

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

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As stipulated by the Chemical Substances Control Law, information on the hazards to human health associated with different chemical substances is collected by the Japanese Ministry of Health, Labour and Welfare. For this study, we reviewed all the information collected, including data on acute toxicity, repeated-dose toxicity, genotoxicity, and reproduction/developmental toxicity for chemical substances and assessed the hazards to human health associated with these chemicals. To clarify and evaluate the validity of each study, we created a dossier (composed of a collection of study data with a detailed summary of the methods, results, and conclusions for each study), using the International Uniform Chemical Information Database (IUCLID). In this fourth annual report, we present the summary hazard information related to potential effects on human health of the following five chemical substances: poly (oxy-1, 2-ethanediyl), alpha, alpha'-[(1-methylethylidene) di-4, 1-phenylene] bis [omega-hydroxy- (CAS: 32492-61-8), 2-chlorobenzoyl chloride (CAS: 609-65-4), 1, 1, 2, 2, 3, 3, 4, 4, 5, 6-decafluoro-5, 6-bis (trifluoromethyl) cyclohexane (CAS: 306-98-9), tetraphenyltin (IV) (CAS: 595-90-4), and 1, 3, 5-tri-*tert*-butylbenzene (CAS: 1460-02-2). The IUCLID dossiers created for these five chemical substances have been made available through the Japan Existing Chemical Data Base. Additional information on the hazards to human health of existing chemical substances will be provided using the same methodology and website when such becomes available.

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

### Introduction

The Japanese Chemical Substances Control Law requires that information on the hazards to human health associated with existing chemical substances be collected and assessed by the Ministry of Health, Labour and Welfare (MHLW)<sup>1)</sup>. This hazard information includes data on acute toxicity, repeated-dose toxicity, genotoxicity, and reproduction/development toxicity. The MHLW has collected information on about 450 chemical substances so far. We had reviewed previous toxicity studies and drafted

initial risk assessments for human health to submit to the OECD Cooperative Chemicals Assessment Programme (the CoCAP) and the OECD High Production Volume Chemicals Programme (the former CoCAP). Documents submitted by Japan detailing the initial assessments for approximately 200 substances were internationally approved and are publicly available at <https://hvpchemicals.oecd.org/ui/Search.aspx>. Due to member country demands for changes to the program's focus, initial risk assessments for existing chemical substances ended in 2013. Instead of submitting risk assessment documents to the OECD, we have published reports on studies of each chemical that was initially targeted by MHLW. We have created a large dossier, using the International Uniform Chemical Information Database (IUCLID)<sup>2)</sup>, a leading database in risk assessment of chemical substances, to process the results of each study and evaluate its

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validity. Each dossier was composed of all study data, including a detailed summary of the methods, results, and conclusions for each.

We have previously presented hazard information on 15 chemicals<sup>3)-5)</sup>. In this fourth annual report, we present summary hazard information for the following five chemical substances: poly (oxy-1, 2-ethanediyl), alpha, alpha'- [(1-methylethylidene) di-4, 1-phenylene] bis [omega-hydroxy- (CAS: 32492-61-8), 2-chlorobenzoyl chloride (CAS: 609-65-4), 1, 1, 2, 2, 3, 3, 4, 4, 5, 6-decafluoro-5, 6-bis (trifluoromethyl) cyclohexane (CAS: 306-98-9), tetraphenyltin (IV) (CAS: 595-90-4), and 1, 3, 5-tri-*tert*-butylbenzene (CAS: 1460-02-2). We consider this task to be of vital importance, as the public needs to receive a wider dissemination of such information. One of the most pressing challenges worldwide in the present moment in the field of risk assessment of chemical substances is avoiding the duplication of assessment work performed by other programs or countries. Information sharing can help prevent unnecessary animal studies and provide global access to meaningful toxicity information. The IUCLID dossiers of these five chemical substances will be available internationally via the Japan Existing Chemical Data Base, accessible at [http://dra4.nihs.go.jp/mhlw\\_data/jsp/SearchPageENG.jsp](http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp)<sup>1)</sup>. The same methodology and website will be used to provide additional information on hazards to human health from existing chemical substances when it becomes available.

