Bull. Natl Inst. Health Sci., **139**, 71-78 (2021) **Technical Data**

Summary information of human health hazard assessment of existing chemical substances (Ⅶ)

磯　貴子，重田善之，村田康允，広瀬　望，井上　薫，山田隆志，広瀬明彦#，松本真理子

Takako Iso, Yoshiyuki Shigeta, Yasumasa Murata, Nozomu Hirose, Kaoru Inoue, Takashi Yamada, Akihiko Hirose#, Mariko Matsumoto

Under the Japanese Chemical Substances Control Law (CSCL), toxicological information for existing chemical substances has been collected by the Japanese Ministry of Health, Labour and Welfare (MHLW). We have reviewed the collected information and taken the initiative to disseminate information via the Japan Existing Chemical Database (JECDB). We have reviewed the toxicological studies and presented a summary of our evaluation of the following five substances: 2,4-dimethylbenzenesulfonic acid (CAS: 88-61-9), 4-methylpyridine (CAS:108-89-4), 4,4'-bis (chloromethyl) biphenyl (CAS: 1667-10-3), 2,2'- [ethylenebis (oxymethylene)] bisoxirane (CAS: 2224-15-9), and 1,4-bis (isopropylamino)-9,10-anthraquinone (CAS: 14233-37-5). The International Uniform Chemical Information Database (IUCLID) dossiers created for these five chemical substances are available at the JECDB.

Keywords: existing chemical substance, toxicological assessment, JECDB, IUCLID

Introduction

The Chemical Substances Control Law (CSCL), was enacted in 1973 to prevent environmental pollution by chemical substances that pose a risk to human health or the environment in Japan. Chemical substances that were already in the market before 1973 are designated existing chemical substances. The Japanese CSCL requires the Japanese Ministry of Health, Labour and Welfare (MHLW) to collect information on human health, including data on acute toxicity, repeated-dose toxicity, genotoxicity, and reproduction/development toxicity, with respect to existing chemical substances[1]. Thus far, the MHLW has gathered information on nearly 450 existing chemical substances. We have assessed these existing chemical substances for health risks and provide information through the Japan Existing Chemical Database (JECDB)[2-7]. Our previous contribution to the Organization for Economic Co-operation and Development (OECD) Cooperative Chemicals Assessment Programme (CoCAP) or the OECD High Production Volume Chemicals Programme (former CoCAP) is also publicly available in the OECD Existing Chemicals Database[8] as we have repeatedly mentioned here. In this seventh report, we present a summary of the toxicological studies conducted for the following five substances: 2,4-dimethylbenzenesulfonic acid (CAS: 88-61-9), 4-methylpyridine (CAS:108-89-4), 4,4'-bis(chloromethyl) biphenyl (CAS: 1667-10-3), 2,2'-[ethylenebis(oxymethylene)] bisoxirane (CAS: 2224-15-9), and 1,4-bis(isopropylamino)-9,10-anthraquinone (CAS: 14233-37-5). Considering the absence of previous toxicological studies on these chemicals, each study was conducted following the OECD Guidelines[9] for the Testing of Chemicals for combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422), bacterial reverse mutation test (OECD TG 471), or *in vitro* mammalian chromosomal aberration test (OECD TG 473) and in accordance with Good Laboratory Practice

To whom correspondence should be addressed:
Akihiko Hirose
Division of Risk Assessment, National Institute of Health Sciences, 3-25-26, Tonomachi, Kawasaki-ku, Kanagawa 210-9501, Japan
Tel.: +81-44-270-6681, Fax: +81-44-270-6703

Standards. The International Uniform Chemical Information Database (IUCLID) dossiers created for these five chemical substances and our series of human health hazard assessments of existing chemical substances[2-7] are accessible through the JECDB. These challenges require initiative in the field of risk assessment of chemical substances.

