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Summary of human health hazard assessment of existing chemical substances (Ⅸ)

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Toxicological information on existing chemical substances has been collected by the Japanese Ministry of Health, Labour and Welfare according to the Japanese Chemical Substances Control Law. In this study, we sought to disseminate information via the Japan Existing Chemical Database (JECDB). We reviewed several toxicological studies and summarized our assessment of the following five substances: 1,3-benzenedicarboxylic acid, dimethyl ester (CAS: 1459-93-4), terpineol (CAS: 8000-41-7), 2-*tert*-butylcyclohexan-1-yl acetate (CAS: 88-41-5), methyl (2-pentyl-3-oxocyclopentyl) acetate (CAS: 24851-98-7), and 2-decyltetradecanol (CAS: 58670-89-6). The International Uniform Chemical Information Database dossiers created for these five chemical substances are also available in the JECDB.

Keywords: existing chemical substance, toxicological assessment, JECDB

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. Existing chemical substances are substances that were already commercially available prior to 1973. The Japanese CSCL mandates that the Japanese Ministry of Health, Labour and Welfare (MHLW) collect information on human health, including data on repeated-dose toxicity, genotoxicity, and reproductive/development toxicity, for existing chemical substances[1]. Thus far, the MHLW has gathered data on almost 450 existing chemical substances. We have evaluated the impact of existing chemical substances on human health[2-9]. 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 (formerly CoCAP) is accessible to the public through the OECD Existing Chemicals Database[10].

In this report, we summarize the toxicological studies conducted on the following five substances: (1) 1,3-benzenedicarboxylic acid, dimethyl ester (CAS: 1459-93-4), (2) terpineol (CAS: 8000-41-7), (3) 2-*tert*-butylcyclohexan-1-yl=acetate (CAS: 88-41-5), (4) methyl(2-pentyl-3-oxocyclopentyl) acetate (CAS: 24851-98-7), and (5) 2-decyltetradecanol (CAS: 58670-89-6). Given the absence of previous toxicological studies on these chemicals, each study was conducted per Japanese guidelines (similar to the OECD Guidelines for the Testing of Chemicals) for a combined repeated-dose toxicity study with the reproductive/developmental toxicity screening test (OECD TG 422), the bacterial reverse mutation test (OECD TG 471) or the *in vitro* mammalian chromosomal aberration test (OECD TG 473) and according to Good Laboratory Practice Standards. The hazard assessment of these chemicals is well described in the International Uniform Chemical Information Database dossiers. Herein, we introduce findings judged to be toxicological effects, which were statistically significant unless stated as a trend was observed. Our series of human health hazard

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assessments of existing chemical substances, including these five chemicals, is accessible via the Japan Existing Chemical Database (JECDB)[1]. We believe that sharing this data would help prevent the unnecessary use of animals in experiments and provide global access to relevant toxicology data.

(1) 1,3-Benzenedicarboxylic acid, dimethyl ester (CAS: 1459-93-4)

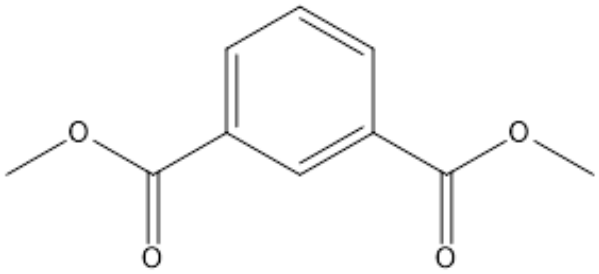

Fig. 1. Structure of 1,3-benzenedicarboxylic acid, dimethyl ester (CAS: 1459-93-4)

Repeated-dose toxicity

A combined repeated-dose toxicity study with a reproductive and developmental toxicity screening test was conducted according to Japanese guidelines for conducting repeated-dose studies combined with reproductive and developmental toxicity screening tests (March 31, 2011; PFSB Notification 0331 No.7). Male and female rats (13 animals/sex/dose) received 1,3-benzenedicarboxylic acid, dimethyl ester (dimethyl isophthalate; DMIP) at 0 (vehicle: corn oil), 62.5, 250, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14-day premating and subsequent mating periods. In contrast, females in the mating group were dosed for 41–45 days, including the 14-day premating, mating, gestation, and lactation periods, and until day 4 of lactation. Five males at the doses of 0 and 1,000 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period. Ten additional females were dosed at 0 and 1,000 mg/kg bw/day as a satellite group. These females were dosed for 42 days without mating, and five females at 0 and 1,000 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period.

