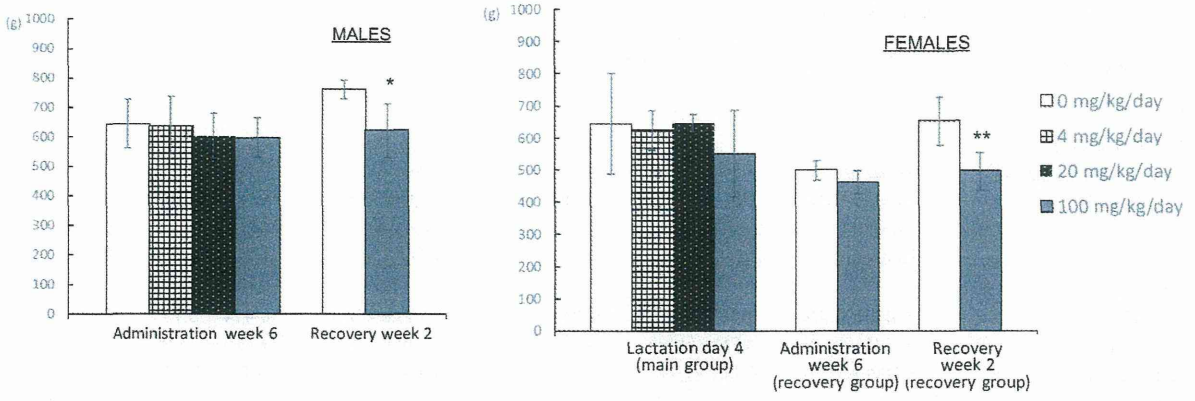

Fig. 4. The hindlimb grip strength of male and female rats in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test for PFHxDA. *: Significantly different from the control, $P \leq 0.05$. **: Significantly different from the control, $P \leq 0.01$.

(data not shown). Serum Cl levels were significantly increased at 100 mg/kg/day in males and at 20 and 100 mg/kg/day in females (Table 4). A significant decrease in serum total bilirubin levels and significant increases in BUN and serum Na levels were also detected in females given 100 mg PFHxDA/kg/day. In males, the absolute and relative liver weights were significantly increased in the 100 mg/kg/day group (Table 4). The relative thyroid weight was significantly increased at 20 and 100 mg/kg/day, with a significant increase also being observed in the absolute weight at 20 mg/kg/day in males. The analysis of serum thyroid-related hormones revealed significantly decreased T_3 in females in all PFHxDA-treated groups. The histopathological examination revealed the centrilobular hypertrophy of hepatocytes in males at 20 mg/kg/day and in both sexes at 100 mg/kg/day (Table 5). Centrilobular fatty changes were also observed in males at 20 and 100 mg/kg/day. No treatment-related histopathological changes were detected in other organs including the thyroid.

A significant decrease was noted in serum total bilirubin levels in both sexes as well as a significant increase in serum Cl level in females in the 100 mg/kg/day group after the 14-day recovery period (Table 4). Serum T_4 levels were significantly decreased in males in the 100 mg/kg/day group. Absolute and relative liver weights in males still remained higher, and in addition, significant decreases were found in absolute and relative adrenal weights in the 100 mg/kg/day group. Histopathologically, the centrilobular hypertrophy of hepatocytes was observed in both sexes as well as centrilobular fatty changes in one male in the 100 mg/kg/day group (Table 5).

Reproductive/developmental toxicity

PFHxDA did not significantly affect any reproductive/developmental parameters (Table 6). Although the body weights of male and female pups on PND 4 were slightly lower in the 100 mg/kg/day group, no significant difference was observed from those in the control group. There were no abnormalities in the general appearance or necropsy findings of neonates.

DISCUSSION

The present study was performed to obtain initial information on the repeated dose and reproductive/developmental toxicity of PFTeDA (C14) and PFHxDA (C16). The results obtained demonstrated that the main toxic target of these compounds was the liver, which was similar to PFUnA (C11), PFDoA (C12), and PFOcDA (C18), which we had examined previously (Hirata-Koizumi *et al.*, 2012; Kato *et al.*, in press; Takahashi *et al.*, 2014).

The hepatic effects of PFCAs in rodents have been attributed, at least partly, to the peroxisome proliferator-activated receptor alpha (PPARα) (Lau *et al.*, 2007; Wolf *et al.*, 2012). PPARα is a nuclear receptor that plays an important role in regulating fatty acid metabolism in tissues such as the liver, kidney, heart, and intestinal mucosa (Corton *et al.*, 2000). In the present study, the blood biochemical examination did not reveal any clear effects on lipid metabolism; however, PFTeDA (C14) and PFHxDA (C16) decreased serum total cholesterol in the 14-day dose finding study performed at higher doses. Although the PPARα agonist activities of PFTeDA and PFHxDA are unknown, PFDoA (C12), which is very similar in

Table 4. Significant changes in blood biochemical parameters, serum thyroid-related hormone levels and organ weights in rats given PFHxDA.

Dose (mg/kg/day)	At the end of the administration period (Main group)				At the end of the recovery period (Recovery group)	
	0	4	20	100	0	100
MALES						
Blood biochemistry						
Total bilirubin (mg/dL)	0.062 ± 0.008	0.056 ± 0.011	0.058 ± 0.015	0.064 ± 0.011	0.064 ± 0.013	0.044 ± 0.005*
BUN (mg/dL)	14.54 ± 0.88	14.52 ± 1.54	14.98 ± 1.60	17.52 ± 2.81	16.16 ± 1.18	15.12 ± 1.31
Na (mEq/L)	144.2 ± 1.6	144.4 ± 1.7	144.4 ± 1.1	145.4 ± 0.9	144.2 ± 1.5	144.4 ± 0.5
Cl (mEq/L)	106.8 ± 1.3	108.2 ± 2.3	107.4 ± 0.9	109.6 ± 1.5*	106.2 ± 1.1	108.0 ± 1.4
Hormonalysis						
T_3 (ng/mL)	0.450 ± 0.070	0.466 ± 0.076	0.390 ± 0.060	0.436 ± 0.119	0.474 ± 0.123	0.452 ± 0.061
T_4 (ng/mL)	69.71 ± 14.91	74.73 ± 10.93	80.05 ± 8.65	71.38 ± 3.83	117.50 ± 15.00	89.25 ± 11.87*
TSH (ng/mL)	3.732 ± 1.491	6.586 ± 2.712	7.064 ± 5.351	9.682 ± 6.029	13.314 ± 5.530	13.564 ± 3.229
Organ weight						
Liver (g)	12.15 ± 1.27	11.81 ± 0.55	12.12 ± 0.85	14.50 ± 0.61**	12.38 ± 1.40	14.62 ± 1.35*
(%)	2.50 ± 0.04	2.45 ± 0.10	2.49 ± 0.15	3.26 ± 0.07**	2.40 ± 0.17	2.97 ± 0.33**
Thyroid (mg)	18.94 ± 1.6	20.58 ± 1.53	24.26 ± 4.28*	22.16 ± 3.26	21.90 ± 3.98	22.40 ± 4.10
(10^{-3}%)	3.94 ± 0.58	4.27 ± 0.34	4.98 ± 0.78*	4.98 ± 0.67*	4.26 ± 0.82	4.54 ± 0.84
Adrenal (mg)	70.0 ± 2.1	58.4 ± 11.9	62.4 ± 9.9	57.8 ± 8.4	70.4 ± 3.8	55.2 ± 6.1**
(10^{-3}%)	14.52 ± 1.43	12.09 ± 2.41	12.91 ± 2.56	13.03 ± 2.03	13.69 ± 0.72	11.17 ± 1.14**
FEMALES						
Blood biochemistry						
Total bilirubin (mg/dL)	0.080 ± 0.007	0.076 ± 0.005	0.072 ± 0. 013	0.060 ± 0.000**	0.084 ± 0.015	0.054 ± 0.011**
BUN (mg/dL)	25.78 ± 2.35	27.82 ± 2.05	28.22 ± 4.41	31.18 ± 1.55*	16.66 ± 1.08	15.50 ± 1.09
Na (mEq/L)	140.8 ± 0.8	142.2 ± 0.8	142.6 ± 1.5	142.8 ± 1.3*	144.6 ± 1.1	145.0 ± 0.7
Cl (mEq/L)	104.0 ± 0.7	105.0 ± 0.7	106.2 ± 1.3*	106.8 ± 1.6**	108.8 ± 0.8	109.8 ± 0.4*
Hormonalysis						
T_3 (ng/mL)	0.734 ± 0.023	0.606 ± 0.036**	0.626 ± 0.068**	0.532 ± 0.040**	0.784 ± 0.143	0.684 ± 0.032
T_4 (ng/mL)	65.48 ± 9.30	65.56 ± 15.86	61.86 ± 7.57	66.36 ± 14.85	46.26 ± 16.70	58.48 ± 7.11
TSH (ng/mL)	4.478 ± 1.454	5.434 ± 5.130	4.408 ± 2.329	8.338 ± 4.661	3.758 ± 0.859	28.772 ± 54.988
Organ weight						
Liver (g)	10.17 ± 0.48	9.70 ± 0.61	10.00 ± 0.81	10.53 ± 0.75	6.95 ± 0.37	7.48 ± 1.00
(%)	3.39 ± 0.12	3.27 ± 0.21	3.35 ± 0.15	3.55 ± 0.20	2.49 ± 0.11	2.71 ± 0.28
Thyroid (mg)	19.28 ± 3.06	15.78 ± 2.95	16.96 ± 3.42	18.04 ± 1.99	18.42 ± 1.97	16.88 ± 3.46
(10^{-3}%)	6.40 ± 0.78	5.30 ± 0.86	5.71 ± 1.27	6.07 ± 0.55	6.60 ± 0.79	6.09 ± 1.01
Adrenal (mg)	76.4 ± 5.8	79.6 ± 6.0	79.4 ± 7.9	75.4 ± 7.9	67.0 ± 3.5	74.2 ± 12.4
(10^{-3}%)	25.44 ± 1.68	26.80 ± 2.00	26.59 ± 2.12	25.43 ± 2.77	23.98 ± 1.11	26.95 ± 4.70

