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

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Under the Chemical Substances Control Law（CSCL）in Japan, initial hazard information for existing chemical substances has been collected by the Ministry of Health, Labour and Welfare, Japan（MHLW）to assess potential initial risks to human health. We have reviewed all collected toxicity information pertaining to acute toxicity, repeated dose toxicity, genotoxicity, and/or reproductive/developmental toxicity and performed hazard assessments. Approximately 150 substances are currently undergoing review and assessment. For clarification and evaluation of each toxicity study, we have created a dossier（a collection of study data containing a detailed summary of the methods, results, and conclusions of each study）in English using the International Uniform Chemical Information Database（IUCLID）version 5. The IUCLID dossier format is widely used and has been accepted as one of the most beneficial formats for providing summarized chemical substance toxicity assessments. In this report, as a contribution to our ongoing hazard assessment activity, we present summary hazard information related to the potential human health effects of the following 5 chemical substances: 4-chlorobenzoyl chloride（CAS: 122-01-0）; benzenesulfonic acid, 4-hydroxy-, tin（2+）salt（CAS: 70974-33-3）; chlorocyclohexane（CAS: 542-18-7）; 1,3-cyclohexanedimethanamine（CAS: 2579-20-6）; and 1,3,5-triazine-2,4,6（1H,3H,5H）-trithione（CAS: 638-16-4）. The IUCLID dossiers created for these 5 chemical substances will be made available via the Japan Existing Chemical Data Base（JECDB）at <http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp>. Additional human health hazard information on existing chemical substances will be provided using the same methodology and website when it is available.

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

Introduction

Under the Chemical Substances Control Law（CSCL）in Japan, hazard information on existing chemical substances has been collected by the Ministry of Health, Labour and Welfare, Japan（MHLW）to assess potential initial risks to human health[1)]. This hazard information includes acute toxicity, repeated dose toxicity, genotoxicity, and/or reproductive/developmental toxicity. To date, the MHLW has collected information on over 400 existing chemical substances. We have reviewed these toxicity studies and drafted initial risk assessments of human health for submission to the OECD Cooperative Chemicals Assessment Programme（CoCAP）or the OECD High Production Volume Chemicals（HPV）programme（former CoCAP）. Initial risk assessments for approximately 250 of the >400 chemical substances have received international agreement following our previous contributions to the OECD programs. Although initial risk assessments for existing chemical substances ended at the CoCAP in 2014 due to member country demands for changes to the program's focus, we have continued to develop hazard assessments for the existing chemical substances initially targeted by MHLW, with the remaining 150 substances either under review or their assessments are in progress. For our assessment

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process, we reviewed the chemical's study reports and created a dossier for clarification and evaluation of each study. Each dossier consisted of a collection of all study data, including a detailed summary of the methods, results, and conclusions for each MHLW study, written in English using the International Uniform Chemical Information Database (IUCLID) version 5[2]. IUCLID software is currently used by Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and other programs such as the OECD CoCAP and EU Biocides programs. Data regarding each chemical substance targeted by multiple programs are stored in IUCLID using the OECD Harmonized Template format. Row data stored in IUCLID are easily exchanged using the program's export and import tools. The IUCLID dossier format appears to be the most beneficial and useful way to provide chemical substance toxicity data. In addition, avoiding duplication of work between programs or countries is one of the most important global challenges. Shared information should help prevent unnecessary animal studies and provide global access to very meaningful toxicity information presented in English, a widely used language.

In this report, summary hazard information is presented for the following 5 existing chemical substances: 4-chlorobenzoyl chloride (CAS: 122-01-0); benzenesulfonic acid, 4-hydroxy-, tin (2+) salt (CAS: 70974-33-3); chlorocyclohexane (CAS: 542-18-7); 1,3-cyclohexanedim ethanamine (CAS: 2579-20-6); and 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione (CAS: 638-16-4). The IUCLID dossiers for these 5 chemical substances will be available from the Japan Existing Chemical Data Base (JECDB) accessible at http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp[1]. Additional human health hazard information on existing chemical substances will be provided using the same methodology and website when it is available.

(1) 4-Chlorobenzoyl chloride (CAS: 122-01-0)

