Name: COMPLETE / SUBSTANCE : 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1 / 4,4'-methylenebis(2,6-di-tert-butylphenol) / 118-82-1 Tue, 13 Dec 2022, 17:17:18+0900 /

Legal entity owner: National Institute of Health Sciences

Printing date: 2022-12-13T17:17:19.007+09:00

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DOSSIER:

UUID: 0
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Author:
Date: 2022-12-13T17:17:18.745+09:00
Remarks:

Dossier header

Dossier submission type

Name
Complete table of contents

Version
core 7.0

Name (given by user)

Dossier subject

Dossier subject
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1 / 4,4'-methylenebis(2,6-di-tert-butylphenol) / 118-82-1

Public name

Submitting legal entity
National Institute of Health Science

Dossier creation date/time
Tue, 13 Dec 2022, 17:17:18+0900

Used in category

LEGAL_ENTITY: National Institute of Health Science

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Dossier UUID:	
Author:	
Date:	2022-11-07T16:24:02.822+09:00
Remarks:	

General information

Legal entity name
National Institute of Health Science

2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

OECD

Health Effects

Repeated dose toxicity: oral

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.001

UUID: 19959dda-809b-4487-9f62-78f8e15a1a5e
Dossier UUID:
Author:
Date: 2022-12-13T17:07:12.026+09:00
Remarks:

Administrative data

Endpoint
short-term repeated dose toxicity: oral

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

A 28-day repeat dose oral toxicity study of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol in rat / Ministry of Health, Labour and Welfare (MHLW), Japan / publication

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

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

Qualifier
according to guideline

Guideline
other: Study Methods on New Chemical Substances (Chemical Substances Control Law of Japan)

GLP compliance
yes

Test material

Specific details on test material used for the study
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

Test animals

Species
rat
common rodent species

Strain
other: SD [crl :CD (SD)]

Sex
male/female

Details on test animals or test system and environmental conditions
TEST ANIMALS
- Source: Charles River Laboratories Japan, Inc. Atsugi
- Age at study initiation: 5 weeks
- Weight at study initiation: Males: 149-168 g ; Females: 135-155 g
- Housing: bracket-type metallic wire-mesh cages (W 260 × D 380 × H 180 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 7 days(male), 8 days(female)

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22.8-24.7°C (acceptable range: 22±3°C)
- Humidity (%): 51-60 % (acceptable range: 55±10 %)
- Air changes: >10 times / hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
olive oil

Details on oral exposure
PREPARATION OF DOSING SOLUTIONS:
Test substance was dissolved in olive oil for injection.

Vehicle
- Name: olive oil (Japanese Pharmacopoeia)
- Lot Number: MI-13
- Manufacturer: Kozakai pharmaceutical Co., Ltd.
- Storage Conditions: Room temperature

Analytical verification of doses or concentrations
yes

Details on analytical verification of doses or concentrations
Test solutions were prepared at least once in 8 days and used within 7 days of preparation.
The test solutions were analyzed concentration after 0days, 4days and 8days preparation.
The results showed that the concentrations were all within the acceptable range.

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.	
8	mg/kg bw/day (actual dose received)
Dose / conc.	
40	mg/kg bw/day (actual dose received)
Dose / conc.	
200	mg/kg bw/day (actual dose received)
Dose / conc.	
1000	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
5 or 10/sex/dose

Control animals
yes, concurrent vehicle

Details on study design
Dose selection rationale: Doses in this test were set based on the results of the following dose setting study: a 14-day repeated dose oral toxicity test (doses: 0 (vehicle), 50, 100, 200, 500,and 1000 mg/kg bw/day).

At 100 mg/kg bw/day, significantly lower total cholesterol levels were observed in males and higher re lative liver weights in females.
At 200 mg/kg bw/day, significantly higher absolute and relative liver weights were observed in fe males.
At 500 mg/kg bw/day, significant increases in relative kidney weights were observed in males and relative liver weights in females.
At 1000 mg/kg bw/day, significantly lower total cholesterol levels and higher relative liver weights were observed in males.
At 1000 mg/kg bw/day, significantly higher absolute and relative liver and kidney weights were o bserved in females.
In this study, the highest dose was 1000 mg/kg bw/day, which is expected to cause toxic effects after repeated administration for 28 days, and the lowest dose was 8 mg/kg bw/day, which is expected to cause no toxic effects.

Therefore, the high dose in this study was set at 1000 mg/kg bw/day, and a low dose of 8 mg/kg bw/ day.

- Rationale for animal assignment (if not random): Body weight-balanced randomization
- Post-exposure recovery period in satellite groups: 14 days

Examinations

Observations and examinations performed and frequency
All animals were subjected to detailed clinical observations once before the start of administration Thereafter, detailed clinical observations were made once a week in dosing and recovery periods.

