Table 1
Maternal findings in rats given DTG on days 6–19 of pregnancy

Dose (mg/kg)	0 (control)	10	20	40
No. of rats	24	24	24	24
No. of pregnant rats	24	24	24	24
Initial body weight	256 ± 13	256 ± 13	256 ± 13	256 ± 13
No. of females showing clinical sign of toxicity				
Death	0	0	0	4
Alopecia	2	2	3	2
Bradypnea	0	0	0	2
Decreased locomotor activity	0	0	1	11**
Mydriasis	0	0	12**	24**
Prone position	0	0	0	3
Salivation	0	0	2	2
Soil of perigenital	0	0	1	4
Tremor	0	0	0	2
Body weight gain during pregnancy (g)[a]				
Days 0–6	40 ± 8	39 ± 8	40 ± 8	39 ± 8
Days 6–15	50 ± 7	49 ± 9	37 ± 11**	23 ± 10**
Days 15–20	77 ± 9	77 ± 9	71 ± 10	47 ± 16**
Days 0–20	167 ± 17	165 ± 21	148 ± 24**	109 ± 21**
Adjusted weight gain[b]	88 ± 15	87 ± 19	77 ± 15	49 ± 17**
Food consumption during pregnancy (g/day)[a]				
Days 0–6	23 ± 2	23 ± 2	23 ± 2	23 ± 2
Days 6–15	26 ± 2	26 ± 2	24 ± 3	20 ± 3**
Days 15–20	28 ± 2	28 ± 3	26 ± 2	22 ± 3**
Days 0–20	25 ± 2	26 ± 2	24 ± 2	21 ± 2**
Weight of gravid uterus (g)[a]	79 ± 10	78 ± 11	72 ± 15	59 ± 10**

[a] Values are given as the mean ± S.D.
[b] Adjusted weight gain refers to maternal weight gain excluding the gravid uterus.
** Significantly different from the control ($p < 0.01$).

3. Results

Table 1 shows the maternal findings in rats given DTG on days 6–19 of pregnancy. At 40 mg/kg bw/day, death was found on day 8 of pregnancy in two females and on days 7 and 19 of pregnancy in one female each. Statistically significant increases in the incidence of mydriasis occurred at 20 and 40 mg/kg bw/day, and in decreased locomotor activity at 40 mg/kg bw/day. Additional findings that appeared to be treatment related, but not statistically significant were decreased locomotor activity at 20 mg/kg bw/day, salivation and soil of the perigenital area at 20 and 40 mg/kg bw/day, and bradypnea, prone position and tremors at 40 mg/kg bw/day. These signs were observed consistently throughout the dosing period and relatively higher incidences of these signs were noted during the early administration period. Maternal body weight gain was significantly decreased on days 6–15 and 0–20 of pregnancy at 20 mg/kg bw/day, and on days 6–15, 15–20 and 0–20 of pregnancy at 40 mg/kg bw/day. Adjusted weight gain, the net weight gain of maternal rats during pregnancy, and the weight of the gravid uterus were also significantly reduced at 40 mg/kg bw/day. At this dose, food consumption was significantly lowered on days 6–15, 15–20 and 0–20 of pregnancy.

Table 2 presents the reproductive findings in rats given DTG on days 6–19 of pregnancy. No dam with total litter loss was observed in any group. No effects of DTG were found on the numbers of corpora lutea and implantations, or the incidence of preimplantation loss. At 40 mg/kg bw/day, a significantly increased incidence of postimplantation loss, a decreased number of live fetuses and lowered weights of male and female fetuses and placentae were noted. The sex ratio of live fetuses was significantly reduced in the DTG-treated groups.

The summarized results of external and internal examinations in fetuses of rats given DTG on days 6–19 of pregnancy are shown in Table 3. No fetuses with external malformations were observed in the control group. One fetus with cleft palate was found at 10 mg/kg bw/day. Fetuses with external malformations were found in 13 out of the 328 fetuses (three out of the 24 litters) at 20 mg/kg bw/day and 33 out of the 251 fetuses (11 out of the 20 litters) at 40 mg/kg bw/day, and significantly increased incidence of the total number of fetuses with external malformations was noted at 40 mg/kg bw/day. Incidences of fetuses with brachydactyly and with short tail were increased at 20 and 40 mg/kg bw/day, and significantly increased incidences were found at 40 mg/kg bw/day. As for internal malformations, one fetus each with microphthalmia in the control and 20 mg/kg bw/day groups, one fetus with dilatation of the lateral ventricles in the control group and one fetus with undescended testes in the 40 mg/kg bw/day were observed. Variations in the internal organs were observed in 11–19 fetuses in all groups. However, no significant differences in the incidences of

Table 2
Reproductive findings in rats given DTG on days 6–19 of pregnancy

Dose (mg/kg)	0 (control)	10	20	40
No. of litters	24	24	24	20
No. of litters totally resorbed	0	0	0	0
No. of corpora lutea per litter[a]	15.7 ± 2.1	14.8 ± 1.6	14.9 ± 1.9	15.3 ± 1.5
No. of implantations per litter[a]	15.3 ± 1.9	14.7 ± 1.8	14.2 ± 2.7	15.2 ± 1.4
% Preimplantation loss per litter[b]	2.4	0.9	5.6	0.9
% Postimplantation loss per litter[c]	3.5	3.4	4.8	16.4**
No. of live fetuses per litter[a]	14.8 ± 1.9	14.2 ± 2.1	13.7 ± 2.9	12.6 ± 1.9**
Sex ratio of live fetuses (male/female)	0.56	0.49*	0.46*	0.46*
Body weight of live fetuses (g)[a]				
Male	3.64 ± 0.17	3.72 ± 0.18	3.59 ± 0.24	3.19 ± 0.31**
Female	3.42 ± 0.16	3.53 ± 0.25	3.41 ± 0.18	3.03 ± 0.26**
Placental weight (g)[a]	0.47 ± 0.04	0.47 ± 0.03	0.50 ± 0.16	0.40 ± 0.04**

[a] Values are given as the mean ± S.D.
[b] (No. of preimplantation embryonic loss/no. of corpora lutea) × 100.
[c] (No. of resorptions and dead fetuses/no. implantations) × 100.
* Significantly different from the control ($p < 0.05$).
** Significantly different from the control ($p < 0.01$).

fetuses with internal malformations and variations were detected between the control and DTG-treated groups.

The summarized results of skeletal examinations in the fetuses of rats given DTG on days 6–19 of pregnancy are presented in Table 4. Fetuses with skeletal malformations were found in one out of the 184 fetuses (one out of the 24 litters) in the control group, one out of the 176 fetuses (one out of the 24 litters) at 10 mg/kg bw/day, 13 out of the 170 fetuses (six out of the 24 litters) at 20 mg/kg bw/day, and 26 out of the 130 fetuses (12 out of the 20 litters) at 40 mg/kg bw/day. Significantly higher incidences of the total number of fetuses with skeletal malformations were observed at 20 and 40 mg/kg bw/day. Incidences of fetuses with absence, fusion or malposition of the caudal vertebrae and with absence or fusion of phalanges were higher at 20 and 40 mg/kg bw/day, and significantly increased incidences of fetuses with these malformations and fetuses with the absence or fusion of metacarpals were found at 40 mg/kg bw/day. Although skeletal variations in the vertebral column, ribs and sternebrae were observed in all groups, no significant differences in the incidences of fetuses with skeletal variations were detected between the control and DTG-treated groups. A significantly delayed ossification, as evidenced by the numbers of sacral and caudal vertebrae, sternebrae, and metatarsi, was also noted at 40 mg/kg bw/day.