(1) 2,4-Dimethylbenzenesulfonic acid (CAS: 88-61-9)

A combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422 (adapted in 1996). Male and female rats (12 animals/sex/dose) were administered 2,4-dimethylbenzenesulfonic acid by gavage at 0 (vehicle: sterile distilled water), 20, 100 and 500 mg/kg bw/day. Males were treated for 42 days, which included a 14-day premating period, 14-day mating period, and subsequent 14-day period, whereas females were treated for 41–46 days, which included a 14-day premating, mating, and gestation periods and through day 4 of lactation. Five males dosed at 0 and 500 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period. Ten additional females at 0 and 500 mg/kg bw/day were allocated to a satellite group and dosed with 2,4-dimethylbenzenesulfonic acid for 42 days without mating. Five females from each satellite group were maintained and examined after a 14-day recovery period.

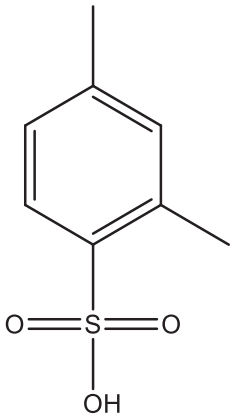

Fig. 1. Structure of 2,4-dimethylbenzenesulfonic acid (CAS: 88-61-9)

Irregular respiration, deep respiration, and dyspnea, which were considered to represent major toxic effects, were observed in all treatment groups at the 500 mg/kg bw/day dose. No deaths were observed in males. One satellite female died at the 500 mg/kg bw/day dose on day 10 of administration, showing erosion and inflammation in the larynx through the upper part of the trachea. Obstruction in the upper trachea by pus and congestion/edema of the lung were also observed, suggesting that the cause of death was suffocation in this female. To investigate obstruction in the nasal cavity, a detailed histopathological examination in the nasal mucosa of all animals was conducted; however, there were no lesions causing obstruction in any animal. An intubation error could not be excluded as the cause of death. Therefore, this death was treatment-related but was not toxicologically relevant. A significant decrease in mean corpuscular hemoglobin levels was observed in males at the 500 mg/kg bw/day dose. A significant decrease in total protein (TP) and albumin levels were observed in males. Significant decreases in TP and increases in aspartate aminotransferase levels were observed in satellite females at a dose of 500 mg/kg bw/day. These hematological and clinical biochemical findings were not associated with other signs, organ weight changes, or histopathological alterations and were confined to the highest dose and no longer apparent after the recovery period. These changes may be treatment-related but were not necessarily adverse. Significant decreases in blood urea nitrogen, creatinine, and phosphorus levels without any other accompanying effects were observed in dams at 500 mg/kg bw/day. These changes were considered treatment-related resulting from pregnancy but were not toxicologically significant.

The no-observed adverse effect level (NOAEL) for the repeated-dose toxicity was 100 mg/kg bw/day based on abnormal respiration at 500 mg/kg bw/day.

In a bacterial reverse mutation assay with *Salmonella typhimurium* (*S. typhimurium*) TA100, TA1535, TA98, and TA1537 and *Escherichia coli* (*E. coli*) WP2*uvrA*/pKM101 (OECD TG 471), 2,4-dimethylbenzenesulfonic acid was negative with and without metabolic activation. In an *in vitro* chromosomal aberration test using Chinese hamster (CHL/IU) cells (OECD TG 473), 2,4-dimethylbenzenesulfonic acid was also negative with and without metabolic activation. Based on these results, 2,4-dimethylbenzenesulfonic was considered non-genotoxic *in vitro*.

In the combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, no treatment-related effects were observed on reproduction/developmental parameters, such as estrous cycle, reproductive performance, implantation index, index, birth index, and external/clinical/gross observation of pups.

The NOAEL for the reproduction/developmental toxicity was 500 mg/kg bw/day (highest dose tested).

(2) 4-Methylpyridine (CAS: 108-89-4)

A combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422 (adapted in 1996). Male and female rats (12 animals/sex/dose) were administered 4-methylpyridine by gavage at 0 (vehicle: sterile distilled water), 5, 20, and 80 mg/kg bw/day. Males were dosed for 28 days, including a 14-day premating period and a subsequent 14-day period. Females were dosed for 42–46 days, including a 14-day premating, mating and gestation periods, and until day 4 of lactation. Six males from each group were allocated to a recovery group and maintained for 14 days after the administration period. Ten additional females were treated at 0 and 80 mg/kg bw/day, and five additional females were treated with 5 and 20 mg/kg bw/day as a satellite group. These females were dosed with 4-methylpyridine for 28 days without mating, and five were treated at 0 and 80 mg/kg bw/day, allocated to a recovery group, and maintained for 14 days after the administration period.