Data are shown as the mean ± S.D.
*: Significantly different from the control group at $P \leq 0.05$.
**: Significantly different from the control group at $P \leq 0.01$.

structure, was recently reported to activate mouse PPARα in transiently transfected COS-1 cells (Wolf *et al.*, 2012) and induce the mRNA levels of the important PPARα target genes, acyl CoA oxidase and CYP4A1, in the rat liver (Zhang *et al.*, 2008; Ding *et al.*, 2009). These findings indicated that PFTeDA and PFHxDA may activate PPARα, which may in turn affect the liver. Regarding the mechanism underlying the hepatotoxicity of PFCAs, many studies have examined PFOA (C8) and showed that PFOA could elicit changes in the liver not only via PPARα activation, but also through PPARα-independent mechanisms (Peters and Gonzalez, 2011). The involvement of other transcription factors such as the constitutive androstane receptor and pregnane X receptor has been implied. Further research is needed to clarify the mechanism involved in the hepatotoxicity of PFCAs including PFTeDA and PFHxDA.

PFTeDA (C14) induced follicular cell hypertrophy in

Table 5. Histopathological findings in the combined repeated dose toxicity study with reproduction/developmental toxicity screening test for PFHxDA in rats.

Dose (mg/kg/day)		At the end of the administration period (Main group)				At the end of the recovery period (Recovery group)	
		0	4	20	100	0	100
MALES							
Number of examined animals		7	12	12	7	5	5
Liver							
- Centrilobular hypertrophy of hepatocytes	+	0	0	5	0 ┐**	0	5**
	++	0	0	0	7 ┘	0	0
- Centrilobular fatty change	+	0	0	2	7**	0	1
FEMALES							
Number of examined animals		12	12	12	12	5	5
Liver							
- Centrilobular hypertrophy of hepatocytes	+	0	0	0	8**	0	1

Values represent the number of animals with findings.
+: Slight change, ++: moderate change
**: Significantly different from the control group at $P \leq 0.01$.
Brackets in the data columns mean that statistical analysis was performed for a total number of animals with findings in consideration of grades.

Table 6. Reproductive/developmental findings in the combined repeated dose toxicity study with the reproduction/developmental screening test for PFHxDA in rats.

Dose (mg/kg/day)		0	4	20	100
Incidence of females with normal estrous cycle[a] (%)		100	100	100	100
Estrous cycle length[a, b] (days)		4.11 ± 0.22	4.18 ± 0.32	4.03 ± 0.09	4.00 ± 0.00
Number of cohabited pairs		12	12	12	12
Couplation index (%)	Males	100	100	100	100
	Females	100	100	100	100
Fertility index (%)		91.7	100	100	100
Gestation index (%)		100	100	100	100
Gestation length[b] (days)		22.3 ± 0.5	22.3 ± 0.5	22.3 ± 0.5	22.2 ± 0.4
Number of pregnant females		11 12	12 12		
Number of corpora lutea[b]		16.5 ± 1.1	17.0 ± 1.2	15.8 ± 1.9	16.1 ± 1.6
Number of implantation sites[b]		16.1 ± 1.4	16.6 ± 1.2	15.3 ± 2.1	15.8 ± 1.6
Number of pups delivered[b]		15.2 ± 1.7	16.0 ± 1.7	14.2 ± 2.2	14.6 ± 2.0
Sex ratio of pups (male pups / all pups)[b]		0.505 ± 0.165	0.413 ± 0.158	0.429 ± 0.140	0.492 ± 0.183
Number of live pups[b]	on PND 0	15.1 ± 1.7	15.8 ± 1.6	14.2 ± 2.2	14.5 ± 2.2
	on PND 4	15.0 ± 1.7	13.1 ± 6.3	14.1 ± 2.3	13.7 ± 2.7
Body weight of male pups[b] (g)					
	on PND 0	6.63 ± 0.58	6.67 ± 0.67	6.75 ± 0.69	6.45 ± 0.46
	on PND 1	7.25 ± 0.56	7.12 ± 1.08	7.33 ± 0.75	6.97 ± 0.80
	on PND 4	10.53 ± 0.85	10.63 ± 1.54	10.67 ± 1.14	9.93 ± 1.24
Body weight of female pups[b] (g)					
	on PND 0	6.27 ± 0.51	6.22 ± 0.60	6.40 ± 0.66	6.08 ± 0.50
	on PND 1	6.91 ± 0.51	6.58 ± 1.07	6.98 ± 0.82	6.59 ± 0.79
	on PND 4	10.05 ± 0.79	9.97 ± 1.36	10.15 ± 1.25	9.43 ± 1.31

a: Data of the main group are shown. No significant changes in estrous cycle normality were found in the recovery group, either.
b: Data are shown as the mean ± S.D.

Table 7. Comparison of the NOAELs for the repeated dose and reproductive/developmental toxicity for long-chain PFCAs.

Chemical name	Carbon number	NOAEL (mg/kg/day)		Reference
		Repeated dose toxicity	Reproductive /developmental toxicity	
PFUnA (perfluoroundacanoic acid)	11	0.1	0.3	Takahashi *et al.*, 2014
PFDoA (perfluorododecanoic acid)	12	0.1	0.5	Kato *et al.*, in press
PFTeDA (perfluorotetradecanoic acid)	14	1	3	Current study
PFHxDA (perfluorohexadecanoic acid)	16	4	100	Current study
PFOcDA (perfluorooctadecanoic acid)	18	40	200	Hirata-Koizumi *et al.*, 2012

The NOAELs were established based on the results of in the combined repeated dose toxicity study with reproduction/developmental toxicity screening tests in rats

the thyroids of males. Although the serum levels of thyroid-related hormones were not analyzed in the present study for PFTeDA, it may be a compensatory response of the thyroid to a decrease in thyroid hormone levels because the structural analogue, perfluorodecanoic acid (PFDeA, C10), was previously reported to reduce serum T_3 and/or T_4 levels in rats (Gutshall *et al.*, 1988; Van Rafelghem *et al.*, 1987; Langley and Pilcher, 1985; Gutshall *et al.*, 1989). In the present study, PFHxDA (C16) did not affect the histopathology of thyroids, but increased the thyroid weight in males and decreased serum T_3 level in females. Although these effects of PFHxDA were not consistent between sexes and lacked clear dose-dependency, our results indicate that PFHxDA may slightly affect the thyroid system through a similar mechanism to PFTeDA (C14) and PFDeA (C10). The findings of mechanistic studies on PFDeA (C10) suggested that reduced serum thyroid hormone levels may result from (1) a displacement in the hormones from plasma protein binding sites, leading to an increase in tissue uptake and turnover (Gutshall *et al.*, 1989), and (2) the enhanced metabolism of thyroid hormones in the liver (Shelby and Klaassen, 2006). In our previous studies, we did not detect any effects of PFUnA (C11), PFDoA (C12), and PFOcDA (C18) on the histopathology or weight of the thyroids (Hirata-Koizumi *et al.*, 2012; Kato *et al.*, in press; Takahashi *et al.*, 2014). Serum hormone levels were not measured in these studies.

We previously reported that PFOcDA (C18) reduced forelimb grip strength in females (Hirata-Koizumi *et al.*, 2012). This effect was not observed at the end of the administration period, but appeared at the end of recovery period in both sexes in studies on PFUnA (C11) and PFDoA (C12) (Kato *et al.*, in press; Takahashi *et al.*, 2014). We considered that the reduction observed in grip strength may reflect the muscle weakness associated with a decrease in food consumption and/or body weight. In the present study, PFTeDA (C14) and PFHxDA (C16) reduced hindlimb grip strength, but not that of the forelimb. As with PFUnA (C11) and PFDoA (C12), the effects of PFHxDA (C16) on grip strength only appeared at the end of the recovery period. Hindlimb grip weakness was not necessarily accompanied by a low body weight. Further studies are required in order to clarify the mechanism responsible.

As for reproductive/developmental toxicity, the only effect observed was an inhibited postnatal body weight gain in pups at a maternal toxic dose of PFTeDA (C14). Similar results were observed in the study on PFHxDA (C16), but these changes were not significant. In our previous studies on long-chain PFCAs, postnatal body weight gain in pups was also inhibited at the highest dose (Hirata-Koizumi *et al.*, 2012; Kato *et al.*, in press; Takahashi *et al.*, 2014). In studies performed on PFDoA (C12) and PFOcDA (C18), such effects were accompanied by more severe reproductive/developmental effects, such as the deaths of dams at the end of pregnancy and stillbirths, and with more severe maternal toxic effects than those observed in the present study. The effect of long-chain PFCAs on postnatal development could be attributed to secondary effects due to maternal toxicity such as a low body weight during the lactation period. If PFTeDA (C14) reduced thyroid hormone levels as speculated above, it may be one cause of impaired postnatal development because Hapon *et al.* (2003) reported that hypothyroidism induced by a propylthiouracyl treatment impaired the growth of pups during the lactation period in rats. When the lipophilic properties of long-chain PFCAs (Inoue *et al.*, 2012) are considered, there is also the possibility that they were transferred via breast milk and affected the pups directly.