4. Discussion

In order to obtain further information on the reproductive and developmental toxicity of DTG, the present study was conducted in compliance with OECD guideline 414 Prenatal Developmental Toxicity Study [16]. DTG was given to pregnant rats during the time of implantation to the term of pregnancy to

Table 3
External and internal examinations in fetuses of rats given DTG on days 6–19 of pregnancy

Dose (mg/kg)	0 (control)	10	20	40
External examination				
Total no. of fetuses (litters) examined	354 (24)	341 (24)	328 (24)	251 (20)
Total no. of fetuses (litters) with malformations	0	1	13 (3)	33 (11)**
Cleft palate	0	1	0	0
Brachydactyly	0	0	8 (3)	31 (11)**
Short tail	0	0	7 (2)	10 (7)**
Internal examination				
Total no. of fetuses (litters) examined	170 (24)	165 (24)	158 (24)	121 (20)
Total no. of fetuses (litters) with malformations	1	0	1	1
Microphthalmia	1	0	1	0
Dilatation of lateral ventricles	1	0	0	0
Undescended testes	0	0	0	1
Total no. of fetuses (litters) with variations	16 (10)	11 (9)	13 (7)	19 (12)
Thymic remnants in neck	13 (10)	8 (7)	12 (7)	17 (11)
Dilated renal pelvis	2 (2)	2 (2)	0	0
Left umbilical artery	1	1	1	2 (2)

** Significantly different from the control ($p < 0.01$).

Table 4
Skeletal examinations in fetuses of rats given DTG on days 6–19 of pregnancy

Dose (mg/kg)	0 (control)	10	20	40
Total no. of fetuses (litters) examined	184 (24)	176 (24)	170 (24)	130 (20)
Total no. of fetuses (litters) with malformations	1	1	13 (6)*	26 (12)**
Split cartelige of thoracic centrum	0	0	1	1
Fused cartilage of cervical vertebral arches	0	1	1	1
Fused cartilage of ribs	1	0	0	0
Absence, fusion or malposition of caudal vertebrae	0	0	8 (3)	10 (8)**
Absence or fusion of phalanges	0	0	5 (3)	18 (9)**
Fusion of metacarpal/metatarsal and phalanx	0	0	0	2 (2)
Absence or fusion of metacarpals	0	0	0	4 (4)*
Shortening of tibia and fibula	0	0	0	1
Total no. of fetuses (litters) with variations	10 (7)	16 (9)	16 (11)	12 (8)
Bipartite ossification of thoracic centrum	0	2 (1)	1	0
Dumbbell ossification of thoracic centrum	0	1	0	0
Unossified thoracic centrum	1	1	0	1
Variation of number of lumbar vertebrae	1	0	0	2 (1)
Wavy ribs	0	1	1	0
Short supernumerary rib	9 (6)	12 (7)	14 (10)	4 (4)
Short 13th rib	0	0	0	2 (2)
Sacralization of lumbar vertebra	0	0	0	2 (1)
Bipartite ossification of sternebra	0	0	1	1
Asymmetry of sternebra	0	0	0	1
Degree of ossification[a]				
No. of sacral and caudal vertebrae	7.3 ± 0.5	7.5 ± 0.5	7.5 ± 0.5	7.0 ± 0.6*
No. of sternebrae	4.6 ± 0.4	4.8 ± 0.5	4.6 ± 0.4	4.2 ± 0.4*
No. of metatarsals	8.0 ± 0.0	7.9 ± 0.3	7.8 ± 0.4	6.7 ± 1.4*

[a] Values are given as the mean ± S.D.
* Significantly different from the control ($p < 0.05$).
** Significantly different from the control ($p < 0.01$).

characterize the effects of DTG on embryonic/fetal development. The findings of the present study confirmed the results of a previous screening study and extended the understanding of the reproductive and developmental toxicity of DTG. The present data showed that the prenatal oral administration of DTG produced maternal toxicity, as evidenced by deaths, neurobehavioral changes, decreased body weight gain and reduced food consumption, and developmental toxicity, as evidenced by a high incidence of postimplantation loss, a decreased number of live fetuses and lower weight of fetuses, and teratogenicity, as evidenced by a higher incidence of fetuses with external and skeletal malformations.

DTG is a specific sigma receptor ligand [3] and sigma receptor ligands can modulate neurotransmissions, including the noradrenergic, glutamatergic and dopaminergic system [10,21,22]. The systemic injection of DTG has been reported to cause neurobehavioral changes in rats [4,6,7,9,22]. The present study shows that the oral administration of DTG also induced neurobehavioral changes at 20 and 40 mg/kg bw/day in pregnant rats. Lowered body weight gain at 20 and 40 mg/kg bw/day and food consumption at 40 mg/kg bw/day were also observed in pregnant rats. These findings indicate that DTG is maternally toxic at 20 mg/kg bw/day and higher.

The sex ratio (males/females) was significantly lowered in all DTG-treated groups. The values for sex ratio were 0.429–0.521 in the background control data for the last 6 years in the laboratory performed present study. Statistically significant changes in the sex ratio observed in the present study were considered to be unrelated to the administration of DTG, because the values for sex ratio in the DTG-treated groups were within the range of the historical control data, no increased embryonic/fetal deaths were detected at 10 and 20 mg/kg bw/day and the control value for the sex ratio was very high in the present study. A decreased number of live fetuses, increased incidence of postimplantation loss, and reduced weights of fetuses and placentae were detected at 40 mg/kg bw/day. A decreased number of live fetuses and increased incidence of postimplantation loss indicate embryonic/fetal lethality, and reduced weights of fetuses and placentae indicate intrauterine growth retardation. These findings indicate that DTG is toxic to embryonic/fetal survival or fetal growth at 40 mg/kg bw/day when administered during the time of implantation to the term of pregnancy.

In our previous reproductive and developmental screening test [15], the total number of fetuses with external malformations, but not individual malformation, was significantly increased at 50 mg/kg. At this dose, oligodactyly and tail anomalies were frequently observed, and the teratogenic effect of DTG was strongly suggested. No malformed fetuses were found at 20 mg/kg bw/day in our previous study. In the present study, morphological examinations in the fetuses of exposed mothers revealed increased incidence of fetuses with external and skeletal malformations at 20 and 40 mg/kg bw/day.

Fetuses with external, internal and/or skeletal malformations and/or variations were found in all groups. The malformations and variations observed in the present study are of the types that occur spontaneously among the control rat fetuses [23–26]. At 40 mg/kg bw/day, significantly higher incidences of the total number of fetuses with external and skeletal malformations were detected, and significantly higher incidences of individual types of external and skeletal malformation were also noted. At 20 mg/kg bw/day, the incidence of the total number of fetuses with skeletal malformations was significantly higher than that of control group. Although the incidence of individual types of skeletal malformation was not significantly increased at 20 mg/kg bw/day, types of external and skeletal malformations observed at this dose were the same as those observed at 40 mg/kg bw/day. Consideration of the sum of these findings suggests that a conservative estimate of the LOAEL for the teratogenic dose of DTG is 20 mg/kg bw/day in rats when administered during the time of implantation to the term of pregnancy. DTG caused suppression of body weight gain and neurobehavioral changes in dams and abnormally morphological development and developmental delay in the offspring of rats at 20 and 40 mg/kg bw/day. Therefore, the teratogenic effects of DTG at doses without maternal toxicity, a selective teratogenicity of DTG, was not found in the current study. There are no available reports in which the developmental toxicity of DTG is assessed in any other animal species. Further studies are needed to confirm the reproductive and developmental toxicity of DTG in additional species. Developmental neurotoxicity and multigeneration studies are also required to support the conclusion of the prenatal hazard of DTG.

In conclusion, DTG caused maternal neurobehavioral changes and decreased body weight gain at 20 mg/kg bw/day and higher, embryonic/fetal deaths and lowered fetal weight at 40 mg/kg bw/day, and increased incidence of fetuses with malformations at 20 mg/kg bw/day and higher when administered during the time of implantation to the term of pregnancy in rats.

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Developmental toxicity of dibutyltin dichloride in cynomolgus monkeys

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Abstract

Dibutyltin dichloride (DBTCl) has been shown to be teratogenic in rats. The present study was conducted to determine the teratogenic potential of DBTCl given to pregnant monkeys during the entire period of organogenesis. Cynomolgus monkeys were dosed once daily by nasogastric intubation with DBTCl at 0, 2.5 or 3.8 mg/kg on days 20–50 of pregnancy, the whole period of organogenesis. The pregnancy outcome was determined on day 100 of pregnancy. In both DBTCl-treated groups, a significant increase in the incidence of pregnant females with soft stool and/or diarrhea, and with yellowish stool was observed. Maternal body weight gain at 3.8 mg/kg and food consumption at 2.5 and 3.8 mg/kg were decreased during the administration period. The survival rate of fetuses at terminal cesarean sectioning was decreased in the DBTCl-treated groups and significantly decreased at 2.5 mg/kg. There were no changes in the developmental parameters of surviving fetuses, including fetal body weight, crown-rump length, tail length, sex ratio, anogenital distance and placental weight, in the DBTCl-treated groups. No external, internal or skeletal malformations were found in the fetuses in any group. Although internal and skeletal variations were found, no difference in the incidence of fetal variation was noted between the control and DBTCl-treated groups. No effect on skeletal ossification was observed in fetuses in the DBTCl-treated groups. The data demonstrate that DBTCl is embryolethal but not teratogenic in cynomolgus monkeys.

