Name: COMPLETE / SUBSTANCE : N-ethyl-1-aminonaphthalene / 118-44-5 / N-ethylnaphthalen-1-amine / 118-44-5 Fri, 16 Dec 2022, 11:01:09+0900 /

Legal entity owner: National Institute of Health Sciences

Printing date: 2022-12-16T11:01:10.640+09:00

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DOSSIER:

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Author:
Date: 2022-12-16T11:01:09.379+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
N-ethyl-1-aminonaphthalene / 118-44-5 / N-ethylnaphthalen-1-amine / 118-44-5

Public name

Submitting legal entity
National Institute of Health Science

Dossier creation date/time
Fri, 16 Dec 2022, 11:01:09+0900

Used in category

LEGAL_ENTITY: National Institute of Health Science

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Author:	
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Remarks:	

General information

Legal entity name
National Institute of Health Science

N-ethyl-1-aminonaphthalene / 118-44-5

OECD

Health Effects

Repeated dose toxicity: oral

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.001

UUID: 6f1c25d9-7cc0-467f-b93b-1058daec97f4
Dossier UUID:
Author:
Date: 2022-12-16T11:00:04.374+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 oral toxicity study of ethyl (1-naphthyl) amine in rats with a recovery period of 2 weeks / 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)

GLP compliance
yes

Test material

Specific details on test material used for the study
Name of test material (as cited in study report): Ethyl (1-naphthyl) amine

Test animals

Species
rat
common rodent species

Strain
other: Sprague-Dawley strain SPF rats [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: 6 weeks
- Weight at study initiation: Males: 211-233 g; Females: 144-169 g
- Housing: bracket-type metallic wire-mesh cages (W 250 × D 350 × H 200 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 9 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22-26 (acceptable range:23±3°C)
- Humidity (%): 39-56 (acceptable range:50±20°C)
- Air changes: 10-15 times / hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
corn oil

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

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 to be used for week 1 or week 4 of administration were analyzed for concentration by HPLC method at Gotemba Laboratory, Bozo Research Center Inc.
The results showed that the concentrations were 98.3 to 103.5% of the nominal concentrations (acceptable range: 100 ± 10% of the nominal value), which were all within the acceptable range.

Duration of treatment / exposure
28 days

Frequency of treatment
Once/day, 7 days/week

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
12	mg/kg bw/day (actual dose received)
Dose / conc.	
60	mg/kg bw/day (actual dose received)
Dose / conc.	
300	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
6 animals/sex/dose as a main dose group,
6 males and 6 females at 0 and 300 mg/kg bw/day as a recovery group (without mating)

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 s etting study: a 14-day repeated dose oral toxicity test (doses: 0, 300, and 1000 mg/kg bw/day).
The main changes observed were death of all males and all females at 1000 mg/kg bw/day and changes in hematological examination etc. in males and females in the 300 mg/kg bw/day group.
Therefore, the high dose in this study was set at 300 mg/kg bw/day, with a middle dose of 60 mg/kg bw/day group and a low dose of 12 mg/kg bw/day, using the common ratio of approximately 5.

- Rationale for animal assignment : Body weight-balanced randomization

- Post-exposure recovery period in recovery 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) , once a day during the recovery period.

DETAILED CLINICAL OBSERVATIONS: yes

BODY WEIGHT: Yes
Time schedule for examinations: Males /females (main/recovery group): All animals were weighed before administration on days 1, 4, 7, 10, 14, 17, 21, 24 and 28 of administration and on days 1, 3, 7, 10 and 14 of recovery, and the day of necropsy (after ca. 16h-fasting).

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):yes
Males /females (main/recovery group): on days 1, 7, 14, 21, and 28 during the administration period, and on days 7 and 14 during the recovery period.

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: Blood was collected on the day of necropsy
- Anaesthetic used for blood collection: Yes (ether)
- Animals fasted: Yes, 16-20h
- How many animals: all animals , 6 sex/dose/group

CLINICAL CHEMISTRY: Yes
Time schedule for collection of blood: Same as hematology
- Animals fasted: Same as hematology
- How many animals: Same as hematology
- Parameters checked in table were examined.

URINALYSIS: Yes
Time schedule for collection of urine: on week 4 of administration period (main/recovery group animals) and week 2 of recovery period (recovery group animals).
- Metabolism cages used for collection of urine: Yes
- Animals fasted: fasting : 4-hour urine samples were collected from animals under fasting but with free access to water,
no fasting : 20-hour urine samples from animals with free access to food and water.
- Parameters checked in table were examined.

Sacrifice and pathology
Detailed macroscopic examination : tissues in the whole body, including the external appearance and those in the cephalic, thoracic and abdominal cavities.

Weight (absolute weight), and the relative weight per 100 g body weight : Brain, adrenals, thymus, spleen, heart, liver, kidneys, testes, epididymides, ovaries, uterus

histopathology: yse
Cerebrum, cerebellum, spinal cord (thoracic), sciatic nerve, eyeballs, pituitary, thyroids, parathyroids, adrenals, thymus, spleen, submandibular lymph node, mesenteric lymph node, heart, trachea, lung (including bronchus), stomach, duodenum, jejunum, ileum (including Peyer’s patches), cecum, colon,

rectum, liver, kidneys, urinary bladder, testes, epididymides, prostate, ovaries, uterus, sternum (including bone marrow), femur (including bone marrow), and femoral skeletal muscle

Statistics
Quantitative items of open field observation, quantitative items of manipulative test, measurements of grip strength and motor activity, body weight (including body weight gain), food consumption, water intake and quantitative items of urinalysis as well as data from hematology and blood chemistry and organ weight data were statistically analyzed between the control and each dose group.
An analysis of variance was conducted by the Bartlett test (level of significance: 1%, two-tailed).
Homogeneous data were analyzed by the Dunnett test while heterogeneous data were analyzed by a Dunnett-type mean rank test between the control and each dose group (levels of significance: 5 and 1%, two-tailed).
For the recovery groups, homogeneity of variance was tested for each group by the F-test (level of significance: 5%, one-tailed). For homogeneous data, the difference in the mean values between the control and treatment groups was analyzed by Student's t-test (levels of significance: 5 and 1%, two-tailed) while heterogeneous data were analyzed by the Aspin-Welch t-test (levels of significance: 5 and 1%, two-tailed)

Results and discussion

Results of examinations

Clinical signs
no effects observed

Description (incidence and severity)
Administration Period :
One female in the 300 mg/kg bw/day group was observed decreased spontaneous movement, soft feces and brown urine, and died on day 4 of administration.
Surviving animals of both sexes showed brown urine during the administration period in the 60 mg/kg bw/day and above groups.
Soft feces were observed sporadically on days 3 to 6 administration in males and females in the 300 mg/kg bw/day group.
Recovery Period :
Brown urine was observed on day 1 of recovery in all males and all females in the 300 mg/kg bw/day group .

These changes were considered to be spontaneous because this change was recovered by withdraw.

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)
Administration Period:
A significantly high value was observed on day 21 of administration in females in the 60 mg/kg bw/day group and a significantly low value was observed on day 7 of administration in males and females in the 300 mg/kg bw/day group and on day 14 of administration in males in the 300 mg/kg bw/day group.
Recovery Period:

A significantly high value was observed on day 14 of recovery in males in the 300 mg/kg bw/day gr oup and throughout the recovery period in females in the 300 mg/kg bw/day group.

These changes were considered to be spontaneous because changes were not dose dependent.

