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.

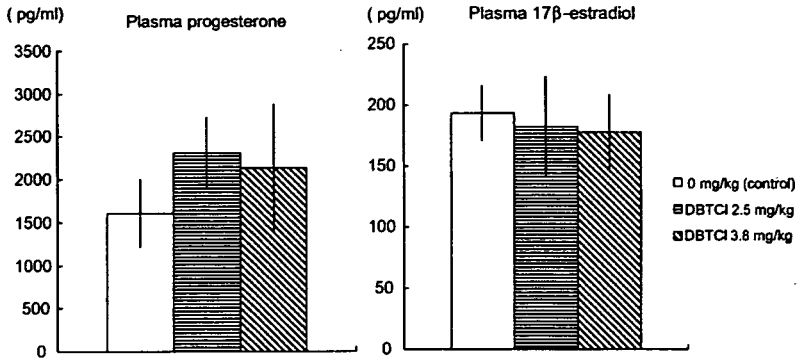

Fig. 1. Plasma progesterone and 17β-estradiol levels in pregnant monkeys given DBTCl on days 20–50 of pregnancy. Blood samples were collected on day 51 of pregnancy, 24 h after the last administration of DBTCl. Values are given as the mean ± S.E.M. of 5–10 monkeys.

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 化学物質対策の動向（第 9 報）

- 第 17 回 OECD 高生産量化学物質初期評価会議（2003 年アローナ）-

Progress on OECD Chemicals Programme (9) - SIAM 17 in Arona, 2003

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要旨：第 17 回 OECD 高生産量化学物質初期評価会議（SIAM17）が 2003 年 11 月にイタリア・アローナで開催された。日本が提出した 6 物質の初期評価文書については全ての評価結果の合意が得られた。本稿では本会議で合意の得られたこれらの物質の初期評価文書について紹介する。

キーワード：OECD、HPV プログラム、SIDS 初期評価会議

Abstract: The 17th Screening Information Data Set (SIDS) Initial Assessment Meeting (SIAM 17) was held in Arona, Italy, hosted by the European Commission. The initial assessment documents of six substances (CAS numbers: 96-29-7,

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118-79-6, 461-58-5, 611-19-8, 6165-51-1, 12125-02-9) at SIAM 17 were submitted by the Japanese Government with or without the International Council of Chemical Associations (ICCA) and all of them were agreed at the meeting. In this report, the documents of these substances are introduced.

Keywords: OECD, HPV program, SIDS Initial Assessment Meeting

1 はじめに

経済協力開発機構 (Organisation for Economic Co-operation and Development: OECD) の加盟各国における高生産量化学物質 (High Production Volume Chemical: HPV) について、1992 年に始まった OECD 高生産量化学物質点検プログラム (HPV Program) により安全性の評価が行われている (長谷川ら 1999a)。日本政府は初回より評価文書を提出しており、第 16 回までの初期評価会議 (Screening Information Data Set (SIDS) Initial Assessment Meeting: SIAM) において結論及び勧告が合意された化学物質のうち、日本政府が担当した評価文書における曝露情報、環境影響及び健康影響については既に紹介してきた (長谷川ら 1999b、2000、2001 ; 高橋ら 2004、2005a、2005b、2006 印刷中)。

国際化学工業協会協議会 (International Council of Chemical Associations: ICCA) による評価文書の原案作成に伴い日本においても 2001 年から、日本政府に加え日本化学工業協会加盟企業も評価文書の原案を作成している。

評価文書は、物性、曝露情報、健康影響及び環境影響に関する記述から構成されている。本稿では第 17 回 SIAM (SIAM17) で合意に至った化学物質名及び日本担当物質の評価文書の概要を紹介する。

2 SIAM17 で合意された化学物質名と日本担当物質の初期評価内容

2003 年 11 月にアローナ (イタリア) で開催された SIAM17 において、26 物質及び 4 カテゴリー (それぞれ 2、10、5 及び 7 物質を含む) 24 物質、計 50 化学物質の初期評価文書が審議され、表 1 に示す物質の初期評価結果および勧告が合意された。SIAM における合意は FW (The chemical is a candidate for further work.) または LP (The chemical is currently of low priority for further work.) として示されている。FW は「今後も追加の調査研究作業が必要である」、LP は「現状の使用状況においては追加作業の必要はない」ことを示す。日本政府が担

