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

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Under the Chemical Substances Control Law in Japan, initial hazard information on existing chemical substances is collected by the Ministry of Health, Labour and Welfare, Japan to assess potential initial risks to human health. This hazard information includes acute toxicity, repeated-dose toxicity, genotoxicity, and/or reproductive/developmental toxicity. In an attempt to disseminate such information more widely, we have been reporting this information. In the present report, a summary of hazard information is presented for the following five existing chemical substances: 2,3,4,4′-tetrahydr oxybenzophenone (CAS: 31127-54-5); 1-propene, tetramer (CAS: 6842-15-5); 4-chlorobenzaldehyde (CAS: 104-88-1); diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate (CAS: 3012-65-5); and 2-nitro-p-cresol (CAS: 119-33-5). Additionally, we created a dossier for the clarification and evaluation of each study in English using the International Uniform Chemical Information Database version 5.

Keywords: hazard assessment, human health, IUCLID, dossier, JECDB

Introduction

Under the Chemical Substances Control Law (CSCL) in Japan, initial hazard information on existing chemical substances is collected by the Ministry of Health, Labour and Welfare (MHLW), Japan to assess potential initial risks to human health[1]. This hazard information includes acute toxicity, repeated-dose toxicity, genotoxicity, and/or reproductive/developmental toxicity. Safety information obtained is essential for not only CSCL assessments but also safe handling of chemicals by users. We had reviewed these toxicity studies and submitted the initial risk assessment documents for 5-10 chemical substances per year to the OECD Cooperative Chemicals Assessment Programme (CoCAP). Although initial risk assessments for chemical substances ended at the CoCAP in 2014, toxicity studies on existing chemical substances are kept on conducting by MHLW. It is necessary to promote a wider dissemination of such information to the public. Thus, we have continued to develop hazard assessments for the existing chemical substances targeted by MHLW, and we have been reporting this information[2]. In the present report, as a contribution to our ongoing hazard assessment study, we present a summary of the hazard information related to the potential human health effects of the following five chemical substances: 2,3,4,4′-tetrahydroxybenzophenon e (CAS: 31127-54-5); 1-propene, tetramer (CAS: 6842-15-5); 4-chlorobenzaldehyde (CAS: 104-88-1); diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate (CAS: 3012-65-5); and 2-nitro-p-cresol (CAS: 119-33-5).

Additionally, we created a dossier (a collection of study data containing a detailed summary of the methods, results, and conclusions of each study) for the clarification and evaluation of each study. Each dossier is written in English using the International Uniform Chemical Information Database (IUCLID) version 53). The IUCLID dossiers for these chemical substances will be available from the Japan Existing Chemical Data Base (JECDB)[1].

(1) 2,3,4,4′-Tetrahydroxybenzophenone (CAS: 31127-54-5)

The acute oral median lethal dose (LD_{50}) for 2,3,4,4′-tetrahydroxybenzophenone was established at > 2,000 mg/kg body weight (bw) in female rats on the basis

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of a study conducted according to the Organisation for Economic Co-operation and Development Test Guideline (OECD TG) 423. The substance caused no deaths or clinical signs of toxicity at 2,000 mg/kg bw.

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed according to OECD TG 422. Male and female rats (12 animals/sex/dose) were administered 2,3,4,4′-tetrahydroxybenzophenone at 0, 100, 300, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14-day pre-mating and mating periods; females were dosed for 41–45 days, including a 14-day pre-mating, mating, and gestation periods and the time until day 4 of lactation. In addition, male and female rats (five animals/sex/dose) were administered 0 and 1,000 mg/kg bw/day for 42 days without mating and examined after a 14-day recovery period. At 1,000 mg/kg bw/day, one female died on day 0 of lactation, salivation was observed in males and a decreased body weight gain was observed in both sexes. Regarding hematology parameters, anemia was observed at the same dose in males. Clinical chemistry studies demonstrated increased inorganic phosphorus at 300 mg/kg bw/day and higher in males. In the thymus, decreased organ weight and atrophy were observed at 300 mg/kg bw/day and higher in females. In the cecum, single cell necrosis of mucosal epithelial cells and diffuse mucosal hyperplasia were observed at 100 mg/kg bw/day and higher in both sexes. In the liver, in both sexes, increased organ weight at 1,000 mg/kg bw/day and decreased vacuolation of the perilobular hepatocytes in a dose-dependent manner at 300 mg/kg bw/day and higher was observed. These changes tended to resolve after the recovery period. On the basis of the findings in the cecum, the lowest observed adverse effect level (LOAEL) for repeated-dose toxicity of 2,3,4,4′-tetrahydroxybenzophenone was determined to be 100 mg/kg bw/day in male and female rats.