Haematological findings
effects observed, treatment-related

Description (incidence and severity)
End of Administration Period:
Significantly low values for red blood cell count and hemoglobin and a significantly high value for reticulocyte percentage were observed in females in the 60 mg/kg bw/day and above groups and in males in the 300 mg/kg bw/day group, a significantly low value for hematocrit was observed in females in the 60 mg/kg bw/day and above groups, significantly high values for mean corpuscular hemoglobin and a significantly low value for fibrinogen was observed in males in the 300 mg/kg bw/ day group, a significantly low value for mean corpuscular hemoglobin concentration was observed in males and females in the 300 mg/kg bw/day group, a significantly reduced value for activated par tial thromboplastin time was observed in females in the 60 mg/kg bw/day group, a significantly high value for the index of lymphocytes and a significantly low value for the index of neutrophils were o bserved in males in the 12 mg/kg bw/day group, a significantly low value for the index of eosinophils was observed in females in the 300 mg/kg bw/day group in differential leukocyte counts and signifi cantly low values for neutrophil count and monocyte count were observed in males in the 60 mg/kg bw/day group in differential count of leukocytes.
End of Recovery Period:
A significantly low value for mean corpuscular hemoglobin concentration was observed in males and females in the 300 mg/kg bw/day group, a significantly low value for reticulocyte percentage was obs erved in females in the 300 mg/kg bw/day group, and a significantly high value for the index of neut rophils in differential leukocyte counts and a significantly high value for the index of neutrophils was observed in males in the 300 mg/kg bw/day group in differential count of leukocytes.

Clinical biochemistry findings
effects observed, treatment-related

Description (incidence and severity)
End of Administration Period:
A significantly low value for AST was observed in males and females in the 12 mg/kg bw/day group, a significantly low value for glucose was observed in males in the 12 and 300 mg/kg bw/day groups, significantly high values for ALT and LDH were observed in males in the 300 mg/kg bw/day group, significantly high values for total cholesterol and phospholipids were observed in males and females in the 60 mg/kg bw/day and above groups, a significantly low value for creatinine was observed in males and females in the 300 mg/kg bw/day group, a significantly low value for potassium was obse rved in females in the 12 mg/kg bw/day group, a significantly high value for calcium was observed in females in the 12 mg/kg bw/day and 300 mg/kg bw/day groups and a significantly high value for total protein was observed in females in the 300 mg/kg bw/day group.

End of Recovery Period:
A significantly high value for albumin was observed in males in the 300 mg/kg bw/day group, and significantly low values for sodium and total protein and a significantly high value for inorganic phosp horus were observed in females in the 300 mg/kg bw/day group.

Urinalysis findings
effects observed, treatment-related

Description (incidence and severity)
Week 4 of Administration:
Brown urine was observed in 3/6 males and 4/6 females in the 60 mg/kg bw/day group, and in all males and all females in the 300 mg/kg bw/day group. In the qualitative items, bilirubin was + (0.5

to 1.5 mL/dL) in 1/12 males in the control group and in 1/6 males in the 12 mg/kg bw/day group, 2+ (1.6 to 5.0 mL/dL) in 1/6 males and 3/6 females in the 12 mg/kg bw/day group, 3+ (5.1 to 10.0 mL/dL) in 3/6 males and 3/6 females in the 12 mg/kg bw/day group and in 1/6 males and 1/6 females in the 60 mg/kg bw/day group, and 4+ (>10.0 mL/dL) in 1/6 males in the 12 mg/kg bw/day group, in 5/6 males and 5/6 females in the 60 mg/kg bw/day group and in all males and all females in the 300 mg/kg bw/day group, and thus bilirubin-positive animals showed an upward trend in males and females in the 12 mg/kg bw/day and above groups. Also, significantly high values for water intake and urine volume and a significantly low value for osmolality were observed in males and females in the 300 mg/kg bw/day group.
Week 2 of Recovery:
There were no abnormalities in the qualitative items in either sex in the 300 mg/kg bw/day group and there were no significant differences in urinary volume, water intake, or osmolality from the control group.

Behaviour (functional findings)
effects observed, treatment-related

Description (incidence and severity)
A significantly low value was observed for 0-10 minutes after starting measurement in males in the 300 mg/kg bw/day group.

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

Description (incidence and severity)
body weight ratios:
Administration Period :
Decreased body weight was observed sporadically in males and females on day 4 administration, and significantly low values were observed in males from day 4 of administration and in females on days 4, and 7 of administration in the 300 mg/kg bw/day group. Also, a significantly high value of body weight gain during the administration period was observed in females in the 12 mg/kg bw/day group and a significantly low value of body weight gain during the administration period was observed in males in the 300 mg/kg bw/day group.
Recovery Period :
Significantly low values were observed throughout the recovery period in males in the 300 mg/kg bw/day group and on days 1 and 3 of recovery in females in the 300 mg/kg bw/day group. Also, a significantly high value of body weight gain during the recovery period was observed in males and females in the 300 mg/kg bw/day group.

Organ weight:
End of Administration Period :
A significantly low value for final body weight was observed in males in the 300 mg/kg bw/day group.
Brain : A significantly high value for relative weight was observed in males in the 300 mg/kg bw/day group.
Heart : A significantly low value for absolute weight was observed in males in the 300 mg/kg bw/day group.
Liver : A significantly high value for relative weight was observed in males in the 300 mg/kg bw/day group and significantly high values for absolute and relative weight were observed in females in the 300 mg/kg bw/day group.
Spleen: A significantly high value for relative weight was observed in males and females in the 300 mg/kg bw/day group.
Kidney : A significantly low value for relative weight was observed in females in the 12 mg/kg bw/day group and significantly high values for absolute and relative weight were observed in males and females in the 300 mg/kg bw/day group.
Testis : A significantly high value for relative weight was observed in the 300 mg/kg bw/day group.
Uterus: A significantly high value for absolute weight was observed in the 12 and 60 mg/kg bw/day groups.

End of Recovery Period :

Liver: A significantly low value for absolute weight was observed in males in the 300 mg/kg bw/day group.
Spleen: A significantly high value for relative weight was observed in males in the 300 mg/kg bw/day group.

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

Description (incidence and severity)
Dead animal (1 female of the 300 mg/kg bw/day group) :
External appearance : Undernourishment was observed.
Lung : Dark discoloration and dark red focus were observed.
Stomach : Distention of forestomach was observed.

End of Administration Period :
Kidney : White focus was observed in 1/6 males in the 60 mg/kg bw/day group.
Lung : Dark red focus was observed in 1/6 males in the 60 mg/kg bw/day group.
Spleen : Dark discoloration was observed in 3/5 females in the 300 mg/kg bw/day group, and en largement was observed in 1/5 females in the 300 mg/kg bw/day group.

End of Recovery Period :
Spleen : Raised focus was observed in 1/6 males in the 300 mg/kg bw/day group.

Effect levels

Key result
false

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
clinical biochemistry
At 60 mg/kg bw/day and higher in female, 300 mg/kg bw/day in male, blood chemistry changes were observed. At 60 mg/kg bw/day and higher in both males and females, histopathological changes in the spleen, etc. were observed. All changes showed disappearance or abatement by cessation of the substance, and thus recovery was observed.
histopathology: non-neoplastic
At 60 mg/kg bw/day and higher in both males and females, histopathological changes in the spleen, etc. were observed. All changes showed disappearance or abatement by cessation of the substance, and thus recovery was observed.

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/PDF118-44-5b.pdf

Applicant's summary and conclusion

Conclusions
The NOAEL of N-ethyl-1-aminonaphthalene was considered to be 12 mg/kg bw/day due to the results of hematological toxicity with related histopathological changes in the spleen observed with 60 mg/kg bw/day.