Keywords: Dibutyltin; Organotin; Teratogenicity; Embryolethality; Monkey

1. Introduction

Organotin compounds are widely used in agriculture and industry. The most important non-pesticidal route of entry of organotin compounds into the environment is through the leaching of organotin-stabilized polyvinyl chloride (PVC) by water [1], and its use in antifouling agents, resulting in the entry of organotin into the aquatic environment [2]. Disubstituted organotin compounds are commercially the most important derivatives, being used as heat and light stabilizers for PVC plastics to prevent degradation of the polymer during melting and the forming of the resin into its final products, as catalysts in the production of polyurethane foams, and as vulcanizing agents for silicone rubbers [3,4]. The identification of dibutyltin (DBT) and tributyltin (TBT) in aquatic marine organisms [5,6] and marine products [7] has been reported. TBT is degraded spontaneously and biochemically via a debutylation pathway to DBT in the environment [8,9]. Organotin compounds are introduced into foods by the use of pesticides and antifoulants and via the migration of tin from PVC materials [4].

We previously demonstrated that tributyltin chloride (TBTCl) during early pregnancy caused early embryonic loss [10–12], and TBTCl on days 10–12 and on days 13–15, but not on days 7–9 of pregnancy, produced fetal malformations in rats [13]. The predominant malformation induced by TBTCl was cleft palate [13,14]. It has been reported that TBT is metabolized to DBT and MBT, and DBT was metabolized to monobutyltin (MBT) [15–17]. DBT is also reported to have toxic effects on reproduction and development in rats [18]. The oral administration of dibutyltin dichloride (DBTCl) during early pregnancy caused early embryonic loss in rats [19–21]. The oral administration of DBTCl to rats throughout the period of organogenesis resulted in a significant increase in the incidence of fetuses with malformations [22], and rat embryos were highly susceptible to the

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teratogenic effects of DBTCl when it was administered on day 7 and 8 of pregnancy [23]. Dibutyltin diacetate (DBTA) [24–28], dibutyltin maleate, dibutyltin oxide, and dibutyltin dilaurate [26] were teratogenic in rats when administered orally. Developmental toxicity studies on butyltins suggest that the teratogenicity of DBT is different from that of tetrabutyltin (TeBT), TBT and MBT in its mode of action because the period of susceptibility to teratogenicity and the types of malformations induced by DBT are different from those induced by TeBT, TBT and MBT [29,30]. DBTCl had dysmorphogenic effects in rat embryos in a whole embryo culture system [31,32]. DBT was detected in rat maternal blood at 100 ng/g and embryos at 720 ng/g at 24 h after gavage of DBTA at 22 mg/kg on day 8 of pregnancy [27]. The dysmorphogenic concentrations of DBTCl in cultured embryos were within the range of levels detected in maternal blood after the administration of a teratogenic dose of DBT. These findings suggest that DBT itself is a causative agent in DBT teratogenesis, which may be due to direct interference with embryos.

As described above, the teratogenic effects of organotin compounds, including DBT, were extensively investigated in rodents [18]. No reports on the assessment of the teratogenicity of DBT in any other species are available. It appears that conclusive evidence in support of the teratogenicity of DBT is still lacking, because the teratogenicity of DBT only has been reported in a single animal species. Studies in non-rodents would be of great value in estimating the teratogenicity of DBT in humans. The present study was conducted to determine the teratogenic potential of DBTCl given to pregnant cynomolgus monkeys during the entire period of organogenesis.

2. Materials and methods

2.1. Animals

Cynomolgus monkeys (*Macaca fascicularis*) were used in this study. The monkeys were obtained from Guangxi Primate Center of China (Guangxi, China) through Guangdong Scientific Instruments and Materials Import/Export Co. (Guangzhou, China). The monkeys were quarantined for 4 weeks, and confirmed to be free from tuberculosis, *Salmonella* and *Shigera*. The animals were maintained in an air-conditioned room at 23.0–29.0 °C, with a relative humidity of 45–58%, under a controlled 12/12 light/dark cycle, with a ventilation rate of 15 air changes/hour, and were housed individually, except during the mating period. The monkeys were fed 108 g/day of diet (Teklad global 25% protein primate diet; Harlan Sprague-Dawley Inc., Madison, USA) and tap water ad libitum from automatic lixit devices. Healthy male and female monkeys were selected for use. Only females showing 25–32 days menstrual cycles were used in the experiment. Each female monkey was paired with a male of proven fertility for three consecutive days between days 11–15 of the menstrual cycle. The visual confirmation of copulation and/or the presence of sperm in the vagina were considered evidence of successful mating. When copulation was confirmed, the

Table 1
Maternal findings in monkeys given DBTCl on days 20–50 of pregnancy

	Dose (mg/kg)		
	0 (control)	2.5	3.8
Number of pregnant females	12	12	10
Number of females showing toxicological signs			
Death	0	0	0
Soft stool/diarrhea	1	12*	10*
Yellowish stool	0	8*	8*
Vomiting	0	3	3
Initial body weight	3.53 ± 0.59	3.49 ± 0.43	3.79 ± 0.36
Body weight gain during pregnancy (g)[a]			
Days 0–20	76 ± 114	42 ± 160	73 ± 142
Days 20–51	57 ± 237	−242 ± 423	−556 ± 526*
Days 51–100	710 ± 162	755 ± 174	848 ± 263
Food consumption during pregnancy (g/day)[a]			
Days 20–21	99 ± 18	93 ± 23	76 ± 33
Days 23–24	91 ± 27	71 ± 31	55 ± 31*
Days 27–28	77 ± 28	47 ± 19*	37 ± 34*
Days 30–31	63 ± 32	33 ± 15*	22 ± 10*
Days 34–35	88 ± 25	53 ± 42	23 ± 17*
Days 37–38	86 ± 28	53 ± 42*	25 ± 24*
Days 41–42	87 ± 27	59 ± 59	36 ± 29*
Days 44–45	95 ± 22	62 ± 40	41 ± 31*
Days 48–49	98 ± 18	70 ± 48	59 ± 44
Days 51–52	94 ± 20	97 ± 24	71 ± 39
Days 55–56	102 ± 12	107 ± 2	100 ± 20
Days 58–59	106 ± 7	108 ± 0	104 ± 10
Days 62–63	106 ± 7	108 ± 0	106 ± 5
Days 80–81	108 ± 0	108 ± 0	108 ± 0
Days 90–91	106 ± 7	108 ± 0	108 ± 0
Days 99–100	108 ± 0	108 ± 0	108 ± 0

[a] Values are given as the mean ± S.D.
* Significantly different from the control, $p < 0.05$.

median day of the mating period was regarded as day 0 of pregnancy. Pregnancy was confirmed on day 18 or 19 of pregnancy by ultrasound (SSD-4000, Aloka Co., Mitaka, Japan) under anesthesia induced by intramuscular injection of 5% ketamine hydrochloride (Sigma Chemical Co., St. Louis, USA). Pregnant females, weighing 2.51–4.50 kg on day 0 of pregnancy, were allocated randomly to three groups, each of 10–12 monkeys, and housed individually. Animal experiments were performed at Shin Nippon Biomedical Laboratories, Ltd. (SNBL; Kagoshima, Japan) during 2004–2005 in compliance with the Guideline for Animal Experimentation (1987) [33], and in accordance with the Law Concerning the Protection and Control of Animals (1973) [34] and the Standards Relating to the Care and Management of Experimental Animals (1980) [35]. This study has been approved by the Institutional Animal Care and Use Committee of SNBL and performed in accordance with the ethics criteria contained in the bylaws of the committee of SNBL.

2.2. Dosing

The monkeys were dosed once daily with DBTCl (lot no. GG01, 98% pure, Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) at 0, 2.5 or 3.8 mg/kg by nasogastric intubation on days 20–50 of pregnancy, i.e., the entire period of organogenesis [36]. Dosing was terminated in the dams in which embryonic/fetal loss occurred. The dosage levels were determined from the results of previous studies in rats, in which DBTCl administered by gavage at 7.6 or 15.2 mg/kg on days 0–3 and days 4–7 of pregnancy caused significant increases in pre- and/or post-implantation embryonic loss in rats [19–21], and in which DBTCl by gavage at 5, 7.5 or 10.0 mg/kg throughout the period of organogenesis resulted in a significant increase in the incidence of fetuses with malformations [22]. DBTCl was dissolved in olive oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The dose volume was adjusted to 0.5 ml/kg of the most recent body weight. The control monkeys received olive oil only.

