Name: OECD_SIDS / SUBSTANCE : 1,3,5-Tri-tert-butylbenzene / 1,3,5-tri-tert-butylbenzene / 1460-02-2 Fri, 16 Dec 2022, 13:58:47+0900 /

Legal entity owner: National Institute of Health Sciences

Printing date: 2022-12-16T13:58:47.156+09:00

Table of Contents

DOSSIER:

UUID: 0
Dossier UUID:
Author:
Date: 2022-12-16T13:58:47.006+09:00
Remarks:

Dossier header

Dossier submission type

Name
OECD SIDS

Version
core 7.0

Name (given by user)

Dossier subject

Dossier subject
1,3,5-Tri-tert-butylbenzene / 1,3,5-tri-tert-butylbenzene / 1460-02-2

Public name

Submitting legal entity
National Institute of Health Science

Dossier creation date/time
Fri, 16 Dec 2022, 13:58:47+0900

Used in category

LEGAL_ENTITY: National Institute of Health Science

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

General information

Legal entity name
National Institute of Health Science

1,3,5-Tri-tert-butylbenzene

General information

Identification

Identification

SUBSTANCE: 1,3,5-Tri-tert-butylbenzene

UUID:	4d00a394-87b4-4ab3-a17c-3627cccd5527
Dossier UUID:	
Author:	
Date:	2022-12-16T13:58:34.349+09:00
Remarks:	

Substance name
1,3,5-Tri-tert-butylbenzene

Legal entity
National Institute of Health Sciences / Kawasaki / Japan

Identification of substance

Reference substance
1,3,5-tri-tert-butylbenzene / 1,3,5-tri-tert-butylbenzene / 1460-02-2 / 215-952-4

EC number	**EC name**
215-952-4	EC Inventory
CAS number	**CAS name**
1460-02-2	
IUPAC name	
1,3,5-tri-tert-butylbenzene	

Role in the supply chain

Manufacturer
false

Importer
false

Only representative
false

Downstream user
false

Assessment approach (assessment entities)

FIXED_RECORD: Assessment approach

UUID: c225b03e-6e1a-3ed0-980a-59682bfcf4ec
Dossier UUID:
Author:
Date: 2019-03-27T10:03:47.000+09:00
Remarks:

Toxicological information

Repeated dose toxicity

Repeated dose toxicity: oral

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.001

UUID:	42ddd009-b975-4656-a208-9af4ba3f8d3f
Dossier UUID:	
Author:	
Date:	2022-12-16T13:56:39.295+09:00
Remarks:	

Administrative data

Endpoint
repeated dose toxicity: oral, other

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 / 1,3,5-Tri-tert-butylbenzene / 1,3,5-tri-tert-butylbenzene / 1460-02-2

Data source

Reference

A combined repeated dose/reproductive developmental toxicity study of 1, 3, 5-Tri-tert-butylbenzene / Ministry of Health, Labor and Welfare, Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)

Deviations
no

GLP compliance
yes

Limit test
no

Test material

Specific details on test material used for the study
- Name of test material (as cited in study report): 1, 3, 5-Tri-tert-butylbenzene
- Analytical purity: 98%
- Storage condition of test material: at a cold (temperature 2-6°C) and dark place, with airtight stopper.
- 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 374 g (335-414 g), female 229 g (205-255 g)
- Housing: Animals were individually housed in bracket-type metallic wire-mesh cages (265W × 426D × 200H mm), Dams were bred individually or with individual littermates in polycarbonate cages with flat floors (265W x 426D x 200H mm) and standard bedding.
- Diet: Solid feed (MR stock: Nosan Corporation) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation period: 12 days
ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22±3 (actual temperature: 22.4-24.4°C)
- Humidity (%): 55±10% (actual humidity: 45-56%)
- Air changes (per hr): >10
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 7:00~19:00)

Administration / exposure

Route of administration
oral: gavage

Vehicle
olive 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

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating
(P)Females: 42-52 days including 14 days pre-mating, mating and gestation periods and the days until day 4 of lactation
Female (no mating, satellite group): 42 days

Frequency of treatment
Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
2	mg/kg bw/day (actual dose received)
Dose / conc.	
10	mg/kg bw/day (actual dose received)
Dose / conc.	
50	mg/kg bw/day (actual dose received)
Dose / conc.	
250	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Main group:12 animals/sex/dose

Satellite (Recovery) group: 5 males/dose and 5 females/dose (0 and 250 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 highest dose of 250 mg/kg bw/day was selected as an expected obvious toxic dose, and the lowest dose of 2 mg/kg bw/day was selected as an expected no toxic dose. The middle dose levels of 10 and 50 mg/kg bw/day were selected.
- Rationale for animal assignment (if not random): Body weight-balanced randomization