Executive summary

A 28-day oral toxicity study (OECD TG407) of N-ethyl-1-aminonaphthalene was conducted in rats (6 or 12/dose/sex). Dose levels were set at 0 (corn oil), 12, 60, and 300 mg/kg bw/day. Treatment was withdrawn for 2 weeks after the end of the administration period to examine the reversibility of the toxic effects, using six animals/sex in the control and 300 mg/kg bw/day groups.

One female in the 300 mg/kg bw/day group died on day 4 of treatment after showing decreased locomotor activity, soft feces, and brown urine. Clinical observation of the survivors revealed brown urine in the males and females in the ≥60 mg/kg bw/day groups, and soft feces in males and females in the 300 mg/kg bw/day group. Low motor activity was observed in males in the 300 mg/kg bw/day group. Body weight and food consumption in both sexes and body weight gain in males were decreased in the 300 mg/kg bw/day group. High values were observed for water intake and urine volume, and low values were observed for osmolality in both sexes at 300 mg/kg bw/day. Hematological toxicity, low values for red blood cell count, hemoglobin, and hematocrit, and/or a high values for reticulocyte percentage, were observed at doses of ≥60 mg/kg bw/day in females and 300 mg/kg bw/day in males.

Blood chemistry analysis showed high levels of total cholesterol and phospholipid compared with the control group in both sexes treated with 60mg/kg bw/day. Treatment with 300 mg/kg bw/day led to low levels of creatinine in both sexes, high levels of alanine transaminase and lactate dehydrogenase in males, and a high levels of total protein in females compared with the control groups. The relative weight of the kidney was increased in both sexes at 300 mg/kg bw/day. Microscopic evaluation showed increased mild extramedullary hematopoiesis in males, and hemosiderin deposition (slight/mild Berlin-blue positive granules) in the spleens of both sexes treated with ≥60 mg/kg bw/day. Hypertrophy of centrilobular hepatocytes of bile duct epithelial cells in the liver was observed in males of the 60 and 300 mg/kg bw/day groups and in females of the 300 mg/kg bw/day group. Increased erythropoiesis was observed in the femurs of males and females and in the sternum in males treated with 300 mg/kg bw/day. These changes were either reduced or no longer observed following withdrawal of treatment. The NOAEL of N-ethyl-1-aminonaphthalene was considered to be 12 mg/kg bw/day due to the results of hematological toxicity with related histopathological changes in the spleen observed with 60 mg/kg bw/day.

ENDPOINT_STUDY_RECORD: Repeated dose toxicity: oral.002

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Dossier UUID:	
Author:	
Date:	2022-12-16T10:57:32.439+09:00
Remarks:	

Administrative data

Endpoint
repeated dose toxicity: oral, other A reproduction/developmental toxicity screening test in rats treated orally

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 / N-ethyl-1-aminonaphthalene / 118-44-5 / N-ethylnaphthalen-1-amine / 118-44-5

Remarks
Toxicity to reproduction.001

Data source

Reference

A reproduction/developmental toxicity screening test in rats treated orally with ethyl (1-naphthyl) / Ministry of Health, Labour and Welfare (MHLW), Japan / publication

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
other: Guideline for reproduction/developmental toxicity screening test in rats (Chemical Substa nces Control Law of Japan)

Deviations
no Housing pressure temporarily fall slightly below the prescribed range. (<30Pa) However, there was no influx of outside air and no abnormalities were observed in the general condition of the animals.

GLP compliance
yes

Test material

Specific details on test material used for the study
Name of test material (as cited in study report): Ethyl (1-naphthyl) amine

Test animals

Species
rat
common rodent species

Strain
other: Sprague-Dawley strain SPF rats [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: 9 weeks
- Weight at study initiation: Males: 339.7-390.2 g, Females: 223.2-254.7 g
- Housing: bracket-type metallic wire-mesh cages (W 291 × D 263 × H 180 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period:15 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21.7-23.2
- Humidity (%): 44.8-62.0
- Air changes: 12 times and over/ hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Details on route of administration
PREPARATION OF DOSING SOLUTIONS: Test substance was dissolved in corn oil for injection.

Vehicle

- Name: Corn oil
- Lot Number: WEK6144, WEF2972
- Manufacturer: Wako pure Chemical Industries, Ltd.
- Storage Conditions: Room temperature

Vehicle
corn oil

Analytical verification of doses or concentrations
yes

Details on analytical verification of doses or concentrations
According to publication “A 28-day oral toxicity study of ethyl (1-naphthyl) amine in rats with a re covery period of 2 weeks, Ministry of Health, Labour and Welfare (MHLW), Japan”
Test solutions were prepared at once in administration period.
The test solutions to be used for Day-1 or last day of administration were analyzed for concentration by HPLC method.
The results showed that the concentrations were 95.6 to 100.1% of the nominal concentrations (acce ptable range: 100 ± 5% of the nominal value), which were all within the acceptable range.

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating and mating periods, and thereafter 14 days
(P) Females: 14 days pre-mating, mating and gestation periods, and the days until day 3 of lactation.

Frequency of treatment
once a day

Doses / concentrations

Dose / conc.	
0	mg/kg bw/day (actual dose received)
Dose / conc.	
12	mg/kg bw/day (actual dose received)
Dose / conc.	
60	mg/kg bw/day (actual dose received)
Dose / conc.	
150	mg/kg bw/day (actual dose received)
Remarks	
from day 14	
Dose / conc.	
300	mg/kg bw/day (actual dose received)
Remarks	
until day-13 (highest dose was reduced to 150 mg/kg bw/day from day 14 because of high mor tality)	

No. of animals per sex per dose
12 animals/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 study: 28-day repeated dose oral toxicity test (doses: 0, 12, 60, 150, and 300mg/kg bw/day).

At 300 mg/kg bw/day, loose stools, decrease of body weights and decrease in food consumption were observed in both sexes of the administration period.
At 300 mg/kg bw/day, one female died on day 4 of the administration.
At 60 mg/kg bw/day and higher, brown urine was observed in both sexes of the administration period.
At 300 mg/kg bw/day, 8 males and 5 females died on day 7 of administration.
The dose was 150 mg/kg bw/day from day 14 of administration to ensure the number of studies in the 300 mg/kg bw/day group.

Therefore, the high dose in this study was set at 300 mg/kg bw/day, with a middle dose of 60 mg/kg bw/day group and a low dose of 12 mg/kg bw/day, using the common ratio of approximately 5.

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

Examinations

Observations and examinations performed and frequency
CAGE SIDE OBSERVATIONS:Yes
Males and females: 2 times/day during the administration period (before and after dosing) , and once on the day of necropsy.

DETAILED CLINICAL OBSERVATIONS: Yes
BODY WEIGHT: Yes

male: Days 0, 7, 14, 21, 28, 35 and 42(the day of necropsy),
female: Days 0, 7 and 14 of administration period, days of 0, 7, 14 and 20 of gestation, days of 0 and 4 of lactation period
Infertile female : Days 21

weight gain:
male : from day 0 to day 42
female : from day 0 to day 14, days from 0 to 20 of gestation, days from 0 to 4 of lactation period

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):yes
male : Days 0, 7, 14, 21, 28, 35 and 42(the day of necropsy)
female : Days 0, 7, 14 and days 0, 7, 14, 18 and 20 of gestation, days 0 and 4 of lactation period

OPHTHALMOSCOPIC EXAMINATION: yes

HAEMATOLOGY: no

CLINICAL CHEMISTRY: no

URINALYSIS: no

Sacrifice and pathology
GROSS PATHOLOGY: yes

At day 42 of administration, the rats were exsanguinated under isoflurane anesthesia.
Body surface, spontaneous pores were observed.