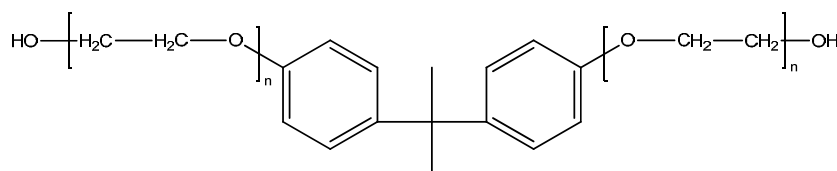
**(1) Poly (oxy-1, 2-ethanediyl), alpha, alpha'- [(1-methylethylidene) di-4, 1-phenylene] bis [omega-hydroxy- (bisphenol A ethoxylate; CAS: 32492-61-8)**

In a study conducted according to OECD TG 423, the acute oral LD<sub>50</sub> of bisphenol A ethoxylate is >2, 000

mg/kg bw in female rats. No deaths were observed in that study after a single dose of 2,000 mg/kg bw (both first and second steps). Restlessness and decreases in locomotor activity were caused.

A combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test was performed in accordance with OECD TG 422. Male and female rats (12 animals/sex/dose) were administered bisphenol A ethoxylate at 0 (vehicle: olive oil), 30, 120, 500, and 1,000 mg/kg bw/day. Including a 14-day pre-mating period and a subsequent mating period, males were dosed for 42 days. Females were dosed for 42-47 days, including 14-day pre-mating, mating, and gestation periods, until lactation day 4. Of the 12 males dosed at 0 and 1,000 mg/kg bw/day, 5 were treated as a recovery group. Five additional females receiving 0 and 1,000 mg/kg bw/day were treated as a satellite group. These females were dosed with bisphenol A ethoxylate for 42 days, without mating, and examined after a 14-day recovery.

Decreased body weight was observed in males in the 1,000 mg/kg bw/day group. Decreases in hemoglobin and hematocrit and increases in the reticulocyte of females in the 1,000 mg/kg bw/day group were observed toward the end of the administration period. The total cholesterol and Ca levels were increased in males receiving 500 and 1,000 mg/kg bw/day and in females at 1,000 mg/kg bw/day. In both sexes, the absolute and/or relative liver weights were increased at 500 mg/kg bw/day and greater. Relative kidney weight were also greater in females at 1,000 mg/kg bw/day. Histopathological analysis also revealed a hypertrophy of centrilobular hepatocytes for both sexes as well as a basophilic tubule of the kidney in males at 500 and 1,000 mg/kg bw/day. Such changes were no longer observed after the recovery period. Judging from the effects on the liver and kidney of



**Fig. 1. Structure of poly (oxy-1,2-ethanediyl), alpha, alpha'- [(1-methylethylidene) di-4,1-phenylene] bis [omega-hydroxy- (CAS: 32492-61-8)**

**[Molar ratio] 1 mole: 0.0%, 2 moles: 5.6%, 3 moles: 16.9%, 4 moles: 23.3%, 5 moles: 21.1%, 6 moles: 15.8%, 7 moles: 8.2%, 8 moles: 4.0%, >= 9 moles: 5.1%**

the bisphenol A ethoxylate at 500 mg/kg bw/day, it was concluded that the NOAEL for the repeated-dose toxicity in rats was 120 mg/kg bw/day.

A bacterial reverse-mutation assay using *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537, and *Escherichia coli* WP 2 *uvrA* (OECD TG 471), negative results were obtained for bisphenol A ethoxylate with or without metabolic activation. However, an *in vitro* chromosomal-aberration test using CHL/IU cells (OECD TG 473) found that structural aberrations increased both with and without metabolic activation. These results led to the conclusion that bisphenol A ethoxylate was clastogenic *in vitro*.

In the combined repeated-dose toxicity study together with a toxicity screening test of reproduction and development (OECD TG 422) described above, reproduction and development were not observed to be affected. The NOAEL for the toxicity of bisphenol A ethoxylate to rat reproduction and development was estimated to be 1,000 mg/kg bw/day (the highest dose tested).

## (2) 2-Chlorobenzoyl chloride (CAS: 609-65-4)

A study conducted according to the OECD TG 423 concluded that acute oral LD<sub>50</sub> of 2-chlorobenzoyl chloride was >2,000 mg/kg bw in female rats. Reactions to the substance included diarrhea, mucus feces, and soiling of the peri-genital or anal fur, no treatment-related deaths ensued after a single dose of 2,000 mg/kg bw (in both the first and second steps).

A combined repeated study of oral-dose toxicity including reproduction/developmental toxicity screening was performed in accordance with OECD TG 422. Male and female rats (12 animals/sex/dose) were administered 2-chlorobenzoyl chloride at 0 (vehicle: corn oil), 40, 200, and 1,000 mg/kg bw/day. The males were dosed for 42 days, including a 14-day

pre-mating period and a subsequent mating period. The females were dosed up to 49 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 5. Out of the 12 males dosed at 0 and 1,000 mg/kg bw/day, 5 were treated as a recovery group. Each five additional females at 0 and 1,000 mg/kg bw/day were dosed with chlorobenzoyl chloride for 42 days without mating and examined after a 14-day recovery period.