CAGE SIDE OBSERVATIONS: Yes
Males and females: 4 times/day during the administration period (before and after dosing, 4 hours after dosing) , once a day during the recovery period.
DETAILED CLINICAL OBSERVATIONS: Yes

BODY WEIGHT: Yes
Body weights were determined on days 1, 7, 14, 21 and 28 of administration.
Body weights were determined on the day of 7 and 14 of recovery.
Body weights were determined on the day of necropsy.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):yes
Food consumption was determined on days 5, 12, 19 and 26 of administration for males.
Food consumption was determined on days 5 and 12 of recovery for males.
Food consumption was determined on days 4, 11, 18 and 25 of administration for females.
Food consumption was determined on days 4 and 11 of recovery for females.
OPHTHALMOSCOPIC EXAMINATION: yes

HAEMATOLOGY: Yes

CLINICAL CHEMISTRY: Yes

URINALYSIS: Yes

Animals fasted: no

Sacrifice and pathology
NECROPSY: yes
[brain, pituitary, eye ball, thyroid, spinal cord, heart, trachea, lung, liver, kidney, thymus, spleen, adrenal gland, stomach, ovary, uterus, bladder, sciatic nerve, lymph node, bone marrow]

HISTOPATHOLOGY: yes

Statistics
The data were analyzed for homogeneity of variance by the Bartlett test. If variances were homogeneous, data was analyzed by the Dunnett test, whereas heterogeneous data was analyzed by the steel test.

Comparisons between the two groups were performed by F-test, followed by Student's t-test if vari ance was uniform, and Aspin-Welch's t-test if variance was not uniform.
Comparisons between multiple groups were performed by Kruskal-Wallis's rank test. When the results showed significant differences, the groups were compared with the control group by the Dunnett type test. Comparisons between the two groups were performed by Mann-Whitney's U-test.

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

Haematological findings
no effects observed

Description (incidence and severity)
At 8 mg/kg bw/day in males and females and at 40 mg/kg bw/day in males, low activated partial thromboplastin times were significantly observed at the end of the dosing period.
At 200 mg/kg bw/day, low values of hemoglobin and hematocrit in males and prothrombin time and activated partial thromboplastin time in females were significantly observed.
At 200 mg/kg bw/day, a significant increase in reticulocyte count was observed in females.
However, no dose relationship was observed for these changes.

Clinical biochemistry findings
effects observed, treatment-related

Description (incidence and severity)
At 40 mg/kg bw/day in females, significantly lower values of cholinesterase were observed at the end of the a dministration period.

At 200 mg/kg bw/day, high levels of LDH and γ-GTP and low total cholesterols and urea-nitrogen were significantly observed in females. However, none of them was dose-related.
At 200 mg/kg bw/day, low creatinine was significantly observed in males. However, the values were within the reference values in the background data and no dose relationship was evident.
A significant increase in AST was observed in males at the end of the recovery period. Low total cholesterol levels were significantly observed in males. In females, high levels of γ-GTP and sodium were significantly observed.
These changes were considered to be spontaneous, because there were not observed and changes were not dose dependent. and recovered by withdraw.

Urinalysis findings
no effects observed

Description (incidence and severity)
At 200 mg/kg bw/day, a significantly higher number of voids per week of dosing were observed in males. However, these changes were not dose-related, and no changes were observed after Week 2. Therefore, these changes were considered incidental.

Behaviour (functional findings)
no effects observed

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

Description (incidence and severity)
At 40 mg/kg bw/day, higher kidneys relative weights were significantly observed in males in animals sacrificed at the end of the administration period.

At 200 mg/kg bw/day, high absolute and relative liver weights were significantly observed in females.
Absolute and relative ovarian and thyroid weights were significantly higher in females than 200 mg/kg bw/day.
At 1000 mg/kg bw/day, a significant increase in relative liver weights was observed in males.
At 1000 mg/kg bw/day, females had significantly lower relative thymic weights.
In the animals sacrificed at the end of the recovery period, high absolute thyroid weights were signif icantly observed in males, but no changes in relative weights were observed.
Higher relative thymus weights and lower relative pituitary weights were significantly observed in females.
These changes were observed in the organs/tissues, but they were thought to be incidental changes based on the incidence of their occurrence and their histopathological profiles.

Gross pathological findings
no effects observed

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

Description (incidence and severity)
At the end of the treatment period, in sacrificed animals:
At 200 mg/kg bw/day, diffuse hyperplasia of mild follicular epithelial cells in the thyroid gland was observed in two females.
At 1000 mg/kg bw/day, diffuse hyperplasia of mild follicular epithelial cells in the thyroid gland was significantly observed in 4 females.

Effect levels

Key result
false

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
clinical biochemistry
organ weights and organ / body weight ratios

Target system / organ toxicity

Key result
false

Critical effects observed
yes

Lowest effective dose / conc.

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

System
cardiovascular

Organ
kidney

Treatment related
yes

Dose response relationship
yes

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/PDF118-82-1b.pdf

Applicant's summary and conclusion

Conclusions
Based on changes in relative kidney weight and blood cholinesterase levels, the NOAEL for repeated-dose toxicity of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol was determined to be 8 mg/kg bw/day in rats.