Abdominal, thoracic, pelvic and cranial cavities, and systemic organs and tissues were observed.

HISTOPATHOLOGY: yes

Male: prostate, seminal vesicles (including coagulation glands), testis, epididymis, gross abnormal site (lung, liver, spleen, small intestine, kidney)
Female (spontaneous labor): ovary, uterus, vagina, gross abnormal site (lung, liver, spleen, kidney)
Female (not pregnancy):. ovary, uterus, vagina, gross abnormal site (lung, spleen, kidney, tail)

Organ weight
Male: testis, epididymis, prostate and seminal vesicles (including clotting glands)
Female: Ovary

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 ($p<0.05$, two-sided, and $p<0.01$).

Results and discussion

Results of examinations

Clinical signs
effects observed, treatment-related

Mortality
mortality observed, treatment-related

Description (incidence)
In 150 mg/kg bw/day(when highest dose 300 mg/kg bw/day), 8 males and 5 female died.

Body weight and weight changes
effects observed, treatment-related

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

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

Haematological findings
not examined

Clinical biochemistry findings
not examined

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

Gross pathological findings
effects observed, treatment-related

Description (incidence and severity)
At 150 mg/kg bw/day (highest dose : 300 mg/kg bw/day), brown urine, loose stools, and staining and paleness of fur were observed in the dead animals (8 males and 5 females).
Irregular respiration and decreased locomotor activity were observed in males, and perinasal staining was observed in females.

Brown urine, loose stools and pallor were observed in sacrificed rats of the 150 mg/kg bw/day group.

28 In a repeated-day study, 1/12 females died at 300 mg/kg bw/day. Loose stools and decreased loc omotor activity were observed.
These may be treatment-related effects.

Brown urine was observed in the 60 mg/kg bw/day group during treatment.

Brown urine is thought to be the result of urinary excretion of N-ethyl-1-aminonaphthalene or its derived substances because it closely resembles the color of N-ethyl-1-aminonaphthalene.
These may be no treatment-related effects.

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

Description (incidence and severity)
At 150 mg/kg bw/day, hepatocellular steatosis or hepatocyte in liver, splenic hemosiderosis, and pro static inflammatory cellular infiltration were observed in males.

At 150 mg/kg bw/day, hemorrhage in the lungs, accumulation of macrophage , increased extramed ullary hematopoiesis in the spleen or hemosiderosis, and uterine lesions were observed in females.

In the 150 mg/kg bw/day group, the observed inflammatory cellular infiltrate of the prostates and le sions in the uterus after delivery were also observed in the control group. Therefore, this was judged no effect of treatment.

At 150 mg/kg bw/day, testicular tubule atrophy was observed in males that did not become pregnant.

At 150 mg/kg bw/day, the seminiferous tubules of other rats (including those that died) were observed no similar abnormalities and the spermatogenic cycles were similar to those of the control group.

In a 28-day repeated dose study, seminiferous tubules were observed no abnormalities. Therefore, it was judged to not be related to N-ethyl-1-aminonaphthalene administration.

Other findings were not dose-related. Only a few findings were observed.
Therefore, they were judged as incidental changes.
In this pathological examination on the reproductive and accessory genital organs, male and female showed no effects by N-ethyl-1-aminonaphthalene administration.

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/PDF118-44-5c.pdf

Applicant's summary and conclusion

Executive summary

The reproductive and developmental toxicity of N-ethyl-1-aminonaphthalene was investigated in rats in accordance with the OECD TG 421 reproductive/developmental toxicity screening test. Rats were treated with N-ethyl-1-aminonaphthalene via oral gavage at doses of 0 (vehicle: corn oil), 12, 60, or 300 (reduced to 150) mg/kg bw/day. Males (12/dose) were treated for 42 days, including a 14-day premating

period and a subsequent mating period, while females (12/dose) were treated over a 14-day premating, mating, gestation, and lactation period (up to on day 3 of lactation). In the highest dose group, eight males and five females died during the premating period; therefore, dose levels were reduced from 300 mg/kg bw/day to 150 mg/kg bw/day from day 14 of dosing. Prior to death, animals showed loose stools, soiled fur, pale skin, irregular respiration, and decreased locomotor activity. Histopathological examination of the dead animals revealed congestion, hemorrhage, and thrombus in various organs, and phosphotungstic acid–hematoxylin positive staining of eosinophilic material was found in the kidneys and lungs. Cause of death was considered to be circulatory disturbance, presumed to be disseminated intravascular coagulation. In the highest dose group, loose stools, pale skin, and irregular respiration were observed in survivors. Body weight and food consumption were decreased during the premating period in males and females in the highest dose group. Histopathological examination in the highest dose group showed fatty changes and centrilobular hepatocellular hypertrophy, and hemosiderin deposition in the spleen in parental males and hemorrhage and macrophage aggregation in the lung and hemosiderin deposition, and increased extramedullary hematopoiesis in the spleen in parental females. Systemic toxicity observed in this study (TG421) at 300 mg/kg bw/day was more severe than that of the 28-day repeated-dose toxicity study (TG407). Age at administration (6 weeks old vs 9 weeks old; TG407 vs TG421) may affect toxic response.

Genetic toxicity in vitro

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.001

UUID:	2a5d1da6-42ca-4417-9a38-3cbd0cad11d9
Dossier UUID:	
Author:	
Date:	2020-10-09T14:06:26.000+09:00
Remarks:	

Administrative data

Endpoint
in vitro gene mutation study in bacteria

Type of information
experimental study

Adequacy of study
key study

Robust study summary
false

Used for classification
false

Used for SDS
false

Reliability
1 (reliable without restriction)

Rationale for reliability incl. deficiencies
guideline study
Reliability 1

Data source

Reference

A reverse mutation test of N-ethyl-1-aminonaphthalene 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
OECD Guideline 471 (Bacterial Reverse Mutation Assay)

in vitro gene mutation study in bacteria

Deviations
no

Qualifier
according to guideline

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

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
N-ethyl-1-aminonaphthalene / 118-44-5

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, the 50 mg/mL solution was diluted 4 times using a common ratio of 4 and a total of 5 dose levels were selected (19.5, 78.1, 313, 1250 and 5000 µg/plate) in the dose-selection test.
In the dose-selection test, growth inhibition by the test substance was observed at 19.5 µg/plate and above for S. typhimurium TA1537 with metabolic activation, at 78.1 µg/plate and above for S. typhimurium TA1535 without metabolic activation, and at 313 µg/plate and above for S. typhimurium TA100, TA98, TA1537 and E. coli WP2 uvrA without metabolic activation and for S. typhimurium TA100, TA1535, TA98 and E. coli WP2 uvrA with metabolic activation. Precipitation of the test substance on the plate was observed at 5000 µg/plate for treatment without metabolic activation. Coloration by the test substance was not observed at any dose level irrespective of the presence or absence of metabolic activation.
Therefore, in the main tests, the lowest dose levels at which cell growth inhibition was observed in the dose-selection test were set as the highest dose levels 19.5 µg/plate for S. typhimurium TA1537 with metabolic activation, 78.1 µg/plate for S. typhimurium TA1535 without metabolic activation and 313 µg/plate for S. typhimurium TA100, TA98, TA1537 and E. coli WP2 uvrA without metabolic activation

and S. typhimurium TA100, TA1535, TA98 and E. coli WP2 uvrA with metabolic activation, and a total of 6 dose levels were selected by 5-step dilution using a common ratio of 2.

