Name: 4-sec-Butyl-2,6-di-tert-butylphenol / 4-sec-butyl-2,6-di-tert-butylphenol / 17540-75-9

Legal entity owner: National Institute of Health Sciences, Japan

Printing date: 2020-10-01T12:24:15.423+09:00

Table of Contents

4-sec-Butyl-2,6-di-tert-butylphenol

CORE

General information

FIXED_RECORD: Assessment approach

UUID:	a253430f-9d8b-3bf9-87dd-d3aa6c1c3e03
Dossier UUID:	
Author:	SuperUser
Date:	2020-03-24T16:11:36.000+09:00
Remarks:	

OECD

Health Effects

Repeated dose toxicity: oral

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.001

UUID: e671a33a-d6fc-4e25-a27f-e5c467cea51f
Dossier UUID:
Author: SuperUser
Date: 2020-10-01T10:36:12.144+09:00
Remarks:

Administrative data

Endpoint
short-term repeated dose toxicity: oral

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
other: The study was conducted in accordance with Test Guidelines and under GLP

Data source

Reference

Twenty-eight-day Repeated Dose Oral Toxicity Test of 4-sec-Butyl-2,6-di-tert-butylphenol in Rats / Ministry of Health, Labour and Welfare (MHLW), Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to

Guideline
OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)

Deviations
no

GLP compliance
yes

Limit test
no

Test material

Test material information
4-sec-butyl-2,6-di-tert-butylphenol

Specific details on test material used for the study
- Analytical purity: 99.0%
- Storage condition of test material: Stored in a refrigerator (2 - 9°C)
- Stability under test conditions: The stability of test material was identified by analysis of the remainder.

Test animals

Species
rat
common rodent species

Strain
other: Crl:CD(SD)

Details on test animals and environmental conditions
TEST ANIMALS
- Source: Charles River Japan, Inc., Atsugi Breeding Center.
- Age at study initiation: 5 weeks old
- Weight at study initiation: male 175.0 g (163-185 g), female 147.7 g (131-162 g)
- Housing: Animals were individually housed in bracket-type metallic wire-mesh cages (260W × 380D × 170H mm)
- Diet: Solid feed (CRF-1: Oriental Yeast Co., ltd.) was given ad libitum.
- Water: Tap water was given ad libitum.
- Acclimation and quarantine period: male 5 days, female 6 days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22±3 (actual temperature: 21-24°C)
- Humidity (%): 50±20% (actual humidity: 38-54%)
- Air changes (per hr): 10-15
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light (light: 8:00~20:00)

Administration / exposure

Route of administration
oral: gavage

Vehicle
corn oil

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

Analytical verification of doses or concentrations
yes

Duration of treatment / exposure
28 days

Frequency of treatment
once a day

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
15	mg/kg bw/day (actual dose received)
Dose / conc.	
60	mg/kg bw/day (actual dose received)
Dose / conc.	
250	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
Control and high-dose group: 12 animals/sex/ (including recovery group of 6 animals/sex/each group)
Low- and middle-dose group: 6 animals/sex/dose

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 15 mg/kg bw /day was selected as an expected no toxic dose. The middle dose levels of 60 mg/kg bw/day were selected.
[14-day preliminary study]
In a dose finding study for a 28 day study, Crl:CD(SD) rats were given 4-sec-Butyl-2,6-di-tert-butylph enol at 0 (corn oil), 15, 60, 250 or 1000 mg/kg bw/day for 14 days. At 250 mg/kg bw/day and higher, increase in the liver weights were observed. At 1000 mg/kg bw/day, tendency decreased body weight gain, tendency decreased food consumption, increase in total bilirubin and BUN, soft stool, dilatation of gastrointestinal tract, dark reddish change of cauda epididymis were observed.

- Rationale for animal assignment (if not random): Body weight-balanced randomization
- Rationale for selecting satellite groups: Reversibility of toxic effects by treatment was examined in re covery test with control- and high-dose groups for both sexes.
- Post-exposure recovery period in satellite groups: 14 days

Examinations

Observations and examinations performed and frequency
CLINICAL OBSERVATIONS: Yes
- Time schedule: every day during the administration (twice a day: am and pm) and recovery periods (twice a day: am and pm).
DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: Once prior to dosing and once during each study week (administration days 7, 14, 21, 28 and recovery days 7, 14).

BODY WEIGHT: Yes
- Time schedule for examinations: Before administration (on days 1, 4, 7, 14, 21 and 28 of the a dministration period, days 7 and 14 of the recovery period) and the necropsy days after completion of every period.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption: Yes. Before administration (on days 1, 4, 7, 14, 21 and 28 of the administration period and days 7 and 14 of the recovery period)
OPHTHALMOSCOPIC EXAMINATION: No
HAEMATOLOGY: Yes
- Time schedule for collection of blood: the after completion of the administration and recovery periods
- Anaesthetic used for blood collection: ether
- Animals fasted: Yes (16-19 hours)
- How many animals: all animals
- Parameters examined included RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet, reti culocyte, PT, APTT, WBC and differential WBC.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: the day after completion of the administration and recovery periods
- Animals fasted: Yes
- How many animals: all animals
- Parameters examined included total protein, protein fraction, albumin, A/G ratio, total bilirubin, glucose, total cholesterol, triglyceride, AST, ALT, ALP, gamma-GTP, BUN, creatinine, Na, K, Cl, Ca and IP.

