Bull. Natl Inst. Health Sci., **135**, 39-44 (2017) **Technical Data**

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

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In Japan, information on the initial hazards associated with existing chemical substances has been collected by the Ministry of Health, Labour and Welfare to assess their potential risks to human health under the Chemical Substances Control Law. We have reviewed all collected information pertaining to acute toxicity, repeated-dose toxicity, genotoxicity, and reproductive/developmental toxicity and assessed the hazards associated with these chemicals. Approximately 150 substances are currently under review and assessment. To clarify and evaluate the validity of each toxicity study, we created a dossier (a collection of study data containing a detailed summary of the methods, results, and conclusions of each study) using the International Uniform Chemical Information Database (IUCLID). In this third annual report, we present summary hazard information related to the potential effects of the following five chemical substances on human health: 4-chloro-m-cresol (CAS: 59-50-7), benzenesulfonamide (CAS: 98-10-2), cyclododeca-1, 5, 9-triene (CAS: 4904-61-4), benzene-1, 2, 4, 5-tetracarboxylic acid (CAS: 89-05-4), and poly[oxy(methyl-1, 2-ethanediyl)], alpha, alpha'-[(1-methylethylidene)di-4, 1-phenylene]bis[omega-hydroxy- (CAS: 37353-75-6). The IUCLID dossiers created for these five chemical substances are made available via the Japan Existing Chemical Data Base. Additional information on the hazards of existing chemical substances for human health is provided using the same methodology and website when it becomes available.

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

Introduction

Under the Japanese Chemical Substances Control Law, information on the hazards associated with existing chemical substances has been collected by the Ministry of Health, Labour and Welfare (MHLW) to assess their potential initial risks to human health[1]. This hazard information includes acute toxicity, repeated-dose toxicity, genotoxicity, and reproductive/developmental toxicity. To date, the MHLW has collected information on over 400 existing chemical substances. We reviewed these toxicity studies and drafted initial assessments of the risk to human health for submission to the OECD Cooperative Chemicals Assessment Programme (CoCAP) or the OECD High Production Volume Chemicals Programme (former CoCAP). Although initial risk assessments for existing chemical substances ended at the CoCAP 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. Approximately 150 substances are currently under review and assessment in this context. For our assessment process, we reviewed the reports on the study of each chemical and created a dossier to clarify and evaluate the validity 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, using the International Uniform Chemical Information Database (IUCLID)[2], a leading database in the field of risk assessment of chemical

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substances.

We previously presented summary hazard information on 10 chemicals[3),4)]. Building on this work, in this third annual report, we present summary hazard information for the following five existing chemical substances: 4-chloro-m-cresol (CAS: 59-50-7), benzenesulfonamide (CAS: 98-10-2), cyclododeca-1, 5, 9-triene (CAS: 4904-61-4), benzene-1, 2, 4, 5-tetracarboxylic acid (CAS: 89-05-4), and poly[oxy(methyl-1,2-ethanediyl)], alpha, alpha'-[(1-methylethylidene)di-4, 1-phenylene]bis[omega-hydroxy- (propoxylated bisphenol A; CAS: 37353-75-6). This work is important because it is necessary to promote a wider dissemination of such information to the public. Avoiding duplication of assessment work between programs or countries is one of the most important global challenges in the field of risk assessment of chemical substances. The sharing of information should help prevent unnecessary animal studies and provide global access to very meaningful toxicity information presented in English. The IUCLID dossiers for these five chemical substances will be made available via the Japan Existing Chemical Data Base (JECDB), accessible at http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp[1)]. Additional information on hazards to human health from existing chemical substances will be provided using the same methodology and website when it becomes available.