Vehicle / solvent
DMSO

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
other: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-a minopropylamino]acridine 2HCl (ICR-191) 2-Aminoanthracene (2AA)

Positive control substance
sodium azide
benzo(a)pyrene

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

Exposure duration: ca. 50 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. For the results of measu rement, mean with standard deviation was also indicated.

Statistics
No statistic method was used for judging of results.

Results and discussion

Test results

Key result
false

Species / strain
other: S. typhimurium TA100, S. typhimurium TA1535, E. coli WP2 uvrA, S. typhimurium TA98, S. typhimurium TA1537

Metabolic activation
with and without

Genotoxicity
negative

Cytotoxicity / choice of top concentrations
cytotoxicity 9.77 µg/plate for TA1537 with S9mix, 78.1 µg/plate for TA1535 without S9mix, 156 µg/plate for TA100, TA98, TA1537 and E. coli WP2 uvrA without S9mix and for TA100, TA1535 and TA98 with S9mix, 313 µg/plate for E. coli WP2 uvrA with S9mix

Vehicle controls validity
valid

Positive controls validity
valid

Additional information on results
TEST-SPECIFIC CONFOUNDING FACTORS
- Precipitation: Precipitation of the test substance on the plate was observed at 5000 µg/plate for treatment without metabolic activation.
Other effects: Coloration by the test substance was not observed at any dose level irrespective of the presence or absence of metabolic activation.

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 conclusion, N-ethyl-1-aminonaphthalene was judged to have no bacterial reverse mutagenic activity (negative) under the conditions of this study.

ENDPOINT_STUDY_RECORD: Genetic toxicity in vitro.002

UUID:	2364a08b-68c9-43ed-8844-2b7ac30fedea
Dossier UUID:	
Author:	
Date:	2019-05-23T14:01:25.000+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 N-ethyl-1-aminonaphthalene / 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 473 (In Vitro Mammalian Chromosomal Aberration Test)
in vitro cytogenicity / chromosomal aberration study in mammalian cells (from 26 September 2014)

Deviations
no

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
N-ethyl-1-aminonaphthalene / 118-44-5

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
[main test]
+S9 mix(short-term treatment): 0, 4.48, 5.60, 7.00, 8.75, 10.9, 13.7 μg/mL
-S9 mix(short-term treatment): 0, 10.8, 16.2, 24.3, 36.5, 54.7 μg/mL
[Confirmation test]
+S9 mix(short-term treatment):0.625, 1.25, 2.5, 5.00, 10.00μg/mL

[cell-growth inhibition test]
+S9 mix(short-term treatment): 0, 13.7, 27.3, 54.7, 109, 219, 438, 875, 1750 μg/mL
-S9 mix(short-term treatment): 0, 13.7, 27.3, 54.7, 109, 219, 438, 875, 1750μg/mL
-S9 mix(24hr-continuous treatment): 0, 13.7, 27.3, 54.7, 109, 219, 438, 875, 1750 μg/ μg/mL
-S9 mix(48hr-continuous treatment): 0, 13.7, 27.3, 54.7, 109, 219, 438, 875, 1750μg/ μg/mL

Cell-growth inhibition test was conducted up to the limited concentration of 1750 μg/mL (10 mM)

-Short term treatment, +S9 mix: concentration of 50% cell-growth inhibition was determined as 13.7μg/mL
-Short term treatment, -S9 mix: concentration of 50% cell-growth inhibition was determined as 40.7 μg/mL

-Continous treatment (24 h): concentration of 50% cell-groth inhibition was determined as 27.3 μg/mL
-Continous treatment (48 h): concentration of 50% cell-groth inhibition was determined as 23.6 μg/mL

Vehicle / solvent
DMSO

Controls

Untreated negative controls
no

Negative solvent / vehicle controls
yes

True negative controls
no

Positive controls
yes

Positive control substance
cyclophosphamide
mitomycin C

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
not used.

Results and discussion

Test results

Key result
false

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

Metabolic activation
with and without

Genotoxicity
positive chromosome aberration tests were reproducible and dose-dependency was observed in the confirmation test, the results were judged comprehensively to be positive.

Cytotoxicity / choice of top concentrations
cytotoxicity 50% cell growth inhibition: below 13.7μg/mL (short)

Vehicle controls validity
valid

Positive controls validity
valid

Key result
false

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

Metabolic activation
with and without

Genotoxicity
negative The chromosome numerical aberration: negative The chromosome numerical aberration inducibility of N-ethyl-1-aminonaphthalene was judged to be negative since the incidence of polypl oidy was less than 5% for all the treatment methods.

Cytotoxicity / choice of top concentrations
cytotoxicity 50% cell growth inhibition: below 13.7μg/mL (short)

Vehicle controls validity
valid

Positive controls validity
valid

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/PDF118-44-5f.pdf

Applicant's summary and conclusion

Conclusions
In the confirmation test, the TA value was 37.0% at 10.0 μg/mL, 16.5% at 5.00 μg/mL, 2.5% at 2.50 μg /mL, 0.5% at 1.25 μg/mL and 1.0% at 0.625 μg/mL. Thus the positive reactions were observed only at dose concentrations which 50% cell growth inhibition was observed. However, since the results in the chromosome aberration tests were reproducible and dose-dependency was observed in the conf irmation test, the results were judged comprehensively to be positive. Since clearly positive results were observed in the short-term treatment with metabolic activation, continuous treatment was not done.

In the positive control group, remarkable induction of chromosome structural aberrations was observed. Therefore, it was judged that the study was conducted appropriately.

Executive summary

It was concluded that *N*-ethyl-1-aminonaphthalene induced chromosome structural aberrations but did not induce chromosome numerical aberrations under the conditions of this study.

Genetic toxicity in vivo

ENDPOINT_STUDY_RECORD: Genetic toxicity in vivo.001

UUID: c13508bf-4a71-4dd0-a903-5aecfdc25da6
Dossier UUID:
Author:
Date: 2022-12-16T10:51:56.120+09:00
Remarks:

Administrative data

Endpoint
genetic toxicity in vivo, other transgenic rodent somatic and germ cell gene mutation assays

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

Transgenic rodent somatic and germ cell gene mutation assays with N-ethyl-1-aminonaphthalene / 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 488 (Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays)

in vivo mammalian somatic and germ cell study: gene mutation

Deviations
no

GLP compliance
yes

Type of assay
transgenic rodent mutagenicity assay
in vivo mammalian germ cell study: gene mutation

Test material

Specific details on test material used for the study
N-ethyl-1-aminonaphthalene / 118-44-5

Test animals

Species
mouse

Strain
C57BL 6JJmsSlc-Tg (gpt delta)
mouse

Sex
male

Details on test animals or test system and environmental conditions
TEST ANIMALS
- Source: Japan SLC, Inc., Inc. Hamamatsu
- Age at study initiation: 10 weeks
- Weight at study initiation: Males: 24.1 - 28.3 g
- Housing: bracket-type metallic wire-mesh cages (W 10 × D 19.6 × H 13.0 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22.9 – 23.2℃
- Humidity (%): 47.7-62.2
- Air changes: >=12 times / hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
corn oil

- Concentration of test material in vehicle: 0, 2.5, 5, 10 and 20 mg/mL
- Amount of vehicle (if gavage or dermal): 10 mL/kg bw

Details on exposure
Dosing solutions were prepared by dissolving the test substance in corn oil. They were used within 8 days

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.	
25	mg/kg bw/day (actual dose received)
Dose / conc.	
50	mg/kg bw/day (actual dose received)
Dose / conc.	
100	mg/kg bw/day (actual dose received)
Dose / conc.	
200	mg/kg bw/day (actual dose received)

No. of animals per sex per dose
6/male/dose

Control animals
yes, concurrent vehicle

Positive control(s)
Benzo[a]pyrene (B[a]P)

Examinations

Tissues and cell types examined
Liver, femur, testis

Details of tissue and slide preparation
Gene mutagenicity (reporter gene: gpt, and red and gam) in liver, bone-marrow and testis was studied in transgenic mice (gpt delta) for the mutagenicity of ethyl (1-naphthyl)amine.