2.3. Observations

The pregnant monkeys were observed for clinical signs of toxicity twice a day during the administration period and once a day during the non-administration period. The body weight was recorded on days 0, 20, 27, 34, 41, 51, 60, 70, 80, 90 and 100 of pregnancy. The food consumption was recorded on days 20, 23, 27, 30, 34, 37, 41, 44, 48, 51, 55, 58, 62, 80 and 90 of pregnancy. Embryonic/fetal heartbeat and growth were monitored using ultrasound under anesthesia induced by intramuscular injection of 5% ketamine hydrochloride on days 25, 30, 35, 40, 50, 60, 70, 80, 90 and 99 of pregnancy. In the dams in which embryonic/fetal cardiac arrest was confirmed by ultrasound, necropsy was performed under anesthesia induced by intraperitoneal injection of pentobarbital Na (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan). The uterus, including the embryo/fetus and placenta and ovaries, was removed from the maternal body and stored in 10% neutral buffered formalin. Dead or aborted embryos/fetuses were morphologically examined.

Terminal cesarean sectioning was performed on day 100 of pregnancy, under anesthesia induced by intramuscular injection of 5% ketamine hydrochloride (0.1–0.2 ml/kg) and inhalation of isoflurane (0.5–2.0%, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan), and contraction was induced with atropine (0.01 mg/kg, Tanabe Seiyaku Co. Ltd., Osaka, Japan). The fetus and placenta were removed from the dams. The placenta was weighed and stored in 10% neutral buffered formalin. Dams that underwent cesarean sectioning were not necropsied.

Fetal viability was recorded, and the fetuses were anesthetized by intraperitoneal injection of pentobarbital Na and euthanized by submersion in saline for 30–40 min at room temperature. Fetuses were sexed and examined for external anomalies after confirmation of the arrested heart-beat. Fetal and placental weights were recorded. The head width, tail length, crown-rump length, chest circumference, paw and foot length, distance between the eyes, umbilical cord length, volume of amniotic fluid and diameters of the primary and secondary placentae were measured. After the completion of external examinations, fetuses were examined for internal anomalies. The peritoneal cavity was opened and the organs were grossly examined. The brain, thymus, heart, lung, spleen, liver, kidneys, adrenal glands and testes/uterus and ovaries were weighed and stored in 10% neutral buffered formalin. The eyeballs, stomach, small and large intestine, head skin and auricles were stored in 10% neutral buffered formalin. Fetal carcasses were fixed in alcohol, stained with alizarin red S [37] and examined for skeletal anomalies. The number of ossification centers of the vertebral column, and lengths of the ossified parts of the humerus, radius, ulna, femur, tibia and fibula were recorded. Histopathological evaluations were performed on single

Table 2
Reproductive and developmental findings in monkeys given DBTCl on days 20–50 of pregnancy

	Dose (mg/kg)		
	0 (control)	2.5	3.8
Number of pregnant females	12	12	10
Number of females with embryonic/fetal loss	1	8*	4
Number of females with live fetuses until terminal cesarean section	11	4*	6
Number of live fetuses at terminal cesarean section	11	4*	6
Sex ratio of live fetuses (male/female)	6/5	1/3	3/3
Body weight of live fetuses (g)			
Male	133 ± 13	125	112 ± 24
Female	118 ± 12	108 ± 20	118 ± 13
Anogenital distance (cm)[a]			
Male	2.0 ± 0.2	1.9	1.7 ± 0.4
Female	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1
Crown-rump length (cm)[a]			
Males	12.8 ± 0.6	12.4	12.4 ± 0.7
Female	12.6 ± 0.4	12.3 ± 0.5	12.6 ± 0.1
Tail length (cm)[a]			
Male	11.8 ± 1.2	11.8	11.4 ± 0.7
Female	11.9 ± 0.8	11.7 ± 1.7	12.4 ± 0.6
Placental weight (g)[a]	42.4 ± 7.2	38.9 ± 6.2	37.5 ± 9.1
Number of a single placenta	1	1	3

[a] Values are given as the mean ± S.D.
* Significantly different from the control, $p < 0.05$.

placentas and accessory spleens after fixation, paraffin embedding, sectioning and staining with hematoxylin and eosin.

2.4. Analysis of plasma steroids hormone levels

Blood samples were collected from the femoral vein on day 51 of pregnancy, 24 h after the last administration of DBTCl. The plasma was separated and stored at −80 °C for the later assay of steroid hormones. Plasma progesterone and 17β-estradiol were measured by Teizo Medical Co. Ltd. (Kawasaki, Japan) using liquid chromatography-electrospray ionization Tandem Mass Spectrometry (LC-MS/MS, Applied Biosystems/MDS SCIEX). The detection limits of plasma progesterone and 17β-estradiol were 10.0 pg/ml and 0.25 pg/ml, respectively. The intra- and inter-assay coefficients of variation for 17β-estradiol were below 6.4 and 8.9%, respectively. The intra- and inter-assay coefficients of variation for progesterone were below 9.0 and 7.9%, respectively.

2.5. Data analysis

The data was analyzed by MUSCOT statistical analysis software (Yukums Co. Ltd., Tokyo, Japan) using the dam or fetus as the experimental unit [38]. Data were analyzed using Bartlett's test [39] for the homogeneity of variance. When the variance was homogeneous, Dunnett's test [40] was performed to compare the mean value in the control group with that in each DBTCl group. When the variance was heterogeneous, the data were rank-converted and a Dunnett-type test [41] was performed to compare the mean value in the control group with that in each DBTCl group. The incidences of maternal and embryonic/fetal deaths and anomalous fetuses were analyzed by Fisher's exact test. The 0.05 level of probability was used as the criterion for significance.

3. Results

Table 1 presents maternal findings in monkeys given DBTCl on days 20–50 of pregnancy. No maternal death occurred in any group. In both DBTCl-treated groups, a significant increase in the incidence of females with soft stool and/or diarrhea, and with yellowish stool was observed. Soft stool and/or diarrhea were observed in one of the 12 females in the control group and in all females of the DBTCl-treated groups. In both groups treated with DBTCl, yellowish stool was noted in eight females and vomiting was observed in three females. Body weight gain on days 0–20, during the pre-administration period, did not significantly differ among the groups. Body weight gain on days 20–50, during the administration period, was lower in the DBTCl-treated groups, and significantly decreased at 3.8 mg/kg. No significant decrease in body weight gain on days 51–100, during the post-administration period, was found in the DBTCl-treated groups. Food consumption during the administration period was significantly reduced at 2.5 mg/kg and higher. Relatively marked decreases in the body weight gain and food consumption were observed in dams showing abortion or embryonic/fetal death.

The reproductive and developmental findings in monkeys given DBTCl on days 20–50 of pregnancy are shown in Table 2. The incidence of females with embryonic/fetal loss was increased in the DBTCl-treated groups, and a significant difference was noted at 2.5 mg/kg. Embryonic/fetal loss was observed in one of the 12 females in the control group, eight of the 12 females in the 2.5 mg/kg group and four of the 10 females in the 3.8 mg/kg group. Abortion occurred on day 30 of pregnancy in the control group, and on day 35, 44, 46, 49 or 60 of pregnancy at 2.5 mg/kg. Embryonic/fetal death was found on day 35, 40 or 64 of pregnancy at 2.5 mg/kg, and on days 38, 40 or 50 (two embryos) of pregnancy at 3.8 mg/kg. External examinations was performed in five of the eight embryonic/fetal losses at 2.5 mg/kg and four of the four embryonic/fetal losses at 3.8 mg/kg, and no anomalies were detected. Eleven, four and six females in the control, 2.5 and 3.8 mg/kg groups, respectively,

Table 3
Morphological findings in fetuses of monkeys given DBTCl on days 20–50 of pregnancy

	Dose (mg/kg)		
	0 (control)	2.5	3.8
Number of fetuses examined	11	4	6
External examination			
Number of fetuses with malformations	0	0	0
Internal examination			
Number of fetuses with malformations	0	0	0
Number of fetuses with variations	0	0	1
Accessory spleen	0	0	1
Skeletal examination			
Number of fetuses with malformations	0	0	0
Number of fetuses with variations	0	1	1
Short supernumerary rib	0	1	1
Degree of ossification[a]			
Number of ossified centers of vertebral column	53.6 ± 0.8	53.0 ± 1.2	54.2 ± 1.0
Skeletal length (mm)[a]			
Humerus	23.6 ± 0.8	23.3 ± 1.3	23.6 ± 1.2
Radius	23.0 ± 1.0	22.3 ± 1.6	23.1 ± 1.7
Ulna	24.6 ± 1.0	23.9 ± 1.5	24.3 ± 2.2
Femur	22.3 ± 1.2	21.8 ± 1.3	22.7 ± 1.6
Tibia	21.5 ± 1.3	20.5 ± 1.7	21.7 ± 1.4
Fibula	19.8 ± 1.0	19.0 ± 1.8	19.9 ± 1.6

[a] Values are given as the mean ± S.D.