Trends in the restriction of body weight gain were observed during gestation and lactation days in females at ≥250 mg/kg bw/day, while food consumption led to decrease.

Under general condition observations, reddish urine was observed in one mating female at 1,000 mg/kg bw/day. This finding was considered an adverse effect because dimethyl terephthalate (CAS: 120-61-6), an analog of DMIP, produces calculi that affect the kidney and urinary bladder[11]. In the urinalysis, urine volume and Na increased in males at 1,000 mg/kg bw/day. In these studies, a tendency to decrease the pH of urine was also observed.

In blood biochemistry, glucose and triglycerides increased in mating and satellite females at 1,000 mg/kg bw/day. Furthermore, bile acid increased in mating females at the same dose.

There were increases in the relative liver and kidney weights of both mating and satellite females at 1,000 mg/kg bw/day. At the same dose, their absolute weights also increased in satellite females. After the recovery period, these changes were no longer detectable.

Based on these results, the no observed adverse effect level (NOAEL) for repeated-dose toxicity was determined to be 250 mg/kg bw/day for males and 62.5 mg/kg bw/day for females.

Reproductive and developmental toxicity

In the screening test described above, the reproductive and developmental toxicity parameters at the highest dose tested were not caused by DMIP. The NOAEL for reproductive and developmental toxicity was considered to be 1,000 mg/kg bw/day.

Genotoxicity

In a bacterial reverse mutation assay conducted with *Salmonella typhimurium* (*S. typhimurium*) TA100, TA98, TA1535, and TA1537 along with *Escherichia coli* (*E. coli*) WP2 *uvrA* according to OECD TG 471, DMIP was found to be negative with and without metabolic activation. In contrast, in an *in vitro* chromosomal aberration test using Chinese hamster (CHL/IU) cells (OECD TG 473), DMIP was found to be positive for structural aberrations with and without metabolic activation. Thus, based on these results, DMIP was considered to be clastogenic *in vitro*.

(2) Terpineol (CAS: 8000-41-7)

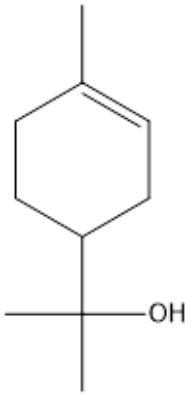

Fig. 2. Structure of terpineol (CAS: 8000-41-7)

Repeated-dose toxicity

A combined repeated-dose toxicity study with a reproductive and developmental toxicity screening test was conducted according to Japanese guidelines for conducting repeated-dose studies combined with reproductive and developmental toxicity screening tests (March 31, 2011; PFSB Notification 0331 No.7). Furthermore, male and female rats (12 animals/sex/dose) were administered terpineol at 0 (vehicle: corn oil), 100, 300, and 1,000 mg/kg bw/day. Males were dosed for 44 days, including a 14-day premating period and subsequent mating period, whereas females in the mating group were dosed for 41–51 days, including the 14-day premating, mating, gestation, and lactation periods, as well as until day 4 of lactation. Five males receiving doses of 0 and 1,000 mg/kg bw/day were assigned to a recovery group and observed for 14 days following the administration period. As a satellite group, 10 additional females were administered doses of 0 and 1,000 mg/kg bw/day. These females were dosed for 44 days without mating, after which five females at 0 and 1,000 mg/kg bw/day was assigned to a recovery group and observed for 14 days.

At 1,000 mg/kg bw/day, four mating females and two satellite females perished or became moribund. Their food consumption and body weight both decreased. In addition to the histopathological changes described later at the same dose, the following histopathological findings were observed: an eosinophilic droplet in the adrenal cortical cell as well as tubular dilatation and regeneration of the papillary collecting duct in the kidney.

Due to death, non-pregnant, or all its pups dead, some results were recorded for only one mating female receiving 1,000 mg/kg bw/day. Thus, findings on them, especially in the hematology, the biochemistry, and organ weights, were for reference and could not be determined as adverse effects of terpineol statistically.