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.
2 groups: The data were analyzed for homogeneity of variance by the F test. If variances were homo geneous, data was analyzed by the Student t test, whereas heterogeneous data was analyzed by the Aspin-Welch t test.

Results and discussion

Test results

Key result
false

Sex
male

Genotoxicity
negative

Vehicle controls validity
valid

Positive controls validity
valid

Additional information on results
[RESULTS OF RANGE-FINDING STUDY]
- Dose range: 75, 150, 300, 600 mg/kg for males
- duration : once a day, 7days
- Clinical signs of toxicity in test animals: Death was observed in 3/3 males at 600 mg/kg, and in 2/3 males 300 mg/kg.
Colored urine was observed in 2 males at 150 mg/kg and 1 male at 75.0 mg/kg.
Therefore, the highest dose in this study was set at 200 mg/kg, with a high dose of 100 mg/kg group, a middle dose of 50 mg/kg group and a low dose of 25.0 mg/kg

[TEST RESULT]
- Body weight and weight changes : no effect observed
- Clinical signs: Irregular respiration was observed in 1/6 male at 200 mg/kg. Brown urine was obs erved in 6/6 males at 200 mg/kg, 6/6 males at 100 mg/kg, and 4/6 males at 50 mg/kg.
- Motor Activity: Low value was observed in 1/6 male at 200 mg/kg.
- Organ weight / body weight ratios: no effect observed
- Gross pathological findings: no effect observed

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/PDF118-44-5t.pdf

Applicant's summary and conclusion

Executive summary

Based on the above results, it was determined that the N-ethyl-1-aminonaphthalene was not mutagenic (negative) to the transgenic mice under the test conditions.

Toxicity to reproduction

ENDPOINT_STUDY_RECORD: Toxicity to reproduction.001

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Dossier UUID:	
Author:	
Date:	2022-12-16T10:55:36.337+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 / N-ethyl-1-aminonaphthalene / 118-44-5 / N-ethylnaphthalen-1-amine / 118-44-5

Remarks
Repeated dose toxicity: oral.002

Data source

Reference

A reproduction/developmental toxicity screening test in rats treated orally with ethyl (1-naphthyl) / Ministry of Health, Labour and Welfare (MHLW), Japan / publication

Data access
data published

Materials and methods

Test guideline

Qualifier
according to guideline

Guideline
other: Guideline for reproduction/developmental toxicity screening test in rats (Chemical Substa nces Control Law of Japan)

Deviations
no Housing pressure temporarily fall slightly below the prescribed range. (<30Pa) However, there was no influx of outside air and no abnormalities were observed in the general condition of the animals.

GLP compliance
yes

Test material

Specific details on test material used for the study
Name of test material (as cited in study report): Ethyl (1-naphthyl) amine

Test animals

Species
rat

Strain
other: Sprague-Dawley strain SPF rats [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: 9 weeks
- Weight at study initiation: Males: 339.7-390.2 g, Females: 223.2-254.7 g
- Housing: bracket-type metallic wire-mesh cages (W 291 × D 263 × H 180 mm)
- Diet: ad libitum
- Water: ad libitum
- Acclimation period:15 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21.7-23.2
- Humidity (%): 44.8-62.0
- Air changes: 12 times and over/ hr
- Photoperiod: 12 hrs dark / 12 hrs light

Administration / exposure

Route of administration
oral: gavage

Vehicle
corn oil

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

Vehicle
- Name: Corn oil
- Lot Number: WEK6144, WEF2972
- Manufacturer: Wako pure Chemical Industries, Ltd.
- Storage Conditions: Room temperature

Details on mating procedure
- M/F ratio : 1/1 (1/2 at 150 mg/kg bw /day, because of deth of male rats)
- Length of cohabitation: up to 2 weeks
- Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0 of pregnancy

Details on analytical verification of doses or concentrations
According to publication “A 28-day oral toxicity study of ethyl (1-naphthyl) amine in rats with a re covery period of 2 weeks, Ministry of Health, Labour and Welfare (MHLW), Japan”
Test solutions were prepared at once in administration period.

The test solutions to be used for Day-1 or last day of administration were analyzed for concentration by HPLC method.

The results showed that the concentrations were 95.6 to 100.1% of the nominal concentrations (a cceptable range: 100 ± 5% of the nominal value), which were all within the acceptable range.

Duration of treatment / exposure
(P) Males: 42 days including 14 days pre-mating and mating periods, and thereafter 14 days
(P) Females: 14 days pre-mating, mating and gestation periods, and the days until day 3 of lactation.

Frequency of treatment
once / day

No. of animals per sex per dose
12 animals/male and female/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 study: 28-day repeated dose oral toxicity test (doses: 0, 12, 60, 150, and 300mg/kg bw/day).

At 300 mg/kg bw/day, loose stools, decrease of body weights and decrease in food consumption were observed in both sexes of the administration period.
At 300 mg/kg bw/day, one female died on day 4 of the administration.
At 60 mg/kg bw/day and higher, brown urine was observed in both sexes of the administration period.
At 300 mg/kg bw/day, 8 males and 5 females died on day 7 of administration.
The dose was 150 mg/kg bw/day from day 14 of administration to ensure the number of studies in the 300 mg/kg bw/day group.

Therefore, the high dose in this study was set at 300 mg/kg bw/day, with a middle dose of 60 mg/kg bw/day group and a low dose of 12 mg/kg bw/day, using the common ratio of approximately 5.

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

Examinations

Parental animals: Observations and examinations
CAGE SIDE OBSERVATIONS: Yes

Males and females: 2 times/day during the administration period (before and after dosing) , and once on the day of necropsy.

DETAILED CLINICAL OBSERVATIONS:Yes

BODY WEIGHT: Yes
male: Days 0, 7, 14, 21, 28, 35 and 42(the day of necropsy),
female: Days 0, 7 and 14 of administration period, days of 0, 7, 14 and 20 of gestation, days of 0 and 4 of lactation period
Infertile female : Days 21

weight gain:
male : from day 0 to day 42
female : from day 0 to day 14, days from 0 to 20 of gestation, days from 0 to 4 of lactation period

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):yes
male : Days 0, 7, 14, 21, 28, 35 and 42(the day of necropsy)
female : Days 0, 7, 14 and days 0, 7, 14, 18 and 20 of gestation, days 0 and 4 of lactation period

Oestrous cyclicity (parental animals)
yes

Sperm parameters (parental animals)
no

Litter observations
no

Postmortem examinations (parental animals)
yes

Postmortem examinations (offspring)
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 ($p<0.05$, two-sided, and $p<0.01$).