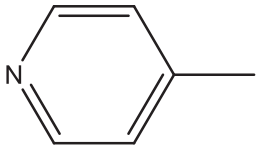

Fig. 2. Structure of 4-methylpyridine (CAS: 108-89-4)

No deaths were observed in the males and satellite females. Transient salivation was observed in all treatment groups at a dose of 80 mg/kg bw/day. This finding was considered to be treatment-related resulting from a substance stimulus property but was not adverse. Significant changes in motor activities, such as decreased vertical counts at a dose of 80 mg/kg bw/day and ambulatory counts at a dose of 20 mg/kg bw/day and above, were recorded in males at the end of the administration period. There were no effects on body weight, food consumption, and clinical chemistry in males and satellite females. Water consumption was significantly higher in parental females during pregnancy at a dose of 80 mg/kg bw/day. Dark red spots in the stomach glandular mucosa were observed by gross pathology in one satellite female at the 80 mg/kg bw/day dose. At the end of the administration period, slight erosion in the glandular stomach was observed in one of six males and one of five satellite females at a dose of 80 mg/kg bw/day. Minimal centrilobular cell infiltration in the liver was observed in one of six and three of six males at 20 and 80 mg/kg bw/day doses, respectively. The absence of these gross or histopathological changes at the end of the recovery period suggests that these changes in the liver and stomach are reversible. These findings in the stomach and liver were treatment-related but were of unknown toxicological significance.

The NOAEL for the repeated-dose toxicity was 5 mg/kg bw/day based on the lower motor activity at a dose of 20 mg/kg bw/day in males.

In a bacterial reverse mutation assay with *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (OECD TG 471), 4-methylpyridine was negative with and without metabolic activation. In an *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473), 4-methylpyridine was also negative with and without metabolic activation. Based on these results, 4-methylpyridine was considered to be non-genotoxic *in vitro*.

In the combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test (OECD TG 422) described above, two parental females died on gestation days 23 and 24 at the 80 mg/kg bw/day dose.

Histopathological changes observed in dead parental females included centrilobular hepatocyte necrosis, apoptosis in the thymus cortex, decreased density of lymphocytes in the spleen and mandibular and mesenteric lymph nodes. Two cases showed a significant deterioration in the whole body during late pregnancy. Five dams delivered only stillbirths or dead pups and were euthanized on days 0 and 1 of lactation at the 80 mg/kg bw/day dose. Treatment-related reduced food consumption and body weight

gain were observed in dams during lactation at the 20 mg/kg bw/day dose. Absolute and relative uterus weights were significantly higher in dams at the 80 mg/kg bw/day dose. For fertility parameters, significant decreases in the number of estrous cases before pairing and significant increases in the length of gestation were observed at the 80 mg/kg bw/day dose. Prolonged delivery period, cannibalism, and faulty nest-building were observed in dams at 80 mg/kg bw/day. In offspring, a significant large number of stillborn pups, reduced number of live pups, and lower survival rates of pups during lactation were observed at the 80 mg/kg bw/day dose. A slight trend toward a decreased number of live pups and viability index on day 4 of lactation at the 20 mg/kg bw/day dose were considered to be toxicologically relevance.

The NOAEL for maternal toxicity was 5 mg/kg/day based on reduced food consumption and body weight gain in the dams during lactation at the 20 mg/kg/day dose. The NOAEL for reproduction/developmental toxicity was 5 mg/kg bw/day based on the decreased number of live pups and viability index observed on day 4 of lactation at the 20 mg/kg bw/day dose.