In clinical observations, transient salivation and decreased feces were observed in males and satellite females at 1,000 mg/kg bw/day. In addition, bradypnea, emaciation, ataxia, and decreased spontaneous movement were observed at the same dose in mating and satellite females. Additionally, a decrease in body weight gain was observed in males at the highest dose.

Males and satellite females receiving 1,000 mg/kg bw/day exhibited an increase in urine volume and a decrease in osmotic pressure accompanied with an increase in water intake, as determined by urine analysis. The excreted electrolytes increased at the conclusion of their recovery period, and these findings remained unchanged.

At 1,000 mg/kg bw/day, male and female satellites exhibited a decrease in hemoglobin (HGB). At the same dose, satellite females exhibited a decrease in hematocrit (Ht) and an increase in mean corpuscular HGB concentration, and fibrinogen (FIB). In blood chemistry, an increase in γ-GTP was observed in males at 1,000 mg/kg bw/day.

In organ weights, absolute and relative liver weight increased in mating females at 300 mg/kg bw/day. The absolute and relative weights of the liver and the kidney increased, and ones of the testes and epididymides decreased in males at 1,000 mg/kg bw/day, which did not restore at the end of the recovery period. Moreover, the absolute and relative weights of the liver, kidney, and adrenal increased in satellite females at the same dose. At the end of the recovery period, their relative liver weight was still higher than that of the control group.

In terms of histopathology, the following observations were made: At 1,000 mg/kg bw/day, the kidneys of males and females exhibited vacuolation of the distal tubule and collecting duct, single cell necrosis of the papillary duct, tubular dilatation, and cell infiltration in the cortex, papillary necrosis, and cell infiltration in the papilla in mating and satellite females, and regeneration of the papillary collecting duct in males. Males receiving ≥300 mg/kg bw/day

and female satellites receiving 1,000 mg/kg bw/day showed tubular regeneration in the cortex. Eosinophilic bodies likely attributable to α2μ-globulin were observed in tubular cells in males at ≥300 mg/kg bw/day. Moreover, vacuolation of the proximal tubule was observed in mating females at 300 mg/kg bw/day. In the urinary bladder, vacuolation of umbrella cells was observed in males at ≥300 mg/kg bw/day and in mating females at 300 mg/kg bw/day.

Additionally, atrophy of umbrella cells and hypertrophy or hyperplasia of transitional epithelium were observed in males and females at 1,000 mg/kg bw/day. In the liver, hypertrophy of the centrilobular hepatocyte was observed in males, and hepatocyte vacuolation in females was observed at 1,000 mg/kg bw/day. In the pancreas, the zymogen granule decreased in females at 1,000 mg/kg bw/day, and there is a tendency toward that in mating females at 300 mg/kg bw/day. Cortical cell vacuolation was observed in the adrenals of females at ≥300 mg/kg bw/day. Examination with electron microscopy revealed that vacuolation of adrenal cortical cells and vacuolation of hepatocytes were caused by the enlargement of mitochondria. At 1,000 mg/kg bw/day, seminiferous tubular atrophy or vacuolation and multinucleated giant cells were observed in the testes. These findings remained at the end of the recovery period. In the epididymides, hypospermia, and cell debris in the lumen were observed at 1,000 mg/kg bw/day and remained at the end of the recovery period.

Based on these results, the NOAEL for repeated-dose toxicity was considered to be 100 mg/kg bw/day.

Reproductive and developmental toxicity

A reproductive and developmental toxicity screening test was conducted with the abovementioned combined screening test.

For the parents, the insemination index decreased at 1,000 mg/kg bw/day, which is considered to be caused by the toxicity of testes and epididymides. In addition, poor nursing was observed in dams at ≥300 mg/kg bw/day and caused the death of all delivered pups in each one dam at 300 and 1,000 mg/kg bw/day.

The effects of the pups at 1,000 mg/kg bw/day cannot be determined because the fertility index was too low (20.0%), and all the pups of one dam were dead due to severe maternal state, whereas no effect was observed at ≤300 mg/kg bw/day.

Based on these results, the NOAELs for reproductive and developmental toxicity were considered to be 300 mg/kg bw/day for male parents and pups and 100 mg/kg bw/day for dams.