Based on the present results, the NOAELs for the repeated dose and reproductive/developmental toxicity were concluded to be 1 and 3 mg/kg/day for PFTe-

DA (C14) and 4 and 100 mg/kg/day for PFHxDA (C16), respectively. When the NOAELs were compared with those of PFUnA (C11), PFDoA (C12), and PFOcDA (C18) from our previous studies, the toxic potency of PFCAs was found to become weaker as the carbon chain length increased from C12 to C18 (Table 7). Since the previous comparative studies on the hepatic effects of PFCAs demonstrated increases in toxic potency due to an increase in the length of carbon chains up to C8 in rodents (Kudo *et al.*, 2006; Permadi *et al.*, 1993), the toxic potency of PFCAs was considered to be the strongest when the carbon length was C8 to C12. A clear chain length-dependent downward trend was observed in the renal elimination of PFCAs with a carbon chain length from C6 to C10 in rats (Ohmori *et al.*, 2003; Kudo *et al.*, 2001), and active renal tubular reabsorption via organic anion transport proteins was considered to be responsible for this (Han *et al.*, 2012). On the other hand, Wolf *et al.* (2008, 2012) reported that PFCAs of longer chain lengths induced more activity from mouse and human PPARα than those of shorter chain lengths up to C9 in transiently transfected COS-1 cells; therefore, not only toxicokinetic, but also toxicodynamic factors may contribute to the chain length-dependent toxicity of PFCAs with carbon chain lengths up to C8. Regarding PFCAs with carbon chain lengths of C11 and above, although no data is currently available to explain the cause of the chain length-dependent differences in toxic potencies, medium chain fatty acids (typically C6-C12) are known to be absorbed better from the gastrointestinal tract than long-chain fatty acids (typically longer than C12) (Ramirez *et al.*, 2001). Considering structural similarities, the gastrointestinal absorption of longer chain PFCAs may be poorer than that of PFCAs with shorter carbon chains. In order to clarify the cause of the differences in the toxic potencies of long-chain PFCAs, we are planning to first analyze serum PFCA levels in rats given different long-chain PFCAs.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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Original Article

A repeated dose 28-day oral toxicity study of β-bromostyrene in rats

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ABSTRACT — To obtain information on the possible repeated-dose oral toxicity of β-bromostyrene and its reversibility, Crl: CD (SD) rats were administered β-bromostyrene through gavage at 0, 30, 125, and 500 mg/kg/day once for 28 days, followed by a 14-day recovery period. In the 500 mg/kg group, decrease in spontaneous movement was observed in all males and females on the first dosing day, and one female rat died on Day 3. There were no significant changes in body weight or food consumption. An increase in urine volume and decrease in urine osmolality were observed in males receiving 125 mg/kg and above, and an increase in urine volume was observed in females receiving 500 mg/kg. On blood biochemical examination, increases in total cholesterol, phospholipids, triglycerides, total protein, albumin, inorganic phosphorus, and/or chlorine were observed in the 125 and/or 500 mg/kg groups. Histopathologically, eosinophilic bodies of tubular cells and/or renal tubular degeneration were observed in the kidneys of males in the 125 and 500 mg/kg groups. In the thyroid, hypertrophy of follicular cells was observed in females receiving 125 mg/kg and above and males receiving 500 mg/kg. Furthermore, centrilobular hepatocellular hypertrophy was observed in both sexes receiving 500 mg/kg. These changes observed at the end of the dosing period disappeared or were reduced after the recovery period. Based on these results, the no-observed-adverse-effect-level of β-bromostyrene was judged to be 30 mg/kg/day for both sexes.

Key words: β-bromostyrene, CAS No. 103-64-0, OECD TG 407, Repeated dose toxicity, Rat, Gavage

INTRODUCTION

Safety information on chemicals is necessary for the proper use and management of chemical substances or products containing them. In Japan, the existing chemicals testing program has been conducted by the government. In the program, the Ministry of Health, Labour and Welfare is conducting safety testing and gathering safety information related to health risks on existing chemicals to which humans may be exposed. β-bromostyrene (CAS No. 103-64-0) is a yellow-clear liquid used as an ingredient in mildly fragrant materials, including soap, detergent, creams, lotions, and perfume (HSDB, 1993). Only limited information is available about the toxicity of β-bromostyrene. It has been reported that the oral 50% lethal dose is 1250 mg/kg in rats (HSDB). Since there is insufficient information on its toxicity and no data available on the actual exposure levels at present, this chemical was selected as an object substance in the existing chemical testing program by the Japanese government. In this paper, we report the result of a 28-day repeated oral administration study of β-bromostyrene.

MATERIALS AND METHODS

The present study was conducted at BoZo Research Center Inc. (Shizuoka, Japan). This study was designed to meet the Japanese Test Guidelines for Toxicology Studies issued by "Notification test methods of New Chemical Substances" (Yakushokuhatsu No. 1121002, Seikyoku No. 2, Kanpokihatsu No. 031121002, last revision November 20, 2006) and OECD Guideline for the Testing of Chemicals (TG 407, adopted on July 27, 1995), and was conducted in compliance with the Good

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Laboratory Practice Standards criteria for test facilities for carrying out tests on new chemical substances, etc. in Japan (Yakushokuhatsu No. 1121003, Seikyoku No. 3, and Kanpokihatsu No. 031121004, last revision April 1, 2005). The use and care of animals complied with the Act on Welfare and Management of Animals (Japanese Animal Welfare Law, Act No. 105, last revision June 22, 2005), Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Announcement No. 88, Ministry of the Environment, Japan, April 28, 2006), and Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006).

Test substance and reagent

β-bromostyrene [lot no. TEYUC, purity 99.6% (cis- and trans-mixture), yellow-clear liquid, CAS No. 103-64-0] was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and kept in a test substance storage room (light shielding and moisture prevention) of the testing facility at approximately 3-9°C. Corn oil (lot no. WKJ3948) as a vehicle was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Animals and husbandry

Specific-pathogen-free Sprague-Dawley rats [Crl: CD (SD)] at 5 weeks of age were purchased from Atsugi Breeding Center of Charles River Japan, Inc. (Kanagawa, Japan). Forty-seven males and 47 females were obtained and individually identified using ear tags. Rats were quarantined and acclimatized to the testing environment for 7 days and assigned to each dose group by stratified random sampling based on body weight. Administration of the test substance was initiated at 6 weeks of age. Body weight ranges for males and females upon initiation of treatment were 182-216 g and 145-171 g, respectively. Animals were individually housed in wire-mesh steel bracket cages (W 250 × D 350 × H 200 mm) and kept in an environmentally-controlled room: temperature, 21-23°C; humidity, 49-66%; ventilation, 10-15 times/hr; and lighting, 12 hr per /day (light on/off, 7:00/19:00). The animals were fed a pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and given tap water through bottle *ad libitum*.

Selection of dose levels

Dose levels of β-bromostyrene were selected based on results obtained from a 14-day dose range-finding study using the same strain of rats (five males and five females per group) at dose levels of 0 (corn oil only), 100, 300, and 1000 mg/kg/day. In the dose range-finding study, all males and females in the 1000 mg/kg group died. Increases in relative liver and kidney weights were observed in the 300 mg/kg group. Therefore, in the present study, the high dose was set at 500 mg/kg/day, and middle and low doses were set at 125 and 30 mg/kg/day, respectively, using common ratio 4.

Experimental design

Rats were administered β-bromostyrene by gavage once daily at 0 (vehicle control), 30, 125, and 500 mg/kg/day for 28 days. There were 12 rats/sex/dose in the 0 and 500 mg/kg groups and 6 rats/sex/dose in the 30 and 125 mg/kg groups; the dosing volume was 5 mL/kg body weight. On the day after the last dosing, six males and six females from each group were euthanized for the assessment of hematology, blood biochemistry, organ weight, and macroscopic and microscopic findings (main group). The respective remaining 6 rats/sex at 0 and 500 mg/kg were kept without treatment for 14 days as a recovery period and then fully examined (recovery group).

Daily observation, functional observation battery, body weight, and food and water consumption

All animals were observed, in their cages, for clinical signs of toxicity 2-3 times daily during the dosing period and once daily during the recovery period. Detailed clinical observations, including observations in the home cage, during handling, and outside of the home cage in an open field, were conducted before the start of dosing and once a week during the dosing and recovery periods. At the end of the dosing and recovery periods, functional observations, including auditory, approach, touch, and tail pinch responses, pupillary and aerial righting reflexes, and landing foot splay, were performed. In addition, grip strengths (fore and hindlimb) were measured using a CPU gauge (model-9502A, Aikoh Engineering Co., Ltd., Osaka, Japan), and motor activity was recorded at 10 min intervals for 1 hr by an activity monitoring system (model NS-AS01, Neuroscience, Inc., Tokyo, Japan). Body weight was recorded before dosing on Days 1, 4, 7, 10, 14, 17, 21, 24, and 28 of the dosing period and on Days 1, 3, 7, 10, and 14 of the recovery period. Food consumption was measured on the same days as body weights. Water consumption was recorded during Week 4 of the dosing period and Week 2 of the recovery period.

Urinalysis, hematology, and clinical biochemistry

Urinalysis was conducted during Week 4 in the dosing period and Week 2 in the recovery period. Urine was

collected for 4 hrs under fasting conditions with water *ad libitum* and analyzed using an AUTION MINI™ AM-4290 (Arkray Inc., Kyoto, Japan) for dipstick parameters, such as pH, proteins, ketone bodies, glucose, occult blood, bilirubin, urobilinogen, color, sediments, and volume. Urine volume and osmolality were measured using an automatic osmometer (Auto & Stat OM-6030, Arkray Inc., Kyoto, Japan) using a 20-hr urine sample collected with food and water *ad libitum*.

