bone in the femur and erosion in the glandular stomach were observed in males, mating females and non-mating females at 360 mg/kg bw/day.

In the recovery study, a significant decrease in red blood cell count, significant increases in mean corpuscular volume and mean corpuscular hemoglobin were observed in males at 360 mg/kg bw/day.

Significant increases in absolute and relative weights of the adrenal gland were observed in non-mating females at 360 mg/kg bw/day.

Genetic toxicity

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

UUID:	e9cedb3e-28b0-4689-9716-b10b5d984a56
Dossier UUID:	
Author:	
Date:	2024-02-19T10:22:04.000+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

Study period: start date
2012-10-12

End date
2013-03-22

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

Reverse mutation test of cis-3-hexenyl salicylate / Ministry of Health, Labor 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 (incl. QA statement)

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

Test material

Test material information
cis-3-Hexenyl salicylate

Specific details on test material used for the study
- Name of test material (as cited in study report): cis-3-Hexenyl salicylate
- Analytical purity: 98.86%
- Storage condition of test material: sealed, cool dark place (actual temperature: 2.9-5.7°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

Test concentrations
Main test 1/2
- TA: 0.31, 0.61, 1.22, 2.44, 4.88, 9.77, 19.5 µg/plate
- WP2uvrA: 313, 625, 1250, 2500, 5000 µg/plate

High dose level used
no

Justification for deviation from the high dose level
Maximum concentration was established based on the result of the range-finding study at co ncentration up to 5000 ug/plate. In this study, growth inhibition was observed for all TA strains at 19.5 µg/plate and above, and not observed for E. coli WP2 uvrA at 5000 µg/plate and below.

Vehicle / solvent
DMSO

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
other:

Remarks
-S9 mix: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) (TA100, WP2uvrA, TA98), Sodium azide (SAZ) (TA1535) and 9-Aminoacridine (9 AA) (TA1537)
+S9 mix: 2-Aminoanthracene (2AA) (TA1535, WP2uvrA), Benzo[a]pyrene (B[a]P) (TA100, TA98, TA1537)

Details on test system and experimental conditions
METHOD OF APPLICATION: Preincubation
DURATION- Preincubation period: 20 min at 37°C
- Exposure duration: Main test 1: 49.5 hrs / Main test 2 : 48hrs
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 100
bacteria

Metabolic activation
with and without

Genotoxicity
negative

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

Vehicle controls validity
valid

Positive controls validity
valid

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: 9.77 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: 9.77 µg/plate and above
+S9 mix: 19.5 µg/plate

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
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

Additional information on results
RANGE-FINDING/SCREENING STUDIES (if applicable):

Concentration: 19.5, 78.1, 313, 1250, 5000 ug/plate with and without S9mix
Growth inhibitions: Growth inhibition was observed in all TA strains at 19.5 µg/plate and above.
Precipitation: No test substance-related precipitation was observed at any concentration with or wi thout metabolic activation.
Genotoxicity: No increase in the number of revertant colonies was observed in any strain with or witho ut metabolic activation.

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/PDF65405-77-8e.pdf

Please also see the attached files (Tables in English)

Overall remarks, attachments

Attachments

Attached (sanitised) documents for publication
R5_65405-77-8_Ames Tables.xlsx / 46.104 KB (application/vnd.openxmlformats-officedocument.spreadsheetml.sheet)

Applicant's summary and conclusion

Conclusions
In a bacterial reverse mutation assay with Salmonella typhimurium (S. typhimurium) TA100, TA98, TA1535, and TA1537 and Escherichia coli (E. coli) WP2 uvrA in accordance with OECD TG 471, cis-3-hexenyl salicylate was negative with and without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

UUID:	df6d3a78-1eff-4ef5-a014-9ec14bf2b70b
Dossier UUID:	
Author:	
Date:	2024-02-19T10:18:47.000+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

Study period: start date
2012-10-18

End date
2013-03-22

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

Chromosome aberration test for cis-3-hexenyl salicylate / Ministry of Health and Labor 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 (before 26 September 2014)

Deviations
no

Qualifier
according to guideline

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

Deviations
no

GLP compliance
yes (incl. QA statement)

Type of assay
in vitro mammalian chromosome aberration test
in vitro cytogenicity / chromosome aberration study in mammalian cells