[14-day preliminary study]
A 14-day repeated dose oral toxicity test (Crl:CD(SD) rats, doses: 0 (olive oil), 10, 30, 100 or 300 mg/kg bw/day). High value of albumin was observed in males at 30 mg/kg bw/day or more. High value of A/G ratio was observed in male at 100 mg/kg bw/day or more, and high value of calcium was observed in females at 100 mg/kg bw/day or more. High value of total cholesterol was observed in males and females at 300 mg/kg/day. High value of ALT, ChE and inorganic phosphorus, low value of chlorine were observed in males at 300 mg/kg bw/day. High value of sodium was observed in females at 300 mg/kg bw/day. Large liver was observed in males at 30 mg/kg bw/day or more, and in females at 100 mg/kg bw/day or more. Increase in liver weight was observed in males at 100 mg/kg bw/day or more, and in females at 300 mg/kg bw/day. Thickening of the forestomachial mucosa was observed in females at 300 mg/kg bw/day.

Examinations

Observations and examinations performed and frequency
CAGE SIDE OBSERVATIONS: Yes
- Time schedule: 3 times/day (am: before and after administration; pm) during the administration period. 2 times/day (am and pm) during the recovery period.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: Once before the start of administration, and once every week by the end of the study period.

BODY WEIGHT: Yes
- Time schedule for examinations:
Males were weighed on Day 1, 7, 14, 21, 28, 35, and 42 of administration, and weighed on Day 7 and 14 of recovery.
Female satellite groups were weighted same frequencies to male recovery groups.
Females in the main groups were weighed on Day 1, 7 and 14 of administration and copulated females were weighed on Day 0, 7, 14 and 20 of gestation, and days 0 and 4 of lactation.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Measurement of food consumption was conducted on all animals at the following frequencies:
Males in the main and recovery groups; on Day 1, 7, 14, 21, 28, 35, and 41 of administration, and on Day 7 and 13 of recovery.
Female satellite groups were weighted same frequencies to male recovery groups.
Females in the main group; on Day 1, 7 and 14 of administration and copulated females were weighed on Day 0, 7, 14 and 20 of gestation, and days 0 and 3 of lactation.

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes

- Time schedule for collection of blood: At the end of administration period, or at the end of recovery period in both sexes
- Anaesthetic used for blood collection: ether
- Animals fasted: Yes
- How many animals: 5 animals/sex/group
- Parameters examined included RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet, reticulocyte, PT, APTT, WBC and differential WBC.

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 examined included total protein, albumin, A/G ratio, total bilirubin, glucose, total cholesterol, triglyceride, phospholipid, AST, ALT, LDH, ChE, ALP, gamma-GTP, BUN, creatinine, Na, K, Cl, Ca and IP.

URINALYSIS OF MALES: Yes
- Time schedule for collection of urine (male only): On Day 37 of administration, and on Day 9 of recovery.
- Metabolism cages used for collection of urine: Yes
- How many animals: 5 animals/group
- Parameters examined included color, cloudy, urine volume, specific gravity, pH, protein, glucose, ketone body, bilirubin, occult blood and urobilinogen.

NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations: On week 6 of the administration period, and on week 2 of the recovery period
- Dose groups that were examined: All dose groups (5 animals/sex/group)
- Battery of functions tested: sensory activity (hearing reaction, eye sight reaction, sense of touch reaction, pain reaction, pupil reflex, righting reflex), grip strength, motor activity

Sacrifice and pathology
GROSS PATHOLOGY: Yes

ORGAN WEIBHT: Yes [brain, thymus, heart, liver, kidney, adrenal gland, spleen, seminal vesicle, testis, epididymis, pituitary, thyroid]

HISTOPATHOLOGY: Yes, [brain, pituitary, spinal cord, thyroid, parathyroid, heart, thymus, trachea, lung, liver, kidney, adrenal, spleen, stomach, small intestine, large intestine, sciatic nerve, bone, bone marrow, lymph nodes (mesenteric and cervical lymph nodes), urinary bladder, testis, seminal vesicle, prostate, epididymis, mammary gland, ovary and uterus.]

Statistics
As for parametric data (grip strength, locomotor activity, body weight, body weight gain, food consumption, hematology and clinical chemistry data, organ weights, quantitative urinalysis data, number of corpora lutea, number of implantation sites, number of pups born, number of pups alive, number of stillborn), the values of means and standard deviations were calculated per group. When more than three groups exist in the test group, Bartlett test for variance was done, and if the variance was homogenous, ANOVA was applied. If the variance was not homogenous or data was non-parametric (differential WBC percentage, qualitative urinalysis data, stages of spermatogenesis, length of the estrous cycle, implantation index, delivery index, live birth index, viability index,), Kruskal-Wallis rank sum test was used. Consequently, if the result was significant, Dunnett multiple comparison or Dunnett typed method was used for detection of statistical significance against control group. When the number of the test group was two, F-test was used as for parametric data.