(1) 4-Chloro-m-cresol (CAS: 59-50-7)

A repeated-dose 28-day oral toxicity study was performed in accordance with the Japanese guidelines (similar to OECD TG 407). Male and female rats (5 or 10 animals/sex/dose) were administered 4-chloro-m-cresol for 28 days at 0 (vehicle: olive oil), 15, 60, 250, and 1,000 mg/kg bw/day. Five out of the 10 males with this administration at 0 and 1,000 mg/kg bw/day were used as a recovery assessment group and examined after a 14-day recovery period.

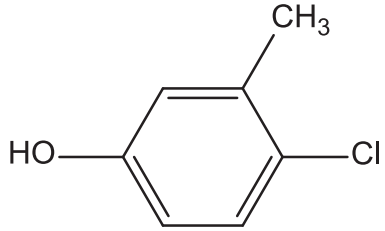

Fig. 1. Structure of 4-chloro-m-cresol

During the administration period, one female died upon administration at 1,000 mg/kg bw/day. Tremor, decrease in locomotor activity, ptosis, prone/side position, abnormal respiratory sound, reddish tear, and salivation were observed in males and females at 1,000 mg/kg bw/day, and decrease in locomotor activity and prone position were also transiently observed at 250 mg/kg/day in males. Significant decreases in body weight, body weight gain, and food consumption were observed in males at 1,000 mg/kg bw/day. At the end of the administration period, significant increases in relative liver weight and incidence of centrilobular hypertrophy were observed in both sexes at 1,000 mg/kg bw/day. Squamous hyperplasia in the forestomach, considered to be a local effect of irritation due to the test substance, was also observed in both sexes at 250 mg/kg bw/day and higher. After the recovery period, these effects on the forestomach tended to resolve. No other effects were observed at the end of the recovery period. Based on the effects of the administration of 4-chloro-m-cresol at 250 mg/kg bw/day on the general condition of the rats, the no-observed-adverse-effect level (NOAEL) for its repeated oral dosing in rats 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*uvr*A (OECD TG 471), negative results were obtained for 4-chloro-m-cresol with or without metabolic activation. Moreover, in an in vitro chromosomal aberration test using CHL/IU cells (OECD TG 473), 4-chloro-m-cresol was clastogenic with and without metabolic activation and also induced polyploidy without metabolic activation. Based on these results, 4-chloro-m-cresol was judged to cause chromosomal aberration in vitro.

A reproduction/developmental toxicity screening test (OECD TG 421) was also performed using rats. In this study, 4-chloro-m-cresol was administered via gavage to 12 animals/sex/dose at 0 (vehicle: corn oil), 35, 150, and 600 mg/kg bw/day. Males were dosed for 42 days, including a 14-day pre-mating period and subsequent mating period. Females were dosed up to 56 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 3. Upon the administration of 4-chloro-m-cresol at 600 mg/kg bw/day, there were changes in general condition and decreases in food consumption and body weight in

both sexes. However, reproductive and developmental parameters were not affected up to 600 mg/kg bw/day. The NOAEL for maternal general toxicity was considered to be 150 mg/kg bw/day, while the NOAEL for rat reproductive and developmental toxicity was determined to be 600 mg/kg bw/day, the highest dose tested.

(2) Benzenesulfonamide (CAS: 98-10-2)

A repeated-dose 28-day oral toxicity study was performed in accordance with the Japanese guidelines (similar to OECD TG 407). Male and female rats (5 or 10 animals/sex/dose) were administered benzenesulfonamide for 28 days at 0 (vehicle: 1 w/v% methyl cellulose solution), 6, 30, and 150 mg/kg bw/day. Five out of 10 males with this administration at 0 and 150 mg/kg bw/day were used as a recovery assessment group and examined after a 14-day recovery period. At 150 mg/kg bw/day, one male died and decrease in locomotor activity was observed in both sexes during the administration period. Male and female rats administered 30 and 150 mg/kg bw/day showed significantly decreased food consumption and body weight. Upon the administration of benzenesulfonamide at 150 mg/kg bw/day, the following effects on the liver were also observed in both sexes: significantly increased relative organ weight with centrilobular hypertrophy and changes in blood chemical parameters. Mineralization was observed in the kidney and lung at 150 mg/kg bw/day in both sexes. Moreover, hyperplasia of transitional cells in the urinary bladder was observed at 30 mg/kg bw/day and higher in both sexes. Sulfonamides are known to produce urinary bladder hyperplasia, but the effect is specific to rats due to urinary composition[5]. At the end of the recovery period, all of the changes except mineralization in the lung had resolved or showed a tendency to resolve. Based on decreases in food consumption and body weight and histopathological changes in the urinary bladder at 30 mg/kg bw/day, the NOAEL of the repeated-dose toxicity was determined to be 6 mg/kg bw/day for male and female rats.