The day after the end of the dosing and recovery periods, blood was collected from the abdominal aorta under deep anesthesia after overnight starvation. One portion of the blood was treated with EDTA-2K and examined using an Advia 120 Hematology System (Siemens Medical Solutions Diagnostics, New York, USA) for hematologic parameters, such as red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte, platelet (PLT), white blood cell (WBC), and differential leukocyte count [lymphocyte (LYMP), neutrophil (NEUT), eosinophil (EOS), basophil (BASO), monocyte (MONO), and large unstained cell (LUC)]. Another blood sample was treated with sodium citrate and analyzed using a coagulometer ACL 100 (Instrumentation Laboratory, Massachusetts, USA) for blood clotting parameters, such as prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen level (FIB).

Serum from the remaining portion of blood was analyzed for alkaline phosphatase (ALP), total cholesterol (T-CHO), triglyceride (TG), phospholipid (PL), total bilirubin (T-BIL), glucose (GLU), blood urea nitrogen (BUN), creatinine (CRNN), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), inorganic phosphorus (P), total protein (TP), albumin (ALB), and albumin/globulin (A/G) ratio. Plasma isolated from heparinized blood was analyzed for aspartate and alanine aminotransferases (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and γ-glutamyl transpeptidase (γ-GTP). Items excluding electrolytes were analyzed using a clinical chemistry automatic analyzer (TBA-120FR, Toshiba Corporation, Tokyo, Japan) and electrolytes were analyzed by an automatic analyzer (PVA-αII, Analytical Instruments, Inc., Massachusetts, USA).

Organ weights, gross necropsy, and histopathology

After blood collection, all animals were sacrificed by exsanguination, and the organs and tissues of the whole body, including external surfaces, head, breast, and abdomen, were observed macroscopically. Next, the brain, adrenals, thymus, spleen, heart, liver, kidneys, testes, epididymides, ovaries, and uterus were removed and weighed. In addition, relative organ weights were calculated from organ/body weight ratios.

The cerebrum, cerebellum, spinal cord (chest), sciatic nerve, pituitary gland, thyroid, parathyroids, adrenal glands, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, trachea, lung (including bronchial), stomach, duodenum, jejunum, ileum (including Peyer's patches), cecum, colon, rectum, liver, kidneys, urinary bladder, testes, epididymides, prostate, ovaries, uterus, sternum (including bone marrow), femur (including bone marrow), and femoral skeletal muscle were fixed in 10% phosphate-buffered formalin. The eyeballs and optic nerves were fixed in phosphate-buffered 3 vol% glutaraldehyde/2.5 vol% formalin, and the testes and epididymides were fixed in Bouin's solution.

Paraffin sections for microscopic examination were routinely prepared and stained with hematoxylin-eosin. In the control and high dose groups sacrificed at the end of the dosing period, all preserved organs were examined under a light microscope. If treatment-related histopathological changes were found, the same tissues were examined for low and middle dose groups and the recovery group.

Data analysis

Parametric data, such as quantitative data in open field observation, functional observation and urinalysis, grip strengths, motor activity, body weight, food and water consumption, hematological and blood biochemistry findings, and organ weights, were analyzed using Bartlett's test for homogeneity of distribution. The Dunnett's multiple comparison test and the Dunnett's-type mean rank sum test were conducted for homogenous and non-homogenous distribution, respectively to compare the control and individual treatment groups. Parametric data obtained during or after the recovery period were analyzed by *F*-test for homogeneity of distribution. For comparison, the Student's *t*-test and the Aspin-Welch's *t*-test were conducted for homogenous and non-homogenous distribution, respectively, (Snedecor and Cochran, 1989; Dunnett, 1955, 1964; Sakuma, 1977, 1981).

RESULTS

General clinical observations

No abnormal clinical signs were observed in either sex receiving 30 or 125 mg/kg during the dosing period. One female rat receiving 500 mg/kg was found dead

on Day 3. In this rat, decrease in spontaneous movement was observed only on the first dosing day. Decreases in spontaneous movement were also observed in all other male and female rats receiving 500 mg/kg on the first dosing day; however, no abnormalities were observed in the general conditions thereafter during the dosing period. No clinical signs were observed in any animal during the recovery period.

Detailed clinical and functional observations

Detailed clinical observations: In the open field observation, a significant increase in rearing counts was observed in males receiving 30 mg/kg only during Week 1 of the dosing period; this was not observed in the higher dose groups. A significantly low number of rearing counts was observed in females receiving 500 mg/kg during Week 4, but this value was equivalent to those during Weeks 1-3 in the same group. During handling, slight salivation was observed in four males and one female during Week 3 and in three males and two females during Week 4 in the 500 mg/kg group. There were no abnormal or significant changes in recovery group rats.

Functional observations: No significant changes were observed in any parameter for either sex receiving the test substance during Week 4 of the dosing period. A significant decrease in landing foot splay was observed in males receiving 500 mg/kg during Week 2 of the recovery period; however, it was determined to be incidental because this sign was not observed during Week 4 of the dosing period.

Grip strength: A significant increase in hindlimb grip strength was observed in females receiving 125 mg/kg during Week 4 of the dosing period. However, this was not observed in the high dose group. A significant decrease in forelimb grip strength was observed in males receiving 500 mg/kg during Week 2 of the recovery period, but this change was not observed during Week 4 of the dosing period.

Motor activity: No significant change was observed in any male or female rats receiving the test substance during Week 4 of the dosing period. During Week 2 of the recovery period, a significant decrease was observed in males receiving 500 mg/kg only 40-50 min after the start of measurement.

Body weight, food consumption, and water consumption

Body weights in females receiving 125 mg/kg were significantly higher at Days 17-24 during the dosing period, but no significant differences were found in the 500 mg/kg groups throughout the study. In the 500 mg/kg group, food consumption significantly decreased for both sexes at Day 4 of the dosing period and in females at Days 7 and 14 of the recovery period. On the other hand, food consumption significantly increased in females at Days 7-21 of the dosing period in the 125 mg/kg group and Days 7, 14-21, and 28 of the dosing period in the 500 mg/kg group. A significant decrease in water consumption was observed in females receiving 125 mg/kg during Week 4 of the dosing period; however, this change was not observed in the high dose group. No significant differences were seen with water consumption for either sex in the recovery group.

Urinalysis

During Week 4 of the dosing period, significant increases in urine volume were observed in males receiving 125 and 500 mg/kg (12.1 ± 3.0 and 13.1 ± 4.4 mL/24 hr, respectively, versus 7.4 ± 3.6 mL/24 hr for control) and in females receiving 500 mg/kg (10.9 ± 3.5 mL/24 hr versus 6.4 ± 3.2 mL/24 hr for control). A significant decrease in urine osmolality was also observed in males receiving 125 and 500 mg/kg (1783 ± 359 and 1665 ± 328 mOsm/kg, respectively, versus 2194 ± 355 mOsm/kg for control). In the sediments, small round epithelial cells were observed in 5/12 males and 1/11 females receiving 500 mg/kg, and this change increased in males compared with the control group. No significant differences were seen in urine volume, osmolality, or qualitative measurements for either sex compared with the control groups during Week 2 of the recovery period.

Hematology

Hematological results are summarized in Table 1. At the end of the dosing period, significant decreases in MCH were observed in males receiving 30 and 500 mg/kg, and significant decreases in MCH concentration were observed in both sexes receiving 500 mg/kg. A significant increase in reticulocytes was also found in females receiving 500 mg/kg. However, these changes were slight, and no clear changes were observed in RBC or HGB. Other significant changes were a reduction in APTT and an increase in FIB in females receiving 125 mg/kg, but these changes were not observed at 500 mg/kg. Therefore, these changes were determined to be incidental. At the end of the recovery period, the only significant changes observed were a decrease in EOSs in males and an increase in MONOs in females. However, these changes were not observed at the end of the dosing period; therefore, these changes were determined to be incidental.

Table 1. Hematological values in the repeated dose 28-day oral toxicity study of β-bromostyrene in rats.