URINALYSIS: Yes
- Time schedule for collection of urine: on weeks 4 of the administration period and weeks 2 of the recovery period.
- Metabolism cages used for collection of urine: Yes
- Animals fasted: No
- How many animals: all animals
- Parameters examined included color, urine volume, specific gravity, pH, protein, glucose, ketone bo dy, bilirubin, occult blood and urobilinogen.

NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations: On week 4 of administration period and week 2 of recovery period.
- Dose groups that were examined: All animals
- Battery of functions tested:
1) Sensory/Reflex test: Visual reactivity, touch reactivity, auditory reactivity, pain reactivity, pro prioceptive reactivity and aerial righting reflex.
2) Measurement of Grip Strength. Grip strength of forelimb and hind limb was measured by CPU gauge (Aikoh Engineering Co., Ltd.).

3) Measurement of Motor Activity.Motor activity was measured by a motor activity sensor for experimental animals SUPERMEX & CompAct (Muromachi Kikai Co., Ltd.). The measurement was conducted for 1 hour, and measured values at 10-minute intervals and from 0 to 60 minutes was collected.

Sacrifice and pathology
GROSS PATHOLOGY: Yes
ORGAN WEIBHT: Yes [brain, pituitary gland, thyroid, adrenal, spleen, heart, liver, kidney, thymus, testis, epididymis, prostate, seminal vescles (including coagulation gland), ovary, uterus]
HISTOPATHOLOGY: Yes [brain (cerebrum, cerebellum and medulla oblongata), pituitary gland, spinal cord, thymus, thyroid, parathyroid, adrenal glands, spleen, heart, tongue, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum (including Peyer's patches), cecum, colon, rectal, mesenteric lymph nodes, submandibular lymph nodes, trachea, lung, kidney, bladder, testis, epididymis, prostate, seminal vesicles (including coagulation glands) , ovary, uterus, vagina, eye, Harder gland, femur (including bone marrow, right) and the sciatic nerve. (see tables in the study report.)]

Statistics
The homoscedasticity was analyzed by Bartlett’s test for data of grip strength, motor activity, body weight, body weight gain, food consumption, urine volume, hematological test, biochemical test, organ weight and organ weight ratio. When homogeneity was recognized, one-way analysis of variance (homogeneous data) or kruskal-wallis (non-homogenous data) was conducted. If a significant difference was detected, as the result of oneway analysis of variance, Dunnett’s method was applied for comparisons between control and individual treatment groups. And in the case of a significant difference was detected on kruskal-wallis, Mann-Whitney’s U-test was applied for the same purpose. The trend by the group was analyzed by Kruskal-Wallis for general appearance, detailed clinical observation, qualitative items of urinary findings, and urine specific gravity. If a significant difference was detected as the result of kruskal-wallis, Mann-Whitney’s U-test as applied for comparisons between control and individual treatment groups.

Results and discussion

Results of examinations

Clinical signs
effects observed, treatment-related

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
effects observed, treatment-related

Behaviour (functional findings)
no effects observed

Immunological findings
not examined

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

Gross pathological findings
effects observed, treatment-related

Neuropathological findings
not examined

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

Details on results
CLINICAL SIGNS AND MORTALITY
[At the dosing period]
Soft feces was observed in both sexes receiving 250 mg/kg bw/day.
[At the recovery period]
There were no changes related to the test substance in any groups.

BODY WEIGHT AND WEIGHT GAIN
There were no changes related to the test substance in any groups during the dosing.

FOOD CONSUMPTION
There were no changes related to the test substance in any groups during the dosing.

HAEMATOLOGY
[At the end of the dosing period]
Prothrombin time was prolonged in males receiving 60 mg/kg bw/day and above. Increased activated partial thromboplastin time was observed in males receiving 60 mg/kg/day and above and females r eceiving 250 mg/kg bw/day.
[At the end of the recovery period]
There were no changes related to the test substance in any groups.

CLINICAL CHEMISTRY
[At the end of the dosing period]
An increase in total cholesterol was observed in females receiving 15 mg/kg bw/day and above and in males receiving 60 mg/kg bw/day and above. An increase in total bilirubin was observed in both sexes receiving 250 mg/kg bw/day, increases in AST, ALT, ALP and BUN, and decreases in K and IP were observed in males receiving 250 mg/kg bw/day, and increases in alfa1-globulin protein fraction % and creatinine were observed in females receiving 250 mg/kg bw/day.
[At the end of the recovery period]
There were no changes related to the test substance in any groups.
URINALYSIS
[At the dosing period]
A decrease in urine pH was observed in females receiving 250 mg/kg bw/day.
[At the recovery period]
There were no changes related to the test substance in any groups.

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

ORGAN WEIGHTS
[At the end of the dosing period]
An increase in relative liver weight was observed in females receiving 15 mg/kg bw/day and above, an increase in absolute liver weight was observed in females receiving 250 mg/kg bw/day.
[At the end of the recovery period]
An increase in relative liver weight was observed in females receiving 250 mg/kg bw/day.

GROSS PATHOLOGY
[At the end of the dosing period]
Cecum: Watery contents were observed in males receiving 60 mg/kg bw/day and above and in females receiving 250 mg/kg bw/day.
[At the end of the recovery period]
There were no changes related to the test substance in any groups.

HISTOPATHOLOGY: NON-NEOPLASTIC
[At the end of the dosing period]
Liver: Slightly centrilobular hypertrophy of hepatocytes were observed in females receiving 250 mg/kg bw/day.
[At the end of the recovery period]
There were no changes related to the test substance in any groups.