Executive summary

The repeated-dose toxicity of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol was investigated in rats according to the OECD TG 407. Male and female rats (5 or 10 animals/sex/dose) were treated with 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol at doses of 0 (vehicle: olive oil), 8, 40, 200, and 1,000 mg/kg bw/day for 28 days. Five out of the 10 animals/sex treated with 0 and 1,000 mg/kg bw/day were assigned as a recovery group.

There were no deaths in either sex. At doses of ≥40 mg/kg bw/day, the relative kidney weight was increased in males, and blood cholinesterase levels were decreased in females. At doses of ≥200 mg/kg bw/day, mean corpuscular volume and mean corpuscular hemoglobin levels were decreased in males, and mean corpuscular hemoglobin concentration was decreased in females. Absolute and relative weights of the thyroid gland were increased, and histopathological analysis showed slight diffuse hyperplasia of follicular cells of the thyroid gland was increased with doses of 200 and 1000 mg/kg bw/day. Absolute and relative weights of the ovary were increased without histopathological changes with doses of ≥200 mg/kg bw/day. These changes were no longer found after the recovery period. Based on changes in relative kidney weight and blood cholinesterase levels, the NOAEL for repeated-dose toxicity of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol was determined to be 8 mg/kg bw/day in rats.

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.002

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Dossier UUID:
Author:
Date: 2022-12-13T17:16:46.046+09:00
Remarks:

Administrative data

Endpoint
short-term repeated dose toxicity: oral

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Cross-reference

Reason / purpose for cross-reference
reference to same study

Related information
OECD / Toxicity to reproduction / Toxicity to reproduction.001 / 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1 / 4,4'-methylenebis(2,6

Remarks
Toxicity to reproduction.001

Data source

Reference

A reproduction/developmental toxicity screening test in rats treated orally with 2,2',6,6'-tetra-ter / Ministry of Health, Labour and Welfare (MHLW), Japan / publication

Data access
data published

Materials and methods

Test guideline

Qualifier according to guideline **Guideline** other: OECD Guideline for Testing of Chemicals 421
Qualifier according to guideline **Guideline** other: Study Methods on New Chemical Substances (Chemical Substances Control Law of Japan)

GLP compliance
yes

Test material

Specific details on test material used for the study
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

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 Laboratories Japan, Inc. Atsugi
- Age at study initiation: 10 weeks
- Weight at study initiation: Males: 392 - 449g; Females: 228 - 274g
- Housing: bracket-type metallic wire-mesh cages (W 254 × D 350 × H 170 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 18 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22-25 (acceptable range:23±3 °C)
- Humidity (%): 38-64 (acceptable range:50±20 %)
- Air changes: 10-15 times / hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
methylcellulose 0.5 w/v% methylcellulose solution

Details on oral exposure
PREPARATION OF DOSING SOLUTIONS: Test substance was dissolved in 0.5 w/v% methylcellulose solution for injection.

Vehicle
- Name: 0.5 w/v% methylcellulose solution (METOLOSE SM-400, Japanese Pharmacopoeia)
- Lot Number: 8105606
- Manufacturer: Shin-Etsu Chemical Co., Ltd.
- Storage Conditions: In well-closed containers, in a cold and dark place [stored in a refrigerator as the cold and dark place (acceptable values: 1-10°C, measured values: 2-9°C)]

Analytical verification of doses or concentrations
yes

Details on analytical verification of doses or concentrations
Test solutions were prepared at least once in 8 days and used within 7 days of preparation.
The test suspensions at all dose concentrations to be used for administration in week 1 or week 6 of administration, dose concentrations and homogeneity were verified by the HPLC method at Gotemba Laboratory, Bozo Research Center Inc. before use for administration.
The results showed that the concentrations were 97.5 to 103.5% and the coefficient of variation in the range from 0.5 to 4.6%, both of which were within the acceptable range (concentration: within 100.0 ± 10.0% of the nominal value, homogeneity: CV within 10.0%)

Duration of treatment / exposure
Males: 42 days (14 days prior to mating, 14 days during the mating period and 14 days after the end of the mating period),
Females: 41 to 50 days (14 days prior to mating and throughout the mating and gestation periods until day 4 of lactation)

Non-pregnant females: 53 days

Frequency of treatment
once a day (7 times/week)

Doses / concentrations

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

No. of animals per sex per dose
12 animals/sex/dose

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, “A 14-day oral gavage toxicity study of 2,2’,6,6’-tetra- tert-butyl-4,4’-methylenediphenol in rats (dose-finding study)”). In that study, there were no toxic changes at 1000 mg/kg, which is the limit test dose prescribed in the OECD Guidelines for Testing of Chemicals 421. Based on these results, the high d ose in this reproduction/developmental toxicity screening test was set at 1000 mg/kg, and the lower doses were set at 300 and 100 mg/kg using the common ratio of approximately 3.