Then, student's t-test or Aspin-Welch's t-test was applied depending on the result of homogeneity of variance. While, as for non-parametric data, Man-Whitney's U-test was applied. Furthermore, as for categorized data (incidence of abnormal findings in clinical observation, detailed observation, sensory functional examination, necropsy and histopathology, copulation index, fertility index, gestation index), Fischer's exact test was used. In the histopathological examination findings, Mann-Whitney's U test was used for graded data, and chi-squared test was used for sex ratio of pups. In any tests, level of significance was set at 5%.

Results and discussion

Results of examinations

Clinical signs
no effects observed

Mortality
no mortality observed

Body weight and weight changes
no effects observed

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

Food efficiency
not examined

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

Ophthalmological findings
not examined

Haematological findings
effects observed, treatment-related

Clinical biochemistry findings
effects observed, treatment-related

Urinalysis findings
no effects observed

Behaviour (functional findings)
no effects observed

Organ weight findings including organ / body weight ratios
effects observed, treatment-related

Gross pathological findings
no effects observed

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

Histopathological findings: neoplastic
not examined

Details on results
CLINICAL SIGNS AND MORTALITY:

Mortality: There was no death.
Clinical signs: There were no effects related to the test substance in any groups at the dosing and recovery periods.
DETAILED CLINICAL OBSERVATIONS, MANIPULATIVE TEST, GRIP STRENGTH TEST AND LOCOMOTOR ACTIVITY MEASUREMENT: There were no changes related to the test substance in any groups at the dosing and recovery periods.

BODY WEIGHT:
There were no changes related to the test substance in any groups at the dosing and recovery periods.

FOOD CONSUMPTION: There were no changes related to the test substance in any groups at the dosing and recovery periods.

HAEMATOLOGY:
[At the end of dosing period]: Decrease in MCHC was observed in females at 250 mg/kg bw/day.
[At the end of recovery period]: Decrease in MCHC was observed in females at 250 mg/kg bw/day.
CLINICAL CHEMISTRY:
[At the end of dosing period]: Increase in total protein, albumin were observed in males and females at 250 mg/kg bw/day. Increase in ALT and cholinesterase activity, decrease in glucose were observed in males at 250 mg/kg bw/day. Decrease in triglyceride were observed in females at 250 mg/kg bw/day.
[At the end of recovery period]: Increase in ALT activity were observed in males at 250 mg/kg bw/day.

URINALYSES OF MALES: There were no changes related to the test substance in any groups at the dosing and recovery periods.
ORGAN WEIGHTS:
[At the end of dosing period]: The relative and absolute weights of the liver increased at 10 mg/kg bw/day and greater doses in females and at 50 mg/kg bw/day and greater doses in males. Increased relative and absolute weights of the kidney in females of the 250 mg/kg bw/day group were also observed.
[At the end of recovery period]: Increased relative and absolute weights of the kidney were observed in males and females at 250 mg/kg bw/day.

GROSS PATHOLOGY: There were no changes related to the test substance in any groups at the end of dosing and recovery periods.

HISTOPATHOLOGY: NON-NEOPLASTIC:
[At the end of dosing period]: Hypertrophy of centrilobular hepatocytes were observed at 10 mg/kg bw/day and greater in females and at 50 mg/kg bw/day and greater in males. Dilatation of the distal/collecting tubules and the hyperplasia of the collecting tubular epithelium in the kidney were observed in females at 250 mg/kg bw/day.
[At the end of recovery period]: Hypertrophy of centrilobular hepatocytes were observed in males and females at 250 mg/kg bw/day.

Effect levels

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
histopathology: non-neoplastic
Hypertrophy of centrilobular hepatocytes were observed at 10 mg/kg bw/day in females
organ weights and organ / body weight ratios
The relative and absolute weights of the liver increased at 10 mg/kg bw/day in females

Any other information on results incl. tables

Figures and Tables (in English) are available in the following full report of the study.

http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF1460-02-2d.pdf

Applicant's summary and conclusion

Executive summary

A combined repeated oral-dose toxicity study with a reproduction/developmental toxicity screening test performed in accordance with OECD TG 422. Male and female rats (12 animals/sex/dose) were administered 1, 3, 5-tri-tert-butylbenzene at 0 (vehicle: olive oil), 2, 10, 50, and 250 mg/kg bw/day. The males were dosed for 42 days, including a 14-day pre-mating period and a subsequent mating period. The females were dosed for 42–52 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 4. Out of the 12 males, 5 were dosed at 0 and 250 mg/kg bw/day and were treated as a recovery group. Five additional females at 0 and 250 mg/kg bw/day were assigned to a satellite group and were dosed 1, 3, 5-tritert-butylbenzene for 42 days without mating, and they were examined after a 14-day recovery period.