Test material

Test material information
cis-3-Hexenyl salicylate

Specific details on test material used for the study
- Name of test material (as cited in study report): cis-3-Hexenyl salicylate
- Analytical purity: 98.86%
- Storage condition of test material: sealed, cool dark place (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
Chinese hamster lung (CHL/IU)
mammalian cell line

Metabolic activation
with and without

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

Test concentrations
Cell growth inhibition study
-S9 mix (short-term treatment): 18.0, 35.9, 71.9, 144, 288, 575, 1150, 2300 ug/mL
+S9 mix (short-term treatment): 18.0, 35.9, 71.9, 144, 288, 575, 1150, 2300 ug/mL
-S9 mix (continuous treatment, 24hr): 18.0, 35.9, 71.9, 144, 288, 575, 1150, 2300 ug/mL

-S9 mix (continuous treatment, 48hr): 18.0, 35.9, 71.9, 144, 288, 575, 1150, 2300 ug/mL

Main study
-S9 mix (short-term treatment): 57.9, 69.4, 83.3, 100, 120 ug/mL
+S9 mix (short-term treatment): 42.7, 64.0, 96.0, 144 ug/mL
-S9 mix (continuous treatment, 24hr): 21.3, 32.0, 48.0, 72.0 ug/mL
-S9 mix (continuous treatment, 48hr): 21.3, 32.0, 48.0, 72.0 ug/mL

High dose level used
no

Justification for deviation from the high dose level
Chromosomal aberration test was carried out at several different doses of test substance selected fr
om the result of cell growth inhibition study.
Cell-growth inhibition study was conducted up to the limited concentration of 2300 µg/mL (10 mM)
In this study, precipitation and more than 50% cell growth inhibition were observed.
(See Additional information on results)

Vehicle / solvent
DMSO

Controls

Negative solvent / vehicle controls
yes

Positive controls
yes

Positive control substance
cyclophosphamide
with metabolic activation
mitomycin C
without metabolic activation

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):
Cell-growth inhibition study was conducted up to the limited concentration of 2300 µg/mL (10 mM)
In this study, precipitation and more than 50% cell growth inhibition were observed.
- Precipitation: above 144 µg/mL (all treatments)
- More than 50% cell growth inhibition:
Short term treatment: above 144 µg/mL
Continuous treatment: above 71.9 µg/mL
- 50% cell-growth inhibition:
Short term treatment (-S9 mix): 97 µg/mL
Short term treatment (+S9 mix): 125 µg/mL
Continuous treatment (24 h): 62 µg/mL
Continuous treatment (48 h): 60 µg/mL

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/PDF65405-77-8.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), cis-3-hexenyl salicylate was negative with or without metabolic activation.

Toxicity to reproduction

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction. 001.

UUID:	adbdf2cb-c3ab-444c-ba31-a0547a123a17
Dossier UUID:	
Author:	
Date:	2025-02-26T15:36:34.655+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

Study period: start date
2012-09-28

End date
2013-03-28

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. / cis-3-Hexenyl salicylate / hex-3-en-1-yl salicylate / 65405-77-8

Data source

Reference

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

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
other:

Version / remarks
Guideline for Combined Repeated Dose Study with the Reproduction /Developmental Toxicity S creening Test in Mammalian Species (Chemical Substances Control Law of Japan)

GLP compliance
yes

Limit test
no

Test material

Test material information
cis-3-Hexenyl salicylate

Specific details on test material used for the study
- Name of test material (as cited in study report): cis-3-Hexenyl salicylate
- Analytical purity: 98.86%
- Storage condition of test material: sealed, cool dark 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: 402 g (366-438 g), Female: 226 g (205-251 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 × 400D × 185H mm) and bedding.
- Diet: Solid feed (NMF: Oriental Yeast Co., ltd.) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation period: 19 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23±3 (actual temperature: 21-23°C)

- Humidity (%): 50±20% (actual humidity: 52-60%)
- 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 14 days
- Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0 of pregnancy