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当した化学物質の初期評価文書の概要を以下に示す。

（1）2-Butanoneoxime (96-29-7)（原案作成：ICCA 日本及び米国企業）

1）曝露状況

本物質は塗料の皮張り防止剤、シリコン樹脂の硬化剤及びウレタンのブロッキング剤として用いられている。本物質を含む製品を用いる場合、吸入により消費者曝露の可能性がある。職業曝露の主要経路は吸入と考えられる。

2）環境影響

本物質が大気に放出された場合、約 63%が大気にとどまり、約 17%が水圏に、約 20%が土壌に分布する。本物質は容易に生物分解しない (OECD TG 301C) が、水生生物における生物濃縮性は低い (生物濃縮係数 BCF : 0.5-5.8、OECD TG 305C)。水生生物に対する急性毒性では、藻類の半数影響濃度 (EC_{50}) は 6.1 mg/L (72 時間、生長阻害 : OECD TG 201)、ミジンコの EC_{50} は 201 mg/L (48 時間、遊泳阻害 : OECD TG 202)、魚類の半数致死濃度 (LC_{50}) は>100 mg/L (96 時間、OECD TG 203) であった。慢性毒性では、藻類の最大無影響濃度 (NOEC) は 1.02 mg/L (72 時間、生長阻害 : OECD TG 201)、ミジンコの NOEC は 100 mg/L (21 日間、繁殖阻害 : OECD TG 211)、魚類の NOEC は 50 mg/L (14 日間、魚類延長毒性試験 : OECD TG 204) であった。

3）健康影響

本物質は消化管と皮膚から速やかに吸収され、速やかに代謝されて尿中に排泄される。

ラットの単回経口投与毒性試験における 50%致死量 (LD_{50}) は 900～2,528 mg/kg であり、毒性症状として全身衰弱、振戦等が認められている。単回経皮毒性試験におけるウサギの経皮 LD_{50} は 1,000～1,800 mg/kg、ラットの単回吸入毒性試験 (OECD TG 403) における LC_{50} は >1,400 ppm (>4,800 mg/m^3) と判定された。

ウサギの皮膚に対して弱い刺激性、眼に対しては強い刺激性が認められた。モルモットにおいて皮膚感作性が認められた。

マウスに 1 日 6 時間、0、3、10、30 及び 100 ppm を週 5 日曝露した 13 週間吸入毒性試験において、10 ppm (36 mg/m^3) 以上で嗅上皮の変性が認められ、無毒性量 (NOAEL) は 3 ppm (10.8 mg/m^3) とされた。マウスに 1 日 6 時間、0、25、100 及び 400 ppm を週 5 日曝露した 4 週間吸入毒性試験において、400 ppm (1440 mg/m^3) で脾臓及び副腎重量の増加、メトヘモグロビンレベルの上昇が認められ、NOAEL は 100 ppm (360 mg/m^3) とされた。ラットに 1 日 6

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時間、0、25、100及び400 ppmを週5日曝露した4週間反復吸入毒性試験において、100 ppm (360 mg/m^3) 以上でメトヘモグロビンレベルの上昇が認められ、NOAELは25 ppm (90 mg/m^3) とされた。

ラットに週5日で13週間、0、25、75及び225 mg/kg/dayを強制経口投与した反復経口投与毒性試験では、25 mg/kg/day以上で溶血性貧血、脾臓及び肝臓の重量増加が認められ、最低毒性量 (LOAEL) は25 mg/kg/dayとされた。また、ラットに週5日で13週間、0、40、125及び400 mg/kg/dayを強制経口投与した反復経口投与神経毒性試験では、40 mg/kg/day以上でメトヘモグロビンレベルの上昇がみられた。400 mg/kg/dayの投与直後にみられた一過性の神経症状以外、神経毒性影響は認められなかった。ラットに0、4、20及び100 mg/kg/dayを強制経口投与した28日間反復経口投与毒性試験では、20 mg/kg/day以上で雌雄に網状赤血球率の上昇及び脾臓への影響(うっ血、髄外造血亢進、ヘモジデリン顆粒増加)が認められ、NOAELは4 mg/kg/dayとされた。雄ラットに0、250及び500 mg/kg/dayを強制経口投与した肝毒性を検討するための28日間反復経口投与毒性試験において、肝ペルオキシソーム増殖は認められなかったが、250 mg/kg/day以上で肝グルタチオンの増加が認められ、LOAELは250 mg/kg/dayとされた。