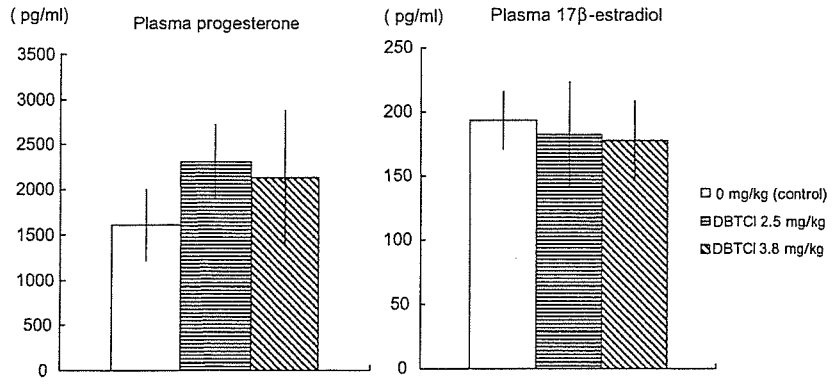

Fig. 1. Plasma progesterone and 17β-estradiol levels in pregnant monkeys given DBTCl on days 20–50 of pregnancy. Blood samples were collected on day 51 of pregnancy, 24 h after the last administration of DBTCl. Values are given as the mean ± S.E.M. of 5–10 monkeys.

had live fetuses at terminal cesarean sectioning. There were no significant differences between the control and DBTCl-treated groups in parameters of fetal growth, such as body weight, crown-rump length and tail length. No significant differences in the head width, chest circumference, paw and foot length, distance between the eyes, umbilical cord length, volume of amniotic fluid and diameters of the primary and secondary placentae were also noted between the control and DBTCl-treated groups (data not shown). No significant differences between the control and DBTCl-treated groups were found in the sex ratio of live fetuses, anogenital distance or placental weight. A single placenta was observed in one dam in the control group, one dam in the 2.5 mg/kg group and three dams in the 3.8 mg/kg group.

Table 3 shows the morphological changes in fetuses of monkeys given DBTCl on days 20–50 of pregnancy. No external, internal or skeletal malformations were found in fetuses in any group. Although internal and skeletal examinations revealed one fetus with an accessory spleen at 3.8 mg/kg, and one fetus with a short supernumerary rib at both 2.5 and 3.8 mg/kg, no difference in the incidence of fetuses with variation was noted between the control and DBTCl-treated groups. There were no differences between the control and DBTCl-treated groups in the number of ossified centers of the vertebral column or length of the humerus, radius, ulna, femur, tibia or fibula.

Although a significant decrease in the absolute weight of the brain and lung, and increase in the relative weight of the spleen were observed in male fetuses at 3.8 mg/kg, no significant difference in the relative weight of the brain and lung or in absolute weight of the spleen was detected between the control and DBTCl-treated groups. There were no differences in absolute and relative weights of the fetal thymus, heart, lung, liver, kidneys, adrenal glands or testes/uterus and ovaries between the control and DBTCl-treated groups (data not shown). Histopathological examinations revealed no abnormalities in single placenta and accessory spleen, and the histological structures of single placenta and accessory spleen were similar to those of normal placenta and spleen.

Plasma progesterone and 17β-estradiol levels are shown in Fig. 1. Although higher levels of plasma progesterone were observed in the DBTCl-treated groups, no statistically significant difference was noted between the control and DBTCl-treated groups. There were no significant differences in the plasma 17β-estradiol levels between the control and DBTCl-treated groups.

4. Discussion

In previous studies, the teratogenic effects of DBT were investigated in rats. The teratogenicity of DBT should be studied using other animal species to gain a better understanding of the developmental toxicity of butyltins. Non-human primates appear to provide an especially appropriate model for teratogenicity testing because of their high ranking on the evolutionary scale [42]. The close phylogenetic relatedness of old world monkeys to humans appears to render them most desirable as models in teratology studies [43]. The similarities in placentation and embryonic development indicate considerable value in the use of monkeys for investigating the developmental toxicity of chemicals [44]. In the present study, we determined the developmental toxicity, particularly the teratogenicity, of DBTCl in monkeys after administration over the entire period of organogenesis.

The doses of DBTCl set in the present study were expected to induce maternal toxicity, such as decreases in maternal body weight gain and food consumption, and were given to monkeys during organogenesis to characterize the effects of DBTCl on embryonic/fetal development. Toxicological sign, as evidenced by the significant increase in the incidence of pregnant females showing soft stool/diarrhea and yellowish stool, was found at 2.5 and 3.8 mg/kg. A significant decrease in the maternal body weight gain accompanied by significantly reduced food consumption was noted at 3.8 mg/kg. A significant decrease in food consumption was also found at 2.5 mg/kg. These maternal findings indicate that more severe adverse effects on pregnant females were noted at 3.8 mg/kg and DBTCl exerts maternal toxicity at 2.5 mg/kg and higher when administered during the entire period of organogenesis in monkeys.

Embryonic/fetal loss was observed in one dam in the control group and eight dams in the 2.5 mg/kg group and four dams in the 3.8 mg/kg group. The increased incidence of pregnant females with embryonic/fetal loss was observed at 2.5 and 3.8 mg/kg, and a significantly increased incidence of these females was found

at 2.5 mg/kg. Embryonic/fetal loss occurred on days 35–64 of pregnancy at 2.5 mg/kg, and on days 38–50 of pregnancy at 3.8 mg/kg. The embryonic mortality during organogenesis in cynomolgus monkeys of 2.4–18.2% has been reported [45]. Binkerd et al. [46] also noted that post-implantation embryonic loss was 5.4% in vehicle control pregnancies in developmental toxicity studies. Average abortion rate in cynomolgus monkeys was 26.1% in control data from 24 teratogenicity studies, and most of the abortions (66.7%) occurred during organogenesis [47]. In the background control data from 1994 to 2004 of the laboratory that performed this study, the post-implantation embryonic loss was 8.8% (29 of the 330 pregnancies). Because the incidence of embryonic/fetal loss in the DBTCl-treated groups was greater than in the historical control values, it was considered to be due to the administration of DBTCl. The data indicate that DBTCl at 2.5 mg/kg was sufficient to induce embryonic/fetal loss and the latter half of organogenesis was more susceptible for DBTCl-induced embryonic loss in cynomolgus monkeys.

We previously reported that DBTCl during early pregnancy caused pre- and post-implantation embryonic loss in pregnant rats [19,20] and that DBTCl suppressed uterine decidualization and reduced the levels of serum progesterone in pseudopregnant rats at doses that induced implantation failure [48]. We also showed that the suppression of uterine decidualization was reversed by administration of progesterone in pseudopregnant rats [48], and that progesterone protected against DBTCl-induced implantation failure [21]. Based on these findings, we hypothesized that the decline in serum progesterone levels was a primary factor for the implantation failure due to DBTCl in rats. However, no significant changes in plasma progesterone levels were noted in monkeys after the administration of DBTCl during organogenesis. The peripheral serum progesterone levels during the first 8 days of pseudopregnancy were essentially similar to those found in pregnant rats, and the serum progesterone levels rose steadily to a peak on day 4 and remained at a plateau of approximately 70 ng/ml until day 8 of pseudopregnancy [49]. In cynomolgus monkeys, plasma progesterone levels had distinct two peaks, one about 15 days postbreeding and another at about days 23–25, the progesterone decline which followed the second peak reached minimal levels (1–2 ng/ml) by about day 45 of pregnancy, and progesterone levels increased gradually throughout the rest of pregnancy with average levels of approximately 4 ng/ml [50]. In our previous study [48], rat blood samples were obtained on day 4 or 9 of pseudopregnancy. At these stages, progesterone levels could be steadily rising or remained at a plateau in pseudopregnant rats. In the present study, blood samples were collected from pregnant monkeys that were carrying their offspring and had not suffered from miscarriage on day 51 of pregnancy. At this stage, progesterone levels could be remained at a nadir in pregnant cynomolgus monkeys. The discrepancy in the effect of DBTCl on serum progesterone levels between rats and monkeys may be explained by the differences in the status and stage of pregnancy. Further studies are required to characterize more precisely the relationship between embryonic loss and maternal progesterone levels in monkeys given DBTCl.