- Rationale for animal assignment (if not random): Body weight-balanced randomization
- Post-exposure recovery period in satellite groups: 14 days

Examinations

Observations and examinations performed and frequency
CAGE SIDE OBSERVATIONS: Yes
Males and females: 3 times/day during the administration period (before and after dosing)

DETAILED CLINICAL OBSERVATIONS: Yes

BODY WEIGHT: Yes
males : days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39 and 42 of administration and the day of necropsy,
females : 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 and the day of necropsy
uncopulated females : days 18, 22 and 25 of administration during the mating period),

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):yes
males : days 1, 4, 8, 11, 15, 32, 36, 39 and 42 of administration
females : days 1, 4, 8, 11 and 15 of administration, days 1, 4, 7, 11, 14, 17 and 20 of gestation and days 2 and 4 of lactation.

OPHTHALMOSCOPIC EXAMINATION:yes

HAEMATOLOGY: no

CLINICAL CHEMISTRY: no

URINALYSIS: no

Sacrifice and pathology
Necropsy:yes
[Detailed macroscopic examination: including the external appearance, head, thorax and abdomen]
histopathology: yes
[testes, epididymides, ovaries, uterus, prostate, seminal vesicle, vagina, macroscopic lesions]

Statistics
Group mean with standard deviation was calculated and subjected to a Dunnett-type mean rank test for the difference in mean rank (levels of significance: 0.05 and 0.01, two-tailed).
For the copulation index, insemination index, fertility index and delivery index, the number of copulated animals, number of males impregnated females, number of pregnant females and the number of f emales that delivered liveborn pups were calculated for each group and analyzed by the chi-square test with Yates’ continuity correction (levels of significance: 0.05 and 0.01, two-tailed). If there were cells whose expected frequency was 5 or below, data were analyzed by Fisher’s exact test (levels of significance: 0.05 and 0.01, two-tailed) .

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

Description (incidence and severity)
In females in the 1000 mg/kg group, significantly low values compared to that of the control group w ere observed on day 15 of administration and on days 1 and 14 of gestation, but since there were no abnormalities in the body weight development in any period, the changes were thought to be of no tox icological significance.

Organ weight findings including organ / body weight ratios
no effects observed

Gross pathological findings
no effects observed

Description (incidence and severity)
The following changes were observed in the organs/tissues listed below, but they were thought to be incidental changes based on the incidence of their occurrence and their pathological profiles:
small testis and epididymis (unilateral) in 1/12 males in the 1000 mg/kg group, white focus in the epididymis in 1/12 males in the 300 mg/kg group, large testis (unilateral) in 1/12 males in the 100 mg/kg group, and diverticulum in the ileum in 1/12 females in the 300 mg/kg group

Histopathological findings: non-neoplastic
no effects observed

Description (incidence and severity)
The some changes were observed in the organs/tissues, but they were thought to be incidental changes based on the incidence of their occurrence and their histopathological profiles.

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/PDF118-82-1c.pdf

Applicant's summary and conclusion

Executive summary

A reproduction/developmental toxicity screening test (OECD TG 421) was conducted to clarify the effects of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol on reproductive and developmental toxicity.

Male rats were treated with 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol at doses of at 0 [vehicle: 0.5% (w/v) methylcellulose solution], 100, 300, and 1,000 mg/kg bw/day for 14 days prior to mating and throughout the mating period until the day before necropsy (42 days), and female rats were treated for 14 days prior to mating and throughout the mating and gestation periods until day 4 of lactation (41–50 days). There were no mortalities with any dose during the treatment period. There were no advserse effects at 1000 mg/kg/day.

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

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Dossier UUID:
Author:
Date: 2022-12-13T17:00:59.643+09:00
Remarks:

Administrative data

Endpoint
in vitro gene mutation study in bacteria

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

A reverse mutation test of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol using bacteria / Ministry of Health, Labour and Welfare(MHLW), Japan / publication

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
other: Study Methods on New Chemical Substances (Chemical Substances Control Law of Japan)

Deviations
no

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
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

Method

Species / strain

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

Species / strain / cell type
E. coli WP2 uvr A
bacteria

Metabolic activation
with and without

Metabolic activation system
S9 mix, SD male rat liver, induced by phenobarbital (PB) and 5,6-benzoflavone (BF)

Test concentrations with justification for top dose
To set the dose levels for the main tests, a total of 8 dose levels were selected (20, 50, 100, 200, 500, 1000, 2000 and 5000 µg/plate) in the dose-selection test.
In the dose-selection test, growth inhibition by the test substance was not observed all dose levels.
Precipitation by the test substance on the plate was observed at 200 µg/plate and above.
Test were set as the highest dose levels 5000 µg/plate for all tested strains with or without metabolic activation, and a total of 6 dose levels were selected using a common ratio of 2.

Vehicle / solvent
acetone

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
9-aminoacridine
sodium azide
other: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) 2-Aminoanthracene (2AA)

Details on test system and experimental conditions
METHOD OF APPLICATION: Preincubation
DURATION
- Preincubation period: 20 min

Exposure duration: ca.48 hours
NUMBER OF REPLICATIONS: 3
DETERMINATION OF CYTOTOXICITY
- Method: Cell growth

Evaluation criteria
If two-fold increase in the number of revertant colonies on the test plates or more was observed in comparison with the number of natural revertant colonies (the negative control) and dose response and reproducibility were noted, or if no clear dose response was observed but there was at least two-fold increase in comparison with the number of natural revertant colonies and reproducibility was obs erved in the two main tests, the test substance was judged to be positive.