In a bacterial reverse mutation assay using *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and *Escherichia coli* WP2*uvrA* (similar to OECD TG 471), 2,3,4,4′-tetrahydroxybenzophenone was negative with or without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) showed positive; however, an *in vivo* micronucleus study (OECD TG 474) showed negative up to the limit dose (2,000 mg/kg bw/day for 2 days) in mice. On the basis of these results, 2,3,4,4′-tetrahydroxybenzophenone was regarded as non-genotoxic *in vivo*.

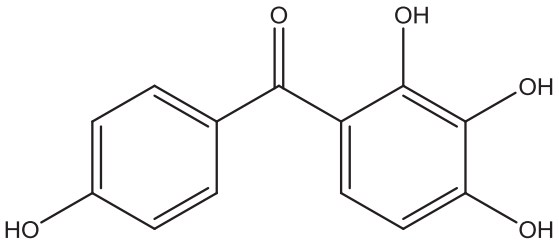

Fig. 1. Structure of 2,3,4,4′-tetrahydroxybenzophenone

In the aforementioned combined repeated oral dose toxicity study (0, 100, 300, and 1,000 mg/kg bw/day) with the reproduction/developmental toxicity screening test (OECD TG 422), no effects were found on reproductive parameters up to 1,000 mg/kg bw/day. The body weights of male and female pups decreased on postnatal day (PND) 4 at 300 mg/kg bw/day and higher, with decreased body weights observed for both sexes on PND 0 at 1,000 mg/kg bw/day. The no observed adverse effect levels (NOAELs) for rat reproductive toxicity and developmental toxicity were determined to be 1,000 mg/kg bw/day and 100 mg/kg bw/day, respectively.

(2) 1-Propene, tetramer (CAS: 6842-15-5)

The acute oral LD_{50} of 1-propene, tetramer was > 2,000 mg/kg bw in female rats based on a study conducted according to OECD TG 423. No deaths were observed at 2,000 mg/kg bw. This substance at 300 mg/kg bw caused diarrhea and at 2,000 mg/kg bw caused decreased locomotor activity, diarrhea, and soiled perineal region.

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed according to a Japanese guideline (similar to OECD TG 422). Male and female rats (12 animals/sex/dose) were administered 1-propene, tetramer at 0, 40, 150, and 600 mg/kg bw/day. Males were dosed for 42 days, including a 14-day pre-mating and mating periods. Females were dosed for 40–45 days, including a 14-day pre-mating, mating, and gestation periods and the time until day 4 of lactation. Five out of 12 males with administered doses of 0 and 600 mg/kg bw/day were evaluated as a 14-day recovery group. In addition, 10 females/dose were administered 0 and 600 mg/kg bw/day for 42 days without mating; they

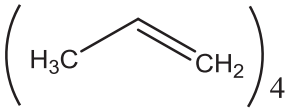

Fig. 2. Structure of 1-propene, tetramer

were examined after the administration period or after a 14-day recovery period. Regarding hematology parameters, anemia was observed at 150 mg/kg bw/day and higher in males, with decreased red blood cell counts at 600 mg/kg bw/day in females without mating. In the kidney, in males, α2u-globulin nephropathy was observed at 40 mg/kg bw/day and higher, with increased kidney weight at 40 mg/kg bw/day and higher and basophilic changes in the tubular epithelium at 150 mg/kg bw/day and higher. Furthermore, necrosis of the tubular epithelium, increased blood urea nitrogen level, and round epithelial cells in urinary sediments were observed in males at 600 mg/kg bw/day. These effects were considered to be caused by α2u-globulin accumulation in the kidney as male rat specific disease, and were not relevant in human health. In the liver, in both sexes, increased liver weight was observed at 150 mg/kg bw/day and higher, with centrilobular hepatocytes hypertrophy at 600 mg/kg bw/day. Furthermore, increases in the α2-globulin fraction, γ-glutamyl transpeptidase, and total cholesterol levels and a decrease in glucose level were observed at 600 mg/kg bw/day in both sexes. In the thyroid, in females, increased thyroid weight and hypertrophy of follicular cells were observed at 600 mg/kg bw/day, with thyroxin level increasing after the recovery period at this dose. Hematology, kidney, and liver, but not thyroid, changes tended to resolve after the recovery period. On the basis of anemia in males and increased liver weight in both sexes, NOAEL for repeated-dose toxicity was determined to be 40 mg/kg bw/day in male and female rats.

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (similar to OECD TG 471), 1-propene, tetramer was negative with and without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) showed negative result with and without metabolic activation. On the basis of these results, 1-propene, tetramer was regarded as non-genotoxic *in vitro*.