Genotoxicity

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

(3) 2-*tert*-Butylcyclohexan-1-yl acetate (CAS: 88-41-5)

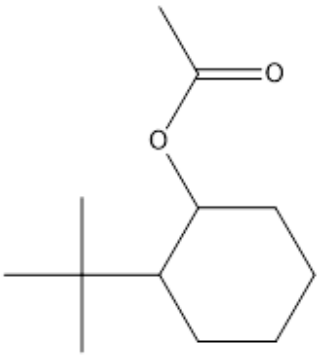

Fig. 3. Structure of 2-*tert*-butylcyclohexan-1-yl acetate (CAS: 88-41-5)

Repeated-dose toxicity

A combined repeated-dose toxicity study with a reproductive and developmental toxicity screening test was performed according to Japanese guidelines for conducting repeated-dose studies combined with reproductive and developmental toxicity screening tests (March 31, 2011; PFSB Notification 0331 No.7). Male and female rats (12 animals/sex/dose) were administered 2-*tert*-butylcyclohexan-1-yl acetate (2BA) at 0 (vehicle: corn oil), 50, 150, and 500 mg/kg bw/day. Males were dosed for 42 days, including a 14-day premating period and subsequent mating period, whereas females in the mating group were dosed for 41–46 days, including the 14-day premating, mating, gestation, and lactation periods, as well as until day 4

of lactation. Five males at doses of 0 mg/kg bw/day and 500 mg/kg bw/day were assigned to a recovery group and monitored for 14 days following the administration period. As a satellite group, 10 additional females were dosed with 0 and 500 mg/kg bw/day. These females were dosed for 42 days without mating, and five females at 0 and 500 mg/kg bw/day were assigned to a recovery group and maintained for 14 days after the administration period.

Deaths were recorded in four females from the mating group at 500 mg/kg bw/day. Three died on gestation days 12–17, and the other died on administration day 10. In addition, three satellite females died at the same dose on days 15–29. Some dead animals showed clinical signs such as clonic convulsions and salivations several hours after dosing and slight restraint of body weight gain. In histopathological findings, diffuse acinar atrophy of the submandibular gland and centrilobular hypertrophy of the hepatocyte were observed.

In clinical observations, transient clonic convulsions and salivations were observed in other live males and females at 500 mg/kg bw/day. Furthermore, there was a decrease in body weight gain during gestation in females and a tendency toward that in males at the same dose.

In urinalysis, tendencies to increase urine volume and decrease osmotic pressure accompanied with an increase in water intake were observed in males at 500 mg/kg bw/day. There was also a notable increase in excreted Cl and an increase in Na and K. The trend of increased water intake, lower osmotic pressure, and increased excreted Cl remained at the end of the recovery period, whereas the increase in Na and K was reduced.

In hematology, the red blood cell (RBC) count decreased in satellite females at 500 mg/kg bw/day. In addition, prolonged prothrombin time (PT) and increased FIB were observed in males at 500 mg/kg bw/day. In blood biochemistry, total bile acid decreased at the same dose in males and satellite females. A decrease in glucose was also observed in males at the highest dose tested.

Absolute and relative liver weights, as well as relative kidney weight, increased in males receiving ≥ 50 mg/kg bw/day. The absolute kidney mass increased in males at 500 mg/kg bw/day. In addition, the relative and absolute weight of the adrenal glands increased in mating females at ≥150 mg/kg bw/day, as well as at 500 mg/kg bw/day. At 500 mg/kg bw/day, the absolute and relative weights of the thyroid and thymus increased in female satellites. At the end of the recovery period, the increase in relative thymus weight in females remained.

Histopathology revealed hypertrophy of the centrilobular hepatocyte in males and females receiving ≥150 mg/kg bw/day. Tubule dilation, granular cast, and interstitial cell infiltration were observed in males at 500 mg/kg bw/day. At the end of the recovery period, the urinary cast remained. Tubule regeneration was observed in males at ≥150 mg/kg bw/day. The eosinophilic body in the tubular cell, which is considered due to α2μ-globulin, was also observed at ≥50 mg/kg bw/day. Vacuolation of the adrenocortical cell was found in mating and satellite females at 500 mg/kg bw/day.

Furthermore, hypertrophy in thyroid follicular cells was observed in males at ≥150 mg/kg bw/day and in mating and satellite females at 500 mg/kg bw/day.

Except for the regeneration of renal tubules, granular urinary casts, increased urine volume, and increased Cl excretion, all of the aforementioned findings were attenuated or disappeared during the recovery period and were reversible.