One pregnant female died on gestation day (GD) 22 and another on GD23, having been administered 200 mg/kg bw/day and 1,000 mg/kg bw/day, respectively. A non-pregnant female also died on day 5 of the administration period. Salivation, diarrhea, mucus feces, and/or soiling of the peri-genital or anal fur were observed in the 200 and 1,000 mg/kg bw/day groups, in both sexes. Decreases in body weight, weight gain, and food consumption were observed in the males of the 1,000 mg/kg bw/day group. Body weight was not affected by chlorobenzoyl chloride treatment in females, but food consumption decreased during the lactation period for the 1,000 mg/kg bw/day group. The relative organ weights of the liver and kidney increased in both sexes in the 1,000 mg/kg bw/day group. After the administration period, (slight to severe) squamous cell hyperplasia of the forestomach was observed at 1,000 mg/kg bw/day for all animals in both sexes, and an increased tendency was observed in males of the 200 mg/kg bw/day group. This change persisted in 3/5 males and 1/4 female after the end of the recovery period, but the grade had recovered to a slight level. Spermatogenic examination of the 1,000 mg/kg bw/day group found a decreased number of Sertoli cells at stages IX–XIV. After the recovery period, no adverse effects were observed, except in the forestomach. Judging from the changes in general condition and death at 200 mg/kg bw/day, the NOAEL for the repeated-dose toxicity of chlorobenzoyl chloride was determined to be 40 mg/kg bw/day in rats.

A bacterial reverse-mutation assay that used *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP 2 *uvrA* (OECD TG 471) was negative for 2-chlorobenzoyl chloride, both with and without metabolic activation. An *in vitro* chromosomal-aberration test using CHL/IU cells (OECD TG 473) also gave negative results, both with and without

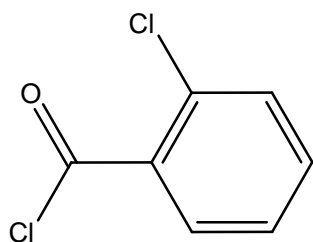


Fig. 2. Structure of 2-chlorobenzoyl chloride (CAS: 609-65-4)

metabolic activation. These findings indicated that 2-chlorobenzoyl chloride was not genotoxic *in vitro* for this population.

The above-described combination of repeated-oral-dose toxicity research and of a reproduction/developmental toxicity screening test (OECD TG 422), included observations of deaths in each one dam, before or during the delivery at 200 or 1,000 mg/kg bw/day, which was considered to be reproduction toxicity. Further, an increased tendency was found for abnormal estrus cyclicity and a tendency of decrease in the fertility index at 1,000 mg/kg bw/day. The body weights of the male and female pups were lower on postnatal days (PND) 0, 1, and 4 at 1,000 mg/kg bw/day. The maternal death at 200 mg/kg bw/day leads to the conclusion that the NOAEL for rat reproduction/developmental toxicity of 2-chlorobenzoyl chloride is 40 mg/kg bw/day.

(3) **1, 1, 2, 2, 3, 3, 4, 4, 5, 6-Decafluoro-5, 6-bis (trifluoromethyl) cyclohexane (CAS: 306-98-9)**

A combined repeated-dose toxicity study with a reproduction/developmental toxicity screening test (OECD TG422) was conducted to specify the general toxic effects of repeated administration of 1, 1, 2, 2, 3, 3, 4, 4, 5, 6-decafluoro-5, 6-bis (trifluoromethyl) cyclohexane and its effects on reproduction and development. The test substance was administered to male rats (12 males/dose) for 14 days before mating and throughout the mating period until the day before necropsy (42 days) and to female rats (12 females/dose) for 14 days before mating and throughout the mating and gestation periods until day 4 of lactation (41 to 45 days) at doses of 0 (vehicle: 0.5w/v% carboxymethylcellulose sodium solution containing

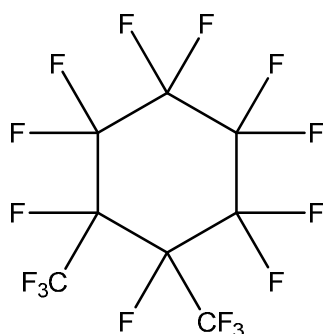


Fig. 3. Structure of 1,1,2,2,3,3,4,4,5,6-decafluoro-5,6-bis (trifluoromethyl) cyclohexane (CAS: 306-98-9)