No effects were found on clinical signs, FOB, body weight, food consumption, or urinalysis. Increase in total protein and albumin were observed in males and females at 250 mg/kg bw/day. Increase in ALT and cholinesterase activity, decrease in glucose were observed in males at 250 mg/kg bw/day. Decrease in triglyceride were observed in females at 250 mg/kg bw/day. The relative and absolute weights of the liver increased at 10 mg/kg bw/day and greater doses in females and at 50 mg/kg bw/day and greater doses in males. Increased relative and absolute weights of the kidney in females of the 250 mg/kg bw/day group were also observed. Histopathological examination showed hypertrophy of centrilobular hepatocytes at 10 mg/kg bw/day and greater in females and at 50 mg/kg bw/day and greater in males; dilatation of the distal/collecting tubules and the hyperplasia of the collecting tubular epithelium in the kidney were observed in females at 250 mg/kg bw/day. These changes were no longer found after the recovery period. The effects of 1, 3, 5-tritert-butylbenzene on the liver at 10 mg/kg bw/day, led to a determination of the NOAEL for repeated-dose toxicity at 2 mg/kg bw/day in rats.

Genetic toxicity

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

UUID:	9fa33584-aa8a-4916-830f-7118b93ec78d
Dossier UUID:	
Author:	
Date:	2019-09-03T16:21:24.000+09:00
Remarks:	

Administrative data

Endpoint
in vitro gene mutation study in bacteria

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

Reverse Mutation Test of 1,3,5-Tri-tert-butylbenzene / 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

GLP compliance
yes

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

Test material

Specific details on test material used for the study
- Lot No.: 08627MD (Sigma-Aldrich corporation)
- Purity: >99.2%
- Solubility: soluble in acetone and insoluble in water and DMSO.
- Physical state: white powder
- Storage condition of test material: room temperature (16-27 degree C)

Method

Species / strain

Species / strain / cell type
S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2
bacteria

Metabolic activation
with and without

Metabolic activation system
S9 mix: Rat liver, induced with phenobarbital and 5,6-benzoflavone

Test concentrations with justification for top dose
Dosage of each strain with or without S9
-S9 mix: 0, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000 µg /plate
+S9 mix: 0, 313, 625, 1250, 2500, 5000 µg /plate

Maximum concentration was established based on the result of the preliminary test at concentration up to 5000 ug/plate.
The growth inhibition was observed without S9 mix at 313 µg/plate and higher in TA100, TA98, and TA1537, and at 1250 µg/plate and higher in TA1535.

Vehicle / solvent
acetone

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
sodium azide
without S9 mix (TA 1535)
benzo(a)pyrene
with S9 mix (TA100, TA98, TA1537)
other: without S9 mix:2-(2-Furyl)-3-(5-nitro -2-furyl)acrylamide (TA100, TA98, WP2uvrA), without S9 mix: ICR-191 (TA1537) with S9 mix: 2-aminoanthracene (TA1535, WP2uvrA)

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

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

Statistics
not used

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 Without S9mix: 1250 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 without S9 mix' >=156 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 without S9 mix' >=156 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 Without S9mix: at 313 µg/plate

Vehicle controls validity
valid

Positive controls validity
valid

Key result
true

Species / strain
E. coli WP2 uvr A pKM 101
bacteria

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
no cytotoxicity nor precipitates, but tested up to recommended limit concentrations

Vehicle controls validity
valid

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. http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF1460 -02 -2e.pdf

Tables (in English) are attachted to this document. Please download the export file to see the Tables.

Overall remarks, attachments

Overall remarks

Genotoxic effects:

With metabolic activation: Negative

Without metabolic activation: Negative

Applicant's summary and conclusion

Executive summary

In a bacterial reverse mutation assay using S. typhimuriumTA100, TA1535, TA98, and TA1537, and E. coli WP2uvrA/pKM101 (OECD TG 471), negative results were obtained for 1,3,5-Tri-tert-butylbenzene with or without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

UUID:	afa7813a-fa19-4a8e-b863-dd5258e2c4a9
Dossier UUID:	
Author:	
Date:	2019-02-18T11:14:59.000+09:00
Remarks:	

Administrative data

Endpoint
in vitro cytogenicity / chromosome aberration study in mammalian cells

Type of information
experimental study

Adequacy of study
key study

Robust study summary
true

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 on 1,3,5-Tri-tert-butylbenzene Cultured Chinese Hamster Cell / 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

Qualifier
according to guideline

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

GLP compliance
yes

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

Test material

Specific details on test material used for the study
- Lot No.: 08627MD (Wako Pure Chemical Corporation)
- Purity: >99.2%
- Solubility: soluble in acetone and insoluble in water, DMSO and ethanol.
- Physical state: white powder
- Storage condition of test material: at room temperature (15-25 degree C)

Method

Species / strain

Species / strain / cell type
other:

Details on mammalian cell type (if applicable)
Chinese hamster lung(CHL/IU) cell

Metabolic activation
with and without

Metabolic activation system
S9 mix: Rat liver, induced with phenobarbital and 5,6- benzoflavone