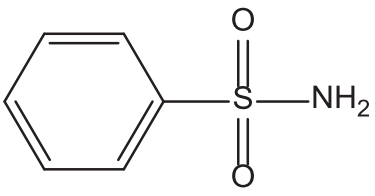

Fig. 2. Structure of benzenesulfonamide

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP2*uvr*A (OECD TG 471), negative results were obtained for benzenesulfonamide with and without metabolic activation. In addition, in an in vitro chromosomal aberration test using CHL/IU cells (OECD TG 473), negative results were again obtained both with and without metabolic activation. These results indicated that benzenesulfonamide is not genotoxic in vitro.

A reproduction/developmental toxicity screening test (OECD TG 421) was also performed using rats. In this study, benzenesulfonamide was administered via gavage to 12 animals/sex/dose at 0 (vehicle: 1 w/v% methyl cellulose solution), 3, 10, and 30 mg/kg bw/day. Males were dosed for 28 days, including a 14-day pre-mating period and subsequent mating period. Females were dosed for 40–53 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 3. Significantly decreased body weight and hyperplasia/hypertrophy of the transitional cells in the urinary bladder were observed at 10 and 30 mg/kg bw/day in males. In females, hyperplasia/hypertrophy of the transitional cells in the urinary bladder was observed upon administration of benzenesulfonamide at doses $\geq$ 3 mg/kg bw/day. Absolute and relative weights of the ovary were also significantly increased at 30 mg/kg bw/day. There were no effects on fertility, but the body weights of pups on postnatal day (PND) 0 and/or 4 were found to be significantly decreased at 30 mg/kg bw/day in both sexes. The NOAEL for the rat reproductive/developmental toxicity of benzenesulfonamide was determined to be 10 mg/kg bw/day based on decreased pup body weights at 30 mg/kg bw/day while the lowest-observed-adverse-effect level for parental general toxicity was 3 mg/kg bw/day, based on the histopathological change in the urinary bladder.

(3) Cyclododeca-1, 5, 9-triene (CAS: 4904-61-4)

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422. Male and female rats (12 animals/sex/dose) were

administered cyclododeca-1, 5, 9-triene at 0（vehicle: corn oil）, 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 42–53 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 4. Five out of 12 males dosed at 0 and 300 mg/kg bw/day were treated as a recovery group. Reduced body weight gain was observed in both sexes in the 300 mg/kg bw/day group. Regarding hematological parameters, prolongation of prothrombin time in males in the 60 and 300 mg/kg bw/day groups as well as activated partial thromboplastin time and a high level of fibrinogen in males in the 300 mg/kg bw/day group were observed. High liver weights were also observed in both sexes at 300 mg/kg bw/day and in females at 60 mg/kg bw/day. Moreover, histopathological analysis revealed hypertrophy of centrilobular hepatocytes at 300 mg/kg bw/day. These changes were either not found or the degree and incidence were reduced after the recovery period. Based on the effects on the liver of cyclododeca-1, 5, 9-triene at 60 mg/kg bw/day, the NOAEL for its repeated-dose toxicity was determined to be 12 mg/kg bw/day in rats.