ラットに飲水中0、312、625、1,250、2,500及び5,000 ppm (およそ、雄に0、25、50、100、175及び280 mg/kg/day、雌に0、30、65、120、215及び335 mg/kg/day) で投与した13週間反復経口投与毒性試験において、625 ppm以上で雌雄に造血細胞の増殖が認められ、NOAELは312 ppm (25 mg/kg/day) とされた。また、雌雄マウスに飲水中0、625、1,250、2,500、5,000及び10,000 ppm (およそ、雄に0、110、200、515、755及び1,330 mg/kg/day、雌に0、145、340、630、1,010及び3,170 mg/kg/day) で投与した13週間反復経口投与毒性試験において、1,250 ppm以上で雄の膀胱の移行上皮過形成がみられ、NOAELは625 ppm (110 mg/kg/day) とされた。

雌雄ラットに交配前2週間及び交配期間、雄では計48日間、雌では妊娠期間及び分娩後哺育3日まで、0、10、30及び100 mg/kg/dayを強制経口投与した経口投与簡易生殖毒性試験 (OECD TG 421) では、10 mg /kg/day以上で雌雄に脾臓のうっ血、色素沈着、髄外造血などがみられた。雄の生殖と児の発生に及ぼす影響は認められず、NOAELは100 mg/kg/day (最高用量) とされた。雌では100 mg/kg/dayで分娩率が低値を示したので、NOAELは30 mg/kg/dayとされた。

ラットの雌雄 (F_0) に交配前10週間から計13週間、0、10、100及び200 mg/kg/dayを強制

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経口投与し、さらに、雌雄 F_1 に交配前 10 週間から計 13 週間、0、10、100 及び 200 mg/kg/day を強制経口投与した二世代繁殖毒性試験では、10 mg/kg/day 以上で F_0 及び F_1 の雌雄に髄外造血やヘモジデリン沈着が認められたが、生殖発生毒性に関する影響は認められず、NOAEL は 200 mg/kg/day (最高用量) とされた。

ラットの妊娠 6-15 日に 0、60、200 及び 600 mg/kg/day を強制経口投与した発生毒性試験 (OECD TG 414) では、60 mg/kg/day 以上で母体の脾臓肥大がみられたが、発生への悪影響は認められず、発生毒性の NOAEL は 600 mg/kg/day とされた。また、ウサギの妊娠 6-18 日に 0、8、14、24 及び 40 mg/kg/day を強制経口投与した催奇形性試験 (OECD TG 414) では、40 mg/kg/day で流産や妊娠ウサギの死亡がみられ、24 mg/kg/day 以上で妊娠ウサギの体重の低下が認められたことから、母体毒性の NOAEL は 14 mg/kg/day、発生毒性の NOAEL は 24 mg/kg/day とされた。

In vitro 及び *in vivo* の遺伝毒性試験の結果から本化学物質は遺伝毒性を示さないと結論された。

2 年間、雌雄ラットに 0、15、75 及び 374 ppm、雌雄マウスに 0、15、75 及び 375 ppm を 1 日 6 時間、週 5 日曝露した反復吸入癌原性試験では、雄ラットの 374 ppm (1,331 mg/m^3) 及び雄マウスの 375 ppm (1,335 mg/m^3) で肝臓がんの増加が認められた。

4) 結論と勧告

本物質はＦＷと勧告され、環境曝露量及び消費者曝露量の追加調査が推奨された。

(2) 2,4,6-Tribromophenol (118-79-6) (原案作成 : ICCA 日本企業)

1) 曝露状況

本物質は難燃性付与剤及びその中間体として用いられている。製品の中間体であれば、消費者曝露は起こりにくいが、本物質の用途から環境に排出する可能性がある。職業曝露の主要経路は吸入及び経皮と考えられる。

2) 環境影響

本物質が大気に放出された場合、約 29%が大気にとどまり、約 21%が水圏に、約 48%が土壌に分布する。本物質は易分解性ではないが、環境中で生物分解し (生物化学的酸素要求量 BOD : 49%)、また、水生生物における生物濃縮性は高くない (BCF : 513)。水生生物に対する急性毒性では、藻類の EC_{50} は 0.76 mg/L (72 時間、生長阻害 : OECD TG 201)、ミジンコの EC_{50} は 0.26 mg/L (48 時間、遊泳阻害 : OECD TG 202)、魚類の LC_{50} は 1.1 mg/L (96 時間、

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OECD TG 203) であった。慢性毒性では、藻類の NOEC は 0.22 mg/L (72 時間、生長阻害 : OECD TG 201)、ミジンコの NOEC は 0.10 mg/L (21 日間、繁殖阻害 : OECD TG 211) であった。