Test concentrations with justification for top dose
0, 156.3, 312.5, 625.0, 1250, 2500 µg/mL (short-term, and continuous first test)
0, 4.883, 93766, 19.53, 39.06, 78.13 µg/mL (continuous, second test)
Cell-growth inhibition test was conducted up to the limited concentration of 2500 µg/mL (10 mM)
Short-term -S9mix: Concentration of 50% cell-growth inhibition was 62.9 µg/mL
Short-term +S9mix: Concentration of 50% cell-growth inhibition was 57.6 µg/mL
Continuous: Concentration of 50% cell-growth inhibition was 36.9 µg/mL

Vehicle / solvent
acetone

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
cyclophosphamide
(with S9 mix)
mitomycin C
(without S9 mix)

Details on test system and experimental conditions
METHOD OF APPLICATION: Exposure duration: [short-term treatment]:6 hrs + 18 hr, [continuous treatment]: 24h
NUMBER OF CELLS EVALUATED: 200 cells /concentration (100 cells/plate x 2)

Evaluation criteria
Positive: total chromosomal aberrations increased >=10% and concentration dependent increase or reproducibility was observed
Equivocal: total chromosomal aberrations increased >=5-10%
Negative: total chromosomal aberrations increased <5%

Statistics
Not used

Results and discussion

Test results

Key result
true

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

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
no cytotoxicity nor precipitates, but tested up to recommended limit concentrations short term treatment

Vehicle controls validity
valid

Positive controls validity
valid

Key result
true

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

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity 78.13 µg/mL (24h)

Vehicle controls validity
valid

Positive controls validity
valid

Any other information on results incl. tables

Figures and Tables (inEnglish) are available in the following full report of the study.

http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF1460-02-2f.pdf

Applicant's summary and conclusion

Conclusions
1,3,5-Tri-tert-butylbenzene did not induce structural aberrations for the short-term study with and without S9 mix. Positive, vehicle and negative control groups were valid.

Executive summary

In anin vitro chromosomal aberration test using CHL/IU cells (OECD TG 473), negative results were obtained with or without metabolic activation for 1,3,5-tri-tert-butylbenzene.

Toxicity to reproduction

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction.001

UUID: 08848368-20dc-42cf-8ef0-bca234fe7ef3
Dossier UUID:
Author:
Date: 2022-12-16T13:57:46.187+09:00
Remarks:

Administrative data

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

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Cross-reference

Reason / purpose for cross-reference
reference to same study

Related information
OECD / Repeated dose toxicity: oral / Repeated dose toxicity: oral.001 / 1,3,5-Tri-tert-butylbenzene / 1,3,5-tri-tert-butylbenzene / 1460-02-2

Data source

Reference

A combined repeated dose/reproductive developmental toxicity study of 1, 3, 5-Tri-tert-butylbenzene / Ministry of Health, Labor and Welfare, Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)

Deviations
no

GLP compliance
yes

Limit test
no

Test material

Specific details on test material used for the study
- Name of test material (as cited in study report): 1,3,5-tri-tert-butylbenzene
- Analytical purity: 98%
- Storage condition of test material: at a cold (temperature 2-6°C) and dark place, with airtight stopper.
- Stability under test conditions: The stability of test material was identified by analysis of the remainder.

Test animals

Species
rat

Strain
other: Crl:CD(SD)

Sex
male/female

Details on test animals or test system and environmental conditions
TEST ANIMALS
- Source: Charles River Japan, Inc., Atsugi Breeding Center.
- Age at study initiation: 10 weeks old
- Weight at study initiation: male 374 g (335-414 g), female 229 g (205-255 g)
- Housing: Animals were individually housed in bracket-type metallic wire-mesh cages (265W × 426D × 200H mm) , Dams were bred individually or with individual littermates in polycarbonate cages with flat floors (265W x 426D x 200H mm) and standard bedding.
- Diet: Solid feed (MR stock: Nosan Corporation) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation period: 12 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22±3 (actual temperature: 22.4-24.4°C)
- Humidity (%): 55±10% (actual humidity: 45-56%)
- Air changes (per hr): >10
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 7:00~19:00)

Administration / exposure

Route of administration
oral: gavage

Vehicle
olive oil

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

Analytical verification of doses or concentrations
yes

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating
(P)Females: 42-54 days including 14 days premating, mating and gestation periods and the days until day 4 of lactation
Female (no mating, satellite group): 42 days

Frequency of treatment
Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
2	mg/kg bw/day (actual dose received)
Dose / conc.	
10	mg/kg bw/day (actual dose received)
Dose / conc.	
50	mg/kg bw/day (actual dose received)
Dose / conc.	
250	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Main group:12 animals/sex/dose
Satellite (Recovery) group: 5 males/dose and 5 females/dose (0 and 250 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 highest dose of 250 mg/kg bw/day was selected as an expected obvious toxic dose, and the lowest dose of 2 mg/kg bw/day was selected as an expected no toxic dose. The middle dose levels of 10 and 50 mg/kg bw/day were selected.
- Rationale for animal assignment (if not random): Body weight-balanced randomization