Decreases in the absolute weights of the brain and lung, and an increase in the relative weight of the spleen, which were observed in male fetuses at 3.8 mg/kg, were not thought to de due to the toxic effects of DBTCl on fetal development, because these changes were not found in female fetuses and differences were not detected in the relative weight of the brain and lung or the absolute weight of the spleen in male fetuses. Any adverse effects on the parameters of fetal growth were also not detected in the surviving fetuses of dams given DBTCl. These findings indicate that DBTCl is not toxic to fetal growth at up to 3.8 mg/kg when administered over the entire period of organogenesis. Placental examinations revealed single placenta in all groups. In the background control data of the laboratory that performed the present study, the incidence of single placenta over a period of 10 years was 0–66.7% (mean = 13.0%, 26 of the 213 pregnancies). Histopathological examinations of single placenta revealed no changes, and the histological structure of single placenta was similar to that of normal placenta. These findings indicate that the single placenta observed in the present study was of no toxicological significance.

In the morphological examinations of the fetuses of exposed dams, a few fetuses with morphological changes were found in the DBTCl-treated groups. An accessory spleen was observed in one fetus at 3.8 mg/kg, and a short supernumerary rib was found in one fetus at both 2.5 and 3.8 mg/kg. In the background control data of the laboratory that performed the present study, the accessory spleen over the last 10 years was not observed. Leemans et al. [51] noted that the exact frequency of accessory spleen is not known, but is estimated to be between 10 and 30% in humans, and the immunohistological structure of the accessory spleen was similar to that of the normal spleens. In the present study, histopathological examinations of the accessory spleen revealed no changes, and the histological structure of accessory spleen was similar to that of the normal spleen. The accessory spleen observed in the present study contained only a minute amount of accessory tissue, and it was not considered to be a malformation. Short supernumerary rib is classified as skeletal variation [52], and the incidence of this change in the historical control data of the laboratory that performed the present study was 13.3% (31 of the 240 fetuses). DBTCl caused no skeletal retardation, as evidenced by no significant changes in the number of ossified centers of the vertebral column or the length of the humerus, radius, ulna, femur, tibia or fibula. Chahoud et al. [53] noted that variations are unlikely to adversely affect survival or health, and might result from a delay in growth or morphogenesis; the fetuses otherwise following a normal pattern of development. Furthermore, morphological examinations of aborted or dead embryos/fetuses in the DBTCl-treated groups revealed no anomalies. Considered collectively, these findings suggest that the morphological changes observed in the fetuses in the present study do not indicate a teratogenic response, and that DBTCl possesses no teratogenic potential in cynomolgus monkeys.

In conclusion, the administration of DBTCl to pregnant cynomolgus monkeys throughout organogenesis had an adverse effect on embryonic/fetal survival, but had no adverse effects on fetal morphological development, even at a maternal toxic

dose level. The data from the present study indicate that DBTCl shows embryonic/fetal lethality in monkeys.

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【特集】

OECDの高生産量化学物質安全性点検プログラムとその実施手順

Introduction to the OECD high production volume (HPV) chemicals programme

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要旨：高生産量化学物質点検プログラム（HPV Programme: High Production Volume Chemicals Programme）は、1992年に開始された国際的な取り組みであり、現在OECD加盟国30ヶ国のうち26ヶ国が本プログラムに参加している。1993年に第1回初期評価会議（SIAM: SIDS, Screening Information Data Set, Initial Assessment Meeting）がフランスのパリで開催されてから、2005年末までに21回のSIAMが行われた。第10回SIAMまでは加盟国政府がスポンサーとなり初期評価を行い、第11回SIAM (2001年)からは国際化学工業協会協議会（ICCA : International Council of Chemical Associations）イニシアティブとして産業界が評価文書の作成に参画している。初期評価は第1回から第21回SIAMまでに574物質について合意されている。日本は第1回SIAM (1993年)から参加し、米国に次いで多くの評価文書を提出してきており、本プログラムの中で重要な働きをしている。本稿ではOECDにおける化学物質対策、特に高生産量化学物質の初期評価について概説した。
キーワード：OECD 化学物質プログラム、高生産量化学物質、SIDS (初期情報データセット)、SIAM (SIDS 初期評価会議)

Abstract: The OECD High Production Volume (HPV) Chemicals Programme started in 1992. Twenty-six countries of the OECD member countries participate in this programme at present. The first SIDS, Screening Information Data Set, Initial Assessment Meeting (SIAM) was held in Paris in 1993, and a total of twenty-one SIAMs were held until the end of 2005. In the first ten SIAMs, the governments of the member countries participated and submitted the SIDS documents of HPV chemicals in this programme. The International Council of Chemical Associations (ICCA) has been participating in this programme since 2001. The SIDS documents of 574 chemical substances have been agreed at the SIAMs. The Japanese government has been participating and submitting the SIDS documents in this programme since the first SIAM. The contribution of Japanese government to this programme is remarkable. This paper summarized the OECD HPV Programme.
Keywords: OECD chemicals programme, High production volume chemical, SIDS (Screening Information Data set), SIAM (SIDS Initial Assessment Meeting)

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はじめに

経済協力開発機構（OECD: Organization for Economic Cooperation and Development）は、世界の150以上ある国のうちの30ヶ国（世界人口の16%、世界総生産額の3分の2、総輸出額の5分の3、海外援助の5分の4）が加盟する市場経済を原則とする先進諸国の集まりであり、政治および軍事を除くあらゆる分野の様々な問題を取りあげて政策提言を行っている（OECD東京センター、2006）。1960年代から化学物質の生産および貿易拡大に伴い環境問題が重要な課題となり、OECDにおいても様々な取り組みがなされてきた。人類が100年ほどの間に創り出し、見つけた化学物質は2千万種類を超えるとされており（西原、2001）、化学物質対策はOECDの環境保健安全プログラム（EHS: Environment, Health and Safety Programme）の環境問題の中でも最も重要なものとなっている。化学物質の安全性確保のための活動の一つとしてOECDでは、1992年から高生産量（HPV: High Production Volume）（定義は5．高生産量化学物質点検プログラムで詳述）化学物質の初期評価を行っている。日本は初回からこの活動に参加している（長谷川ら、1999, 2001; 江馬、2005ab）。HPV化学物質は現在安全性点検の最優先物質となっており、データの取得および初期評価がOECD加盟国で分担されて行われている。本稿ではOECDの化学物質の安全性対策、特にHPV化学物質の安全性点検についての取り組みについて紹介する。

1．OECDの概要

米国の欧州復興支援策「マーシャル・プラン」の受け入れ体制として、1948年にパリにOECDの前身である欧州経済協力機構（OEEC: Organization for European Economic Cooperation）が設立された。その後の欧州の経済復興に伴いOEECは発展的に改組され、1961年に経済協力開発機構（OECD: Organisation for Economic Co-operation and Development）が設立された。日本は1964年に21番目のOECD正式加盟国となっている（経済産業省、2006a；外務省、2006）。OECDはその条約に明記された3つの目的、すなわち、経済成長、発展途上国援助および多角的な自由貿易の拡大を柱として活動してきた。また、その後の国際社会・経済の多様化に伴って活動目的が拡大し、環境、エネルギー、農林水産、科学技術、教育、高齢化および年金・健康保険制度等の社会・経済の広範な分野で活動を行っている。

OECDは政策協調の場であり、活動形態は加盟国間の意見・情報交換を主体とし、自由な討議を通じて国際的公正さについて共通の認識を持ち、また、各国の政策の調和を図ることを目的としており、理事会およびその他の組織の活動はコンセンサス方式によりすすめられている。OECDの意志決定機関として理事会があり、閣僚レベルが参加する閣僚理事会は年一回開催され、常任代表による通常理事会が頻繁に開催されている。執行委員会は加盟国の常駐代表によって構成され、理事会を補佐し、理事会の決定事項を執行する。各種委員会は加盟国の代表により構成され、年次作業計画を作成し、作業部会や専門家グループの補佐を受けながら広範な分野の研究調査を行っている。また、ビジネス界代表からなる経済産業諮問委員会（BIAC: Business and Industry Advisory Committee）および労働組合諮問委員会（TUAC: Trade Union Advisory Committee）が採択される方針に対して見解を述べることができる仕組みがある。

2．環境保健安全プログラムの歴史と概要

OECDには三大目的の任務を担う経済政策委員会、貿易委員会および開発援助委員会を含めて全体で20以上の委員会が多岐に渡る分野で活動している。それらの委員会の一つとして環境