Based on these findings, the lowest observed adverse effect level for repeated-dose toxicity of 2BA was determined to be 50 mg/kg bw/day for males, and the NOAEL was considered to be 50 mg/kg bw/day for females.

Reproductive and developmental toxicity

In conjunction with the abovedescribed combined repeated-dose toxicity screening test, a screening for reproductive and developmental toxicity was conducted. Postnatally, on days 0–4 at 500 mg/kg bw/day, inhibited body weight gain was observed in pups. No additional reproductive and developmental effects were identified.

Thus, the NOAEL for reproductive and developmental toxicity was considered to be 500 mg/kg bw/day for the parent animals and 150 mg/kg bw/day for the pups.

Genotoxicity

In a bacterial reverse mutation assay with *S. typhimurium* TA100, TA98, TA1535, and TA1537, as well as *E. coli* WP2 *uvrA* according to OECD TG 471, 2BA was negative with and without metabolic activation. Furthermore, 2BA was negative in an *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473), with or without metabolic activation. Based on these results, 2BA was considered to be nongenotoxic *in vitro*.

(4) Methyl (2-pentyl-3-oxocyclopentyl) acetate (CAS: 24851-98-7)

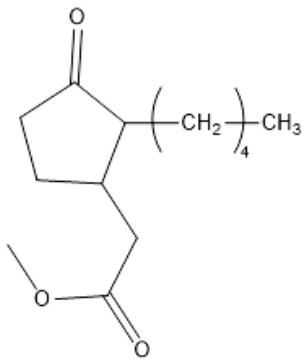

Fig. 4. Structure of methyl (2-pentyl-3-oxocyclopentyl) acetate (CAS: 24851-98-7)

Repeated-dose toxicity

A combined repeated-dose toxicity study with a reproductive and developmental toxicity screening test was conducted according to Japanese guidelines for conducting repeated-dose studies combined with reproductive and developmental toxicity screening tests (March 31, 2011; PFSB Notification 0331 No.7). Male and female rats (12 animals/sex/dose) were administered methyl (2-pentyl-3-oxocyclopentyl) acetate at the following doses: 0 (vehicle: corn oil), 100, 300, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14-day premating period and a subsequent mating period, whereas females in the mating group were dosed for 41–46 days, including the 14-day premating, mating, gestation, and lactation periods, as well as until day 4 of lactation. Five males receiving doses of 0 and 1,000 mg/kg bw/day were assigned to a recovery group and observed for 14 days after the administration period. As a satellite group, 10 additional females were administered doses of 0 and 1,000 mg/kg bw/day. These females were dosed for 42 days without mating, and five females at 0 and 1,000 mg/kg bw/day were assigned to a recovery group and observed for 14 days following the administration period.

In clinical observations, transient slight salivation was observed in males and females at 1,000 mg/kg bw/day. There is an increase in food consumption in them, whereas food consumption transiently with restriction of body weight gain in males on day 2 administration at the same dose.

In urinalysis, increase in water intake was observed in males and female satellites at 1,000 mg/kg bw/day. This is accompanied with increase in urine volume in males at 1,000 mg/kg bw/day and decreased osmotic pressure in satellite females at the same dose.

In hematology, a decrease in RBC, HGB, or Ht was observed in satellite females at 1,000 mg/kg bw/day. In blood chemistry, a decrease in Cl and an increase in alkaline phosphatase were observed in males at 1,000 mg/kg bw/day. At the same dose, a decrease in glucose and an increase in total cholesterol (T-CHO) and BUN were observed in mating females, whereas a decrease in Cl and glucose and an increase in alanine aminotransferase, T-CHO, triglycerides, and phospholipids (PL) were observed in satellite females.

Relative liver weights increased in males at ≥ 300 mg/kg bw/day, and absolute and relative liver weights also increased in mating females at ≥300 mg/kg bw/day and in satellite females at 1,000 mg/kg bw/day. In addition, an increase in relative kidney weight was observed in both males and mating females at 1,000 mg/kg bw/day. Furthermore, an increase in absolute kidney weights was observed in female satellites.

At 1,000 mg/kg bw/day, histopathology revealed focal tubular dilatation in the kidneys of mating females. Furthermore, there was hypertrophy of centrilobular hepatocytes in males and mating females at ≥300 mg/kg bw/day. These changes were reduced or no longer observed after the withdrawal of treatment.