The acute oral LD_{50} for 4-chlorobenzoyl chloride was established at >2,000 mg/kg bw in female rats on the basis of a study conducted according to the OECD Test Guideline (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 the OECD TG 422. Male and female rats (12 animals/sex/dose) were administered 4-chlorobenzoyl chloride at 0, 20, 100, and 500 mg/kg bw/day. Males were dosed for 42 days, including a 14 day pre-mating period and subsequent mating period; whereas females were dosed for 42-48 days, including the 14 day pre-mating, mating, and gestation periods, and the time until day 4 of lactation. Five out of 12 males at 0 and 500 mg/kg bw/day were used as a recovery assessment group. In addition, 10 females/dose were administered 0 and 500 mg/kg bw/day for 42 days without mating and examined after the administration period or after a 14 day recovery period. At 500 mg/kg bw/day, the absolute and relative thymus weights had decreased in the mating group females. Relative kidney weight increased in males at 500 mg/kg bw/day. Histopathological examination revealed basophilic changes in the tubular cells of kidneys from males and both mating and non-mating females, and tubular dilatation, granular casts, and fibrosis was observed in male kidneys. Atrophy of the thymus was observed in all mating females, including the control group; however, the incidence was particularly high in the 500 mg/kg bw/day group. Furthermore, histopathological changes were observed in the stomach, including intercellular edema in squamous cells and cell infiltration or hyperplasia of the forestomach mucosa, in males and mating and non-mating females. Forestomach erosion and ulceration were present in one mating female administered 500 mg/kg bw/day. These histopathological changes tended to resolve after the 14 day recovery period. Based on the effects of 4-chlorobenzoyl chloride on the thymus, kidney, and stomach, the no observed adverse effect level (NOAEL) for repeated oral dosing 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* (OECD TG 471), 4-chlorobenzoyl chloride was negative with or

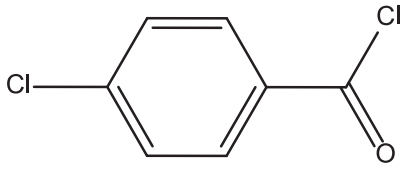

Fig. 1. Structure of 4-chlorobenzoyl chloride

without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) was negative with or without metabolic activation. Based on these results, 4-chlorobenzoyl chloride was regarded as non-genotoxic *in vitro*.

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422), reproductive parameters were not affected up to 500 mg/kg bw/day. The body weights of pups on postnatal day (PND) 0 and PND 4 were decreased in pups of both sexes following 500 mg/kg bw/day dosing. The NOAELs for rat reproductive toxicity and developmental toxicity were determined to be 500 mg/kg bw/day and 100 mg/kg bw/day, respectively.

(2) Benzenesulfonic acid, 4-hydroxy-, tin (2+) salt (CAS: 70974-33-3)

The acute oral LD_{50} of benzenesulfonic acid, 4-hydroxy-, tin (2+) salt was >2,000 mg/kg bw in female rats based on a study conducted according to the OECD TG 423. No deaths were observed at 2,000 mg/kg bw. The substance caused transient salivation at 300 mg/kg bw, and the transient effects of dirty nose, loose stool, and no feces at 2,000 mg/kg bw.

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed according to the OECD TG 422. Male and female rats (12 animals/sex/dose) were administered benzenesulfonic acid, 4-hydroxy-, tin (2+) salt at 0, 12, 60, and 300 mg/kg bw/day. Males were dosed for 42 days, including a 14-day pre-mating period and subsequent mating period. Females were dosed for 41-51 days, including 14 day pre-mating, mating, and gestation periods, and the time until lactation day 4. Five animals/sex/dose administered 0 and 300 mg/kg bw/day were treated as a recovery group and examined after a 14 day recovery period. Salivation was observed after 4 weeks of administration in males at 300 mg/kg bw/day. After the administration period, rats administered 300 mg/kg bw/day showed decreased hemoglobin and hematocrit levels in females and increased serum alanine transaminase levels in males. By gross pathology, both sexes exhibited thickening of the limiting ridge of the stomach and dilatation of the cecum at 300 mg/kg bw/day. Upon histopathological examination, both sexes had minimal hypertrophy of the duodenal mucosal epithelia at 300 mg/kg bw/day. These changes resolved after the recovery period. Based on the changes in the blood and gastrointestinal organs, the NOAEL of repeated dose toxicity was determined to be 60 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* (OECD TG 471), the benzenesulfonic acid, 4-hydroxy-, tin (2+) salt was negative with and without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) was positive, both with and without metabolic activation. Based on these results, benzenesulfonic acid, 4-hydroxy-, tin (2+) salt was regarded as clastogenic *in vitro*.

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, there were no effects on reproductive and developmental parameters at 300 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of benzenesulfonic acid, 4-hydroxy-, tin (2+) salt was determined to be 300 mg/kg bw/day, the highest dose tested.

(3) Chlorocyclohexane (CAS: 542-18-7)

The acute oral LD_{50} of chlorocyclohexane was >2,000 mg/kg bw in female rats following a study conducted according to the OECD TG 423. The substance caused no deaths or clinical signs of toxicity up to 2,000 mg/kg bw.