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政策委員会（EPOC: Environment Policy Committee）がある。EPOCは環境問題の関心の高まりを受けて、1970年に科学政策委員会から独立し環境委員会として設立され、その後の気候変動等の環境問題に対する関心の高まりを背景として、1992年に組織を強化して現在の組織となり、広範な分析作業や政策提言等によって各国の環境保護水準の向上を目指している。EPOC傘下では化学品作業部会をはじめ4部会により各分野の活動が行われている（図1）（環境省、2006）。

日本の化学物質の審査および製造等の規制に関する法律（化審法）、米国の有害物質規制法（TSCA: Toxic Substances Control Act）、欧州連合（EU）の危険な物質の分類、包装および表示に関する理事会指令67/548/EECの第6次修正理事会指令等、各国の化学物質の規制に関する法令制定に対応して1978年に化学品規制特別プログラム管理委員会が設置され、EPOCおよび化学品作業部会の協力のもとで作業を行うこととされ、合同部会が1983年以降開催されている。その後バイオテクノロジー分野への対応をも含めて全体を環境保健安全プログラム（EHS）と呼ぶようになり、現在に至っている。EHSの前身である化学品プログラムは1971年に設立され、当初はヒトの健康や環境に有害なPCBや水銀等の特定の化学物質を対象としていたが、1970年代半ばからは新規化学物質が市場に出る前に各国が試験やリスク管理ができるような共通の方法の開発に取り組み始め、1980年代にはリスク評価方法、リスク管理手法、事故の防止・対策および事故後の対応に関するプロジェクトが始まり、また、生産量の多い既存化学物質の調査が開始された。1990年代には農薬、バイオテクノロジー製品および環境汚染物質排出移動登録（PRTR: Pollutant Release and Transfer Register）についてのプロジェクトが開始されている。

現在、EHSにおいては、化学産業およびバイオテクノロジー産業によって生産され、市場で売買される製品で、環境、経済、健康と生活水準、世界貿易、地域産業および農産物に影響を及ぼすものに関するプログラムについて、主に化学品の試験と評価、既存化学品に対する協力、化学品のリスク管理の3つのテーマのもとに活動が展開されている。

3．化学物質プログラムの概要

化学産業は世界で最も大きな産業の一つであり、化学産業による生産額は年間1兆5千億米ドル、工業製品の世界貿易額の約9％を占めている（環境省、2006）。OECD加盟国は化学製品の75%を生産しており、化学物質を可能な限り安全に生産、使用および廃棄することを確保する責任があることから、1970年代の終わりころからOECD加盟国政府は有害性試験結果およびリスク評価に基づいて化学品の規制をしてきた。EHSプログラムは、実験動物福祉の精神を考慮に入れた上で高品質な化学物質の試験および評価方法の確立、化学物質管理の効率性および有効性の向上、化学物質および化学製品の取引における非関税障壁の最小化を目的としている。化学物質に関するテーマはEHSプログラムの環境問題の中でも最も重要なものとなっている。

現在、EHSのプログラムの下では、下記に示す12のサブプログラムが運営されている（図1）（環境省、2006）。

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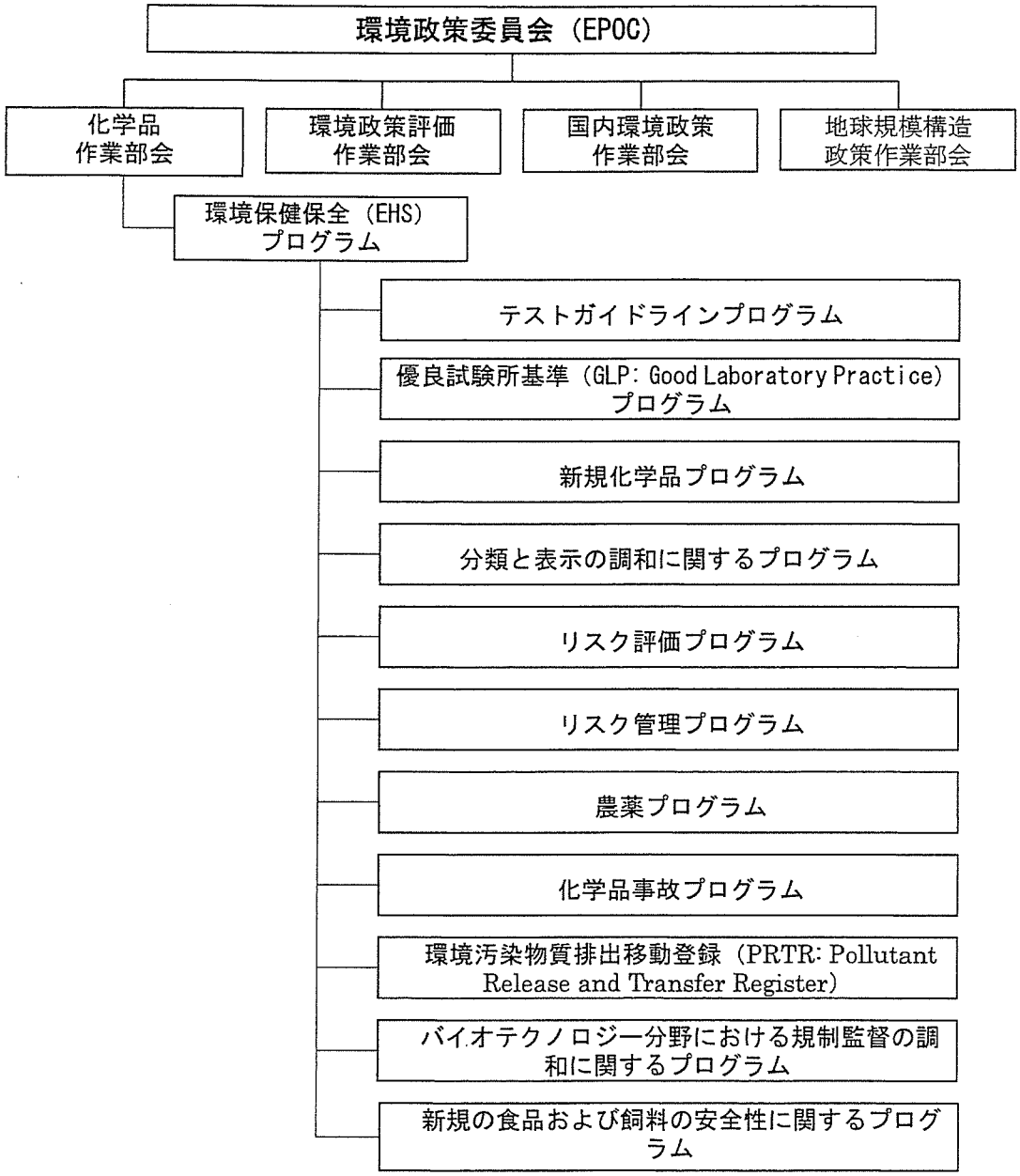

図1．OECDの環境保健安全（EHS）プログラム

テストガイドラインプログラム：物理化学的性状（Test Guideline 101-121、融点、沸点、蒸気圧、水溶解度等）、生態系における影響（Test Guideline 201-217、藻類、ミジンコ、魚類、鳥類試験等）、分解性と蓄積性（Test Guideline 301-308）およびヒト健康影響（Test Guideline402-425, 429, 451-453, 471, 473-486）等についての試験法ガイドラインが公表されている。科学の進展と共にこれらのガイドラインの改訂作業が行われ、また、テストガイドラインのガイダンスドキュメントの作成も行われている。

優良試験所基準（GLP: Good Laboratory Practice）プログラム：国際調和と基準の利用拡大のための活動が行われており、1981年にはじめてGLP基準が公表された。化学物質の届

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出および登録の目的で規制機関に提出される試験結果に十分に良質かつ正確であることを確実にするために試験実施機関における管理、試験実施および報告等に関する基準を定めている。

新規化学品プログラム:企業が新たに上市をする新規化学物質の評価を行う際の時間、資金、人的資源の削減と情報交換の簡易化のために化学物質の電子届出フォームを開発している。

既存化学品プログラム:新規化学物質の届出制度が整備される以前に上市され有害性評価が不十分な化学物質に関する取り組みがなされている(4.既存化学物質点検の項で詳述する)。