Effect levels

Key result
true

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male

Basis for effect level
gross pathology
Watery contents of cecum were observed in males receiving 60 mg/kg bw/day and above.

Any other information on results incl. tables

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

https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF17540-75-9b.pdf

Applicant's summary and conclusion

Conclusions
Based on the findings in haematological examination, clinical chemistry, organ weight, and gross pat hology, the NOAEL for repeated-dose toxicity of 4-sec-butyl-2,6-di-tert-butylphenol was determined to be 15 mg/kg bw/day.

Executive summary

The repeated-dose toxicity of 4-sec-butyl-2,6-di-tert-butylphenol was assessed with the use of rats. Male and female rats (6 animals/sex/dose) were administered with 0 [vehicle: corn oil], 15, 60, and 250 mg/kg bw/day of 4-sec-butyl-2,6-di-tert-butylphenol for 28 days. Six of the 12 animals/sex that have received 0 and 250 mg/kg bw/day were selected to be part of a 14-day recovery group.

No treatment-related deaths were noted for both sexes. Clinical signs have shown that the doses did not affect the manipulative test, body weight, and food consumption. In general appearance, the male and female groups receiving 250 mg/kg bw/day had soft feces. The urinalysis shows the urine pH in females receiving 250 mg/kg bw/day wassignificantlydecreased. Hematological parameters, such as prothrombin time in males in the ≥60 mg/kg bw/day group as well as activated partial thromboplastin time in males in the ≥60 mg/kg bw/day group and in females in the 250 mg/kg bw/day group, were significantly prolonged. Blood chemistry analysis has shown that total cholesterol level in females receiving ≥15 mg/kg bw/day and in males receiving ≥60 mg/kg bw/day and total bilirubin level in both sexes receiving 250 mg/kg bw/day significantly increased. Contrarily, the levels of potassium (K) and inorganic phosphorus (IP) significantly decreased in male rats receiving 250 mg/kg bw/day. A gross pathological examination has shown watery content of cecum in males receiving 60 mg/kg bw/day and in both sexes receiving 250 mg/kg bw/day. Soft feces, watery content of cecum, and decreases of K and IP levels were discovered, suggesting that the digestive tract is affected. There has been significantly increase in the relative weight of the liver of females receiving ≥15 mg/kg bw/day and the absolute weight of the liver of females receiving 250 mg/kg bw/day. Histopathological findings declared a slight hypertrophy of centrilobular hepatocytes in females receiving 250 mg/kg bw/day. These changes were no longer observed after the recovery period, showing that they were reversible.Founded on these effects of the cecumat 60 mg/kg bw/day in males, the no-observed-adverse-effect level (NOAEL) for repeated-dose toxicity of 4-sec-butyl-2,6-di-tert-butylphenol was 15 mg/kg bw/day in rats.

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

UUID: 0446cc20-0c98-4c91-8609-5eee0e1f8326
Dossier UUID:
Author: SuperUser
Date: 2020-03-26T15:44:02.660+09:00
Remarks:

Administrative data

Endpoint
in vitro gene mutation study in bacteria

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
other: OECD Test Guideline study under GLP condition

Data source

Reference

Reverse Mutation Test of 4-sec-butyl-2,6-di-tert-butylphenol on Bacteria. / MHLW, Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to

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

Deviations
no

Qualifier
according to

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

Deviations
no

GLP compliance
yes

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

Test material

Test material information
4-sec-butyl-2,6-di-tert-butylphenol

Specific details on test material used for the study
Purity 99.0%

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 pKM 101
bacteria

Metabolic activation
with and without

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

Test concentrations with justification for top dose
-S9 mix:
156, 313, 625, 1250, 2500, 5000 µg/plate (TA100, TA1535, TA98, TA1537 strains)
156, 313, 625, 1250, 2500, 5000 µg/plate (WP2uvrA/pKM101 strain)
+S9 mix:
156, 313, 625, 1250, 2500, 5000 µg/plate (TA100, TA1535, TA98, TA1537 strains)
156, 313, 625, 1250, 2500, 5000 µg/plate (WP2uvrA/pKM101 strain)

Maximum concentration was established based on the result of the preliminary test at concentration up to 5000 ug/plate. In this test, no growth inhibition was observed at any dose.

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

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
other:

Remarks
-S9 mix: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA 100, TA98 and WP2 uvrA/pKM101), sod ium azide (TA1535) and 9-aminoacridine hydrochloride monohydrate (TA1537). +S9 mix: 2-ami noanthracene (TA100, TA1535, WP2 uvrA/pKM101, TA98 and TA1537)

Details on test system and experimental conditions
METHOD OF APPLICATION: Preincubation
DURATION- Preincubation period: 20 min at 37°C
- Exposure duration:49 hrs

NUMBER OF PLATES: 3
NUMBER OF REPLICATIONS: 2
DETERMINATION OF CYTOTOXICITY
- Method: other: growth inhibition

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

Statistics
no

Results and discussion

Test results

Key result
true

Species / strain
other: S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 bacterias

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

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

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

Remarks on result
other: all strains/cell types tested Migrated from field 'Test system'.