3) 健康影響

本物質は消化管から速やかに吸収され、速やかに代謝されて主に尿中に排泄される。

ラットの単回経口投与毒性試験 (OECD TG 401) での LD_{50} は 1,486 mg/kg、ラットの単回経皮投与毒性試験 (OECD TG 402) での LD_{50} は 2,000 mg/kg 以上、ラットの単回吸入毒性試験での LC_{50} は 50 mg/L と報告されている。

ウサギの皮膚に対して刺激性は認められないが、眼に対しては中程度の刺激性がみられた。モルモットにおいて皮膚感作性が認められた。

ラットに交配前 2 週間及び交配期間を含め、雄では計 48 日間、雌では分娩後哺育 3 日まで、0、100、300 及び 1,000 mg/kg/day を強制経口投与した反復投与毒性・生殖発生毒性併合試験 (OECD TG 422) では、300 mg/kg/day 以上の雌雄で流涎がみられ、さらに雄で血清クレアチニンの高値が認められたことから、反復投与毒性の NOAEL は 100 mg/kg/day とされた。最高用量の 1,000 mg/kg/day で雌雄の生殖能に及ぼす影響は認められないが、哺育 4 日の生存率と哺育 0 及び 4 日の体重が低値を示したことから、児では 1,000 mg/kg/day で発育抑制が認められ、生殖発生毒性の NOAEL は 300 mg/kg/day とされた。

細菌を用いる復帰突然変異試験では陰性であった。チャイニーズ・ハムスター培養細胞を用いる染色体異常試験では、連続処理では陰性であったが、S9 mix 存在下及び非存在下の短時間処理では染色体異常の誘発作用が認められたことから、染色体異常試験では陽性と判定された。しかしながら、*in vivo* でのマウスの小核試験では投与可能な最高用量においても陰性であったことから、本物質は *in vivo* では遺伝毒性を発現しないと結論された。

4) 結論と勧告

本物質はＦＷと勧告され、職業曝露量や殺菌剤としての使用量の追加調査が推奨された。

(3) Cyanoguanidine (461-58-5) (原案作成 : ICCA 日本企業)

1) 曝露状況

本物質はメラミンやグアニジン塩などの製造原料、化学肥料や爆薬などの原料、エポキシ樹脂硬化剤、安定剤、医薬品、合成洗剤、粘度調整剤として用いられている。また、間接食品添加物として米国食品医薬品局の承認を得ている。職業曝露の主要経路は吸入及び経皮と考えら

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れる。また、本物質を含む製品から、吸入及び経皮経路による消費者曝露の可能性がある。

2）環境影響

本物質が水圏に放出された場合、大気や土壌には分布しない。大気や土壌に放出された場合、主に水圏と土壌に分布する。本物質は容易に生物分解しないが、水生生物における生物濃縮性は低い（BCF：<3.1、OECD TG 305C）。水生生物に対する急性毒性では、藻類の EC_{50} は935 mg/L、NOECは171 mg/L（72時間、生長阻害：OECD TG 201）、ミジンコの EC_{50} は>1,000 mg/L（48時間、遊泳阻害：OECD TG 202）、魚類の LC_{50} は>100 mg/L（96時間、OECD TG 203）であった。慢性毒性では、ミジンコのNOECは25.0 mg/L（21日間、繁殖阻害：OECD TG 211）、魚類の LC_{50} は>100 mg/L（14日間、魚類延長毒性試験：OECD TG 204）であった。

3）健康影響

ラットの単回経口投与毒性試験での LD_{50} は30,000 mg/kg以上と報告されている。

モルモットの皮膚に対して刺激性が認められた。モルモットにおいて皮膚感作性はみられなかった。

ラットに交配前2週間及び交配期間を含め、雄では計44日間、雌では分娩後哺育3日まで、0、40、200及び1,000 mg/kg/dayを強制経口投与した反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では、最高用量の1,000 mg/kg/dayでも反復投与毒性及び生殖発生毒性に関する影響は認められず、反復投与毒性と生殖発生毒性のNOAELは1,000 mg/kg/dayとされた。

細菌を用いる復帰突然変異試験及びチャイニーズ・ハムスター培養細胞を用いる染色体異常試験では陰性あったことから、本物質は遺伝毒性を発現しないと結論された。

ラットに2年間0、2.5及び5.0%（雄では0、837.2及び1958.6 mg/kg/day、雌では0、1001.3及び2169.2 mg/kg/day）を混餌投与した発がん性試験において、腫瘍発生率の上昇は認められなかった。