In the aforementioned combined repeated oral dose toxicity study (0, 40, 150, and 600 mg/kg bw/day) with the reproduction/developmental toxicity screening test (OECD TG 422), no effects of this substance on reproductive and developmental parameters were observed at 600 mg/kg bw/day. NOAEL for the rat reproductive/developmental toxicity of 1-propene, tetramer was determined to be 600 mg/kg bw/day, the highest dose tested.

(3) 4-Chlorobenzaldehyde (CAS: 104-88-1)

In a 28-day repeated-dose toxicity test performed according to OECD TG 407, male and female rats (6 animals/sex/dose) were administered 4-chlorobenzaldehyde at 0, 8, 40, 200, and 1,000 mg/kg bw/day. In addition, both sexes (6 animals/sex/dose) were administered 0 and 1,000 mg/kg bw/day of this substance for 28 days and examined after a 14-day recovery period. At 1,000 mg/kg bw/day, transient salivation and tremors were observed in both sexes, decreases in body weight gain and grip strength of forearms were observed in males, and a decrease in the locomotor activity was observed in females. At this dose, increases in serum aspartate aminotransferase and alanine aminotransferase levels were observed in both sexes, whereas decreases in total protein and β-globulin fraction were observed in males, and increases in alkaline phosphatase and triglyceride levels were observed in females. Increased liver weight and decreased ovary weight were also observed at 1,000 mg/kg bw/day in females. Histopathological examinations revealed hyperostosis metaphysis of the femur in females at 200 mg/kg bw/day. Furthermore, at 1,000 mg/kg bw/day, hyperkeratosis of the mucosal epithelium in the forestomach, grade enhancement of regeneration of the tubular epithelium, dilatation of the renal tubules in the cortex, and cyst-like extension of the collecting duct in the kidney, and hyperostosis metaphysis of the femur were observed in both sexes. Additionally in males at 1,000 mg/kg bw/day,

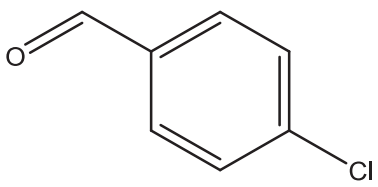

Fig. 3. Structure of 4-chlorobenzaldehyde

degeneration of nerve fibers in the sciatic nerve and atrophy of muscle fibers in the skeletal muscle were observed. And in females at 1,000 mg/kg bw/day, squamous cell hyperplasia of the boundary edge in the stomach was observed. These changes tended to resolve after the recovery period. On the basis of these effects, NOAELs for repeated-dose toxicity were determined to be 200 mg/kg bw/day and 40 mg/kg bw/day in male and female rats, respectively.

A reproduction/developmental toxicity screening test was performed according to OECD TG 421. Male and female rats (12 animals/sex/dose) were administered 4-chlorobenzaldehyde at 0, 40, 200, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14 day pre-mating and mating periods. Females were dosed for 42–45 days, including a 14-day pre-mating, mating, and gestation periods and the time until day 4 of lactation. Nine males and seven females died with tremors and decreased locomotor activity at 1,000 mg/kg bw/day by day 9 of administration. In this study, NOAEL for repeated-dose toxicity was determined to be 200 mg/kg bw/day in male and female rats.

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (OECD TG 471), 4-chlorobenzaldehyde was negative with or without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) showed positive result with and without metabolic activation. On the basis of these results, 4-chlorobenzaldehyde was regarded as clastogenic *in vitro*.

In the aforementioned reproduction/developmental toxicity screening test (0, 40, 200, and 1,000 mg/kg bw/day) (OECD TG 421), administration of 4-chlorobenzaldehyde at 1,000 mg/kg bw/day was halted because of the frequent deaths in male and female rats. No effects of this substance on reproductive and developmental parameters were observed at 200 mg/kg bw/day. NOAEL for the rat reproductive/developmental toxicity of 4-chlorobenzaldehyde was determined to be 200 mg/kg bw/day.

(4) Diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate (CAS: 3012-65-5)

The acute oral LD_{50} of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate was > 2,000 mg/kg bw in female rats based on a study conducted according to OECD TG 423. No deaths were observed at 2,000 mg/kg bw. This substance caused mucoid stools at 2,000 mg/kg bw.

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed according to OECD TG 422. Male and female rats (12 animals/sex/dose) were administered diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate at 0, 100, 300, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14-day pre-mating and mating periods. Females were dosed for 41–47 days, including a 14-day pre-mating, mating, and gestation periods and the time until day 4 of lactation. Five animals/sex/dose administered 0 and 1,000 mg/kg bw/day were treated as the recovery group and examined after a 14-day recovery period. After the administration period, squamous cell hyperplasia of the boundary edge in the stomach was observed at 1,000 mg/kg bw/day in both sexes. This change resolved after the recovery period. On the basis of the observed stomach changes, NOAEL for repeated-dose toxicity was determined to be 300 mg/kg bw/day in male and female rats.