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/PDF17540-75-9e.pdf

Please also see the attached files (Tables in English)

Overall remarks, attachments

Attached background material

Attached document
17540759.xlsx / 21.361 KB (application/vnd.openxmlformats-officedocument.spreadsheetml.sheet)

Applicant's summary and conclusion

Conclusions
Interpretation of results (migrated information): negative
In a bacterial reverse mutation assay using Salmonella typhimurium TA100, TA1535, TA98, and TA 1537, and Escherichia coli WP2uvrA/pKM101 (OECD TG 471), 4-sec-butyl-2,6-di-tert-butylphenol was negative with or without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

UUID: fbf35256-3f54-428c-910d-ced8387052fb
Dossier UUID:
Author: SuperUser
Date: 2020-03-17T14:56:12.000+09:00
Remarks:

Administrative data

Endpoint
in vitro cytogenicity / chromosome aberration study in mammalian cells

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
other: OECD Test Guideline study under GLP condition

Data source

Reference

In Vitro Chromosomal Aberration Test of 4-sec-butyl-2,6-di-tert-butylphenol on Cultured Chinese Hams... / MHLW, Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to

Guideline
OECD Guideline 473 (In Vitro Mammalian Chromosome Aberration Test)
in vitro cytogenicity / chromosome aberration study in mammalian cells

Deviations
no

Qualifier
according to

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

Deviations
no

GLP compliance
yes

Type of assay
other: in vitro mammalian chromosome aberration test

Test material

Test material information
4-sec-butyl-2,6-di-tert-butylphenol

Specific details on test material used for the study
Purity 99.0%

Method

Species / strain

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

Metabolic activation
with and without

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

Test concentrations with justification for top dose
Cell growth inhibition study
-S9 mix (short-term treatment): 10.2, 20.5, 40.9, 81.9, 164, 328, 655, 1310, 2620 ug/mL (IC50=15.1 ug/mL)
+S9 mix (short-term treatment): 10.2, 20.5, 40.9, 81.9, 164, 328, 655, 1310, 2620 ug/mL (IC50=29.0 ug/mL)
-S9 mix (continuous treatment, 24hr): 10.2, 20.5, 40.9, 81.9, 164, 328, 655, 1310, 2620 ug/mL (IC50=14.4 ug/mL)
Main study
-S9 (short-term treatment): 2.5, 5, 10, 13.3, 16.7 20 ug/mL
+S9 (short-term treatment): 5, 10, 20, 26.7, 33.3 ug/mL
+S9 (short-term treatment) (confirmatory test): 10, 20, 26.7, 33.3, 40 ug/mL

-S9 (continuous treatment, 24hr): 2.5, 5, 10, 13.3, 16.7, 20 ug/mL
-S9 (continuous treatment, 24hr) (confirmatory test): 4, 6, 8, 10, 11, 12, 13, 14, 15 ug/mL

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

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
other: [-S9]: mitomycin C; [+S9]: benzo(a)pyrene

Details on test system and experimental conditions
METHOD OF APPLICATION: Exposure duration: [short-term treatment]: 6 hrs + 18 hr, [continuous treatment]: 24 hr
SPINDLE INHIBITOR: Colcemid
STAIN: Giemsa stain (2 v/v%) for 20 min.
NUMBER OF REPLICATIONS: 2
NUMBER OF CELLS EVALUATED: 100 + 100 cells /concentration
DETERMINATION OF CYTOTOXICITY
- Method: relative total growth

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

Statistics
no

Results and discussion

Test results

Key result
true

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

Metabolic activation
without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

Key result
true

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

Metabolic activation
with

Genotoxicity
other: equivocal

Cytotoxicity / choice of top concentrations
cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

Positive controls validity
valid

Additional information on results
Figures and Tables (in English) are available in the following full report of the study.
https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF17540-75-9f.pdf

Applicant's summary and conclusion

Conclusions
Interpretation of results (migrated information):
equivocal with metabolic activation
negative without metabolic activation
The in vitro chromosomal aberration test using CHL/IU cells (OECD TG 473) was equivocal with metabolic activation.

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction.001

UUID: f2efea51-6b6b-4539-a905-c1f8ce22b37a
Dossier UUID:
Author: SuperUser
Date: 2020-10-01T10:57:18.448+09:00
Remarks:

Administrative data

Endpoint
screening for reproductive / developmental toxicity

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

Reproduction/Developmental Toxicity Screening Test on 4-sec-butyl-2,6-di-tert-butylphenol / MHLW, Japan / study report

Data access
data published

Materials and methods

Test guideline

Qualifier
according to

Guideline
OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)

GLP compliance
yes

Test animals

Species
rat

Strain
other: Crl:CD(SD)

Sex
male/female

Details on test animals and environmental conditions
Sixty two male and 62 female Note) Sprague-Dawley strain SPF rats [Crl:CD(SD), Atsugi Breeding Center, Charles River Laboratories Japan, Inc.] were purchased at 8 weeks of age and quarantined/acclimated for 18 days counting the day of receipt as day 1 of acclimation and including the quarantine period of 3 days.
The body weight range at the start of administration was 405 to 494 g (mean body weight: 451 g) for males and 227 to 272 g (mean body weight: 244 g) for females.

Animals were housed individually and subsequently in male-female pairs during the mating period in bracket-type metallic wire-mesh cages (W 254 × D 350 × H 170 mm: Lead Engineering Co., Ltd.) in an animal room (Room No. 905) where the temperature was maintained at 23 ± 3°C (measured values: 21-25°C), the relative humidity at 50 ± 20% (measured values: 42-65%), the air ventilation at 10 to 15 times per hour and 12-hour lighting per day (07:00 to 19:00). From day 17 of gestation to day 4 of lactation, animals were housed in the unit of litter in plastic Econ cages (W 340 × D 400 × H 185 mm: CLEA Japan, Inc.) with bedding (Whiteflakes: Charles River Laboratories Japan, Inc.).