Statistics
No statistic method was used for judging of results.

Results and discussion

Test results

Key result
false

Species / strain
S. typhimurium TA 1535
bacteria

Metabolic activation
with and without

Genotoxicity
negative tested up to max concentration

Cytotoxicity / choice of top concentrations
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

True negative controls validity
not examined

Positive controls validity
valid

Key result
false

Species / strain
S. typhimurium TA 1537
bacteria

Metabolic activation
with and without

Genotoxicity
negative tested up to max concentration

Cytotoxicity / choice of top concentrations
cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

True negative controls validity
not examined

Positive controls validity
valid

Key result
false

Species / strain
S. typhimurium TA 98
bacteria

Metabolic activation
with and without

Genotoxicity
negative tested up to max concentration

Cytotoxicity / choice of top concentrations
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

True negative controls validity
not examined

Positive controls validity
valid

Key result
false

Species / strain
S. typhimurium TA 100
bacteria

Metabolic activation
with and without

Genotoxicity
negative tested up to max concentration

Cytotoxicity / choice of top concentrations
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

True negative controls validity
not examined

Positive controls validity
valid

Additional information on results
TEST-SPECIFIC CONFOUNDING FACTORS
- Precipitation: Precipitation was observed on plates with concentration of 200 µg/plate or more with and without metabolic activation.
RANGE-FINDING/SCREENING STUDIES:
In range-finding studies, growth inhibition by the test substance was no observed at all dose 20 µg/plate and above for all tested strains.

COMPARISON WITH HISTORICAL CONTROL DATA:
In all test conditions and in all tested strains, the number of revertant colonies of solvent controls and positive controls were within the range of historical control data.

Any other information on results incl. tables

Tables in English are attached.

Applicant's summary and conclusion

Executive summary

In a bacterial reverse mutation assay using S. typhimuriumTA100, TA1535, TA98, and TA1537 and E.coli WP2uvrA, 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol was negative with or without metabolic activation.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

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Author:
Date: 2020-10-09T15:01:44.000+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
guideline study
Reliability 1

Data source

Reference

Chromosome aberration test in cultured chinese hamster cells treated with 2,2',6,6'-tetra-tert-butyl / Ministry of Health, Labour and Welfare(MHLW), Japan / publication

Data access
data published

Materials and methods

Test guideline

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
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

Method

Species / strain

Species / strain / cell type
Chinese hamster lung (CHL/IU)
mammalian cell line

Metabolic activation
with and without

Metabolic activation system
S9 mix, SD male rat liver, induced by phenobarbital (PB) and 5,6-benzoflavone (BF)

Test concentrations with justification for top dose
+S9 mix(short-term treatment): 0, 250, 500, 1000 µg/mL
-S9 mix(short-term treatment): 0, 250, 500, 1000µg/mL
-S9 mix(24hr-continuous treatment): 0, 250, 500, 1000 µg/mL
-S9 mix(48hr-continuous treatment): 0, 250, 500, 1000µg/ µg/mL

Cell-growth inhibition test was conducted up to the limited concentration of 1000 µg/mL (10 mM)
-Short term treatment and continous treatment, 50% cell-growth inhibition was not observed any dose (0, 250, 500, 1000 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
N-ethyl-N-nitro-N-nitrosoguanidine
benzo(a)pyrene

Details on test system and experimental conditions
METHOD OF APPLICATION:

Exposure duration: [continuous treatment]: 24, 48 hrs [short-term treatment]:6 hrs + 18 hr
SPINDLE INHIBITOR: Colcemid
NUMBER OF REPLICATIONS: 2
NUMBER OF CELLS EVALUATED: 200 cells / dose
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 cells with chromosomal aberrations: Negative (-): < 5%; equivocal (±): 5 -10% ; positive (+): > 10%.
Finally, the substance is positive when the incidence is considered to be dose-related and reprod ucible.

Statistics
no

Results and discussion

Test results

Key result
false

Species / strain
Chinese hamster lung (CHL/IU)
mammalian cell line

Metabolic activation
with and without

Genotoxicity
negative :tested up to max concentration

Cytotoxicity / choice of top concentrations
no cytotoxicity

Vehicle controls validity
valid

Untreated negative controls validity
not examined

True negative controls validity
not examined

Positive controls validity
valid

Any other information on results incl. tables

Tables in English are attached.

Applicant's summary and conclusion

Executive summary

It was concluded that 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol is negative for chromosome numerical aberration and chromosome structural aberration under the conditions of this study.