(3) 4,4'-Bis (chloromethyl) biphenyl (CAS: 1667-10-3)

A combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422 (adapted in 1996). Male and female rats (12 animals/sex/dose) were administered 4,4'-bis(chloromethyl) biphenyl by gavage at 0 (vehicle: 0.5 w/v% methylcellulose in water), 62.5, 250, and 1,000 mg/kg bw/day. Males were dosed for 28 days, including a 14-day premating period and a subsequent 14-day period. Females were dosed for 42–46 days, including a 14-day premating, mating, and gestation periods and until day 4 of lactation. Six males from each group were allocated to a recovery group and maintained 14 days after the administration period. Ten additional females treated at a dose of 0 and 1,000 mg/kg bw/day and five additional females at 62.5 and 250 mg/kg bw/day were treated as a satellite group. These females were dosed with 4,4'-bis(chloromethyl) biphenyl for 28 days without mating, and five of the females at the 0 and 1,000 mg/kg bw/day dose were allocated to a recovery group and maintained for 14 days after the administration period.

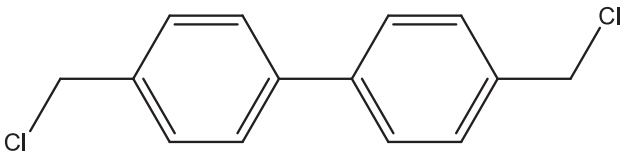

Fig. 3. Structure of 4,4'-bis(chloromethyl)biphenyl (CAS: 1667-10-3)

There were no treatment-related effects on mortality, clinical or functional observation battery (FOB) signs of toxicity, body weight, food consumption, urinary parameters, or hematology in all treatment groups. There were alterations in blood clinical chemistry parameters consisting of a significant increase in alanine aminotransferase (ALT), significant decrease in triglycerides (TG) in males at the 1,000 mg/kg bw/day dose, and significant increases in the albumin/globulin ratio in satellite females at the 250 mg/kg bw/day dose and above at the end of the administration period. A significant increased absolute and relative thyroid weights were observed in satellite females at the 1,000 mg/kg bw/day dose. There were neither histopathological change in the thyroid nor T3/T4/TSH levels in the high-dose female group; therefore, it was considered to be marginal.

According to these results, the NOAELs for the repeated-dose toxicity were 250 mg/kg bw/day in males based on increased ALT and decreased TG at the 1,000 mg/kg bw/day dose and 62.5 mg/kg bw/day in females based on an albumin/globulin ratio change at doses of 250 mg/kg bw/day and above.

A bacterial reverse mutation assay with *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (OECD TG 471) was performed with and without metabolic activation. 4,4'-bis(chloromethyl) biphenyl showed positive results in TA100, TA1535, TA98, and WP2*uvrA* regardless of metabolic activation. As for TA1537, positive results were obtained with metabolic activation. An *in vitro* chromosomal aberration test using OECD TG 473 in CHL/IU cells showed positive results for structural aberrations with and without metabolic activation. Based on the positive results in both studies, 4,4'-bis(chloromethyl)biphenyl was considered genotoxic *in vitro*.

In the combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test described above, no treatment-related effects were

observed on reproduction/developmental parameters. The NOAEL for reproduction/developmental toxicity was 1,000 mg/kg bw/day (highest dose tested).

(4) 2,2'- [Ethylenebis (oxymethylene)] bisoxirane (CAS: 2224-15-9)

A combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422 (adapted in 1996). Male and female rats (12 animals/sex/dose) were administered 2,2'-[ethylenebis (oxymethylene)] bisoxirane by gavage at 0 (vehicle: sterile distilled water), 12.5, 50, and 200 mg/kg bw/day. Males were dosed for 28 days, including a 14-day premating period and a subsequent 14-day period. Females were dosed for 42–46 days, including a 14-day premating, mating, and gestation periods and until day 4 of lactation. Six males from each group were allocated to a recovery group and maintained for 14 days after the administration period. Ten additional females at doses of 0 and 200 mg/kg bw/day and five additional females at doses of 12.5 and 50 mg/kg bw/day were treated as a satellite group. These females were treated with 2,2'-[ethylenebis(oxymethylene)] bisoxirane for 28 days, without mating, and five of the females at doses of 0 and 200 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period.