Dose (mg/kg/day)	At the end of the dosing period				At the end of the recovery period	
	0	30	125	500	0	500
Males						
No. of animals	6	6	6	6	6	6
RBC (×10⁴/μL)	795 ± 31	817 ± 29	816 ± 31	831 ± 38	855 ± 15	873 ± 39
HGB (g/dL)	16.1 ± 0.5	15.8 ± 0.5	16.0 ± 0.6	15.9 ± 0.5	15.9 ± 0.4	16.1 ± 0.4
HCT (%)	43.7 ± 1.7	43.5 ± 1.4	43.9 ± 1.6	44.2 ± 1.9	44.2 ± 1.1	44.9 ± 1.2
MCV (fL)	55.0 ± 1.4	53.2 ± 0.9	53.9 ± 1.5	53.2 ± 1.2	51.7 ± 1.2	51.6 ± 2.3
MCH (pg)	20.2 ± 0.5	19.3 ± 0.4*	19.6 ± 0.6	19.2 ± 0.6**	18.5 ± 0.4	18.5 ± 0.8
MCHC (g/dL)	36.8 ± 0.3	36.3 ± 0.3	36.4 ± 0.2	36.1 ± 0.6*	35.9 ± 0.4	35.9 ± 0.3
Reticulocyte (%)	2.1 ± 0.5	1.9 ± 0.1	2.0 ± 0.2	1.9 ± 0.3	1.8 ± 0.4	1.8 ± 0.4
PLT (×10⁴/μL)	130.8 ± 6.2	130.1 ± 8.5	121.7 ± 13.5	122.3 ± 16.2	114.2 ± 13.8	128.3 ± 11.8
PT (sec)	14.3 ± 1.0	13.7 ± 1.0	13.6 ± 1.0	15.8 ± 1.5	15.9 ± 3.3	16.1 ± 1.5
APTT (sec)	22.8 ± 3.1	20.8 ± 1.9	20.3 ± 1.8	23.7 ± 2.2	24.7 ± 2.1	23.9 ± 1.6
FIB (mg/dL)	332 ± 25	334 ± 26	332 ± 31	360 ± 26	292 ± 27	331 ± 38
WBC (×10²/μL)	104.5 ± 21.1	100.0 ± 12.4	118.1 ± 26.6	119.3 ± 10.2	89.7 ± 19.5	110.4 ± 31.9
Differential leukocyte count (%)						
LYMP	79.2 ± 3.3	82.1 ± 3.4	78.7 ± 7.8	80.0 ± 3.7	79.3 ± 2.9	77.2 ± 6.5
NEUT	17.7 ± 3.1	14.7 ± 3.3	17.7 ± 7.5	16.0 ± 3.9	16.6 ± 3.0	19.4 ± 6.1
EOS	0.7 ± 0.2	1.0 ± 0.5	0.8 ± 0.3	0.7 ± 0.2	1.4 ± 0.4	0. 9 ± 0.2**
BASO	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
MONO	1.5 ± 0.5	1.4 ± 0.2	1.9 ± 0.8	2.2 ± 0.4	1.9 ± 0.4	1.7 ± 0.6
LUC	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.4	0.5 ± 0.2	0.5 ± 0.2
Females						
No. of animals	6	6	6	6	6	5
RBC (×10⁴/μL)	802 ± 31	796 ± 38	794 ± 37	827 ± 46	825 ± 21	861 ± 51
HGB (g/dL)	15.7 ± 0.2	15.7 ± 0.5	15.6 ± 0.5	15.7 ± 0.4	15.8 ± 0.5	15.9 ± 0.7
HCT (%)	42.0 ± 0.5	42.0 ± 1.5	42.0 ± 1.7	43.3 ± 1.1	42.5 ± 1.1	43.2 ± 2.1
MCV (fL)	52.5 ± 2.1	52.7 ± 1.3	53.0 ± 1.0	52.5 ± 1.8	51.6 ± 1.5	50.2 ± 0.8
MCH (pg)	19.6 ± 0.7	19.7 ± 0.6	19.7 ± 0.6	19.0 ± 1.0	19.1 ± 0.6	18.5 ± 0.4
MCHC (g/dL)	37.3 ± 0.4	37.5 ± 0.6	37.2 ± 0.5	36.2 ± 0.6**	37.1 ± 0.4	36.8 ± 0.4
Reticulocyte (%)	1.2 ± 0.3	1.5 ± 0.3	1.4 ± 0.4	1.7 ± 0.2*	1.3 ± 0.4	1.2 ± 0.4
PLT (×10⁴/μL)	142.9 ± 14.1	136.9 ± 9.8	138.4 ± 9.9	130.6 ± 9.2	137.2 ± 14.4	131.9 ± 8.7
PT (sec)	11.5 ± 0.4	11.3 ± 0.6	11.1 ± 0.5	11.6 ± 0.7	12.2 ± 0.5	12.1 ± 0.7
APTT (sec)	17.7 ± 1.6	16.2 ± 1.3	15.4 ± 1.4*	16.1 ± 1.8	18.2 ± 1.7	20.6 ± 2.0
FIB (mg/dL)	220 ± 19	234 ± 20	256 ± 12**	243 ± 18	214 ± 15	246 ± 40
WBC (×10²/μL)	78.5 ± 9.4	83.2 ± 16.5	82.3 ± 10.7	91.5 ± 18.6	74.6 ± 19.7	87.3 ± 23.5
Differential leukocyte count (%)						
LYMP	81.9 ± 4.8	75.5 ± 8.1	79.9 ± 8.9	78.5 ± 8.7	77.9 ± 6.2	75.8 ± 4.6
NEUT	14.3 ± 4.7	20.2 ± 8.4	16.1 ± 8.5	17.1 ± 8.8	18.4 ± 5.9	19.8 ± 5.5
EOS	1.2 ± 0.4	1.5 ± 0.7	1.1 ± 0.3	1.3 ± 0.6	1.4 ± 0.6	1.4 ± 0.8
BASO	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
MONO	1.7 ± 0.7	2.0 ± 0.7	2.0 ± 0.9	2.2 ± 1.4	1.4 ± 0.3	2.3 ± 0.7*
LUC	0.7 ± 0.2	0.6 ± 0.3	0.7 ± 0.4	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2

Values are expressed as the mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$ versus control.
RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; PT, prothrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; WBC, white blood cells; LYMP, lymphocytes; NEUT, neutrophils; EOS, eosinophils; BASO, basophils; MONO, monocytes; LUC, large unstained cells.

Table 2. Clinical biochemistry values of the repeated dose 28-day oral toxicity study of β-bromostyrene in rats.

Dose (mg/kg/day)	At the end of the dosing period				At the end of the recovery period	
	0	30	125	500	0	500
Males						
No. of animals	6	6	6	6	6	6
AST (IU/L)	65 ± 3	57 ± 4	56 ± 7*	57 ± 8*	66 ± 3	64 ± 6
ALT (IU/L)	28 ± 3	27 ± 3	24 ± 3	23 ± 4*	30 ± 3	27 ± 3
LDH (IU/L)	73 ± 19	60 ± 11	52 ± 8*	75 ± 17	51 ± 6	54 ± 6
γ-GTP (IU/L)	1 ± 1	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
ALP (IU/L)	704 ± 186	647 ± 112	667 ± 95	645 ± 116	530 ± 100	542 ± 76
T-CHO (mg/dL)	52 ± 13	54 ± 12	67 ± 12	67 ± 13	62 ± 16	69 ± 17
TG (mg/dL)	50 ± 33	71 ± 26	72 ± 35	54 ± 18	45 ± 19	52 ± 18
PL (mg/dL)	88 ± 21	93 ± 14	110 ± 19	108 ± 15	96 ± 15	108 ± 20
T-BIL (mg/dL)	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
GLU (mg/dL)	137 ± 17	142 ± 7	137 ± 11	137 ± 12	129 ± 11	127 ± 21
BUN (mg/dL)	12 ± 1	13 ± 1	11 ± 1	11 ± 1	14 ± 1	13 ± 1
CRNN (mg/dL)	0.25 ± 0.03	0.22 ± 0.03	0.22 ± 0.01	0.21 ± 0.03*	0.25 ± 0.02	0.25 ± 0.01
Na (mmol/L)	143 ± 1	143 ± 1	143 ± 2	143 ± 1	145 ± 1	145 ± 1
K (mmol/L)	4.7 ± 0.2	4.8 ± 0.2	4.5 ± 0.2	4.9 ± 0.2	4.6 ± 0.2	4.6 ± 0.2
Cl (mmol/L)	112 ± 2	110 ± 2	111 ± 2	111 ± 1	110 ± 1	110 ± 2
Ca (mg/dL)	9.5 ± 0.1	9.7 ± 0.3	9.9 ± 0.2*	9.9 ± 0.2**	9.7 ± 0.2	9.7 ± 0.2
P (mg/dL)	7.8 ± 0.4	8.4 ± 0.6	8.3 ± 0.3	9.7 ± 0.8**	7.4 ± 0.6	7.7 ± 0.3
TP (g/dL)	5.9 ± 0.1	6.0 ± 0.2	5.9 ± 0.2	6.4 ± 0.3**	6.3 ± 0.3	6.3 ± 0.3
ALB (g/dL)	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.1**	2.9 ± 0.1	2.9 ± 0.1
A/G	0.87 ± 0.04	0.89 ± 0.05	0.88 ± 0.05	0.85 ± 0.04	0.86 ± 0.08	0.86 ± 0.05
Females						
No. of animals	6	6	6	6	6	5
AST (IU/L)	63 ± 6	61 ± 7	56 ± 3	55 ± 8	61 ± 7	57 ± 6
ALT (IU/L)	25 ± 3	24 ± 5	21 ± 3	19 ± 3	25 ± 5	22 ± 2
LDH (IU/L)	60 ± 17	58 ± 17	60 ± 16	57 ± 12	52 ± 12	52 ± 10
γ-GTP (IU/L)	1 ± 0	1 ± 1	1 ± 0	2 ± 1	1 ± 0	1 ± 0
ALP (IU/L)	334 ± 90	407 ± 166	377 ± 66	362 ± 114	304 ± 63	241 ± 46
T-CHO (mg/dL)	41 ± 7	53 ± 19	72 ± 19*	92 ± 18**	63 ± 8	83 ± 23
TG (mg/dL)	10 ± 4	14 ± 8	18 ± 6	19 ± 5*	17 ± 7	27 ± 8*
PL (mg/dL)	77 ± 11	91 ± 23	113 ± 23*	141 ± 23**	113 ± 16	137 ± 20
T-BIL (mg/dL)	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
GLU (mg/dL)	98 ± 11	110 ± 19	115 ± 10	114 ± 9	107 ± 9	117 ± 18
BUN (mg/dL)	15 ± 1	16 ± 3	14 ± 2	12 ± 2	18 ± 2	16 ± 3
CRNN (mg/dL)	0.28 ± 0.03	0.28 ± 0.05	0.27 ± 0.03	0.23 ± 0.04	0.31 ± 0.03	0.27 ± 0.05
Na (mmol/L)	142 ± 1	142 ± 1	142 ± 1	141 ± 1	143 ± 1	143 ± 1
K (mmol/L)	4.9 ± 0.2	4.7 ± 0.3	4.7 ± 0.3	4.6 ± 0.2	4.6 ± 0.2	4.3 ± 0.3
Cl (mmol/L)	113 ± 2	114 ± 1	114 ± 1	116 ± 1*	113 ± 1	111 ± 2
Ca (mg/dL)	9.8 ± 0.3	9.7 ± 0.1	9.8 ± 0.4	10.1 ± 0.3	9.9 ± 0.3	10.1 ± 0.3
P (mg/dL)	7.7 ± 0.5	7.7 ± 0.4	7.5 ± 0.5	7.2 ± 1.0	7.0 ± 0.3	7.3 ± 0.7
TP (g/dL)	6.1 ± 0.3	6.1 ± 0.2	6.1 ± 0.3	6.5 ± 0.2*	6.5 ± 0.3	6.7 ± 0.1
ALB (g/dL)	2.9 ± 0.2	2.9 ± 0.1	3.0 ± 0.2	3.1 ± 0.1	3.1 ± 0.1	3.2 ± 0.1
A/G	0.92 ± 0.04	0.91 ± 0.07	0.95 ± 0.06	0.90 ± 0.04	0.91 ± 0.02	0.90 ± 0.05