Administration / exposure

Route of administration
oral: gavage

Vehicle
corn oil

Details on exposure
Corn oil was selected as the vehicle since the stability of the test article in corn oil was favorable in the study for validation of the analytical method for determination of the test article concentration in test solutions and stability (Study No. A-2262) which was conducted before this study.

Details on mating procedure
Males and females in the same dose group of the main groups were co-housed overnight on a one-to-one basis after the end of the pre-mating administration period. Copulation was considered successful if the formation of vaginal plugs or presence of sperm in vaginal smears was confirmed the following morning. The length of the mating period for the same male and female was 14 days at maximum.
Delivery and delivery/lactation status: All copulated females were allowed to deliver spontaneously and examined for any abnormality of delivery. Dams which completed delivery were observed for clearance of placenta and amnion, and the end of delivery was designated as day 0 of lactation. Dams were then allowed to nurse their liveborn pups until day 4 of lactation and examined for lactation status using the gathering of pups, nesting and lactating as indicators.

Analytical verification of doses or concentrations
yes

Details on analytical verification of doses or concentrations
For the test solutions at all dose concentrations to be used for administration in week 1 or week 6 of administration, dose concentrations were verified by the HPLC method at Gotemba Laboratory, Bozo

Research Center Inc. before use for administration. In the results, the percentage of the test article concentration in each dose concentration to the nominal concentration was in the range from 94.2 to 102.1%, which was within the acceptable range (concentration: within 100.0 ± 10.0% of the nominal value)

Duration of treatment / exposure
Males: 42 days Females: 42-46 days from 14 days before mating to day 4 of lactation

Frequency of treatment
Once a day

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day
Dose / conc.	
12	mg/kg bw/day
Dose / conc.	
60	mg/kg bw/day
Dose / conc.	
300	mg/kg bw/day

No. of animals per sex per dose
The number of animals per group was 12 animals of each sex

Control animals
yes, concurrent vehicle

Details on study design
The dose levels of this study were selected based on the results of the previously conducted study (dose-finding study). In the dose-finding study, death occurred at 1000 mg/kg and effects on general condition, hematology and blood chemistry examinations at 300 mg/kg and a high value in liver wei ght at 100 mg/kg and above. At 100 and 300 mg/kg, there were no clear effects from administration of the test article on body weight or food consumption. In the dose-finding study, there were mort alities in 1000 mg/kg group. In the 300 mg/kg group, some changes were observed in clinical observ ation, hematological examination and blood chemistry examination, and organ weight of the liver sho wed high value in the 100 mg/kg group and above. In the 100 and 300 mg/kg groups, there were no changes which were thought to be effects from administration of the test article in body weight or fo od consumption. Based on these results, the high dose in this reproduction/developmental toxicity screening test was set at 300 mg/kg, and the lower doses were set at 60 and 12 mg/kg using the common ratio of approximately 5.

Positive control
no

Examinations

Parental animals: Observations and examinations
Clinical observation performed and frequency: General condition was observed 3 times a day during the administration period (before dosing, and immediately after and approximately 2 hours after dosing).
Body weights were determined on days 1 (before dosing), 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 42 of administration for males and on days 1, 4, 8, 11, 15 of administration, days 0, 4, 7, 11, 14, 17 and 20 of gestation, days 0 and 4 of lactation for females and the day of necropsy in males and females.
Food consumption was determined on days 1, 4, 8, 11, 15, 32, 36, 39 and 42 of administration for males and on days 1, 4, 8, 11 and 15 of administration and on days 1, 4, 7, 11, 14, 17 and 20 of gestation and days 2 and 4 of lactation in females, but it was not determined during the mating period for males and females.

Oestrous cyclicity (parental animals)
Vaginal smear pictures were classified as proestrus, estrus, metestrus and diestrus and examined for the frequency of estrus and interval between estruses (estrous cycle).

Postmortem examinations (parental animals)
Dams which delivered pups were exsanguinated on day 5 of lactation. The numbers of corpora lutea and implantation sites were counted.
Necropsy: Detailed macroscopic examination was conducted on the organs/tissues throughout the body of each animal, including the external appearance, head, thorax and abdomen.
Measurement of organ weighs: The testis and epididymis were determined.
Histopathological examination: The testis, epididymis, prostate and seminal vesicle in males at 0 and 300 mg/kg groups, the ovary, uterus and vagina in females at 0 and 300 mg/kg groups In addition, all subject organs/tissues of dead animals, all the animals that had not delivered by day 25 of gestation, dams with total litter loss during the lactation period and all gross pathological lesions of all animals were examined.

Postmortem examinations (offspring)
Examination of liveborn pups: The numbers of liveborn pups and stillborn pups were counted on the day of birth. After liveborn pups were examined for any external abnormality, sexed and weighed, dams were allowed to nurse their pups. Liveborn pups were observed for mortality once daily until day 4 after birth. All liveborn pups were exsanguinated after measurement of body weight on day 4 after birth, necropsied and examined for any abnormality in organs/tissues, including those in the head, thorax and abdomen. Individual body weights of liveborn pups were recorded, and the average body weight per litter was calculated by sex.
Pathological examination were performed.

Statistics
Dunnett's test for continuous data and Dunnett-type mean rank test for quantal data were used.