A combined repeated oral dose toxicity study and reproduction/developmental toxicity screening test was performed according to the OECD TG 422. Male

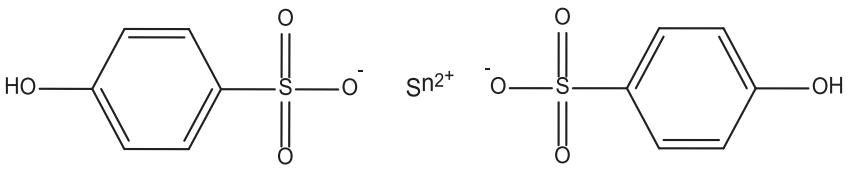

Fig. 2. Structure of benzenesulfonic acid, 4-hydroxy-, tin (2+) salt

and female rats (12 animals/sex/dose) were administered chlorocyclohexane at 0, 10, 60, and 300 mg/kg bw/day. Males were dosed for 42 days, including a 14 day pre-mating period and subsequent mating period. Females were dosed for up to 55 days, including 14 day pre-mating, mating, and gestation periods, and the time until lactation day 4. Five out of 12 males dosed at 0 and 300 mg/kg bw/day were treated as a recovery group. In addition, 5 females/dose 0 and 300 mg/kg bw/day groups were dosed for 42 days without mating and examined after the recovery period. At 300 mg/kg bw/day, increased salivation and decreased body weight gain were observed in both sexes. Absolute and relative kidney weights increased and hyaline droplet formation in the proximal tubular epithelium increased in males administered 300 mg/kg bw/day. Hyperplasia of the urinary bladder mucosal epithelium was observed in males administered 60 and 300 mg/kg bw/day and in females administered 300 mg/kg bw/day. Among these changes, increased relative kidney weight in males and hyperplasia of the urinary bladder mucosal epithelium in females persisted after the recovery period. Based on these effects in the kidney and urinary bladder, the NOAELs for repeated dose toxicity were determined to be 10 mg/kg bw/day and 60 mg/kg bw/day in male and female rats, respectively.

In a bacterial reverse mutation assay using *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537, and *Escherichia coli* WP2*uvrA*/pKM101 (OECD TG 471), chlorocyclohexane was negative with or without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) was negative with and without metabolic activation. Based on these results, chlorocyclohexane was regarded as non-genotoxic *in vitro*.

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, there were no effects on reproductive and developmental parameters at 300 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of chlorocyclohexane was determined to be 300 mg/kg bw/day, the highest dose tested.

(4) 1,3-Cyclohexanedimethanamine (CAS: 2579-20-6)

The acute oral LD_{50} of 1,3-cyclohexanedimethanamine was >300-2,000 mg/kg bw in female rats based on a study conducted according to the OECD TG 423. No deaths were observed after a single dose of 300 mg/kg bw (first and second steps); however, all 3 animals tested died at 2,000 mg/kg bw (third step). At 2,000 mg/kg bw, the substance caused irregular respiration, bradypnea, hypothermia, ptosis, prone position, supine position, crouching position, and decreased locomotor activity.

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed as described in the OECD TG 422. Male and female rats (12 animals/sex/dose) were administered 1,3-cyclohexanedimethanamine at 0, 10, 60, and 300 mg/kg bw/day. Males were dosed for 42 days, including a 14 day pre-mating period and subsequent mating period, whereas females were dosed for up to 52 days, including 14 day pre-mating, mating, and gestation periods, and the time until lactation day 4. Five out of 12 males at 0 and 300 mg/kg bw/day were treated as a recovery group. In addition, 5 females/dose administered 0 and 300 mg/kg bw/day were dosed for 42 days without mating and examined after the recovery period. One male died in the 300 mg/kg bw/day group. At this dose, salivation was observed in both sexes, and decreased body weight gain was observed in males. The relative and absolute weights of the adrenal gland in males and relative weights of the kidneys and adrenal gland in females increased in the 300 mg/kg bw/day groups. Upon histopathological examination, inflammatory cell infiltration, focal hyperkeratosis, focal squamous cell hyperplasia, and ulceration in the forestomach in both sexes, and atrophy of seminiferous tubules of the testis in males were

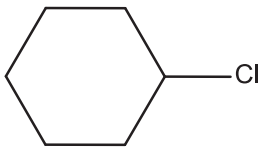

Fig. 3. Structure of chlorocyclohexane

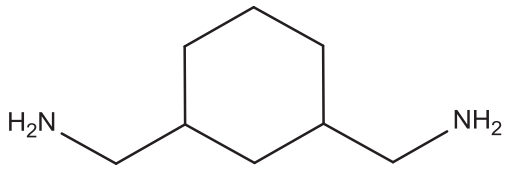

Fig. 4. Structure of 1,3-cyclohexanedimethanamine

observed at 300 mg/kg bw/day. All of these changes resolved after the recovery period. Based on the decreased body weight gain and histopathological changes in the forestomach, the NOAEL for the male and female rat repeated dose toxicity of 1,3-cyclohexanedimethanamine was determined to be 60 mg/kg bw/day.