Values are expressed as the mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$ versus control.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; T-CHO, total cholesterol; TG, triglycerides; PL, phospholipids; T-BIL, total bilirubin; GLU, glucose; BUN, blood urea nitrogen; CRNN, creatinine; TP, total protein; ALB, albumin; A/G, albumin/globulin ratio.

Clinical biochemistry

Clinical biochemistry results are summarized in Table 2. At the end of the dosing period, T-CHO and PL levels were significantly increased in rats receiving 125 mg/kg and above, and TG levels were also significantly increased in females receiving 500 mg/kg. Slight but significant decreases were found in AST activity at doses of 125 and 500 mg/kg, in ALT activity at 500 mg/kg, and in LDH activity at 125 mg/kg in males. In the 500 mg/kg group, there was a significant increase in TP levels for either sex, a significant increase in ALB levels in males, and a significant decrease in CRNN in males. Furthermore, significant increases were observed in Cl in females and P in males receiving 500 mg/kg, as well as Ca in males receiving 125 and 500 mg/kg. After the recovery period, TG levels remained significantly high in females in the 500 mg/kg group, but other changes observed at the end of the dosing period were not detected.

Organ weights

Significant effects by the test substance were observed in the liver and kidneys at the end of the dosing period. Increases were found in absolute and relative liver weights at 500 mg/kg in both sexes along with an increase in absolute liver weight at 125 mg/kg in females, an increase in relative liver weight at 125 mg/kg in males, increases in absolute and relative kidney weights at 500 mg/kg in males, and an increase in relative kidney weights at 500 mg/kg in females (Table 3). The significant increase in relative liver weight remained after the recovery period in females receiving 500 mg/kg. Other significant changes were decreases in relative brain weight at 125 mg/kg in females, relative spleen weight at 30 mg/kg in males, and absolute testes weight at 500 mg/kg at the end of dosing period, as well as an increase in relative heart weight at 500 mg/kg in females at the end of the recovery period. However, these changes were slight and/or lacked dose-dependency.

Gross necropsy

In the female rat which received 500 mg/kg that was found dead on Day 3 of the dosing period, there were excess fluids in the abdominal and thoracic cavities, an enlarged liver, and dark red foci in the glandular stomach. In animals sacrificed as scheduled, enlargement of the liver was observed in three males and two females receiving 500 mg/kg at the end of the dosing period and one male receiving 500 mg/kg at the end of the recovery period. Other findings included dark red foci in the lung in one control female and one male receiving 125 mg/kg, dark red foci in the glandular stomach in one female each receiving 125 and 500 mg/kg, and unilateral small thyroids in one female each receiving 30 and 500 mg/kg at the end of the dosing period.

Histopathology

In the dead 500 mg/kg group female, atrophy of Peyer's patch in the ileum, dilation of the renal tubes with centrilobular necrosis and congestion in the liver, focal hemorrhage and accumulation of foamy cells in the lung, atrophy of the mesenteric and submandibular lymph nodes, an increase in hematopoiesis and atrophy of white pulp in the spleen, erosion in the glandular stomach, atrophy of the thymus, and a remnant of ultimobranchial bodies in the thyroid were observed.

Histopathological changes observed in other animals are summarized in Tables 4 and 5. Significant effects of the test substance were observed in the liver and thyroids of both sexes and kidneys of males at the end of the dosing period. In the kidneys, a minimal to mild degree of eosinophilic bodies in tubular cells was observed in two males receiving 125 mg/kg and all males receiving 500 mg/kg. Mild degeneration of renal tubular and minimal hyaline casts was also observed in males in the 500 mg/kg group. In the liver, minimal to mild centrilobular hypertrophy of hepatocytes was observed in all males and females receiving 500 mg/kg. In the thyroids, minimal hypertrophy of follicular cells was observed in one female receiving 125 mg/kg and two males and five females receiving 500 mg/kg.

At the end of the recovery period, significant effects of the test substance were observed in the kidneys and thyroids of males. A minimal degree of eosinophilic bodies in renal tubular cells was observed in three males at 500 mg/kg. Minimal regeneration of renal tubules was observed in three males of the control group and four males of 500 mg/kg group, and mild regeneration was observed in two males of the 500 mg/kg group; the incidence and severity of regeneration were slightly increased in the 500 mg/kg group. Minimal hypertrophy of follicular cells in the thyroid was observed in one male at 500 mg/kg. Other changes at the end of the dosing and/or recovery periods observed in the heart, rectum, kidneys, liver, prostate, skeletal muscle, spleen, stomach, thyroid, and urinary bladder were considered to be incidental findings due to the apparent situation or histopathological properties.

DISCUSSION

This study examined the toxicity of β-bromostyrene and its reversibility. Crl: CD (SD) rats were administered

Table 3. Absolute and relative organ weights in the repeated dose 28-day oral toxicity study of β-bromostyrene in rats.

Item	Dose (mg/kg/day)	At the end of the dosing period				At the end of the recovery period			
		Males		Females		Males		Females	
		Liver	Kidney (R + L)	Liver	Kidney (R + L)	Liver	Kidney (R + L)	Liver	Kidney (R + L)
Absolute (g)	0	10.50 ± 2.05	2.53 ± 0.25	6.26 ± 0.76	1.67 ± 0.15	11.80 ± 1.27	2.97 ± 0.37	6.29 ± 0.62	1.77 ± 0.11
	30	12.02 ± 1.31	2.84 ± 0.33	6.69 ± 0.74	1.71 ± 0.21	-	-	-	-
	125	12.08 ± 1.45	2.88 ± 0.25	7.42 ± 0.45*	1.91 ± 0.18	-	-	-	-
	500	14.83 ± 1.49**	3.26 ± 0.34**	9.37 ± 0.79**	1.85 ± 0.13	12.06 ± 1.85	2.98 ± 0.26	7.38 ± 1.10	1.89 ± 0.28
Relative (g/100 g BW)	0	2.94 ± 0.20	0.72 ± 0.07	2.83 ± 0.19	0.76 ± 0.04	2.83 ± 0.13	0.71 ± 0.05	2.55 ± 0.10	0.72 ± 0.04
	30	3.26 ± 0.15	0.77 ± 0.05	2.96 ± 0.22	0.76 ± 0.09	-	-	-	-
	125	3.32 ± 0.29*	0.79 ± 0.04	3.11 ± 0.22	0.80 ± 0.06	-	-	-	-
	500	4.31 ± 0.24**	0.95 ± 0.08**	4.34 ± 0.23**	0.86 ± 0.07*	3.00 ± 0.22	0.75 ± 0.08	3.10 ± 0.31*	0.79 ± 0.09

Values are expressed as the mean ± standard deviation of six rats. *$P < 0.05$ and **$P < 0.01$ versus control.
BW, body weight; R, right; L, left.

Table 4. Histopathological findings in the repeated dose 28-day oral toxicity study of β-bromostyrene in rats at the end of the dosing period.