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP2*uvr*A/pKM101（OECD TG 471）, negative results were obtained for cyclododeca-1, 5, 9-triene with or without metabolic activation. In an in vitro chromosomal aberration test using CHL/IU cells（OECD TG 473）, cyclododeca-1, 5, 9-triene was clastogenic with metabolic activation and had weak potential to induce polyploidy with metabolic activation. Based on these results, cyclododeca-1, 5, 9-triene was judged to cause chromosomal aberration in vitro.

In the combination of a repeated-oral-dose toxicity study and a reproduction/developmental toxicity screening test（OECD TG 422）described above, a significantly low value of the number of liveborn pups was observed in the 300 mg/kg bw/day group. No other effects were observed for fertility and development. The NOAEL for the rat reproductive/developmental toxicity of cyclododeca-1, 5, 9-triene was determined to be 60 mg/kg bw/day, at which maternal general toxicity was observed as described above.

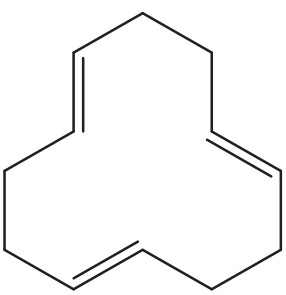

Fig. 3. Structure of cyclododeca-1, 5, 9-triene

(4) Benzene-1, 2, 4, 5-tetracarboxylic acid (CAS: 89-05-4)

A combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test was performed as described in OECD TG 422. Male and female rats（12 animals/sex/dose）were administered benzene-1, 2, 4, 5-tetracarboxylic acid at 0（vehicle: 0.5 w/v% methyl cellulose solution）, 100, 300, and 1,000 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 41–46 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 4. Five out of 12 males administered benzene-1, 2, 4, 5-tetracarboxylic acid at 0 and 1,000 mg/kg bw/day were treated as a recovery group and examined after a 14-day recovery period. Regarding the findings of clinical observation, soft feces were observed in males and females of the 1,000 mg/kg bw/day group. Upon histopathological examination, hyperplasia of the squamous epithelium at the limiting ridge, considered to be due to irritation by the test substance, was found in the stomach of males of the 1,000 mg/kg bw/day group at the end of the administration period. Reversibility was observed for this lesion at the end of the recovery period. Based on the effects in the gastrointestinal tract, the NOAEL for local effects on rat regarding the repeated-dose toxicity of 1,3-cyclohexanedimethanamine was determined to be 300 mg/kg bw/day.

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP2*uvr*A/pKM101（OECD TG 471）, negative results were obtained for benzene-1, 2, 4, 5-tetracarboxylic acid with or without metabolic activation. Moreover, in an in vitro chromosomal aberration test using CHL/IU cells（OECD TG

473), negative results were again obtained with or without metabolic activation. Based on these findings, benzene-1, 2, 4, 5-tetracarboxylic acid was regarded not to be genotoxic in vitro.

In the combined repeated oral dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) described above, benzene-1, 2, 4, 5-tetracarboxylic acid had no effects on reproductive and developmental parameters at 1,000 mg/kg bw/day. The NOAEL for the rat reproductive/developmental toxicity of benzene-1, 2, 4, 5-tetracarboxylic acid was thus regarded as 1,000 mg/kg bw/day, the highest dose tested.

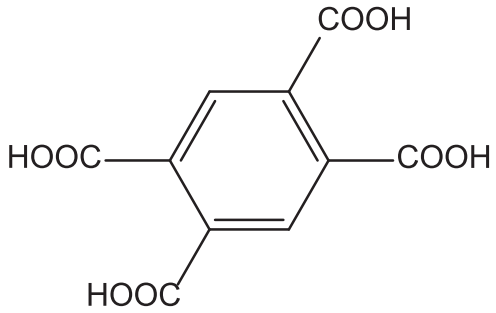

Fig. 4. Structure of benzene-1, 2, 4, 5-tetracarboxylic acid

(5) Poly[oxy(methyl-1, 2-ethanediyl)], alpha, alpha'-[(1-methylethylidene)di-4, 1-phenylene] bis[omega-hydroxy- (propoxylated bisphenol A; CAS: 37353-75-6).