Analytical verification of doses or concentrations
yes

Details on analytical verification of doses or concentrations
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 98.4 to 108.3% 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: 42 days including 14 days pre-mating
Females (mating group): 41-49 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.	
40	mg/kg bw/day (actual dose received)
Dose / conc.	
120	mg/kg bw/day (actual dose received)
Dose / conc.	
360	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Mating group: 12 animals/sex/dose (0, 40, 120 and 360 mg/kg bw/day)
Non-mating group: 10 females/dose (0 and 360 mg/kg bw/day)
Recovery group: 5 males/dose in the mating group and 5 females/dose in the non-mating groups (0 and 360 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 360 mg/kg, which is the intermediate dose between the intermediate and low doses of the preliminary st udy, and the intermediate and low doses were divided by a common ratio of 3, to 120 and 40 mg/kg respectively.

[14-day preliminary study]
A 14-day repeated dose oral toxicity test in Crl: CD(SD) rats, doses: 0, 250, 500, and 1000 mg/kg bw/ day). In the 1000 mg/kg bw/day group, all males and females died from 3 to 6 days of treatment. Clinical signs of the animals included decreased motor activity, decreased feces, pale auricle and limbs, decreased respiration rate and decreased body weight with low food consumption. Necropsy revealed small thymus and spleen, dark red focus in the glandular stomach or the forestomach, eleva ted lesion of the forestomach, dark red contents in the small intestine, white focus in the epididymis, and pale auricle and limbs. In the 500 mg/kg dose group, one female showed decreased motor activ ity, decreased feces, and pale pinna and limbs on day 7 and died on day 8. Necropsy revealed dark re d contents in the small intestine, and pale pinna and limbs. In surviving cases, transient weight loss or suppression of body weight gain with low food consumption was observed in the early phase of t reatment, but there were no abnormalities of the clinical symptoms. Increased reticulocyte percentag e, AST and liver weight in males and females, increased ALT, and decreased glucose and the thymus weight in males, and increased the heart weight in females were observed. Necropsy revealed dark red focus in the lung in males. In the 250 mg/kg dose group, transient low food consumption in males on day 4, increased AST in males and females, and dark red focus in the lung in males were observed.

- 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 admi nistration, 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, Day 6 after the start of 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 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 and 15 of administration (and Day 22 for unmated females), 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 and 42 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 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.
Females in the non-mating group: Days 2, 8, 15, 30, 36 and 42 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.
* Neutrophil, eosinophil, basophil, lymphocyte, monocyte and large unstained cells.

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 10 to 11 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: On the final week of administration (Day 37 of administration), and on the final week of recovery (Day 9 of recovery).
Females in the mating group: LD 4 (Day 41 to Day 45 of administration)
Females in the non-mating group: On the final week of administration (Day 37 of administration), and on the final week of recovery (Day 9 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, pu pillary 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.

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 (including bone marrows)*, femur (inclu ding bone marrows), femoral skeletal muscle, and Individual identification site (pinna with ear tag)*]
Asterisked organs and tissues are fixed and stored only.

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 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, 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
not examined

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
effects observed, treatment-related

Description (incidence and severity)
At 360 mg/kg bw/day, trend towards decreases in gestation index and delivery index, increased gestation length, decreased number of implantation sites, trend towards an increased number of stillborns, decreased number of liveborns, vestigial tail, complete spinal rachischisis, and exencephaly were observed. Poor suckling was observed in one dam during the lactation period, and in three dams all nursing infants died.

Details on results (P0)

See 7.5.1 Repeated dose toxicity.001

Reproductive function / performance:
No effects were observed in the 360 mg/kg dose group of males.
Trend towards decreases in gestation index and delivery index, increased gestation length, decreased number of implantation sites, trend towards an increased number of stillborns, decreased number of liveborns, vestigial tail, complete spinal rachischisis, and exencephaly were observed in the 360 mg/kg dose group of mating females. Poor suckling was observed in one dam during the lactation period, and in three dams all nursing infants died. The poor suckling was thought to be due to the stunted growth of the infants.

Effect levels (P0)

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male

Basis for effect level
histopathology: non-neoplastic
Increased trabecular bone in the femur and erosion in the glandular stomach at 360 mg/kg bw/day.