Organs / Findings	Sex	Males				Females			
	Dose (mg/kg/day)	0	30	125	500	0	30	125	500
	Number	6	6	6	6	6	6	6	6
Heart									
Focal myocarditis	Minimal	1	-	-	0	0	-	-	0
Intestine, rectum									
Submucosal cell infiltration	Minimal	1	-	-	0	0	-	-	0
Kidney									
Tubular regeneration	Minimal	2	3	2	3	1	-	-	2
Eosinophilic body in tubular cells	Minimal	0	0	2	0	0	-	-	0
	Mild	0	0	0	6	0	-	-	0
Hyaline cast	Minimal	0	0	0	3	1	-	-	0
Interstitial mineralization	Minimal	1	5	4	4	4	-	-	2
Interstitial cell infiltration	Minimal	2	1	1	3	3	-	-	0
Tubular degeneration	Mild	0	0	0	2	0	-	-	0
Liver									
Periportal vacuolation of hepatocytes	Minimal	1	0	0	0	1	2	1	1
	Mild	0	0	0	0	3	2	0	1
Extramedullary hematopoiesis	Minimal	0	1	1	0	0	0	0	1
Granuloma	Minimal	0	0	0	0	0	0	0	1
Microgranuloma	Minimal	4	4	4	6	6	5	5	1
	Mild	0	0	0	0	0	0	0	1
Central hypertrophy of hepatocytes	Minimal	0	0	0	5	0	0	0	5
	Mild	0	0	0	1	0	0	0	1
Prostate									
Lymphocyte infiltration	Minimal	3	-	-	5	-	-	-	-
	Mild	1	-	-	0	-	-	-	-
Skeletal muscle									
Cell infiltration	Minimal	1	-	-	0	0	-	-	0
Spleen									
Increased hematopoiesis	Minimal	1	-	-	1	0	-	-	0
Glandular stomach									
Erosion	Minimal	0	-	-	0	0	-	1[a)]	1
Thyroid									
Ectopic thymus	Minimal	2	0	0	0	0	0	0	0
Interstitial cell infiltration	Minimal	0	0	0	1	0	0	0	0
Remnant of ultimobranchial body	Minimal	2	1	2	1	2	2	0	3
Hypertrophy of follicular cells	Minimal	0	0	0	2	0	0	1	5
Urinary bladder									
Submucosal cell infiltration	Minimal	0	-	-	1	0	-	-	0

-, not examined

[a)] Number of examined animals was one in which dark red foci was grossly observed in the glandular stomach.

β-bromostyrene in corn oil through gavage at doses of 0, 30, 125, and 500 mg/kg/day for 28 days, and control and high dose groups were followed over a 14-day recovery period.

One female receiving 500 mg/kg was found dead on Day 3 of the dosing period. Decrease in spontaneous movement was observed in this rat on the first dosing day, but no clinical signs were observed thereafter. Gross and histopathological examinations revealed various changes in the dead rat; however, the cause of death was unclear. In surviving animals, decreases in spontaneous movements were observed only on the first dosing day, and salivation during handling was sporadically observed during Weeks 3 and 4 in both sexes in the 500 mg/kg group.

Table 5. Histopathological findings in the repeated dose 28-day oral toxicity study of β-bromostyrene in rats at the end of the recovery period.

Organs / Findings	Sex	Males		Females	
	Dose (mg/kg/day)	0	500	0	500
	Number	6	6	6	5
Kidney					
Tubular regeneration	Minimal	3	4	-	-
	Mild	0	2	-	-
Eosinophilic body in tubular cells	Minimal	0	3	-	-
Hyaline cast	Minimal	0	1	-	-
Interstitial mineralization	Minimal	3	5	-	-
Liver					
Periportal vacuolation of hepatocytes	Minimal	1	0	1	2
Microgranuloma	Minimal	6	6	5	5
Thyroid					
Ectopic thymus	Minimal	1	0	0	0
Remnant of ultimobranchial body	Minimal	2	1	0	1
Hypertrophy of follicular cells	Minimal	0	1	0	0

-, not examined

Although food consumption in the 125 and 500 mg/kg groups were higher or lower than in the control group, toxicologically significant effects on body weight were not found throughout the study.

β-Bromostyrene clearly affected the kidneys in males; eosinophilic bodies in tubular cells, hyaline casts, and/or tubular degeneration were observed in the 125 and 500 mg/kg groups, and tubular regeneration was found after the recovery period. In the 500 mg/kg group, small round epithelial cells were found in the urine, which is indicative of renal tubular lesions. Increased urine volume and decreased urine osmolality are considered to be due to disturbances in tubular function. Interestingly, histopathological changes were observed only in males. Although slight but significant increases in urine volume and relative kidney weight were observed in females in the 500 mg/kg group, males were more susceptible to renal toxicity induced by β-bromostyrene. However, similar changes can be induced in females at higher doses.

In the present study, toxicological effects on the liver and thyroid were also observed. Hepatocyte hypertrophy was observed in both sexes in the 500 mg/kg group. Increased serum T-CHO, TG and PL levels indicate that β-bromostyrene affected lipid metabolism at 125 and 500 mg/kg in females. In the thyroid, follicular cell hypertrophy was found in the 125 and 500 mg/kg groups, which may be a compensatory response to increased hepatic metabolism of thyroid hormones.

In summary, β-bromostyrene affected the kidneys, liver, and thyroid of rats at doses of 125 mg/kg and above in our 28-day oral toxicity study. It was determined that the no-observed-adverse-effect-level of β-bromostyrene was 30 mg/kg/day for both sexes. Since all changes observed at the end of the dosing period disappeared or were reduced during the 14-day recovery period, toxic effects caused by β-bromostyrene are considered to be reversible.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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【特集】

OECD 化学物質共同評価プログラム：第 6 回化学物質共同評価会議概要

OECD Cooperative Chemicals Assessment Programme:
Summary of 6th Cooperative Chemicals Assessment Meeting

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要旨：第 6 回 OECD 化学物質共同評価会議が、2014 年 9 月 30 日-10 月 3 日にフランスのパリで開催された。この会議では計 136 物質（初期評価：132 物質；選択的初期評価：4 物質）について審議され、titanium dioxide（CAS: 13463-67-6）を除く全ての物質に合意が得られた。日本は、政府作成の物質カテゴリー：Methyl- and Ethylcyclohexane（CAS:108-87-2、1678-91-7)および経済産業諮問委員会原案作成の trimethylsilanol（CAS: 1066-40-6）の初期評価文書、また 1,2-dichloro-4-(chloromethyl)benzene (CAS: 102-47-6)、1-naphthol-4-sulfonic acid sodium salt (CAS: 6099-57-6)、Disperse Red 206 (CAS: 26630-87-5)の計 3 物質の選択的初期評価文書を提出し合意された。本稿では、第 6 回化学物質共同評価会議の討議の概要を報告する。
キーワード：経済協力開発機構、化学物質共同評価会議、有害性評価

Abstract: The 6th Cooperative Chemicals Assessment Meeting was held in Paris, France on 30th September to 3rd October 2014. The initial assessment documents of 136 substances (SIDS Initial Assessment: 132 substances; Initial Targeted Assessment: 4 substances) were discussed, and the conclusions of the assessments for all substances except titanium dioxide (CAS: 13463-67-6) were approved at the meeting. Japan submitted the SIDS initial assessment documents for two substances, Methyl and Ethylcyclohexane (CAS: 108-87-2 and 1678-91-7), as a category assessment, and one substance, trimethylsilanol (CAS: 1066-40-6), as a single chemical assessment with BIAC (Business and Industry Advisory Committee). Japan also submitted the initial targeted assessment documents for three substances, 1,2-dichloro-4-(chloromethyl)benzene (CAS: 102-47-6), 1-naphthol-4-sulfonic acid sodium salt (CAS: 6099-57-6), and Disperse Red 206 (CAS: 26630-87-5). This paper reports the summary of the 6th Cooperative Chemicals Assessment Meeting.
Keywords: OECD, Cooperative Chemicals Assessment Meeting, Hazard Assessment

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はじめに

経済協力開発機構（**OECD**: Organisation for Economic Co-operation and Development）では、市場にある全ての化学物質の環境影響および人健康影響に係る初期有害性情報を収集・評価する「化学物質共同評価プログラム（**CoCAP**：Cooperative Chemicals Assessment Programme）」を 2011 年より行っており、化学物質共同評価会議（**CoCAM**：Cooperative Chemicals Assessment Meeting）が年 2 回開催されてきた。本プログラムでは、化学物質の有害性の初期評価に必要なスクリーニング情報データセット（**SIDS**: Screening Information Data Set）を全て満たしている SIDS 評価と、有害性評価に最も関連の強い一つもしくは複数のエンドポイントに焦点を絞って評価する選択的評価（**TA**：Targeted Assessment）のどちらかの区分により、化学物質の有害性を評価してきた。日本は、化学物質の審査及び製造等の規制に関する法律（化審法）の基に収集した情報を、SIDS エンドポイントを満たしている場合は SIDS 初期評価として、SIDS エンドポイントを満たしていない場合は、化審法の評価を基に人健康影響、物理化学的性状および曝露情報の一部に限定して選択的初期評価として提出している。

OECD 加盟国または企業が作成した初期評価文書の原案は、スポンサー（スポンサー国、またはスポンサー国が定まらない場合には **BIAC**(Business and Industry Advisory Committee、経済産業諮問委員会)を通じて提出され審議を受けている。審議のためには、初期評価プロファイル（**SIAP**: SIDS Initial Assessment Profile）、初期評価レポート（**SIAR**: SIDS Initial Assessment Report）および網羅的資料集（**Dossier**）の 3 文書の一式または、選択的初期評価プロファイル（**ITAP**: Initial Targeted Assessment Profile）、選択的初期評価レポート（**ITAR**: Initial Targeted Assessment Report）および Dossier の 3 文書の一式の提出が必要である。Dossier は **IUCLID**（International Uniform Chemical Information Database）というデータベースのソフトウェアを用いて作成されているが、出力方法をエクスポートファイルにすることによって、生データのやり取りが可能となる。したがって、最終的にはエクスポートファイルの提出も必要となっている。

第 6 回 CoCAM は 2014 年 9 月 30 日-10 月 3 日にフランスのパリで開催され、OECD 加盟国、非加盟国の中国およびロシア、産業界からの出席者で行われた。日本からは政府専門家の 3 名とオブザーバー2 名が出席した。本プログラムは、化学物質の初期評価を行うプログラムから評価手法の開発などに視点を当てたプログラムに変更することが決定しており、今回の第 6 回 CoCAM は、初期評価を行う最後の会議となった。本稿では第 6 回 CoCAM での討議内容として、2013 年 10 月に実施された第 5 回 CoCAM 以降の進捗状況、初期評価文書の審議結果および本プログラムの全般的な懸案事項に関する討議内容について報告する。本稿は第 6 回 CoCAM の会議報告書（OECD 2014）を参照して作成した。