Based on these effects, the NOAEL for repeated-dose toxicity was considered 100 mg/kg bw/day in males and females.

Reproductive and developmental toxicity

A reproductive and developmental toxicity

screening test was conducted with the combined repeated-dose toxicity screening test described above.

For the parents, there was trends of abnormal estrous cycle and lower delivery index in female rats at 1,000 mg/kg bw/day. At the same dose, prolonged labor time was observed on one dam.

On postnatal day 0, the body weight of both males and females decreased at 1,000 mg/kg bw/day.

Based on these results, the NOAELs for reproductive and developmental toxicity were considered to be 300 mg/kg bw/day for pups and dams and 1,000 mg/kg bw/day for male parents.

Genotoxicity

In a bacterial reverse mutation assay with *S. typhimurium* TA100, TA98, TA1535, and TA1537 and *E. coli* WP2 *uvrA* according to OECD TG 471, methyl (2-pentyl-3-oxocyclopentyl) acetate was negative both with and without metabolic activation. In an *in vitro* chromosomal aberration assay utilizing CHL/IU cells (OECD TG 473), methyl (2-pentyl-3-oxocyclopentyl) acetate was also negative both with and without metabolic activation. Based on these results, methyl (2-pentyl-3-oxocyclopentyl) acetate was considered to be nongenotoxic *in vitro*.

(5) 2-Decyltetradecanol (CAS: 58670-89-6)

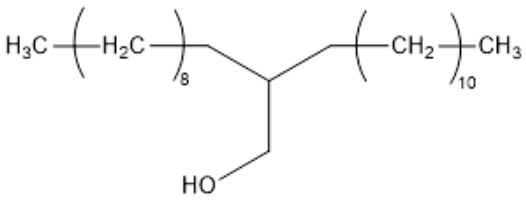

Fig. 5. Structure of 2-decyltetradecanol (CAS: 58670-89-6)

Repeated-dose toxicity

A combined repeated-dose toxicity study with a reproductive and developmental toxicity screening test was conducted according to Japanese guidelines for conducting combined repeated-dose studies with reproductive and developmental toxicity screening tests (March 31, 2011; PFSB Notification 0331 No.7). Male and female rats (12 animals/sex/dose) received 2-decyltetradecanol (2-DT) by gavage at 0 (vehicle: corn oil), 62.5, 250, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14-day premating period and a subsequent mating period, whereas females in the mating group were dosed for 41–55 days, including the 14-day premating, mating, and gestation periods, and until day 4 of lactation. Five males at doses of 0 and 1,000 mg/kg bw/day were allocated to a recovery group and maintained for 14 days after the administration period. Ten additional females were dosed at 0 and 1,000 mg/kg bw/day as a satellite group; these females were dosed for 42 days without mating, and five females at 0 and 1,000 mg/kg bw/day were allocated to a recovery group and observed for 14 days after the administration period.

There were no significant effects on clinical observations, body weight, and food consumption. In urinalysis, occult blood was observed at 1,000 mg/kg bw/day in one male. Furthermore, the urine volumes and excreted electrolytes (Na, K, and Cl) decreased in satellite females administered 1,000 mg/kg bw/day.

In hematology, prolonged PT and a trend toward an increase in activated partial thromboplastin time were observed in males at the end of dosing period at ≥ 250 mg/kg/day. After administering 1,000 mg/kg bw/day, these effects diminished but persisted at the end of the recovery period. In biochemistry, an increase in activated LDH was also observed in them. No treatment-related effects on organ weight or histopathology was observed.

Based on these results, NOAELs for repeated-dose toxicity were considered to be 62.5 mg/kg bw/day in males and 250 mg/kg bw/day in females.

Reproductive and developmental toxicity

The abovementioned screening test showed no adverse effects on reproductive and developmental parameters at the highest dose tested. Therefore, the NOAEL for reproductive and developmental toxicity was determined to be 1,000 mg/kg bw/day.

Genotoxicity

In a bacterial reverse mutation assay with *S. typhimurium* TA100, TA98, TA1535, and TA1537, along with *E. coli* WP2 *uvrA* according to OECD TG 471, 2-DT was negative with and without metabolic activation. In an *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473), 2-DT was also found to be negative with and without metabolic activation. Based on these results, 2-DT was considered

nongenotoxic *in vitro*.

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