Reproductive indices
no. of copulated animals, no. of males impregnated females, no. of pregnant females, no. of females that delivered liveborn pups, estrous cycle, gestational length, no.of corpora lutea, no. of implantation sites, total no. of liveborn and stillborn pups, no. of liveborn pups, sex ratio on day 0 and day 4 after birth, copulation index (no. of copulated animals / no. of animals housed together x 100), insemination index (no. of males that impregnated females / no. of copulated males x 100), fertility index (no. of pregnant females / no. of copulated females x 100)

Offspring viability indices
delivery index (no. of females delivered liveborn pups / no. of pregnant females x 100), implantation index (no. of implantation sites / no. of corpora lutea x100), stillbirth index (no. of stillborn pups / total no. of liveborn and stillborn pups x 100), index of external abnormalities (no. of pups with external abnormalities / no. of liveborn pups x 100), live birth index (no. of liveborn pups / no. of implantation sites x 100), and viability index on day 4 after birth (no. of surviving pups on day 4 after birth / no. of liveborn pups x 100)

Results and discussion

Results: P0 (first parental generation)

General toxicity (P0)

Clinical signs
effects observed, treatment-related

Description (incidence and severity)
In males in the 300 mg/kg group, soft feces were observed in 5-8 animals sporadically from week 4 of administration onward, and fracture of incisors in 1 animal in week 6 of administration. In females of the 300 mg/kg group, there were no abnormalities in clinical signs during the pre-mating administratio n period, but soft feces were observed sporadically in 1-3 animals from day 9 of gestation onward and one female each died on day 22 and day 23 of gestation showing hypothermia and/or emaciation. O ne female showed pale skin on day 1 of lactation and soft feces and emaciation on day 2 of lactation and another female showed soft feces on day 0 of lactation, hypothermia and emaciation on day 1 of lactation and pale skin and hemorrhage from the vaginal opening on day 2 of lactation and all pups of these two females died by day 2 of lactation.

Mortality
mortality observed, treatment-related

Description (incidence)
In the 300 mg/kg group, 1 female died on day 22 and day 23 of gestation.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
In males in the 300 mg/kg group, body weight was lower than that of the control group from day 8 of administration, and the body weight on days 36, 39 and 42 of administration was significantly lower than that of the control group and body weight gain during the administration period was significantly lower than that of the control group.

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

Description (incidence and severity)
Females in the 300 mg/kg group showed a significantly low value compared to that of the control grou p on day 8 of administration, a significantly high value on day 4 and a significantly low value on day 20 of gestation, but there were no significant differences in food consumption between the control group and this group during the lactation period.

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

Description (incidence and severity)
Males showed low body weight on the day of necropsy and males and females showed a high value in the weight of the liver in the 300 mg/kg group, and males showed a high value in the weight of the liver in the 60 mg/kg group.

Gross pathological findings
effects observed, treatment-related

Description (incidence and severity)
In the females in the 300 mg/kg group that died, a female showed undernourishment, dilatation of the cecum, enlargement of the liver and small thymus, but the other female showed no macroscopic

abnormalities. Both females had implantations. For the dams that had total litter loss in the 300 mg/kg group, undernourishment, pale skin, dilatation of the cecum, dark coloration of the kidney, small thymus, dark red focus in the eye, dark coloration of Harderian gland, dark coloration of the lung, small sublingual gland and submandibular gland, dark red focus in the forestomach and small splen were observed. In males that were necropsied on schedule, dilatation of the cecum was observed in the 300 mg/kg group. In females that were necropsied on schedule, enlargement of the liver was observed in the 300 mg/kg group.

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

Description (incidence and severity)
Dead animals showed mild hypertrophy of centrilobular hepatocytes and minimal vacuolation of centriolbular hepatocytes in the liver, minimal hypertrophy of bile duct epithelial cells and severe atrophy of the thymus. Among the dams that had total litter loss, the dams in the 300 mg/kg group showed hypertrophy of bile epithelium, vacuolation of centrilobular hepatocytes and necrosis of centrilobular hepatocytes in the liver, acute tubular necrosis, interstitial inflammatory cell infiltration and glomerular thrombus in the kidney, atrophy of the thymus, diffuse mucosal hyperplasia of the cecum, ulcer and hyperplasia of squamous epithelium in the forestomach, increased porphyrin in Hardelian gland, atrophy of submandibular gland, atrophy of the spleen, cell infiltration and thrombus in the lung, and focal retinal hemorrhage in the eye. In the animals that were sacrificed on schedule, males and females showed hypertrophy of centrilobular hepatocytes in the liver in the 60 and 300 mg/kg groups and bile duct epithelial cell hypertrophy in the liver in the 300 mg/kg group. Otherwise the animal in the 300 mg/kg group showed single cell necrosis and diffuse hyperplasia in cecum mucosa.

Reproductive function / performance (P0)

Reproductive function: oestrous cycle
no effects observed

Reproductive performance
no effects observed

Effect levels (P0)

Key result
false

Dose descriptor
NOAEL

Effect level

12	mg/kg bw/day (nominal)

Based on
test mat.

Sex
male

Basis for effect level
organ weights and organ / body weight ratios
increased liver weight
histopathology: non-neoplastic
hypertrophy of centrilobular hepatocytes in the liver

Key result
true

Dose descriptor
NOAEL

Effect level

60 mg/kg bw/day (nominal)

Based on
test mat.