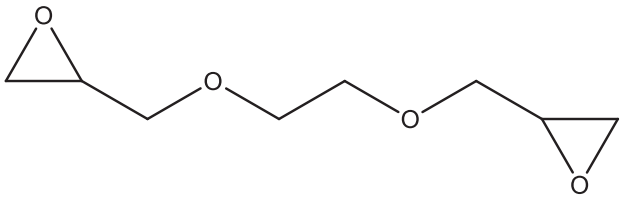

Fig. 4 . Structure of 2,2'-[ethylenebis (oxymethylene)] bisoxirane (CAS: 2224-15-9)

No deaths were observed during the study. Salivation after gavage was observed during exposure in all treatment groups at the 200 mg/kg bw/day dose. These findings were transient and considered to be a response to a substance stimulus property. No treatment-related effects were observed on mortality, locomotor activity, or urinary parameters. Significant low body weights were observed in males at doses of 50 mg/kg bw/day and above and in satellite and parental females at a dose of 200 mg/kg bw/day. Significant decreases in food consumption were observed in all treatment groups at 200 mg/kg bw/day during the administration period. Significant increases in water consumption in satellite females were observed at the 200 mg/kg bw/day dose throughout the treatment period and on day 2 of the recovery period. Significant increases in reticulocytes and prolonged prothrombin time at the 200 mg/kg bw/day dose and significant increases in platelets and differential lymphocyte/neutrophil counts at doses of 50 mg/kg bw/day and above were observed in satellite females at the end of the administration period. Although the toxicological meanings of these changes in blood were unclear, we conservatively considered these changes as adverse effects. Significant decreases in ALT at the 200 mg/kg bw/day dose and in TG at doses of 50 mg/kg bw/day and above were observed in males at the end of the administration period. A significant decrease in absolute uterus weight was observed in satellite females at the 50 mg/kg bw/day dose but was not considered to be treatment-related because of a poor dose dependency and within the range of historical control data provided by the facility. During the histopathological examination, mild squamous epithelium hyperplasia of the forestomach at the 200 mg/kg bw/day dose in both sexes and slight ulcer of the glandular stomach at a dose of 50 mg/kg bw/day in females was observed. The lesions in the forestomach and glandular stomach remained slight at the end of the recovery period in the high-dose group for both sexes.

The NOAEL for the repeated-dose toxicity was 12.5 mg/kg bw/day for both sexes based on low body weight and decreased TG in males, increased platelets, differential lymphocyte/neutrophil count, and glandular stomach ulcers in females at doses of 50 mg/kg bw/day and above.

A bacterial reverse mutation assay with *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (OECD TG 471) was performed with and without metabolic activation. The results were positive in TA100, TA1535, and TA98 regardless of metabolic activation. Positive results were obtained for TA1537 without metabolic activation, and for WP2*uvrA* with metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells

(OECD TG 473) was conducted, and the incidence of structural aberration was elevated with and without metabolic activation. Based on the positive results in both studies, 2,2'-[ethylenebis(oxymethylene)] bisoxirane was considered genotoxic *in vitro*.

In the combined repeated-dose toxicity study using the reproduction/developmental toxicity screening test (OECD TG 422) described above, no treatment-related effects were observed on the number of estrous cases before pairing and copulation index. A markedly decreased fertility index was observed at the 50 mg/kg bw/day dose described next. The number of pregnant females was 0 out of 12 at the 200 mg/kg bw/day dose and 7 out of 12 in the 50 mg/kg bw/day dose. Significant increases in the length of gestation, decreased number of corpora lutea, implantation scars, implantation index, and gestation index were observed in dams at the 50 mg/kg bw/day dose. Moreover, a significant decrease in the relative uterus and ovary weights at the 50 mg/kg bw/day dose and a significant decrease in absolute and relative ovary weights were recorded at the 12.5 mg/kg bw/day dose in dams. The toxicity for dams occurred at a lower dose compared with the dose that induced a repeated-dose toxicity in females described above. No treatment-related effects on the testes, epididymis, ventral prostate, or seminal vesicles were detected in males. A significant decrease in pups born and the number of live pups on postnatal day (PND) 0 and 4 were observed at the 50 mg/kg bw/day dose in offsprings.