In a bacterial reverse mutation assay using *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537, and *Escherichia coli* WP2*uvrA*/pKM101 (OECD TG 471), 1,3-cyclohexanedimethanamine was negative with or without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) was positive without metabolic activation. However, an *in vivo* micronucleus study (OECD TG 474) was negative up to the maximum tolerated dose (500 mg/kg bw/day for 2 days) in mice. Based on these results, 1,3-cyclohexanedimethanamine was regarded as non-genotoxic *in vivo*.

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, there were no effects on reproductive and developmental parameters at 300 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of 1,3-cyclohexanedimethanamine was regarded as 300 mg/kg bw/day, the highest dose tested.

(5) 1,3,5-Triazine-2,4,6 (1H,3H,5H)-trithione (CAS: 638-16-4)

The acute oral LD_{50} of 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione was >300-2,000 mg/kg bw in female rats based on a study conducted according to the OECD TG 423. No deaths were observed after a single dose of 300 mg/kg bw (first and second steps); however, all 3 animals died at 2,000 mg/kg bw (third step). The substance caused dyspnea, crawling, decumbence, and lid closure at 2,000 mg/kg bw.

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed according to the OECD TG 422. Male and female rats (12 animals/sex/dose) were administered 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione at 0, 62.5, 125, and 250 mg/kg bw/day. Males were dosed for 48 days, including a 14 day pre-mating period and subsequent mating period. Females were dosed for up to 54 days, including 14 day pre-mating, mating, and gestation periods, and the time until lactation day 4. Five out of

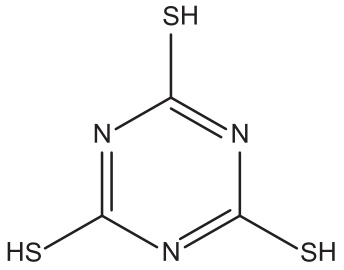

Fig. 5. Structure of 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione

12 males at 0 and 250 mg/kg bw/day were treated as a recovery group. In addition, 5 females/dose administered 0 and 250 mg/kg bw/day were dosed for 42 days without mating and were treated as a recovery group. One male and one female died after 250 mg/kg bw/day dosing. Clinical signs of toxicity included black areas on the pinna, dark purple coloration at the distal end of the tail, reddish urine, induration of the scrotum, and nodules of the tail, pinna, and scrotum in the 250 mg/kg bw/day group. Transient salivation was observed in males at 125 mg/kg bw/day and in both sexes at 250 mg/kg bw/day. At 250 mg/kg bw/day, food consumption and body weight gain were decreased in males and non-mating females. Red blood cells were observed in the urinary sediment from 6 males in the 250 mg/kg bw/day group. In the blood, hematocrit and albumin were decreased in males at 250 mg/kg bw/day. Gross pathological changes were observed in the tail, pinna and scrotum, and the histopathological examination revealed granulation tissues with multinucleated giant cells and inflammatory cell infiltration in the subcutis of the tail, pinna, and scrotum. In the kidney, papilla necrosis and edema were observed in males at doses of 62.5 mg/kg bw/day and higher, and in females at 250 mg/kg bw/day. Deposition of brown pigment in the basophilic tubule cortex was observed in both sexes at doses of 62.5 mg/kg bw/day and higher. In the adrenal grand, diffuse hypertrophy of the fascicular cells was observed in both sexes at doses of 62.5 mg/kg bw/day and higher. The histopathological changes observed in the kidneys and adrenal grand did not resolve after the recovery period. Based on the effects of dosing on the kidney and adrenal gland, the LOAEL for the male and female rat repeated dose toxicity of 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione was determined to be 62.5 mg/kg bw/day.

In a bacterial reverse mutation assay using *Salmonella*

typhimurium TA100, TA1535, TA98, and TA1537, and *Escherichia coli* WP2*uvrA* (OECD TG 471) 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione was negative with or without metabolic activation. An *in vitro* chromosomal aberration test using CHL/IU cells (OECD TG 473) was positive with and without metabolic activation. However, an *in vivo* micronucleus study (OECD TG 474) was negative up to the maximum tolerated dose (1,000 mg/kg bw/day for 2 days) in mice. Based on these results, 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione was considered to be non-genotoxic *in vivo*.

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, infertility was observed in 3 females at 250 mg/kg bw/day. The number of corpora lutea decreased in rats given 250 mg/kg bw/day. No effects were observed in any pups. The NOAEL for the rat reproductive/developmental toxicity of 1,3,5-triazine-2,4,6 (1H,3H,5H)-trithione was determined to be 125 mg/kg bw/day based on infertility and a decrease in corpora lutea.

References

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2) European chemicals agency (ECHA), IUCLID 5 International Uniform Chemical Information Database available at http://iuclid.eu/ (April, 2015)