Sex
female

Basis for effect level
reproductive performance
A tendency of lowing in the delivery index, the number of liveborn, and live birth index and the tendency of high stillborn rate were observed in 300 mg/kg bw/day group. The tendency of low viability index on postnatal day 4 was seen in the 300 mg/kg bw/day group.

Key result
false

Dose descriptor
NOAEL

Effect level

300 mg/kg bw/day

Based on
test mat.

Sex
male

Basis for effect level
reproductive performance
no effects

Results: F1 generation

General toxicity (F1)

Mortality / viability
no mortality observed

Body weight and weight changes
no effects observed

Gross pathological findings
no effects observed

Effect levels (F1)

Key result
false

Dose descriptor
NOAEL

Generation
F1

Effect level

300	mg/kg bw/day

Based on
test mat.

Sex
male/female

Basis for effect level
other: no effects on development

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/PDF17540-75-9c.pdf

Applicant's summary and conclusion

Conclusions
There was no effect of the test substance in the parental males and pup at 300 mg/kg bw/day, the NOAEL of paternal and developmental toxicities was 300 mg/kg bw/day group. On the other hand, as seen on the effects on general condition of dams and delivery at 300 mg/kg bw/day, the NOAEL of the maternal toxicity was 60 mg/kg bw/day. In conclusion, the overall NOAEL for the reproductive/developmental toxicity of4-sec-butyl-2,6-di-tert-butylphenolin this study was 60 mg/kg bw/day.

Executive summary

The reproduction/developmental toxicity screening test of 4-sec-butyl-2,6-di-tert-butylphenol was assessed in rats in accordance to the OECD test guideline (TG) 421. In this study, 4-sec-butyl-2,6-di-tert-butylphenol was administered via gavage at 0 [vehicle: corn oil], 12, 60 and 300 mg/kg bw/day. Males (12/dose) were then treated for 42 days, which include a 14-day premating period and subsequent mating period, while females (12/dose) were treated for 42–46 days, including 14-day premating, mating, and gestation periods until lactation day 4. There were no effects on fertility but a tendency of lowing in the delivery index, the number of liveborn, and live birth index and the tendency of high stillborn rate were observed in 300 mg/kg bw/day group. The tendency of low viability index on postnatal day 4 was seen in the 300 mg/kg bw/day group. Because there was no effect of the test substance in the parental males and pup at 300 mg/kg bw/day, the NOAEL of paternal and developmental toxicities was 300 mg/kg bw/day group. On the other hand, as seen on the effects on general condition of dams and delivery at 300 mg/kg bw/day, the NOAEL of the maternal toxicity was 60 mg/kg bw/day. In conclusion, the overall NOAEL for the reproductive/developmental toxicity of4-sec-butyl-2,6-di-tert-butylphenolin this study was 60 mg/kg bw/day.

DOMAIN

SUBSTANCE: 4-sec-Butyl-2,6-di-tert-butylphenol

UUID: b2fce83d-cfa7-43d6-a1fc-c5819c7f46d7
Dossier UUID:
Author: SuperUser
Date: 2020-10-01T10:58:29.033+09:00
Remarks:

Substance name
4-sec-Butyl-2,6-di-tert-butylphenol

Public name
4-sec-Butyl-2,6-di-tert-butylphenol

Legal entity
National Institute of Health Sciences, Japan

Contact persons

Person
Hirose, Akihiko; National Institute of Health Sciences

Last name
Hirose

First name
Akihiko

Organisation
National Institute of Health Sciences

Department
Division of Risk Assessment

Title
Dr.

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 official MHLW opinions or any other regulatory policies.

Identification of substance

Reference substance
4-sec-butyl-2,6-di-tert-butylphenol / 4-sec-butyl-2,6-di-tert-butylphenol / 17540-75-9 / 241-533-0

EC number	EC name
241-533-0	EC Inventory
CAS number	**CAS name**
17540-75-9	

IUPAC name

4-sec-butyl-2,6-di-tert-butylphenol

Role in the supply chain

Manufacturer
false

Importer
false

Only representative
false

Downstream user
false

References

LEGAL_ENTITY: National Institute of Health Sciences, Japan

UUID: 0952b3b9-2d0c-4bc8-925e-b069be7789b7
Dossier UUID:
Author: SuperUser
Date: 2020-02-19T14:42:16.272+09:00
Remarks:

General information

Legal entity name
National Institute of Health Sciences, Japan

REFERENCE_SUBSTANCE: 4-sec-butyl-2,6-di-tert-butylphenol

UUID: ECB5-4be93424-903c-4590-bb54-0a3185f73206
Dossier UUID:
Author: SuperUser
Date: 2007-05-10T18:00:00.000+09:00
Remarks:

General information

Reference substance name
4-sec-butyl-2,6-di-tert-butylphenol

Inventory

Inventory name
4-sec-butyl-2,6-di-tert-butylphenol

Inventory
EC

Inventory number
241-533-0

CAS number
17540-75-9

Molecular formula
C18H30O

Description

Reference substance information

IUPAC name
4-sec-butyl-2,6-di-tert-butylphenol

Synonyms

Identity Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-
Identity Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-
Identity Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-

CAS information

CAS number
17540-75-9

Related substances

Group / category information
DSL Category: Organics
USEPA Category: Neutral Organics;Phenols