The lowest-observed-adverse-effect level (LOAEL) for maternal toxicity was identified as 12.5 mg/kg bw/day based on the treatment-related ovary weight change in dams occurring at the lowest dose tested. The NOAEL for reproduction/developmental toxicity was also 12.5 mg/kg bw/day based on the reproductive/developmental parameter changes and decreased pup viability at the 50 mg/kg bw/day dose.

(5) 1,4-Bis (isopropylamino)-9,10-anthraquinone (CAS: 14233-37-5)

A combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422 (adapted in 1996). Male and female rats (12 animals/sex/dose) were administered 1,4-bis(isopropylamino)-9,10-anthraquinone by gavage at 0 (vehicle: 0.5 w/v% methylcellulose in water), 12, 60, and 300 mg/kg bw/day. Males were dosed for 42 days, which included a 14-day premating period, a 14-day mating period, and a subsequent 14-day period. Females were dosed for 41–46 days, which included a 14-day premating, mating, and gestation periods and until day 4 of lactation. Five males at the 0 and 300 mg/kg bw/day doses were allocated to a recovery group and maintained for 14 days after the administration period. Ten additional females at the 0 and 300 mg/kg bw/day doses were treated as a satellite group. These females were dosed with 1,4-bis(isopropylamino)-9,10-anthraquinone for 42 days, without mating, and five females from each group were allocated to a recovery group and maintained for 14 days after the administration period.

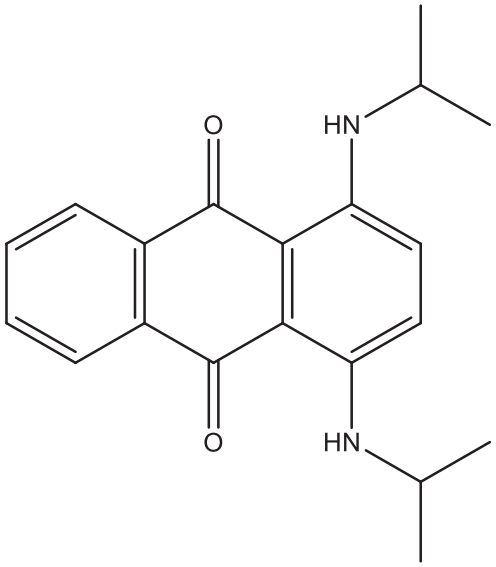

Fig. 5. Structure of 1,4-bis(isopropylamino)-9,10-anthraquinone (CAS: 14233-37-5)

No treatment-related effects were observed with respect to mortality, FOB findings, urinary parameters, hematology, or clinical biochemistry in all treatment groups. Body weight and food consumption was unchanged in males and satellite females during treatment. During the administration period, blue feces/purplish urine in treatment animals at and above the 60 mg/kg bw/day dose and blue-colored skin in males at and above the 60 mg/kg bw/day dose and in dams at and above the 12 mg/kg bw/day dose were observed. After the recovery period, the colored urine or skin were rarely observed. Those color changes reflected the test substance, a blue dye, and

were treatment-related but of dubious toxicological relevance. Significant increases in relative liver weight at the 300 mg/kg bw/day dose and absolute and relative liver weights at the 60 mg/kg bw/day dose in males were observed at the end of the administration period. There were significant increases in absolute/relative liver and adrenal glands in satellite females at the 300 mg/kg bw/day dose and significant decreases in absolute/relative spleen and absolute ovary weight in satellite females at the 300 mg/kg bw/day dose at the end of the administration period. After the recovery period, no relevant differences in organ weight were observed. Blue discoloration in the adipose tissue/skin in both sexes for the treatment animals were observed in gross pathology at the end of the administration period and disappeared after the recovery period, except in high-dose animals, indicating that the reversible effects were not clearly toxicologically relevant. A dose-dependent increased incidence of minimal/mild hepatocyte centrilobular hypertrophy was observed in both sexes at doses of 60 mg/kg bw/day and above at the end of the administration period. However, the lesions disappeared in males, and minimal lesion remained in satellite females after the recovery period following a dose of 300 mg/kg bw/day.