[14-day preliminary study]
A 14-day repeated dose oral toxicity test (Crl:CD(SD) rats, doses: 0 (olive oil), 10, 30, 100 or 300 mg/kg bw/day). High value of albumin was observed in males at 30 mg/kg bw/day or more. High value of A/G ratio was observed in male at 100 mg/kg bw/day or more, and high value of calcium was observed in females at 100 mg/kg bw/day or more. High value of total cholesterol was observed in males and females at 300 mg/kg/day. High value of ALT, ChE and inorganic phosphorus, low value of chlorine were observed in males at 300 mg/kg bw/day. High value of sodium was observed in females at 300 mg/kg bw/day. Large liver was observed in males at 30 mg/kg bw/day or more, and in females at 100 mg/kg bw/day or more. Increase in liver weight was observed in males at 100 mg/kg bw/day or more, and in females at 300 mg/kg bw/day. Thickening of the forestomachial mucosa was observed in females at 300 mg/kg bw/day.

Examinations

Parental animals: Observations and examinations
CAGE SIDE OBSERVATIONS: Yes
- Time schedule: 3 times/day (am: before and after administration; pm) during the administration period. 2 rimes/day (am and pm) during the recovery period.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: Once before the start of administration, and once every week by the end of the study period.

BODY WEIGHT: Yes
- Time schedule for examinations:
Males were weighed on Day 1, 7, 14, 21, 28, 35, and 42 of administration, and weighed on Day 7 and 14 of recovery.
Female satellite groups were weighted same frequencies to male recovery groups.
Females in the main groups were weighed on Day 1, 7 and 14 of administration and copulated females were weighed on Day 0, 7, 14 and 20 of gestation, and days 0 and 4 of lactation.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes
Measurement of food consumption was conducted on all animals at the following frequencies:
Males in the main and recovery groups; on Day 1, 7, 14, 21, 28, 35, and 41 of administration, and on Day 7 and 13 of recovery.
Female satellite groups were weighted same frequencies to male recovery groups.
Females in the main group; on Day 1, 7 and 14 of administration and copulated females were weighed on Day 0, 7, 14 and 20 of gestation, and days 0 and 3 of lactation.

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: At the end of administration period, or at the end of recovery period in both sexes
- Anaesthetic used for blood collection: ether
- Animals fasted: Yes
- How many animals: 5 animals/sex/group
- Parameters examined included RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet, reticulocyte, PT, APTT, WBC and differential WBC.

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 examined included total protein, albumin, A/G ratio, total bilirubin, glucose, total cholesterol, triglyceride, phospholipid, AST, ALT, LDH, ChE, ALP, gamma-GTP, BUN, creatinine, Na, K, Cl, Ca and IP.

URINALYSIS OF MALES: Yes
- Time schedule for collection of urine (male only): On Day 37 of administration, and on Day 9 of recovery.
- Metabolism cages used for collection of urine: Yes
- How many animals: 5 animals/group
- Parameters examined included color, cloudy, urine volume, specific gravity, pH, protein, glucose, ketone body, bilirubin, occult blood and urobilinogen.

NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations: On week 6 of the administration period, and on week 2 of the recovery period
- Dose groups that were examined: All dose groups (5 animals/sex/group)
- Battery of functions tested: sensory activity (hearing reaction, eye sight reaction, sense of touch reaction, pain reaction, pupil reflex, righting reflex), grip strength, motor activity

Oestrous cyclicity (parental animals)
Vaginal smears were collected from all females in the main groups and microscopically examined every day from the day after the start of administration until the day copulation was confirmed.

Sperm parameters (parental animals)
Parameters examined in all P male parental generations: testis weight, epididymis weight, histopathological examinations for testes, epididymides, seminal vesicle including coagulating gland 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, weight gain, physical or behavioral abnormalities.

Postmortem examinations (parental animals)
METHOD OF SACRIFICED: All animals were sacrificed by exsanguination under ether anesthesia.

SACRIFICE: Male animals: On Day 42, Maternal animals: on Day 5 of lactation, and Male recovery and female satellite animals: on next Day 14 of recovery.

GROSS PATHOLOGY: Yes

ORGAN WEIBHT: Yes [brain, thymus, heart, liver, kidney, adrenal gland, spleen, seminal vesicle, testis, epididymis, pituitary, thyroid]

HISTOPATHOLOGY: Yes, [brain, pituitary, spinal cord, thyroid, parathyroid, heart, thymus, trachea, lung, liver, kidney, adrenal, spleen, stomach, small intestine, large intestine, sciatic nerve, bone, bone marrow, lymph nodes (mesenteric and cervical lymph nodes), urinary bladder, testis, seminal vesicle, prostate, epididymis, mammary gland, ovary and uterus.]

Postmortem examinations (offspring)
SACRIFICE

- The F1 offspring were sacrificed at 4 days of age.