1．第 5 回 CoCAM 以降の進捗状況

(1) 初期評価文書の公開状況

CoCAM で合意された初期評価文書(SIAP/ITAP)は、ハザード評価タスクフォース(以下、タスクフォース)および「OECD 化学品委員会および化学品・農薬・バイオテクノロジー作業部会合同会合」に提出して承認を得る。承認が得られた SIAP/ITAP については、OECD が既存化学物質データベース(OECD 2015)を通じて公開している。一方、文書作成を行った国または企業は CoCAM での審議をもとにその他の最終版の初期評価文書（SIAR/ITAR、Dossier およびエクスポートファイル）を作成し、CoCAM 後 3 ヶ月を目途に OECD 事務局に提出する。最終版の初期評価文書の提出が 6 ヶ月以上滞っている場合、スポンサーは状況説明と提出予定期日を示す必要がある。

SIAP/ITAP の公開後、最終文書が整い次第 SIAR/ITAR、Dossier およびエクスポートファイルについても、OECD の既存化学物質データベースより入手が可能となる。第 5 回 CoCAM で合意された初期評価文書は、全て公開の許可が得られた。第 4 回 CoCAM で合意された「物

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質カテゴリー：Monomeric Chlorosilanes」についても、スポンサー国（US/ICCA (International Council of Chemical Associations、国際化学工業協会協議会））が一部内容の訂正を行っていたが、第 5 回 CoCAM の文書と共に公開の許可が得られた。第 6 回 CoCAM 開催時に OECD のデータベースで公開されている物質数は、本プログラムの前身となる「OECD 高生産量化学物質（HPV：High Production Volume Chemicals）点検プログラム」での評価物質を含め、計 984 であった。なお、HPV 点検プログラムの歴史および CoCAP へと移行した経緯については、松本他が報告しているので参照されたい（松本他, 2012）。

2．第 6 回 CoCAM での審議状況

（1）初期評価文書・選択的初期評価文書の審議結果

初期評価文書および選択的初期評価文書の審議は、スポンサーが評価文書の原案を OECD の電子掲示板であるクリアスペースに掲載し、クリアスペース上で行うコメントの提出、コメントへの返答、コメントに応じた SIAP/ITAP の修正という事前討議および CoCAM での対面討議で行われる。第 6 回 CoCAM での初期評価文書の審議は、クリアスペースでの事前討議を基に修正した SIAP/ITAP を用いて行われた。日本は、政府作成の物質カテゴリー：Methyl- and Ethylcyclohexane (CAS:108-87-2、1678-91-7)および BIAC 作成の trimethylsilanol (CAS: 1066-40-6) の初期評価文書、また 1,2-dichloro-4-(chloromethyl)benzene (CAS: 102-47-6)、1-naphthol-4-sulfonic acid sodium salt (CAS: 6099-57-6)、Disperse Red 206 (CAS: 26630-87-5)の計 3 物質の選択的初期評価文書を提出し合意された。この会議では計 136 物質（初期評価：132 物質；選択的初期評価：4 物質）について審議され、titanium dioxide (CAS: 13463-67-6) を除く全ての物質に合意が得られた。付表には、今回の会議で審議された物質を示した。また、本会議で討議された物質は次の通りであった。下記以外の物質は事前討議を反映した SIAP/ITAP がそのまま、もしくは文字句の修正のみで合意された。

1) 物質カテゴリー：t-Butyl and t-Amyl Derived Alkyl Peroxyesters (5CAS)

オランダ/ICCA が担当した本物質カテゴリーについては、カテゴリーを構成する 5 物質中 2 物質は反応性が高く、各種試験に分解を防ぐための溶媒が用いられている点について審議された。溶媒の有無が毒性に与える影響は明確には分からないものの、本カテゴリー内の試験結果では、溶媒の有無による差は見られていない。しかし、溶媒不使用の試験情報のみでは、カテゴリー評価として情報量が不足するため、溶媒を用いた試験も採用することとした。溶媒の使用は毒性評価に問題はないと考えられたが、SIAP に溶媒使用の有無を明記することになった。また、スポンサー国は SIAR についても同様の修正を行うことを約束し、初期評価文書の合意に至った。

2) 物質カテゴリー：Soluble cobalt salts (8CAS)

オランダ/ICCA が担当した本物質カテゴリーについては、カテゴリーを構成する 8 物質の比較対象として掲載していた物質の情報を、添付文書として SIAP から独立させることで合意された。また、現在、生殖発生毒性試験を行っていることを SIAP に記載し、情報が得られ次第 SIAP を改定することで合意が得られた。

3) 物質カテゴリー：Aliphatic acids (78CAS)

イタリア/ICCA が担当した本物質カテゴリーは 78 物質からなり、本プログラム内で審議された物質カテゴリーとしては最も構成物質数の多い初期評価文書であった。環境影響の中で特に生分解性の潜在力について審議された後に合意に至った。

4) 物質カテゴリー：Methyl and ethylcyclohexane (2CAS)

日本が担当した本物質カテゴリーについては、腎臓に対する影響が雄ラット特有のα2u グロブリン腎症によるものか否かをより明確に示す必要があるとの意見があり、免疫組織染色の結

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果の詳細を追記した。一方、環境影響における生態濃縮試験の結果では、メチルシクロヘキサンとエチルシクロヘキサンの間で 10 倍の差があることに対して討議されたが、理由は不明なもののカテゴリーとして問題があることではないと結論され合意に至った。

5) Hexamethyldisiloxane（CAS: 107-46-0)

第 1 回 CoCAM で US/ICCA が提出し合意された本物質の初期評価文書については、BIAC が吸入試験による発生毒性の試験と生態濃縮についての情報を再評価し、今回の会議で再審議された。ヒト健康影響については、前回合意された発生毒性の NOAEC を変更したが、今回の対面会議の結果、第 1 回 CoCAM で合意された NOAEC に戻すことで合意が得られた。BIAC は、2 世代試験の結果で示される児の体重減少は軽微であり、生物学的毒性を反映したものではないと評価したが、用量依存性のある毒性として採用すべきであると結論された。また、環境影響については、フガシティーモデルの値を基に考えた食物連鎖における生態濃縮能に対する記述を削除することで合意が得られた。

6) Titanium Dioxide（CAS: 13463-67-6)

韓国が担当した本物質に対する初期評価文書は、第 3 回 CoCAM に提出され、第 4 回 CoCAM で合意に至った。その後のタスクフォースの承認を受ける段階になって、BIAC が人健康影響に対する知見が不足していることに懸念を呈した。第 4 回 CoCAM の行われた時期に BIAC 及び欧州化学物質生態毒性および毒性センター（**ECETOC**: European Central for Ecotoxicology and Toxicology of Chemicals）ではスタッフの交代があり、第 3 回 CoCAM の後に加えられた初期評価文書の変更点などを把握することが出来ていなかった。BIAC は遺伝毒性試験、生殖発生毒性試験、発がん性に関する情報を追加する必要があるとし、初期評価文書の改訂案を提示した。今回の会議では吸入による発がん性影響の追加分については合意が得られたが、遺伝毒性、生殖発生毒性の情報については、一部の Dossier が提出されていないことを理由に合意に至らなかった。OECD 事務局は、韓国が検証出来るように、遺伝毒性試験と生殖発生毒性試験の報告書を韓国に渡すよう BIAC に提案した。カナダ、ドイツ、オランダは改訂作業を続けることを支持した。しかし、会議後に韓国は「現行の CoCAP が終わりを迎えるこの局面に改訂作業のために人員を割くことが難しい」と OECD 事務局に報告した。更に、韓国は第 4 回 CoCAM で合意された文書を最終版として公開することを提案した。本件に関しては、BIAC がスポンサーとなって改訂作業を行うことに合意が得られれば、CoCAM 後に書面審議という形で引き続き作業が行えるだろうと結論された。また、韓国は引き続きスポンサーとなるためには、予算を確保するために OECD 事務局から正式に韓国政府に対する依頼状が必要であることを伝えた。

（2）化学物質共同評価プログラムにおける全般的な懸案事項の討議結果

1) 化学物質ハザード分類の試験的な作業

CoCAM では、**GHS**（The Globally Harmonized System of Classification and Labelling of Chemicals：化学品の分類および表示に関する世界調和システム)分類の作成および調和に対する試験的な作業を行っており、過去 3 回の CoCAM で審議をしてきた（松本他、2014a,b,c）。今回の会議では、2-vinylpyridine (CAS: 100-69-6)の GHS 分類について、分類作業を行った有志国（デンマーク、フランス、日本、オランダ、ロシア、スイス）の統一見解が得られるように討議を行った。会議に先立って、統一見解が得られていなかった９つのエンドポイント（急性毒性：経皮、急性毒性：経口、刺激性：眼、刺激性：皮膚、皮膚感作性、遺伝毒性、生殖発生毒性、特定標的臓器毒性：単回、特定標的臓器毒性：反復投与）について調整を行ったが、2 つのエンドポイント（特定標的臓器毒性：単回、特定標的臓器毒性：反復投与）については、合意が得られず今回の会議で討議された。二つのエンドポイントの内、特定標的臓器毒性：単回については、会議出席者の間で合意が得られたが、特定標的臓器毒性：反復投与については、

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