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction.001

UUID: 937c2fdf-81ea-4c17-90e5-a51010a56e45
Dossier UUID:
Author:
Date: 2020-10-09T11:16:03.000+09:00
Remarks:

Administrative data

Endpoint
screening for reproductive / developmental toxicity

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.002 / 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1 / 4,4'-methylenebis(2,6

Remarks
Repeated dose toxicity: oral.002

Data source

Reference

A reproduction/developmental toxicity screening test in rats treated orally with 2,2',6,6'-tetra-ter / Ministry of Health, Labour and Welfare (MHLW), Japan / publication

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

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

Deviations
no

GLP compliance
yes

Test material

Specific details on test material used for the study
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

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 Laboratories Japan, Inc. Atsugi
- Age at study initiation: 10 weeks
- Weight at study initiation: Males: 392 - 449g; Females: 228 - 274g
- Housing: bracket-type metallic wire-mesh cages (W 254 × D 350 × H 170 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 18 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22-25 (acceptable range:23±3 °C)
- Humidity (%): 38-64 (acceptable range:50±20 %)
- Air changes: 10-15 times / hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
other: 0.5 w/v% methylcellulose solution

Details on mating procedure
M/F ratio per cage:1:1
- Length of cohabitation:up to 14 days
- Proof of pregnancy: [vaginal plug / sperm in vaginal smear] referred to as [day 0] of pregnancy

Analytical verification of doses or concentrations
yes

Details on analytical verification of doses or concentrations
Test solutions were prepared at least once in 8 days and used within 7 days of preparation.
The test suspensions at all dose concentrations to be used for administration in week 1 or week 6 of administration, dose concentrations and homogeneity were verified by the HPLC method at Gotemba Laboratory, Bozo Research Center Inc. before use for administration.
The results showed that the concentrations were 97.5 to 103.5% and the coefficient of variation in the range from 0.5 to 4.6%, both of which were within the acceptable range (concentration: within 100.0 ± 10.0% of the nominal value, homogeneity: CV within 10.0%)

Duration of treatment / exposure
Males: 42 days (14 days prior to mating, 14 days during the mating period and 14 days after the end of the mating period),
Females: 41 to 50 days (14 days prior to mating and throughout the mating and gestation periods until day 4 of lactation)

Non-pregnant females: 53 days

Frequency of treatment
once a day (7 times/week)

Doses / concentrations

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

No. of animals per sex per dose
12 animals/sex/dose

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, “A 14-day oral gavage toxicity study of 2,2',6,6'-tetra- tert-butyl-4,4'-methylenediphenol in rats (dose-finding study)”). In that study, there were no toxic changes at 1000 mg/kg, which is the limit test dose prescribed in the OECD Guidelines for Testing of Chemicals 421. Based on these results, the high d ose in this reproduction/developmental toxicity screening test was set at 1000 mg/kg, and the lower doses were set at 300 and 100 mg/kg using the common ratio of approximately 3.

- Rationale for animal assignment (if not random): Body weight-balanced randomization
- Post-exposure recovery period in satellite groups: 14 days

Examinations

Parental animals: Observations and examinations
Males and females: 3 times/day during the administration period (before and after dosing)

Observation for completion of delivery (measurement and observation on day 0 of lactation) was con ducted twice daily from day 21 of gestation to day 25 of gestation.

Oestrous cyclicity (parental animals)
yes

Sperm parameters (parental animals)
no

Litter observations
no

Postmortem examinations (parental animals)
Necropsy:yes
[Detailed macroscopic examination: including the external appearance, head, thorax and abdomen]
histopathology: yes
[testes, epididymides, ovaries, uterus, prostate, seminal vesicle, vagina, macroscopic lesions]

Postmortem examinations (offspring)
yes
examine for abnormalities in organs/tissues in the external, thoracic and abdominal regions.

Statistics
mean value with standard deviation : [Body weight, body weight gain (from day 1 to day 42 of adminis tration for males, from day 1 to day 15 of administration, from day 0 to day 20 of gestation, and from day 0 to day 4 of lactation for females, and from day 0 to day 4 after birth for liveborn pups), food consumption, number of estruses, estrous cycle, number of elapsed days until copulation, length of gestation period, number of corpora lutea, number of implantation sites, number of pups alive, sex ratio (on day 0 and day 4 after birth) and organ weight (including body weight at necropsy)]

group mean with standard deviation was calculated and subjected to a Dunnett-type mean rank test for the difference in mean rank (levels of significance: 0.05 and 0.01, two-tailed): [stillbirth index, in dex of external abnormalities, live birth index and viability index on day 4 after birth]
chi-square test with Yates’ continuity correction (levels of significance: 0.05 and 0.01, two-tailed): [the copulation index, insemination index, fertility index and delivery index, the number of copulated ani mals, number of males impregnated females, number of pregnant females and the number of females that delivered liveborn pups were calculated for each group]

If there were cells whose expected frequency was 5 or below, data were analyzed by Fisher's exact test (levels of significance: 0.05 and 0.01, two-tailed)