Reproductive indices
Copulation index (%) = (No. of animals with successful copulation / no. of animals mated) × 100
Fertility index (%) = (No. of pregnant animals / no. of animals with successful copulation) × 100
Gestation index (%) = (No. of dams with live offspring / no. of pregnant female) × 100
Implantation index (%) = (No. of implantation sites/No. of corpora lutea) × 100
Delivery index (%, Mean±S.D.) = (No. of offspring delivered / no. of implantation sites) × 100
Live birth index (%, Mean±S.D.) = (No. of live offspring on day 0 / no. of offspring delivered) × 100

Sex ratio of total number of offspring at birth = No. of male offspring at birth /total number of off spring at birth
Sex ratio of live offspring at birth = No. of male live offspring at birth /total number of live offspring at birth
Sex ratio of live offspring on day 4 = No. of male live offspring on day 4/total number of live offspring on day 4
Sex ratio of total number of offspring at birth (M/Total,litter) = No. of male offspring /total number of offspring, litter at birth
Sex ratio of live offspring at birth (M/Total,litter) = No. of male live offspring /total number of live offs pring, litter at birth
Sex ratio of live offspring on day 4 (M/Total,litter) = No. of male live offspring /total number of live of fspring, litter on day 4

Offspring viability indices
Viability index on day 4 (%, Mean±S.D.) = (No. of live offspring on day 4 / no. of live offspring on day 0) × 100

Results and discussion

Results: P0 (first parental generation)

General toxicity (P0)

Clinical signs
effects observed, treatment-related

Description (incidence and severity)
At 150 mg/kg bw/day (highest dose : 300 mg/kg bw/day), brown urine, loose stools, and staining and paleness of fur were observed in the dead animals (8 males and 5 females).
Irregular respiration and decreased locomotor activity were observed in males, and perinasal staining was observed in females.
Brown urine, loose stools and pallor were observed in sacrificed rats of the 150 mg/kg bw/day group.

1/12 females died at 300 mg/kg bw/day. Loose stools were observed.
Brown urine was observed in the 60 mg/kg bw/day group during treatment.

Brown urine is thought to be the result of urinary excretion of N-ethyl-1-aminonaphthalene or its derived substances because it closely resembles the color of N-ethyl-1-aminonaphthalene.
These may be no treatment-related effects.

Mortality
mortality observed, treatment-related

Body weight and weight changes
effects observed, treatment-related

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

Description (incidence and severity)
in disgust of food,
150 mg/kg bw day, and 300 mg/kg bw day

Ophthalmological findings
not examined

Haematological findings
not examined

Clinical biochemistry findings
not examined

Urinalysis findings
no effects observed

Description (incidence and severity)
observed brown urine.
Brown urine was considered the brown which is color of N-ethyl-1-aminonaphthalene.
These may be no treatment-related effects.

Behaviour (functional findings)
not examined

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

Gross pathological findings
no effects observed

Histopathological findings: non-neoplastic
no effects observed

Description (incidence and severity)
At 150 mg/kg bw/day, hepatocellular steatosis or hepatocyte in liver, splenic hemosiderosis, and pro static inflammatory cellular infiltration were observed in males.
At 150 mg/kg bw/day, hemorrhage in the lungs, accumulation of macrophage , increased extramedu llary hematopoiesis in the spleen or hemosiderosis, and uterine lesions were observed in females.
In the 150 mg/kg bw/day group, the observed inflammatory cellular infiltrate of the prostates and lesi ons in the uterus after delivery were also observed in the control group. Therefore, this was judged no effect of treatment.
At 150 mg/kg bw/day, testicular tubule atrophy was observed in males that did not become pregnant.
At 150 mg/kg bw/day, the seminiferous tubules of other rats (including those that died) were obs erved no similar abnormalities and the spermatogenic cycles were similar to those of the control gro up.
In a 28-day repeated dose study, seminiferous tubules were observed no abnormalities. Therefore, it was judged not to be related to N-ethyl-1-aminonaphthalene administration.
Other findings were not dose-related. Only a few findings were observed.
Therefore, they were judged as incidental changes.

In this pathological examination on the reproductive and accessory genital organs, male and female showed no effects by N-ethyl-1-aminonaphthalene administration.

Reproductive function / performance (P0)

Reproductive function: oestrous cycle
no effects observed

Description (incidence and severity)
Serial estrous quiescence was observed in one rat each in the control and 150 mg/kg bw/day groups. This result is considered to be spontaneous that is not related to N-ethyl-1-aminonaphthalene administration. The estrous cycle is no effects by N-ethyl-1-aminonaphthalene administration.

Reproductive function: sperm measures
not examined

Reproductive performance
no effects observed

Description (incidence and severity)
The mating ability was no effects by N-ethyl-1-aminonaphthalene administration.
At 150 mg/kg bw/day, reduced fertility was observed.
Two infertile females mated with one male, testicular atrophy and azoospermia in the epididymis w ere observed histopathologically in this male at 150 mg/kg bw/day.
The cause of infertility is considered to be due to the male and is not considered to be an effect of N-ethyl-1-aminonaphthalene administration.
In the 150 mg/kg bw/day group, all females mated with the other males conceived. From this result, there is no effect by N-ethyl-1-aminonaphthalene administration on the fertility.

Delivery:
Abnormal parturition status (dystocia) was observed in one rat in the control group. The N-ethyl-1-aminonaphthalene dose group was not observed and was therefore considered incidental.
There were no treatment-related effects on the duration of gestation, number of corpora lutea, numb er of implantation sites, number of pups born, number of pups born on day 0 of lactation, and number of live pups born on day 4 of lactation in dams.

Effect levels (P0)

Key result
false

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
reproductive performance
At 150 mg/kg bw/day (highest dose), reproductive and developmental toxicities showed no tr eatment-related effects.

Key result
false

Dose descriptor
NOAEL

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
histopathology: non-neoplastic
At 150 mg/kg bw/day (highest dose : 300 mg/kg bw/day), circulatory disturbance suspected to be disseminated intravascular coagulation (Disseminated Intravascular Coagulation, DIC) was sugges ted in dead rats.

Results: F1 generation

General toxicity (F1)

Clinical signs
no effects observed

Description (incidence and severity)
At 60 mg/kg bw/day, abdominal trauma was observed in one male, and skin ulcers were observed at necropsy on day 4 of lactation in this rat. This is considered to be due to the bite of the dams and the refore it is considered to be no effect of N-ethyl-1-aminonaphthalene treatment.

Mortality / viability
mortality observed, non-treatment-related

Description (incidence and severity)
All pups per a litter in the control group died during the lactation period, but it was considered an incidental occurrence because it was the control group.

Body weight and weight changes
effects observed, treatment-related

Description (incidence and severity)
At 150 mg/kg bw/day, low value of body weight was observed on day 4 of lactation in both sexes. This suggests postnatal growth depression.

At 60 mg/kg bw/day, a small body size was observed in several males. However, number of incidence was low and a small body size was also observed in the control group. Based on this result, it is cons idered that there is no effect of the N-ethyl-1-aminonaphthalene administration on body size.

Gross pathological findings
no effects observed

Histopathological findings
not examined

Effect levels (F1)

Key result
false

Dose descriptor
NOAEL

Generation
F1

Effect level

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

Based on
test mat.

Sex
male/female

Basis for effect level
body weight and weight gain
At 150 mg/kg bw/day, low value of body weight was observed in pups (F1) of both sexes on day 4 of lactation.

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/PDF118-44-5c.pdf

Applicant's summary and conclusion

Conclusions
The NOAEL of reproductive and developmental toxicity was considered to be 60 mg/kg bw/day based on the decreased body weight observed in pups.