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

HISTOPATHOLOGY / ORGAN WEIGTHS
- Not examined.

Statistics
As for parametric data (grip strength, locomotor activity, body weight, body weight gain, food consu mption, hematology and clinical chemistry data, organ weights, quantitative urinalysis data, number of corpora lutea, number of implantation sites, number of pups born, number of pups alive, number of stillborn), the values of means and standard deviations were calculated per group. When more than three groups exist in the test group, Bartlett test for variance was done, and if the variance was ho mogenous, ANOVA was applied. If the variance was not homogenous or data was non-parametric (differential WBC percentage, qualitative urinalysis data, stages of spermatogenesis, length of the estrous cycle, implantation index, delivery index, live birth index, viability index,), Kruskal-Wallis rank s um test was used. Consequently, if the result was significant, Dunnett multiple comparison or Dunnet t typed method was used for detection of statistical significance against control group. When the nu mber of the test group was two, F-test was used as for parametric data.
Then, student's t-test or Aspin-Welch's t-test was applied depending on the result of homogeneity of variance. While, as for non-parametric data, Man-Whitney's U-test was applied. Furthermore, as fo r categorized data (incidence of abnormal findings in clinical observation, detailed observation, se nsory functional examination, necropsy and histopathology, copulation index, fertility index, gestation index), Fischer's exact test was used. In the histopathological examination findings, Mann-Whitney's U test was used for graded data, and chi-squared test was used for sex ratio of pups. In any tests, level of significance was set at 5%.

Reproductive indices
Estrous cycle: Mean days from metaeatrus I (III) to next III.
Copulation index (%) = (No. of pairs with successful copulation/No. of pairs mated) × 100
Fertility index (%) = (No. of pregnant females/No. of pairs with successful copulation) × 100
Gestation index (%) = (No. of females with live pups/No. of pregnant females) × 100
Implantation index (%) = (No. of implantation sites/No. of corpora lutea) × 100
Delivery index (%) = (No. of pups born/No. of implantation sites) × 100
Live birth index (%) = (No. of live pups on day 0/No. of pups born) × 100
Sex ratio =Total number of male pups/Total number of female pups

Offspring viability indices
Viability index (%) = (No. of live pups on day 4/No. of live pups on day 0) × 100

Results and discussion

Results: P0 (first parental generation)

General toxicity (P0)

Clinical signs
no effects observed

Mortality
no mortality observed

Body weight and weight changes
no effects observed

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

Food efficiency
not examined

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

Ophthalmological findings
not examined

Haematological findings
effects observed, treatment-related

Description (incidence and severity)
See 7.5.1

Clinical biochemistry findings
effects observed, treatment-related

Description (incidence and severity)
See 7.5.1

Urinalysis findings
no effects observed

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

Gross pathological findings
no effects observed

Neuropathological findings
not examined

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

Description (incidence and severity)
See 7.5.1

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)
The numbers of pups alive were lower on PND 0 and 4 at 250 mg/kg bw/day; the delivery index and live-birth index were lower than for the control. At the same dose level, the body weights of the live p ups also decreased on PND 0 and 4

Effect levels (P0)

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
reproductive performance
The numbers of pups alive were lower on PND 0 and 4 at 250 mg/kg bw/day; the delivery index and live-birth index were lower than for the control. At the same dose level, the body weights of the live p ups also decreased on PND 0 and 4

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Mortality / viability
mortality observed, treatment-related

Description (incidence and severity)
The numbers of pups alive were lower on PND 0 and 4 at 250 mg/kg bw/day.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
The body weights of the live pups decreased on PND 0 and 4 at 250 mg/kg bw/day.

Gross pathological findings
no effects observed

Effect levels (F1)

Key result
true

Dose descriptor
NOAEL

Generation
F1

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
viability
The numbers of pups alive were lower on PND 0 and 4 at 250 mg/kg bw/day.
body weight and weight gain
The body weights of the live pups decreased on PND 0 and 4 at 250 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.

http://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF1460-02-2d.pdf

Applicant's summary and conclusion

Executive summary

In a repeated-oral-dose toxicity study and a reproduction/developmental toxicity screening test (OECD TG 422), as described above, the numbers of pups alive were lower on PND 0 and 4 at 250 mg/kg bw/day; the delivery index and live-birth index were lower than for the control. At the same dose level, the body weights of the live pups also decreased on PND 0 and 4. The developmental toxicity at 250 mg/kg bw/day led to the conclusion that the NOAEL for the rat reproduction/developmental toxicity of 1, 3, 5-tritert-butylbenzene should be determined at 50 mg/kg bw/day; at this point, parental general toxicity was observed.