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (OECD TG 471), diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate was negative with or without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) showed negative result with or without metabolic activation. On the basis of these results, diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate was regarded as non-genotoxic *in vitro*.

In the aforementioned combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (0, 100, 300, and 1,000 mg/kg bw/day) (OECD TG 422), no effects of this substance on reproductive and developmental parameters were

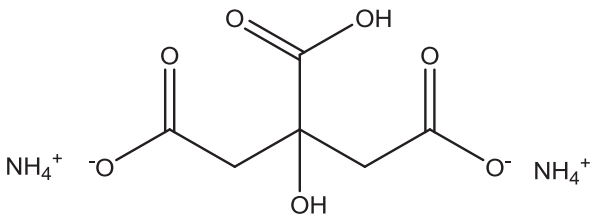

Fig. 4. Structure of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate

observed at 1,000 mg/kg bw/day. NOAEL for the rat reproductive/developmental toxicity of diammonium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate was regarded as 1,000 mg/kg bw/day, the highest dose tested.

(5) 2-Nitro-p-cresol (CAS: 119-33-5)

A 28-day repeated-dose toxicity test was performed according to the Japanese guideline (similar to OECD TG 407). Male and female rats (5 animals/sex/dose) were administered 2-nitro-p-cresol at 0, 15, 60, 250, and 1,000 mg/kg bw/day. In addition, both sexes (5 animals/sex/dose) were administered 0 and 1,000 mg/kg bw/day of this substance for 28 days and examined after a 14-day recovery period. At 250 mg/kg bw/day and higher, sedation and ptosis were observed in both sexes. Increase in the liver weight was observed at 250 mg/kg bw/day and higher in females and at 1,000 mg/kg bw/day in males. Furthermore, increases in the kidney weight in males and spleen weight in both sexes were observed at 1,000 mg/kg bw/day. Histopathological examinations revealed hypertrophy of hepatocytes at 250 mg/kg bw/day and higher in females. At 1,000 mg/kg bw/day, increase in the extramedullary hematopoiesis and brown pigmentation in the spleen was observed in both sexes. Additionally in males, hypertrophy of hepatocytes in the liver was observed at 1,000 mg/kg bw/day. Moreover, an increase in hyaline droplets containing α2u-globulin in the renal proximal tubular epithelium in the kidney was observed in males at the same dose. These changes, except brown pigmentation in the spleen, tended to resolve after the recovery period. On the basis of these effects, NOAEL for repeated-dose toxicity was determined to be 60 mg/kg bw/day in male and female rats.

A reproduction/developmental toxicity screening test was performed according to OECD TG 421. Male and female rats (12 animals/sex/dose) were administered 2-nitro-p-cresol at 0, 60, 250, and 1,000 mg/kg bw/day. Males were dosed for 42 days, including a 14 day pre-mating and mating periods. Females were dosed for 42–47 days, including a 14 day pre-mating, mating, and gestation periods, and the time until lactation day 4. At 1,000 mg/kg bw/day, ptosis and decreased locomotor activity were observed in both sexes. At the same dose, histopathological

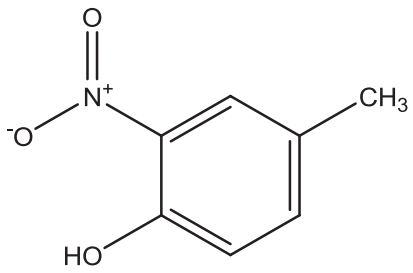

Fig. 5. Structure of 2-nitro-p-cresol

examinations revealed centrilobular hypertrophy of hepatocytes in the liver and increased extramedullary hematopoiesis in the spleen in both sexes. On the basis of these changes, NOAEL for repeated-dose toxicity was determined to be 250 mg/kg bw/day in male and female rats.

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2*uvrA* (OECD TG 471), 2-nitro-p-cresol was negative with or without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) showed positive result with metabolic activation. However, the result of an *in vivo* micronucleus study (OECD TG 474) were negative up to the maximum tolerated dose (1,000 mg/kg bw/day for 2 days) in mice. On the basis of these results, 2-nitro-p-cresol was regarded as non-genotoxic *in vivo*.

In the aforementioned reproduction/developmental toxicity screening test (0, 60, 250, and 1,000 mg/kg bw/day) (OECD TG 421), no effects of this substance on reproductive and developmental parameters were observed at 1,000 mg/kg bw/day. NOAEL for the rat reproductive/developmental toxicity of 2-nitro-p-cresol was determined to be 1,000 mg/kg bw/day, the highest dose tested.

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