Molecular and structural information

Molecular formula
C18H30O

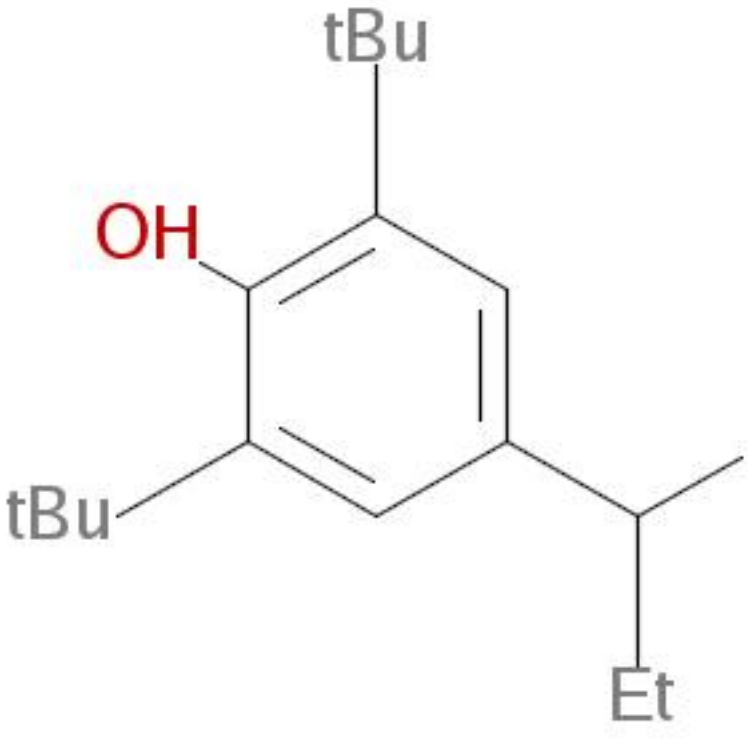
Molecular weight

262.4302

SMILES notation
CCC(C)c1cc(c(O)c(c1)C(C)(C)C)C(C)(C)C

InChI
InChI=1/C18H30O/c1-9-12(2)13-10-14(17(3,4)5)16(19)15(11-13)18(6,7)8/h10-12,19H,9H2,1-8H3

Structural formula



TEST_MATERIAL_INFORMATION: 4-sec-butyl-2,6-di-tert-butylphenol

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Dossier UUID:
Author: SuperUser
Date: 2019-12-17T14:59:36.000+09:00
Remarks:

Name
4-sec-butyl-2,6-di-tert-butylphenol

CONTACT: Hirose, Akihiko; National Institute of Health Sciences

UUID: 4293b0a1-fb1d-47d7-a0f7-2f93622aeb27

Dossier UUID:

Author: SuperUser

Date: 2020-02-20T15:27:47.410+09:00

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 official MHLW opinions or any other regulatory policies.

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Department
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Title
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Remarks
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LITERATURE: In Vitro Chromosomal Aberration Test of 4-sec-butyl-2,6-di-tert-butylphenol on Cultured Chinese Hamster Cells.

UUID: 22775324-3017-4f58-8b64-05b57fe2b4ac
Dossier UUID:
Author: SuperUser
Date: 2019-12-18T15:24:12.000+09:00
Remarks:

General information

Reference Type
study report

Title
In Vitro Chromosomal Aberration Test of 4-sec-butyl-2,6-di-tert-butylphenol on Cultured Chinese Hamster Cells.

Author
MHLW, Japan

Bibliographic source
Japan Existing Chemical Data Base (JECDB) https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF17540-75-9f.pdf

Testing facility
Safety Research Institute for Chemical Compounds Co., Ltd.

Report no.
SR08225

LITERATURE: Reproduction/Developmental Toxicity Screening Test on 4-sec-butyl-2,6-di-tert-butylphenol

UUID: 243fe436-3b37-405c-815a-22caee3481e2
Dossier UUID:
Author: SuperUser
Date: 2020-02-18T11:14:20.057+09:00
Remarks:

General information

Reference Type
study report

Title
Reproduction/Developmental Toxicity Screening Test on 4-sec-butyl-2,6-di-tert-butylphenol

Author
MHLW, Japan

Year
2011

Bibliographic source
https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF17540-75-9c.pdf

Testing facility
BoZo Recearch Center

LITERATURE: Reverse Mutation Test of 4-sec-butyl-2,6-di-tert-butylphenol on Bacteria.

UUID: ae56849c-be2d-49f5-95a4-20dbe725402a
Dossier UUID:
Author: SuperUser
Date: 2019-12-17T14:46:26.000+09:00
Remarks:

General information

Reference Type
study report

Title
Reverse Mutation Test of 4-sec-butyl-2,6-di-tert-butylphenol on Bacteria.

Author
MHLW, Japan

Bibliographic source
Japan Existing Chemical Data Base (JECDB) https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF17540-75-9e.pdf

Testing facility
Safety Research Institute for Chemical Compounds Co., Ltd.

Report no.
SR08224

LITERATURE: Twenty-eight-day Repeated Dose Oral Toxicity Test of 4-sec-Butyl-2,6-di-tert-butylphenol in Rats

UUID: 225d52ac-2e56-4309-b957-5acf7680b7cd
Dossier UUID:
Author: SuperUser
Date: 2020-03-23T15:54:17.000+09:00
Remarks:

General information

Reference Type
study report

Title
Twenty-eight-day Repeated Dose Oral Toxicity Test of 4-sec-Butyl-2,6-di-tert-butylphenol in Rats

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

Bibliographic source
Japan Existing Chemical Data Base (JECDB) https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF17540-75-9b.pdf

Testing facility
Safety Research Institute for Chemical Compounds Co., Ltd.

Report no.
SR08226