The NOAEL for the repeated-dose toxicity was 12 mg/kg bw/day based on weight and histopathological changes in the liver for both sexes at a dose of 60 mg/kg bw/day.

A bacterial reverse mutation assay with *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (OECD TG 471) was performed with and without metabolic activation. 1,4-bis (isopropylamino)-9,10-anthraquinone showed positive results in TA98 and TA1537 with metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) showed positive results for numerical aberrations with and without metabolic activation. Based on the positive results in both studies, 1,4-bis(isopropylamino)-9,10-anthraquinone was considered genotoxic *in vitro*.

In the combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, treatment-related decreases in nursing behavior were observed in dams and were dose-dependent. Also, body weights on lactation day 4 at the 300 mg/kg bw/day dose and food consumption at doses of 12 mg/kg bw/day and above during lactation were significantly lower. Atrophy of the thymus occurred in a dose-dependent manner in dams including controls, which may be associated with the stress of pregnancy. The thymus alterations in high-dose dams were considered to be treatment-related because of the increased incidence and grade. No treatment-related effects were observed on estrous cycle, mating/fertility index, or delivery data. Among the offspring, there were no treatment-related changes in external abnormalities or sex ratio. All pups died during the lactation period in 11, 2, 1, and 1 litters at the 300, 60, 12, and 0 mg/kg bw/day doses, respectively. Pup viability was statistically lower at doses of 60 mg/kg bw/day and above and was considered to be toxicologically relevant. A markedly low body weight of pups was observed at doses of 12 mg/kg bw/day and above. The gross pathological findings of the pups of both sexes were skin/adipose tissue discoloration to blue on PND 4 in all treatment groups, but no treatment-related histopathological changes were observed in the liver, kidney, skin, or adipose tissue.

The LOAEL for the maternal and reproductive/developmental toxicity was determined to be 12 mg/kg bw/day based on decreased nursing behavior and food consumption in dams during lactation, low body weight, and low viability of pups at the 12 mg/kg bw/day dose, which was the lowest dose tested. Reproductive/developmental effects observed at the lower dose induced systemic toxicity.

References

1) Japan Existing Chemical Data Base (JECDB) available at https://dra4.nihs.go.jp/mhlw_data/jsp/SearchPage.jsp (February, 2021)

2) Matsumoto M, Kobayashi K, Takahashi M, Hirata-Koizumi M, Ono A, Hirose A: Summary information of human health hazard assessment of existing chemical substances (Ⅰ), *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*. 2015; 133: 42-47

3) Takahashi M, Matsumoto M, Yamada T, Ono A, Hirose A: Summary information of human health hazard assessment of existing chemical

substances（Ⅱ）, *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*. 2016; 134: 79-83
4）Matsumoto M, Iso T, Yamaguchi H, Igarashi T, Yamada T, Hirose A: Summary information of human health hazard assessment of existing chemical substances（Ⅲ）, *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*. 2017; 135: 39-44
5）Matsumoto M, Iso T, Igarashi T, Tanabe S, Inoue K, Hirose A: Summary information of human health hazard assessment of existing chemical substances（Ⅳ）, *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*. 2018; 136: 108-113
6）Matsumoto M, Iso T, Igarashi T, Tanabe S, Inoue K, Hirose A: Summary information of human health hazard assessment of existing chemical substances（Ⅴ）, *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*. 2019; 137: 66-72
7）Shigeta Y, Iso T, Inoue K, Yamada T, Hirose A, Matsumoto M: Summary information of human health hazard assessment of existing chemical substances（Ⅵ）, *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*. 2020; 138: 33-39
8）OECD Existing Chemicals Database available at https://hpvchemicals.oecd.org/ui/Search.aspx（February, 2021）
9）OECD Guidelines for the Testing of Chemicals available at https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788（February, 2021）