0.1v/v% Tween 80), 100, 300, and 1,000 mg/kg bw/day. Furthermore, a 14-day recovery period was set for each five males from the control and the 1,000 mg/kg bw/day groups. Five additional females at 0 and 1,000 mg/kg bw/day were dosed with the test substance for 42 days without mating and were examined after a 14-day recovery period. No effects were observed from the administration of the test article in clinical signs, the functional observation battery (FOB: detailed clinical signs, manipulative test, grip strength, motor activity), body weight, food consumption, urinalysis, blood chemistry, or organ weight. At the end of the administration period, in hematological examination, low values of hemoglobin and hematocrit were found in males in the 1,000 mg/kg bw/day group. Histopathological examination showed minimal hypertrophy of centrilobular hepatocytes in males in the 1,000 mg/kg bw/day group. At the end of the recovery period, the changes observed were no longer observed. The changes in the hematological and histopathological examinations at 1,000 mg/kg bw/day indicated that the NOAEL of repeated-dose toxicity is 300 mg/kg bw/day in rats.

A bacterial reverse-mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP 2 *uvrA*/pKM101 (OECD TG 471), the results obtained for 1, 1, 2, 2, 3, 3, 4, 4, 5, 6-decafluoro-5, 6-bis (trifluoromethyl) cyclohexane were negative, with and without metabolic activation. An *in vitro* chromosomal-aberration test that used CHL/IU cells (OECD TG 473), 1, 1, 2, 2, 3, 3, 4, 4, 5, 6-decafluoro-5, 6-bis (trifluoromethyl) cyclohexane was again negative, both with and without metabolic activation. These results show that 1, 1, 2, 2, 3, 3, 4, 4, 5, 6-decafluoro-5, 6-bis (trifluoromethyl) cyclohexane was non-genotoxic *in vitro*.

In the above-described OECD TG 422 study, no effects were observed for reproduction or development. The NOAEL for rat reproduction/developmental toxicity of 1, 1, 2, 2, 3, 3, 4, 4, 5, 6-decafluoro-5, 6-bis (trifluoromethyl) cyclohexane was estimated to be 1,000 mg/kg bw/day (the highest dose tested).

(4) **Tetraphenyltin (IV) (CAS: 595-90-4)**

The combination of a study of repeated-oral-dose toxicity and a reproduction/developmental toxicity screening test was performed in accordance with

OECD TG 422. Tetraphenyltin (IV) was administered to male and female rats (12 animals/sex/dose) at 0 (vehicle: olive oil), 4, 20, 100, and 500 mg/kg bw/day. The males were dosed for 42 days, including a 14-day pre-mating period and a subsequent mating period. The females were dosed for 42–54 days, including 14-day pre-mating, mating, and gestation periods and until lactation day 4. Of the 12 males in the control and 500 mg/kg bw/day groups, 5 were treated as a recovery group. Additionally, five females per dose were administered the test substance at 0 and 500 mg/kg bw/day for 42 days without mating; they were examined after a 14-day recovery period (satellite group).

No adverse effects were observed in clinical characteristics, FOB, body weight, food consumption, urinalysis, hematology, clinical biochemistry, organ weight, or pathology after the administration and recovery periods for any of the treated groups. In a 14-day preliminary study of tetraphenyltin (IV) in rats, relative liver weights were found to increase by 3 to 1,000 mg/kg bw/day in males and by 6 to 1,000 mg/kg bw/day in females. For this reason, the largest dose tested in this main study was expected to cause overt toxicity. No clear reason was adduced why no adverse effects on the liver were observed in this study, but age at necropsy and duration of administration might each have affected the response. These data indicate that the NOAEL of repeated-dose toxicity for tetraphenyltin (IV) should be estimated to be 500 mg/kg bw/day (the highest dose tested).

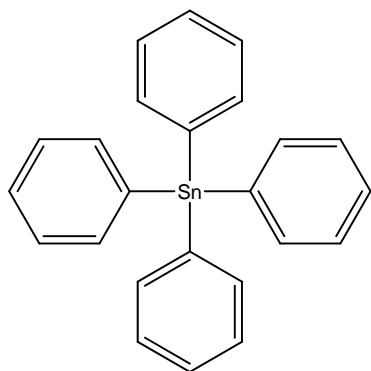


Fig. 4. Structure of tetraphenyltin (IV) (CAS: 595-90-4)

A bacterial reverse-mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP2 *uvrA* (OECD TG 471), found negative results for tetraphenyltin (IV), with or

without metabolic activation. An *in vitro* chromosomal-aberration test that used CHL/IU cells (OECD TG 473), clastogenicity was observed, with or without metabolic activation, although polyploidy was not observed. These findings indicated that tetraphenyltin (IV) should be considered to be clastogenic *in vitro*.