Executive summary

The reproductive and developmental toxicity of N-ethyl-1-aminonaphthalene was investigated in rats in accordance with the OECD TG 421 reproductive/developmental toxicity screening test. Rats were treated with N-ethyl-1-aminonaphthalene via oral gavage at doses of 0 (vehicle: corn oil), 12, 60, or 300 (reduced to 150) mg/kg bw/day. Males (12/dose) were treated for 42 days, including a 14-day premating period and a subsequent mating period, while females (12/dose) were treated over a 14-day premating, mating, gestation, and lactation period (up to on day 3 of lactation). In the highest dose group, eight males and five females died during the premating period; therefore, dose levels were reduced from 300 mg/kg bw/day to 150 mg/kg bw/day from day 14 of dosing. Prior to death, animals showed loose stools, soiled fur, pale skin, irregular respiration, and decreased locomotor activity. Histopathological examination of the dead animals revealed congestion, hemorrhage, and thrombus in various organs, and phosphotungstic acid–hematoxylin positive staining of eosinophilic material was found in the kidneys and lungs. Cause of death was considered to be circulatory disturbance, presumed to be disseminated intravascular coagulation. In the highest dose group, loose stools, pale skin, and irregular respiration were observed in survivors. Body weight and food consumption were decreased during the premating period in males and females in the highest dose group. Histopathological examination in the highest dose group showed fatty changes and centrilobular hepatocellular hypertrophy, and hemosiderin deposition in the spleen in parental males and hemorrhage and macrophage aggregation in the lung and hemosiderin deposition, and increased extramedullary hematopoiesis in the spleen in parental females. Systemic toxicity observed in this study (TG421) at 300 mg/kg bw/day was more severe than that of the 28-day repeated-dose toxicity study (TG407). Age at administration (6 weeks old vs 9 weeks old; TG407 vs TG421) may affect toxic response. The reproductive organs and fertility was not affected in rats treated with N-ethyl-1-aminonaphthalene. On PND 4, male and female pups in the highest dose group showed decreased body weight. The NOAEL of reproductive and developmental toxicity was considered to be 60 mg/kg bw/day based on the decreased body weight observed in pups.

DOMAIN

Substance

SUBSTANCE: N-ethyl-1-aminonaphthalene / 118-44-5

UUID: ae0d95ca-8dbb-4b99-9744-ed64e15434b6
Dossier UUID:
Author:
Date: 2022-12-16T11:01:00.556+09:00
Remarks:

Substance name
N-ethyl-1-aminonaphthalene / 118-44-5

Legal entity
National Institute of Health Sciences / Kawasaki / Japan

Identification of substance

Reference substance
ethyl(1-naphthyl)amine / N-ethylnaphthalen-1-amine / 118-44-5 / 204-250-3

EC number	EC name
204-250-3	EC Inventory
CAS number	**CAS name**
118-44-5	
IUPAC name	
N-ethylnaphthalen-1-amine	

Role in the supply chain

Manufacturer
false

Importer
false

Only representative
false

Downstream user
false

References

Reference Substances

REFERENCE_SUBSTANCE: ethyl(1-naphthyl)amine

UUID: ECB5-cce991a5-cb7c-4975-9dcd-0088955f2e92
Dossier UUID:
Author:
Date: 2007-05-10T18:00:00.000+09:00
Remarks:

Reference substance name
ethyl(1-naphthyl)amine

IUPAC name
N-ethylnaphthalen-1-amine

Inventory

Inventory number

Inventory name
ethyl(1-naphthyl)amine

Inventory
EC Inventory

Inventory number
204-250-3

CAS number
118-44-5

Molecular formula
C12H13N

Description

CAS number
118-44-5

Synonyms

Synonyms

Identity
1-Naphthalenamine, N-ethyl-

Identity
1-Naphthalenamine, N-ethyl-

Molecular and structural information

Molecular formula
C12H13N

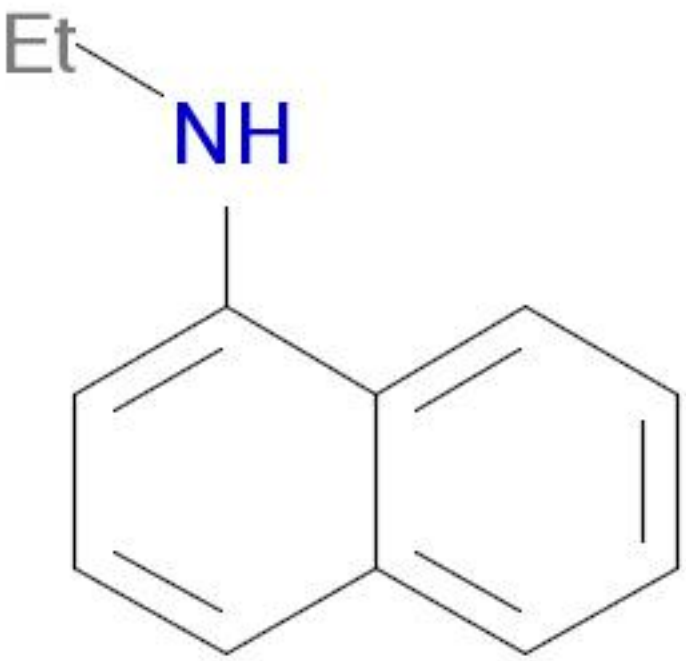
Molecular weight

171.2383

SMILES notation
CCNc1cccc2ccccc12

InChI
InChI=1/C12H13N/c1-2-13-12-9-5-7-10-6-3-4-8-11(10)12/h3-9,13H,2H2,1H3

Structural formula



Literatures

LITERATURE: A 28-day oral toxicity study of ethyl (1-naphthyl) amine in rats with a recovery period of 2 weeks

UUID:	5e11cb9c-533b-4be8-95b5-368a319f5e2a
Dossier UUID:	
Author:	
Date:	2022-12-14T15:25:54.542+09:00
Remarks:	

General information

Reference Type
publication

Title
A 28-day oral toxicity study of ethyl (1-naphthyl) amine in rats with a recovery period of 2 weeks

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

Year
2011

LITERATURE: A reproduction/developmental toxicity screening test in rats treated orally with ethyl (1-naphthyl) amine

UUID: ba29b72f-6571-4830-81b0-86d50b9cf12a
Dossier UUID:
Author:
Date: 2022-12-14T15:28:45.465+09:00
Remarks:

General information

Reference Type
publication

Title
A reproduction/developmental toxicity screening test in rats treated orally with ethyl (1-naphthyl) amine

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

Year
2014

LITERATURE: A reverse mutation test of N-ethyl-1-aminonaphthalene using bacteria

UUID:	f3eae3e6-8413-4918-b183-b92bf62380cf
Dossier UUID:	
Author:	
Date:	2019-05-22T11:09:10.000+09:00
Remarks:	

General information

Reference Type
publication

Title
A reverse mutation test of N-ethyl-1-aminonaphthalene using bacteria

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

Year
2009

LITERATURE: Chromosome aberration test in cultured chinese hamster cells treated with N-ethyl-1-aminonaphthalene

UUID: 20fbe7bd-be16-4443-9227-cdce0a4afa8c
Dossier UUID:
Author:
Date: 2019-05-22T11:14:40.000+09:00
Remarks:

General information

Reference Type
publication

Title
Chromosome aberration test in cultured chinese hamster cells treated with N-ethyl-1-aminon aphthalene

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

Year
2010

LITERATURE: Transgenic rodent somatic and germ cell gene mutation assays with N-ethyl-1-aminonaphthalene

UUID:	9ab4cb75-310f-4b59-8d5d-c7a8693f3756
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Author:	
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General information

Reference Type
publication

Title
Transgenic rodent somatic and germ cell gene mutation assays with N-ethyl-1-aminonaphthalene

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

Year
2014

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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Identifiers

Other IT system identifiers

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

ID
16558402024DIV750