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
mortality
Three mating females died on gestation and lactation period at the 360 mg/kg bw/day group.
organ weights and organ / body weight ratios
Significant increases in absolute and relative weights of the liver were observed in non-mating females at 360 mg/kg bw/day.
histopathology: non-neoplastic
Increased trabecular bone in the femur and erosion in the glandular stomach in mating/non-mating females at 360 mg/kg bw/day.

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male

Basis for effect level
reproductive performance
No effects observed.

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
female

Basis for effect level
reproductive performance
A trend towards decreases in gestation index and delivery index, increased gestation length, decreased number of implantation sites, trend towards an increased number of stillborns, decreased number of liveborns, vestigial tail, complete spinal rachischisis, and exencephaly were observed in females at 360 mg/kg bw/day. In addition, at this dose, poor suckling was observed in one dam during the lactation period, and in three dams all nursing infants died. The poor suckling was thought to be due to the stunted growth of the infants.

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Mortality / viability
mortality observed, treatment-related

Description (incidence and severity)
A trend towards a decrease in the live birth index and decreased viability index on postnatal day 4 were observed at 360 mg/kg bw/day.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
Decreases in female and male body weight at 0 and 4 days of age and weight gains during this period were observed at 360 mg/kg bw/day.

Gross pathological findings
no effects observed

Details on results (F1)

In the offspring, death of all nursing infants in three dams, a trend towards decreased live birth index, decreased viability index on postnatal day 4, decreases in female and male body weights at 0 and 4 days of age, and weight gains during this period were observed at 360 mg/kg bw/day.

Effect levels (F1)

Key result
true

Dose descriptor
NOAEL

Generation
F1

Effect level

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

Based on
test mat.

Sex
not specified

Basis for effect level
mortality
In the 360 mg/kg bw/day, death of all nursing infants in three dams, a trend towards decreased live birth index and increased number of stillborns, and decreased viability index on postnatal day 4 and number of liveborns were observed.
body weight and weight gain
In the 360 mg/kg bw/day, decreases in female and male body weights at 0 and 4 days of age, and weight gains during this period were observed.
other:
In the 360 mg/kg bw/day, a vestigial tail was seen in one birth, complete spinal rachischisis were s een in six births in three dams, and an exencephaly was seen in one fetus from a dam that died durin g delivery at 23 days of gestation.

Overall reproductive toxicity

Key result
false

Reproductive effects observed
yes

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/PDF65405-77-8d.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, increased gestation length, and decreased gestation index and delivery in dex, and intrauterine viability of the offspring were observed at 360 mg/kg bw/day. With regard to e ffects on pups, external abnormalities, and decreased development and viability were observed at the 360 mg/kg bw/day.
The NOAELs for the rat reproductive/developmental toxicity of cis-3-hexenyl salicylate in rats were r egarded as 360 mg/kg bw/day for males, and 120 mg/kg bw/day for females and pups.

References

Reference Substances

REFERENCE_SUBSTANCE: (Z)-3-hexenyl salicylate

UUID: ECB5-e9ff20fd-3df4-4f0d-b3a0-1e7a59db64d6
Dossier UUID:
Author:
Date: 2023-08-01T13:42:02.000+09:00
Remarks:

Reference substance name
(Z)-3-hexenyl salicylate

IUPAC name
hex-3-en-1-yl salicylate

Inventory

Inventory number

Inventory name
(Z)-3-hexenyl salicylate

Inventory
EC Inventory

Inventory number
265-745-8

CAS number
65405-77-8

Molecular formula
C13H16O3

Description

CAS number
65405-77-8

Synonyms

Synonyms

Identity
Benzoic acid, 2-hydroxy-, 3-hexenyl ester, (Z)-

Identity
Benzoic acid, 2-hydroxy-, (3Z)-3-hexenyl ester

Molecular and structural information

Molecular formula
C13H16O3

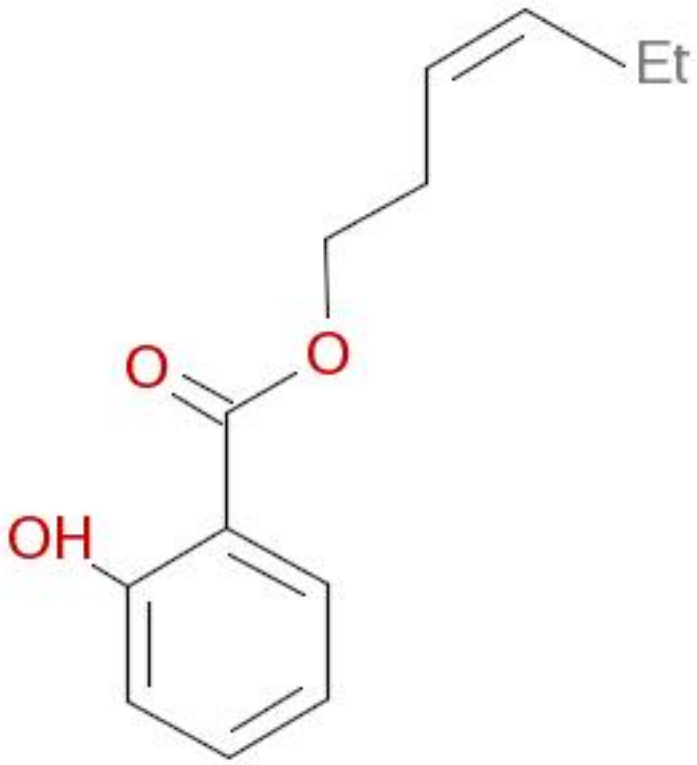
Molecular weight

220.2643

SMILES notation
CC\C=C/CCOC(=O)c1ccccc1O

InChI
InChI=1/C13H16O3/c1-2-3-4-7-10-16-13(15)11-8-5-6-9-12(11)14/h3-6,8-9,14H,2,7,10H2,1H3

Structural formula



Related substances

Group / category information
DSL Category: Organics

Test Materials

TEST_MATERIAL_INFORMATION: cis-3-Hexenyl salicylate

UUID: a0a5270d-5826-4c98-8e08-c12d92198bc1
Dossier UUID:
Author:
Date: 2023-08-03T15:41:02.000+09:00
Remarks:

Name
cis-3-Hexenyl salicylate

Composition

Composition

Type
Constituent

Reference substance
(Z)-3-hexenyl salicylate / hex-3-en-1-yl salicylate / 65405-77-8 / 265-745-8

EC number	EC name
265-745-8	EC Inventory
CAS number	**CAS name**
65405-77-8	
IUPAC name	
hex-3-en-1-yl salicylate	

Composition / purity: other information
other: 98.86%

Other characteristics

Test material form
liquid

Literatures

LITERATURE: Chromosome aberration test for cis-3-hexenyl salicylate

UUID: 991174fe-e76a-4b38-bce2-3b6f9e8ccb72
Dossier UUID:
Author:
Date: 2024-02-09T10:46:35.000+09:00
Remarks:

General information

Reference Type
study report

Title
Chromosome aberration test for cis-3-hexenyl salicylate

Author
Ministry of Health and Labor Welfare (MHLW) Japan

Year
2013

Bibliographic source
Japan Existing Chemical Database(JECDB)
https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF65405-77-8f.pdf

Testing facility
Bozo Research Center Inc.

Report date
2013-03-22

Report number
T-G056

LITERATURE: Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of cis-3-Hexenyl salicylate by oral administration in rats

UUID: be48249e-8287-459d-a226-d116ad6bd234
Dossier UUID:
Author:
Date: 2024-02-14T17:04:10.000+09:00
Remarks:

General information

Reference Type
study report

Title
Combined repeated dose toxicity study with the reproductive/developmental toxicity screening test of cis-3-Hexenyl salicylate by oral administration in rats

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/PDF65405-77-8d.pdf

Testing facility
BoZo Research Center Inc.

Report date
2013-05-22

Study number
R-1101

LITERATURE: Reverse mutation test of cis-3-hexenyl salicylate

UUID:	9633ec92-9c67-4f46-b7db-6fa844dc42dc
Dossier UUID:	
Author:	
Date:	2024-02-14T16:54:18.000+09:00
Remarks:	

General information

Reference Type
study report

Title
Reverse mutation test of cis-3-hexenyl salicylate

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

Year
2013

Bibliographic source
Japan Existing Chemical Database(JECDB)
https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF65405-77-8e.pdf

Testing facility
Bozo Research Center Inc.

Report date
2013-03-22

Report number
T-1108