References

Reference Substances

REFERENCE_SUBSTANCE: 1,3,5-tri-tert-butylbenzene

UUID: ECB5-0252de27-51d6-49c9-b482-64c900eadbb2
Dossier UUID:
Author:
Date: 2007-05-10T18:00:00.000+09:00
Remarks:

Reference substance name
1,3,5-tri-tert-butylbenzene

IUPAC name
1,3,5-tri-tert-butylbenzene

Inventory

Inventory number

Inventory name
1,3,5-tri-tert-butylbenzene

Inventory
EC Inventory

Inventory number
215-952-4

CAS number
1460-02-2

Molecular formula
C18H30

Description

CAS number
1460-02-2

Synonyms

Synonyms

Identity
1,_3,_5-Tri-tert-butylbenzene

Molecular and structural information

Molecular formula
C18H30

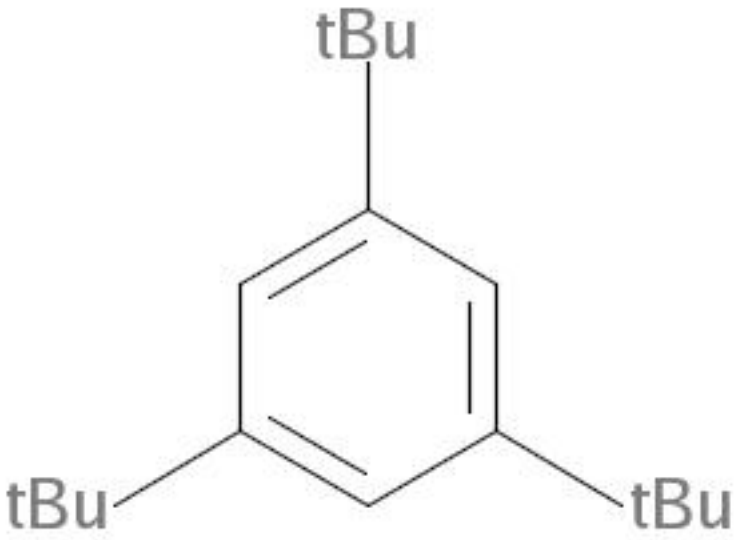
Molecular weight

246.4308

SMILES notation
CC(C)(C)c1cc(cc(c1)C(C)(C)C)C(C)(C)C

InChI
InChI=1/C18H30/c1-16(2,3)13-10-14(17(4,5)6)12-15(11-13)18(7,8)9/h10-12H,1-9H3

Structural formula



Related substances

Group / category information
USEPA Category: Neutral Organics

Literatures

LITERATURE: A combined repeated dose/reproductive developmental toxicity study of 1, 3, 5-Tri-tert-butylbenzene by oral administration in rats.

UUID:	9ce9c808-fda7-488e-94bb-d2effb4b0496
Dossier UUID:	
Author:	
Date:	2019-03-22T10:25:28.000+09:00
Remarks:	

General information

Reference Type
study report

Title
A combined repeated dose/reproductive developmental toxicity study of 1, 3, 5-Tri-tert-butylbenzene by oral administration in rats.

Author
Ministry of Health, Labor and Welfare, Japan

Bibliographic source
Japan Existing Chemical Data Base (JCDB)

Testing facility
Research institute for animal science in biochemistry and toxicology (RIAS)

Report number
07-111

LITERATURE: In Vitro Chromosomal Aberration Test of on 1,3,5-Tri-tert-butylbenzene Cultured Chinese Hamster Cells

UUID: b5165e02-1336-48d7-b3c2-16582d1ccaad
Dossier UUID:
Author:
Date: 2019-02-18T10:54:41.000+09:00
Remarks:

General information

Reference Type
study report

Title
In Vitro Chromosomal Aberration Test of on 1,3,5-Tri-tert-butylbenzene Cultured Chinese Hamster Cell s

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

Bibliographic source
http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp

Testing facility
BoZo Research Center

LITERATURE: Reverse Mutation Test of 1,3,5-Tri-tert-butylbenzene

UUID:	0be57198-4f9b-463a-9a10-22a144172cde
Dossier UUID:	
Author:	
Date:	2019-02-18T09:49:29.000+09:00
Remarks:	

General information

Reference Type
study report

Title
Reverse Mutation Test of 1,3,5-Tri-tert-butylbenzene

Bibliographic source
http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp

Testing facility
BoZo Research Center

Legal Entities

LEGAL_ENTITY: National Institute of Health Sciences

UUID: IUC4-b036ff75-0f3c-323b-b200-ed5f46cf5101
Dossier UUID:
Author:
Date: 2022-11-07T15:49:29.000+09:00
Remarks:

General information

Legal entity name
National Institute of Health Sciences

Remarks
Disclaimer: The contents in this document were created based on the MHLW (Ministry of Health, Labour and Welfare) peer reviewed study reports (in Japanese) in JECDB (Japan Existing Chemical Database) at http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp. Authorship is in the Division of Risk Assessment, the National Institute of Health Sciences, and the contents do not reflect any o fficial MHLW opinions or any other regulatory policies.

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Country
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Identifiers

Other IT system identifiers

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

ID
16558402024DIV750