Reproductive indices
Copulation index (%) = (No. of copulated animals / No. of animals housed together) × 100
Insemination index (%) = (No. of males impregnated females / No. of copulated males) × 100
Fertility index (%) = (No. of pregnant females / No. of copulated females) × 100
Gestation period (days) = Number of days from day 0 of gestation to the day of delivery
Delivery index (%) = (No. of females delivered liveborn pups / No. of pregnant females) × 100
Implantation index (%) = (No. of implantation sites / No. of corpora lutea) × 100
Stillbirth index (%) = (No. of stillborn pups / Total No. of liveborn and stillborn pups) × 100
Index of external abnormalities (%) = (No. of pups with external abnormalities / No. of liveborn pups) × 100
Live birth index (%) = (No. of liveborn pups / No. of implantation sites) × 100
Sex ratio on day 0 after birth = No. of liveborn males / No. of liveborn pups
Sex ratio on day 4 after birth = No. of males alive on day 4 after birth / No. of pups alive on day 4 after birth

Offspring viability indices
Viability index on day 4 after birth (%) = (No. of surviving pups on day 4 after birth / No. of liveborn pups) × 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

Description (incidence and severity)
In females in the 1000 mg/kg group, significantly low values compared to that of the control group w ere observed on day 15 of administration and on days 1 and 14 of gestation, but since there were no abnormalities in the body weight development in any period, the changes were thought to be of no tox icological significance.

Haematological findings
not examined

Clinical biochemistry findings
not examined

Urinalysis findings
not examined

Behaviour (functional findings)
not examined

Organ weight findings including organ / body weight ratios
no effects observed

Gross pathological findings
no effects observed

Description (incidence and severity)
The following changes were observed in the organs/tissues listed below, but they were thought to be incidental changes based on the incidence of their occurrence and their pathological profiles:
small testis and epididymis (unilateral) in 1/12 males in the 1000 mg/kg group, white focus in the epididymis in 1/12 males in the 300 mg/kg group, large testis (unilateral) in 1/12 males in the 100 mg/kg group, and diverticulum in the ileum in 1/12 females in the 300 mg/kg group

Histopathological findings: non-neoplastic
no effects observed

Description (incidence and severity)
The some changes were observed in the organs/tissues, but they were thought to be incidental changes based on the incidence of their occurrence and their histopathological profiles.

Reproductive function / performance (P0)

Reproductive function: oestrous cycle
no effects observed

Reproductive function: sperm measures
not examined

Reproductive performance
no effects observed

Effect levels (P0)

Key result
false

Dose descriptor
NOAEL

Effect level

1000 mg/kg bw/day (actual dose received)

Based on
test mat.

Sex
male/female

Remarks on result
other: no effects on reproduction

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Mortality / viability
mortality observed, non-treatment-related

Description (incidence and severity)
The number of pups that died during the lactation period was 7 in the control group, and 1, 2 and 9 in the 100, 300 and 1000 mg/kg groups, respectively, and there was no significant difference in the viability index on day 4 after birth between the control group and any test article administration group.

Body weight and weight changes
no effects observed

Effect levels (F1)

Key result
false

Dose descriptor
NOAEL

Generation
F1

Effect level

1000 mg/kg bw/day (actual dose received)

Based on
test mat.

Sex
male/female

Remarks on result
other: no effect on development

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/PDF118-82-1c.pdf

Applicant's summary and conclusion

Conclusions
The NOAEL of 4,4'-methylenebis(2,6-di-tert-butylphenol) for reproductive and developmental toxicity was determined to be 1,000 mg/kg/day (the highest dose tested).

Executive summary

A reproduction/developmental toxicity screening test (OECD TG 421) was conducted to clarify the effects of 4,4'-methylenebis(2,6-di-tert-butylphenol) on reproductive and developmental toxicity. Male

rats were treated with 4,4'-methylenebis(2,6-di-tert-butylphenol) at doses of at 0 [vehicle: 0.5% (w/v) methylcellulose solution], 100, 300, and 1,000 mg/kg bw/day for 14 days prior to mating and throughout the mating period until the day before necropsy (42 days), and female rats were treated for 14 days prior to mating and throughout the mating and gestation periods until day 4 of lactation (41–50 days). There were no mortalities with any dose during the treatment period. There were no effects on reproductive toxicity (fertility and reproductive organs) and developmental toxicity up to the highest dose. The NOAEL of 4,4'-methylenebis(2,6-di-tert-butylphenol) for reproductive and developmental toxicity was determined to be 1,000 mg/kg/day (the highest dose tested).

DOMAIN

Substance

SUBSTANCE: 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

UUID: c4a3c724-6cba-4624-ae5f-b2bd2dc4cfc9
Dossier UUID:
Author:
Date: 2022-12-13T17:16:54.832+09:00
Remarks:

Substance name
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 118-82-1

Legal entity
National Institute of Health Sciences / Kawasaki / Japan

Identification of substance

Reference substance
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol / 4,4'-methylenebis(2,6-di-tert-butylphenol) / 118-82-1 / 204-279-1

EC number	EC name
204-279-1	EC Inventory

CAS number	CAS name
118-82-1	

IUPAC name
4,4'-methylenebis(2,6-di-tert-butylphenol)

Role in the supply chain

Manufacturer
false

Importer
false

Only representative
false

Downstream user
false

References

Reference Substances

REFERENCE_SUBSTANCE: 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

UUID: ECB5-40091664-5cd2-4b47-95b6-198f2e35e7cc
Dossier UUID:
Author:
Date: 2007-05-10T18:00:00.000+09:00
Remarks:

Reference substance name
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

IUPAC name
4,4'-methylenebis(2,6-di-tert-butylphenol)

Inventory

Inventory number

Inventory name
2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

Inventory
EC Inventory

Inventory number
204-279-1

CAS number
118-82-1

Molecular formula
C29H44O2

Description

CAS number
118-82-1

Synonyms

Synonyms

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

Identity
Phenol, 4,4'-methylenebis[2,6-bis(1,1-dimethylethyl)-

Identity
Phenol, 4,4'-methylenebis[2,6-bis(1,1-dimethylethyl)-

Molecular and structural information

Molecular formula
C29H44O2

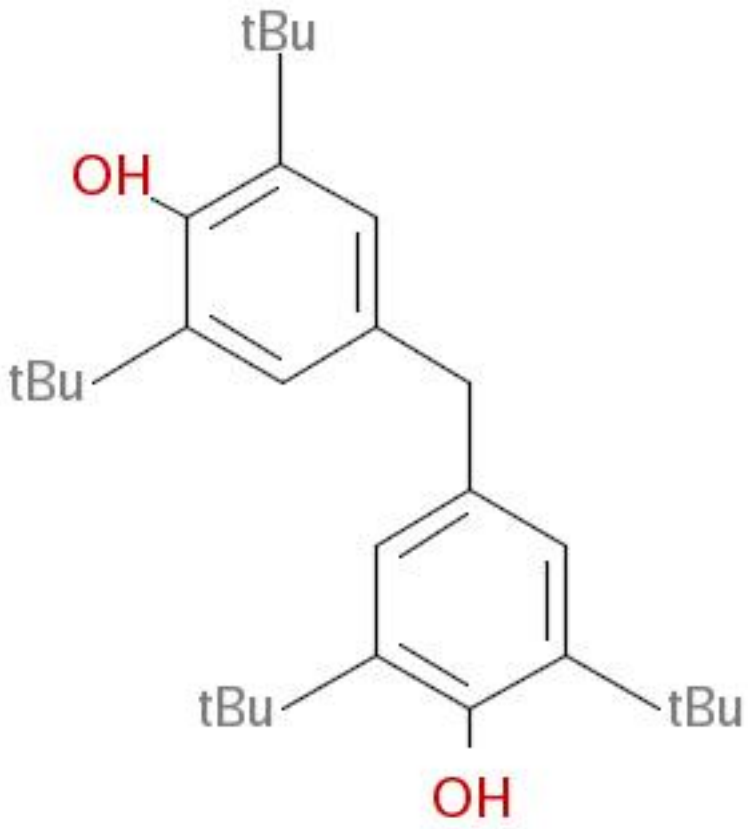
Molecular weight

424.6585

SMILES notation
CC(C)(C)c1cc(Cc2cc(c(O)c(c2)C(C)(C)C)C(C)(C)C)cc(c1O)C(C)(C)C

InChI
InChI=1/C29H44O2/c1-26(2,3)20-14-18(15-21(24(20)30)27(4,5)6)13-19-16-22(28(7,8)9)25(31)23(17-19)29(10,11)12/h14-17,30-31H,13H2,1-12H3

Structural formula



Related substances

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

Literatures

LITERATURE: A 28-day repeat dose oral toxicity study of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol in rat

UUID:	f37fd311-479b-4fca-b7b9-0ac8548efa1e
Dossier UUID:	
Author:	
Date:	2019-05-22T11:25:03.000+09:00
Remarks:	

General information

Reference Type
publication

Title
A 28-day repeat dose oral toxicity study of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol in rat

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

Year
2010

LITERATURE: A reproduction/developmental toxicity screening test in rats treated orally with 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

UUID: 94cbeb6e-5aa1-4b9f-b4d8-92b4f7e5e123
Dossier UUID:
Author:
Date: 2019-03-27T06:49:02.000+09:00
Remarks:

General information

Reference Type
publication

Title
A reproduction/developmental toxicity screening test in rats treated orally with 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

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

Year
2012

LITERATURE: A reverse mutation test of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol using bacteria

UUID:	7620b1e7-84d8-4e14-b39e-a28aca544fbe
Dossier UUID:	
Author:	
Date:	2019-03-27T05:44:24.000+09:00
Remarks:	

General information

Reference Type
publication

Title
A reverse mutation test of 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol using bacteria

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

Year
2010

LITERATURE: Chromosome aberration test in cultured chinese hamster cells treated with 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

UUID: f94b33a7-1c0b-4d80-a883-4d98ff599dd4
Dossier UUID:
Author:
Date: 2019-05-22T11:37:43.000+09:00
Remarks:

General information

Reference Type
publication

Title
Chromosome aberration test in cultured chinese hamster cells treated with 2,2',6,6'-tetra-tert-butyl -4,4'-methylenediphenol

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

Year
2010

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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210-9501

Town
Kawasaki

Region / State
Kanagawa

Country
Japan
JP

Identifiers

Other IT system identifiers

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

ID
16558402024DIV750