4）結論と勧告

本物質はＬＰと勧告された。

（4）1-Chloro-2-(chloromethyl)-Benzene (611-19-8)（原案作成：ICCA日本企業）

1）曝露状況

本物質は農薬の中間体として用いられている。本物質は製品の中間体であり、消費者曝露は起こりにくい。職業曝露の主要経路は吸入及び経皮と考えられる。

2）環境影響

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本物質が水圏に放出された場合、約 74%が水圏にとどまり、約 12%が大気に、約 8%が沈殿物、約 7%が土壌に分布する。本物質が大気に放出された場合、約 64%が大気にとどまり、約 35%が土壌に分布する。本物質が土壌に放出された場合、大気や水圏には分布しない。本物質は容易に生物分解しないが、水生生物における生物濃縮性は低いと考えられる。水生生物に対する急性毒性では、藻類の EC_{50} は 0.78 mg/L (72 時間、生長阻害 : OECD TG 201)、ミジンコの EC_{50} は 0.38 mg/L (48 時間、遊泳阻害 : OECD TG 202)、魚類の LC_{50} は 0.27 mg/L (96 時間、OECD TG 203) であった。慢性毒性では、藻類の NOEC は 0.045 mg/L (72 時間、生長阻害 : OECD TG 201)、ミジンコの NOEC は 0.020 mg/L (21 日間、繁殖阻害 : OECD TG 211) であった。

3) 健康影響

ラットの単回経口投与毒性試験における LD_{50} は 350～951 mg/kg であった。単回経皮毒性試験においてウサギの経皮 LD_{50} は 1,700～2,200 mg/kg、ラットの経皮 LD_{50} は 2,000 mg/kg 以上、ラットの単回吸入毒性試験 (OECD TG 403) での LC_{50} は 2.8 mg/L と判定された。主に本物質の投与部位 (胃、皮膚、肺) に刺激による組織学的損傷が引き起こされた。

ウサギの皮膚と眼に対して刺激性が認められた。

ラットに 1 日 6 時間、0、0.01、0.03 及び 0.10 mg/L を週 5 日曝露した 4 週間反復吸入毒性試験 (OECD TG 412) では、0.10 mg/L で肺重量の増加、鼻粘膜、気管及び気管支の損傷、気管気管支リンパ節のリンパ組織過形成が認められ、NOAEL は 0.03 mg/L と判定された。

ラットに交配前 2 週間及び交配期間を含め、雄では計 45 日間、雌では分娩後哺育 3 日まで、0、2、10 及び 50 mg/kg/day を強制経口投与した反復投与毒性・生殖発生毒性併合試験 (OECD TG 422) では、10 mg/kg/day 以上の雄、50 mg/kg/day の雌に前胃壁の肥厚、扁平上皮の増生、びらん及び潰瘍が認められ、反復投与毒性の NOAEL は雄で 2 mg/kg/day、雌で 10 mg/kg/day とされた。生殖発生毒性に関する影響は認められず、生殖発生毒性の NOAEL は 50 mg/kg/day (最高用量) とされた。

細菌を用いる復帰突然変異試験では S9 mix 存在及び非存在下で陰性であったが、S9 mix 非存在下で弱い陽性を示す結果もみられた。チャイニーズ・ハムスター培養細胞を用いる染色体異常試験では、細胞毒性を示す用量において S9 mix 存在及び非存在下で陽性であった。しかし、*in vivo* でのマウスの小核試験では投与可能な最高用量において陰性であったことから、本物質は *in vivo* では遺伝毒性を発現しないと結論された。

4) 結論と勧告

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本物質はＬＰと勧告された。

（５） 1,4-Dimethyl-2-(1-phenylethyl)benzene (6165-51-1)（日本政府作成）

１）曝露状況

本物質は PCBs の代替物質として用いられ、感圧紙用染料やコンデンサーオイルとして使用されている。本物質を含む製品からの、吸入及び経皮経路による消費者曝露の可能性がある。職業曝露の主要経路は吸入及び経皮と考えられる。