分類と表示の調和に関するプログラム:1992年にリオデジャネイロで開催された地球サミットでの議論の結果、有害化学物質の分類方法の国際的調和を図ることを目的として創設された。OECDと国際労働機関(ILO: International Labour Organization)の共同開発による化学品の分類および表示に関する世界調和システム(GHS: The Globally Harmonized System of Classification and Labelling of Chemicals)は2002年「持続可能な開発」について討議されたヨハネスブルグ世界首脳会議で優れた業績として評価され、国連社会経済理事会により採択された。

リスク評価プログラム:化学物質の曝露評価に関して、特定の産業における化学物質の排出量推計シナリオの作成、コンピューター計算モデルとモニタリング結果を用いた曝露評価のためのガイダンス作成、農薬の職業曝露の評価ガイダンス作成および報告の一貫性・透明性を高めるために環境・職業・消費者曝露を報告するためのフォーマットの作成等のプロジェクト、また、化学物質の有害性評価を改善するためのQSARs(定量的構造活性相関:Quantitative Structure-Activity Relationships)に関するプロジェクトが進行している。

リスク管理プログラム:リスクを最小限に抑えながら、社会が化学製品の便益を享受できるようにするための管理方法の検定に関するプロジェクトであり、持続可能な化学の促進に関する活動では環境にやさしい化学製品および製法の開発につながる科学の進歩を支援している。

農薬プログラム:農業用防除剤(農薬)および非農業用防除剤(バイオサイド:殺生物剤)を対象とし、試験および評価法の調和、ワークシェアリングとリスク削減を促進することを目的としている。農薬プログラムでは、化学農薬および生物農薬の評価におけるOECD加盟国の協力体制の支援、ヒトおよび環境に害を及ぼさない生物農薬(フェロモン、微生物および天敵農薬)に関するデータ要求項目の調和、農薬削減等のための活動を展開している。また、バイオサイドプログラムでも農薬プログラムに類似した活動を進めている。

化学品事故プログラム:有害物質の使用者、取扱者および化学工場の労働者と近隣住民に関わるテーマに取り組み、OECD加盟国での化学品事故の防止、事故発生時の対応の支援のための活動を行っている。1992年に初版が公表された「化学品事故防止・対策・対応のためのOECD指導原則:公共機関、産業界、労働者、その他のためのガイダンス」は化学事故防止・管理のあらゆる面の指針となっている。各国が化学品事故の情報を共有できる体制の構築を支援し、また、化学品事故防止と地域に対応した特殊な問題の分析を行っている。

環境汚染物質排出移動登録(PRTR: Pollutant Release and Transfer Register):汚染物質の排出に関するデータベースの構築と改善、情報の公開を要請した1992年のリオデジャネイロ地球サミットの勧告に対応して、本プログラムが創設された。PRTRとは有害性化学物質の

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発生源から環境中への排出量、あるいは、廃棄物に含まれて事業所外に運び出されたときの移動量に関するデータを把握し、集計し、公表する仕組みであり、汚染状況を把握するためにOECD加盟国が使用している制度であり、様々な人々への情報の提供を可能にするものである。

バイオテクノロジー分野における規制監督の調和に関するプログラム：バイオテクノロジーの環境に対する安全性に関する問題を取り扱っている。

新規の食品および飼料の安全性に関するプログラム：バイオテクノロジーの食品と飼料の安全性に関する問題を取り扱っている。

バイオテクノロジーの安全性に関する上記の２つのプログラムでは、OECD加盟国が遺伝子組み換え生物（GMO: Genetically Modified Organism）の潜在的なリスクを評価し高水準の安全性を確保するのを支援すること、各国におけるGMO産物の規制の過程についての対話や相互理解を促進することおよび非関税貿易障壁をなくすことの三つの目的の基に活動している。

GMOがヒトや動物の健康および環境に及ぼしうる潜在的なリスクを特定するための科学的知見の共通の基盤の構築、OECD加盟国における遺伝子組み換え産物の規制および商品化に関する情報を掲載しているウエブサイトの「バイオトラック・オンライン・データベース」の維持、OECD加盟国と開発途上国の専門家がともに課題に取り組むことのできるワークショップや会議の開催、同じ科学的知見を使用しながら遺伝子組み換え植物に関する判断がOECD加盟国間で異なる状況と理由を明らかにする、の４つの分野で活動を展開している。

４．既存化学物質点検

既存化学物質については数量が圧倒的に多いにもかかわらず、有害性評価が十分になされていないまま利用されており、迅速なリスク評価が急務となっており、1987年の第3回ハイレベル会合において、既存化学物質の調査、評価、管理に各国が協力して取り組むことが合意された。これを受けて、同年のオタワワークショップで、HPVプロジェクト、クリアリングハウス、EXICHEMデータベースを活動の柱として既存化学物質を系統的に点検することとなった（環境省、2005）。

HPV化学物質プロジェクト:（５．高生産化学物質安全性点検プログラムの項で詳述する）。

クリアリングハウス:懸念のある特定の化学物質に関する共同作業の可能性をより詳細に調べるために、加盟各国は自主的に関心を持つ化学品に関して先導的な立場をとり、中心的な機関（クリアリングハウス）として機能し、当該化学物質に関する各国の情報を集約、交換する活動を行っている。クリアリングハウスは、ボランティア国が情報収集、交換のセンターとして活動を行おうとするものであり、集められたデータは、リスク管理の推進やIPCSの環境保健クライテリアの作成にも役立っている。

EXICHEM データベース：加盟各国が特定の既存化学物質を調査する上での協力の機会をつくり易くすることおよび関心を有する国々がそれぞれの活動について情報交換、交渉をし易くすることにある。従って、本データベースの利用により各国政府、機関が個々に実施している安全性点検などの情報をOECDに集約することができ、安全性試験の重複を防ぐとともに同一物質の安全性評価対策における協力関係の促進にも役立っている。

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5．高生産量化学物質点検プログラム

高生産量化学物質点検プログラム（HPV Programme: High Production Volume Chemicals Programme）は、1991年のOECD理事会での既存化学物質の点検とリスク削減のための協力に関する決定に基づいて、1992年から開始されている国際的な取り組みであり、現在OECD加盟国30ヶ国のうち26ヶ国が本プログラムに参加している（OECD 2006a）。当初1年当たり1,000トン以上の生産量が2ヶ国以上あるいは1ヶ国で年間の生産量が10,000トン以上の化学物質のうち有害性情報の少ないものがHPVの対象とされていたが、その後、1993年にEUの既存化学物質のリスク評価制度が設けられたことに対応して、1ヶ国（または1地域）が年間1,000トン以上生産している化学物質に変更された（経済産業省　2006b）。1990年版のOECDのHPVリストには1,592物質が登録されていた。現在のOECDのHPVリスト（The 2004 OECD List of High Production Volume Chemicals）には、OECD加盟国で年間1,000トン以上生産または輸入されている4,843物質が登録されており、うち1,000物質以上については分担する各加盟国と企業がすでに決まっている（OECD 2006b）。

1993年に第1回初期評価会議（SIAM: SIDS, Screening Information Data Set, Initial Assessment Meeting）がフランスのパリで開催されてから、年に2回の会議が開催され、2006年4月までに22回のSIAMが行われてきた。第10回SIAMまで加盟国政府がスポンサーとなり初期評価を行ってきた。第11回SIAM（2001年）からは国際化学工業協会協議会（ICCA : International Council of Chemical Associations）が自主的なイニシアティブを開始したのに伴い、その後評価文書の作成に協力している。ICCAイニシアティブにおいてはICCAが中心となり各国の協会を取りまとめている。実務的には米国化学工業界（ACC）、欧州化学工業界（CEFIC）および日本化学工業協会が主体となって、自主的に1,000物質を目標に有害性情報を収集、評価し、各国政府を通じてOECD事務局に初期評価文書を提出している（菅原、2005）。表1に第22回SIAMまでに審議された物質数を示した。

表1．　SIAMで審議された物質数

HPV		化学物質数	
		総数**	ICCA
情報収集・レビュー中の物質		434	307
SIDS試験計画が提出され、レビュー中の物質		69	62
初期評価文書の草案がCDGに掲載された物質		15	2
SIAM 22で審議された物質		83	78
SIAM 1-21で未採択の物質		16	7
SIAM 1-21で採択された物質	最終文書がOECD事務局に提出されていない物質	167	138
	最終文書がOECD事務局に提出された物質	26	23
	最終文書がOECDのWebサイトに掲載された物質	52	46
	最終文書がUNEPより出版された物質*	272	124
	最終文書がEUより出版された物質*	63	0
		1191	787

*: 6物質はUNEPおよびEUの両方から出版されている。

**: 51物質の非HPVを含む。

(OECD 2006c)

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