The acute oral LD_{50} of propoxylated bisphenol A was >2,000 mg/kg bw in female rats based on a study conducted according to OECD TG 423. No deaths were observed after a single dose of 2,000 mg/kg bw (both first and second steps). The substance caused salivation and restlessness immediately after dosing and decreases in locomotor activity and diarrhea 1 hour after dosing or later.

A combination of a repeated-oral-dose toxicity study and a reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422. Male and female rats (12 animals/sex/dose) were administered propoxylated bisphenol A at 0 (vehicle: olive oil), 30, 120, and 500 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 42–53 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 4. Five out of 12 males administered propoxylated bisphenol A at 0 and 500 mg/kg bw/day were treated as a recovery group. In addition, five females per dose were administered it at 0 and 500 mg/kg bw/day for 42 days without mating; they were examined after a 14-day recovery period. Death was observed in one dam at 500 mg/kg bw/day. Clinical signs of toxicity in the 500 mg/kg bw/day group included decrease in locomotor activity, emaciation, salivation, and ptosis. During the administration period, body weight gain was also significantly decreased in males at 500 mg/kg bw/day. In addition, at 500 mg/kg bw/day, absolute and/or relative weights of the liver were significantly increased in both sexes, and albumin was significantly decreased in males; further, total cholesterol level and incidence of centrilobular hypertrophy of hepatocytes increased in both sexes. Total protein level also significantly decreased in males of the groups administered propoxylated bisphenol A at 120 mg/kg bw/day or higher. Moderate-to-severe dilations of the small intestinal lacteals were observed in both sexes of the 120 and 500 mg/kg bw/day groups. At the end of the recovery period, an increased relative liver weight was observed in females, and slight dilation of the small intestinal lacteals was observed in both sexes. Based on the histopathological changes in the small intestine and low blood protein level at 120 mg/

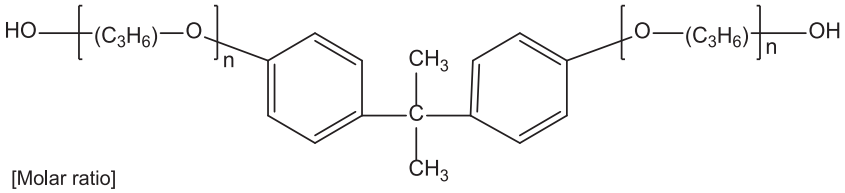

Fig. 5. Structure of poly[oxy(methyl-1, 2-ethanediyl)], alpha, alpha'-[(1-methylethylidene)di-4, 1-phenylene] bis[omega-hydroxy-

kg bw/day, the NOAEL for the rats regarding the repeated-dose toxicity of propoxylated bisphenol A was determined to be 30 mg/kg bw/day.

In a bacterial reverse mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP2*uvr*A (OECD TG 471), negative results were obtained for propoxylated bisphenol A with or without metabolic activation. In addition, in an in vitro chromosomal aberration test using CHL/IU cells (OECD TG 473), negative results were again obtained with or without metabolic activation. Based on these findings, poly[oxy(methyl-1,2-ethanediyl)],alpha,alpha'-[(1-methylethylidene)di-4,1-phenylene]bis[omega-hydroxy- was considered not to be genotoxic in vitro.

In the approach described above combining a repeated-oral-dose toxicity study with a reproduction/developmental toxicity screening test (OECD TG 422), a prolonged estrus cycle was observed upon the administration of propoxylated bisphenol A at 500 mg/kg bw/day. The body weights of pups on PND 0 or 4 were also found to be significantly decreased at 500 mg/kg bw/day in both sexes. The NOAEL for the rat reproductive/developmental toxicity of propoxylated bisphenol A was determined to be 120 mg/kg bw/day.

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