２）環境影響

本物質が土壌や大気に放出された場合は主に土壌に分布し、水圏に放出された場合は主に沈殿物に分布する。本化学物質は容易に生物分解しない（OECD TG 301C）が、水生生物における生物濃縮性は高くない（生物濃縮係数 BCF：760-620、OECD TG 305）。水生生物に対する急性毒性では、藻類の EC_{50} は 0.93-1.54 mg/L 以上（72 時間、生長阻害：OECD TG 201）、ミジンコの EC_{50} は 0.25 mg/L（48 時間、遊泳阻害：OECD TG 202）、魚類の LC_{50} は 0.31 mg/L（96 時間、OECD TG 203）であった。慢性毒性では、藻類の NOEC は 0.047-0.73 mg/L（72 時間、生長阻害：OECD TG 201）、ミジンコの NOEC は 0.009 mg/L（21 日間、繁殖阻害：OECD TG 211）であった。

３）健康影響

ラットの単回経口投与毒性試験（OECD TG 401）では、最高用量 2,000 mg/kg の投与後 1～2 日に、雄 1 匹及び雌 2 匹の死亡が認められ、LD_{50} は 2,000 mg/kg 以上と考えられた。

ラットに交配前 2 週間及び交配期間を含め、雄では計 47 日間、雌では分娩後哺育 3 日まで、0、12.5、50 及び 200 mg/kg/day を強制経口投与した反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では、雄では 12.5 mg/kg/day 以上で副腎の重量低値及び束状帯細胞萎縮がみられ、雌では 200 mg/kg/day で肝重量の高値及び小葉中心性肝細胞肥大が認められた。これらの結果から、反復投与毒性における雄の LOAEL は 12.5 mg/kg/day、雌の NOAEL は 50 mg/kg/day とされた。生殖発生毒性に関する影響は認められず、生殖発生毒性の NOAEL は 200 mg/kg/day（最高用量）とされた。

細菌を用いる復帰突然変異試験及びチャイニーズ・ハムスター培養細胞を用いる染色体異常試験では陰性あったことから、本物質は遺伝毒性を発現しないと結論された。

４）結論と勧告

本物質はＦＷと勧告され、溶剤や PCB 代替物としての使用、または、本物質を含む紙のリサ

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イクル過程に基づく、環境曝露量、職業曝露量及び消費者曝露量の追加調査が推奨された。

（6）Ammonium chloride (12125-02-9)（原案作成：ICCA 日本企業）

1）曝露状況

本物質は主に水田用肥料として使用されている。吸入及び経皮により消費者曝露の可能性がある。職業曝露の主要経路は吸入及び経皮と考えられる。

2）環境影響

環境に放出された場合、本物質はアンモニウムイオン及び塩化物イオンとなり水圏に分布する。生物分解に関するデータは無いが、アンモニア（NH_3 または NH_4+）は生物が利用する前に様々な細菌によって無機化され、亜硝酸イオン（NO_2-）となる。また、NH_3、NH_4+、Cl-は生物の共通構成要素である。水生生物に対する急性毒性では、藻類の EC_{50} は 1,300 mg/L (5 日間、生長阻害)、ミジンコの LC_{50} は 101 mg/L (48 時間、遊泳阻害)、魚類の LC_{50} は 96.2-218 mg/L (96 時間) であった。慢性毒性では、藻類の NOEC は 26.8 mg/L (10 日間、生長阻害)、ミジンコの NOEC は 14.6 mg/L (21 日間、繁殖阻害)、魚類の NOEC は 8.0-23.9 mg/L (28 または 44 日間) であった。

3）健康影響

本物質は消化管から速やかに吸収され、肝臓でアミノ酸やタンパク質の合成に利用される。

単回経口投与毒性試験では、LD_{50} は 1,630 mg/kg (雄ラット)、1,220 mg/kg (雌ラット)、1,300 mg/kg (雄マウス) と判断された。毒性症状として、ラットでは呼吸困難、無欲、異常姿勢が認められ、雄マウスでは下痢、チアノーゼ、よろめき歩行が認められた。

ウサギの皮膚及び眼に対して中程度の刺激性が認められた。モルモットにおいて皮膚感作性はみられなかった。

雄ラットに 12,300 ppm (684 mg/kg/day) を 70 日間混餌投与した反復投与毒性試験では、毒性影響は認められず、NOAEL は 684 mg/kg/day とされた。また、ラットの妊娠 7-10 日に 8.9 mg/kg/day を強制経口投与した試験では、母体毒性及び発生毒性に対する影響は認められなかった。

細菌を用いる復帰突然変異試験では陰性であった。S9 mix 非存在下で行われたチャイニーズ・ハムスター培養細胞を用いる染色体異常試験では陽性であったが、これは本物質の酸性度に起因した結果と判定された。*in vivo* でのマウスの小核試験では投与可能な最高用量においても陰性であったことから、*in vivo* において遺伝毒性は示さないと結論された。

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