The approach described above combined a repeated-oral-dose toxicity study with a reproduction/developmental toxicity screening test (OECD TG 422). No adverse effects were found on reproduction or development. Thus, the NOAEL for the rat reproduction/developmental toxicity of tetraphenyltin (IV) was estimated to be 500 mg/kg bw/day (the highest dose tested).

#### (5) 1, 3, 5-Tri-*tert*-butylbenzene (CAS: 1460-02-2)

A combined repeated oral-dose toxicity study with a reproduction/developmental toxicity screening test performed in accordance with OECD TG 422. Male and female rats (12 animals/sex/dose) were administered 1, 3, 5-tri-*tert*-butylbenzene at 0 (vehicle: olive oil), 2, 10, 50, and 250 mg/kg bw/day. The males were dosed for 42 days, including a 14-day pre-mating period and a subsequent mating period. The females were dosed for 42–52 days, including 14-day pre-mating, mating, and gestation periods, and until lactation day 4. Out of the 12 males, 5 were dosed at 0 and 250 mg/kg bw/day and were treated as a recovery group. Five additional females at 0 and 250 mg/kg bw/day were assigned to a satellite group and were dosed 1, 3, 5-tri-*tert*-butylbenzene for 42 days without mating, and they were examined after a 14-day recovery period.

No effects were found on clinical signs, FOB, body weight, food consumption, or urinalysis. Increased levels of ALT, total protein, albumin, and Ca were

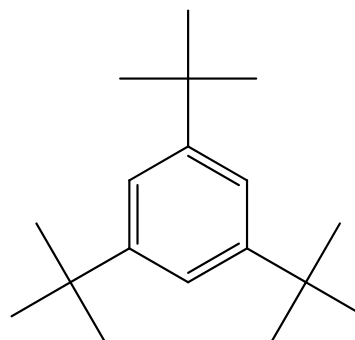


Fig. 5. Structure of 1,3,5-tri-*tert*-butylbenzene (CAS: 1460-02-2)

found, as were decreases in levels of glucose and triglyceride in males and females at 250 mg/kg bw/day. In males of the 250 mg/kg bw/day group, plasma cholinesterase activity was high. The relative and absolute weights of the liver increased at 10 mg/kg bw/day and greater doses in females and at 50 mg/kg bw/day and greater doses in males. Increased relative and absolute weights of the kidney in females of the 250 mg/kg bw/day group were also observed. Histopathological examination showed hypertrophy of centrilobular hepatocytes at 10 mg/kg bw/day and greater in females and at 50 mg/kg bw/day and greater in males; dilatation of the distal/collecting tubules and the hyperplasia of the collecting tubular epithelium in the kidney were observed in females at 250 mg/kg bw/day. These changes were no longer found after the recovery period. The effects of 1, 3, 5-*tert*-butylbenzene on the liver at 10 mg/kg bw/day, led to a determination of the NOAEL for repeated-dose toxicity at 2 mg/kg bw/day in rats.

A bacterial reverse-mutation assay using *S. typhimurium* TA100, TA1535, TA98, and TA1537, and *E. coli* WP2 *uvrA* (OECD TG 471), brought negative results for 1, 3, 5-*tert*-butylbenzene, with and without metabolic activation. In an *in vitro* chromosomal-aberration test that used CHL/IU cells (OECD TG 473), 1, 3, 5-*tert*-butylbenzene was again negative, both with and without metabolic activation. These results indicated that 1, 3, 5-*tert*-butylbenzene should be judged non-genotoxic *in vitro*.

In a repeated-oral-dose toxicity study and a reproduction/developmental toxicity screening test (OECD TG 422), as described above, the numbers of pups alive were lower on PND 0 and 4 at 250 mg/kg

bw/day; the delivery index and live-birth index were lower than for the control. At the same dose level, the body weights of the live pups also decreased on PND 0 and 4. The developmental toxicity at 250 mg/kg bw/day led to the conclusion that the NOAEL for the rat reproduction/developmental toxicity of 1, 3, 5-*tert*-butylbenzene should be determined at 50 mg/kg bw/day; at this point, parental general toxicity was observed.

## References

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