Maternal and Fetal Toxicity of Dimethyltin in Rats

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The maternal and fetal toxicity of dimethyltin chloride (DMTC) was examined in two teratological studies in pregnant Wistar rats. In one study, the animals were treated by oral gavage with DMTC at doses of 0, 5, 10, 15, or 20 mg/kg/day on gestational days 7–17. In the second study, animals were treated by oral gavage at doses of 0, 20 or 40 mg/kg/day DMTC on two or three consecutive days at one of four different periods of gestation (gestational days 7–9, 10–12, 13–15 or 16–17). Caesarean sections were performed on day 20 of gestation in both studies. In the first study, vaginal bleeding, tremor and convulsions were observed in animals treated at 20 mg/kg/day after day 15 of gestation. Of ten dams treated with 20 mg/kg/day DMTC, two died and one exhibited total resorption. While a increase in the incidence of cleft palate was found in the fetuses of animals treated with 20 mg/kg/day DMTC, the dams so treated exhibited severe clinical signs of toxicity. Animals treated in the second study with doses of 20 or 40 mg/kg/day at one of four periods of gestation had a reduction in the adjusted body weight gain but not in gravid uterus weight and did not show any evidence of teratogenic effect at either dose and period tested. These studies suggest that DMTC did not produce teratogenic effects at dose levels where no maternal toxicity was observed. It was suggested that under the conditions of the first study, since no signs of maternal or fetal toxicity could be detected up 10 mg/kg/day DMTC, this dose was chosen to represent the no-observed-adverse-effect level (NOAEL).

Key words —— cleft palate, dimethyltin, developmental toxicity, fetal toxicity, maternal toxicity, organotin

INTRODUCTION

Over the past 5 decades, large quantities of organotin compounds, primarily the tri-, and dialkyl derivatives, have been employed globally in a broad expanse of applications including: principally polyvinyl chloride (PVC) stabilization at general levels of 0.5–2.0%, antifouling agents in marine paints, polyurethane foams, agrochemicals, catalysts, in the glass strengthening and paper industries and as molluscicides.[1–5] The primary uses of orgnotins are differentiated upon structural criteria.[1–6] Dimethyltin is currently used in applications including: stabilization of PVC potable-water piping, in providing abrasion resistance and bursting strength of glass containers, and as catalysts for the curing of electrocoated paints.[1–3]

There is no recent figure for the amount of organotins entering the environment and presently understanding of their reactions in the environment is limited.[6] The broad utilization of dialkyltin compounds including dimethyltin has necessitated concern due to their varied sources of entry into the environment, principally their detection in the aquatic environment and concentrations in fish and shellfish.[2,3,7] Mono-, di- and/or trimethyltin compounds have been detected in fresh water, tap water, seawater, sediment, algae, shellfish and fish.[2,3,7–11] Leaching of organotins from PVC material has been considered one of the principal sources of environmental dimethyltin compounds.[2–4,7] Additionally, the methylation of inorganic tin in the aquatic environment has given rise to dimethyltin compounds.[2,3,12–14] Environmental micro- or macroorganisms including *Pseudomonas* spp. has also been found to convert inorganic tin to methyl tin species, including dimethyltin in a variety of laboratory incubation studies.[13–15]

The principal environmental and toxicological concern to date has focused on and resulted in the restriction in the use of tri-substituted organotin compounds, *e.g.*, the trimethyltin compounds which are neurotoxicants[16,17] and tri-*n*-butyltin compounds which display endocrine disrupting activity,[18] and which exhibit extremely high toxic effects on non-target aquatic organisms.[2,4,5,18]

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Di-*n*-butyltin compounds have particularly warranted scrutiny and concern because of their overall environmental ubiquity,[2,3,7] contamination of food,[19,20] and the teratogenic effects noted in experimental animals.[21–24] However, it nevertheless remains important to evaluate the teratological effects of other disubstituted organotins such as dimethyltin. Noland *et al*.[25] reported that dimethyltin dichloride (DMTC) administered orally to pregnant Sprague-Dawley rats was absorbed from the intestine and transferred across the placenta to the fetus. The majority of the tin received as dimethyltin was transferred to the pups during gestation rather than lactation. However the teratogenicity of DMTC has not been evaluated. In the studies to be presented, two teratological studies were performed to evaluate the teratogenicity of DMTC. In the first study, the induction of cleft palate was observed in the fetuses from dams treated orally by gavage with the highest dose of DMTC administered on days 7–17 of gestation, however the dams with malformed fetuses exhibited severe maternal toxicity. Therefore in the second study, pregnant females were treated with relative higher doses of DMTC at shorter periods of gestation in order to reduce maternal toxicity.

MATERIALS AND METHODS

Chemical —— DMTC (purity > 99.0%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals —— Four-week-old Wistar rats of either sex were purchased from CLEA Japan Inc. (Tokyo, Japan). Animals were individually housed in a room with constant day/night cycle (lights on from 7:00 to 19:00 hr) at 23 ± 2°C temperature and 60 ± 20% relative humidity. They were fed commercial laboratory chow (NMF; Oriental Yeast Co., Ltd. Tokyo, Japan) and tap water *ad lib*. and studied at three months of age. A female rat was paired overnight with a male of the same age and the day on which sperm was observed in the vaginal smears was designated as day 0 of gestation.

Treatment with DMTC —— Two teratological studies were performed. In the first teratological study (Study I), DMTC was administered to pregnant animals during the organogenetic phase of gestation (days 7–17 of gestation). Mated females were randomly assigned to five groups of ten rats each. DMTC was dissolved in saline and administered to pregnant females by oral gavage at 0, 5, 10, 15 or 20 mg/kg/day on days 7–17 of gestation. The volume of vehicle was held constant at 2 ml/kg based on maternal body weight.

In the second teratological study (Study II), mated females were randomly assigned to eight groups of 8–11 rats each. DMTC was administered by oral gavage to pregnant animals at 0, 20, or 40 mg/kg/day for two to three consecutive days at one of four different periods of gestation (gestational days 7–9, 10–12, 13–15 and 16–17). The group treated with DMTC at 20 mg/kg/day on days 16–17 was not tested. Control animals were administered an appropriate volume of vehicle without DMTC on days 10–12 of gestation.

Observations —— All procedures were substantially the same in the two teratological studies. Maternal body weight and food intake were measured daily. Pregnant females were observed daily for clinical signs of toxicity and were sacrificed by overdose of ether anesthesia on day 20 of gestation. The position and the number of living fetuses and implantation loss in the uterus, and the number of corpora lutea, were recorded. Uteri with total resorption were isolated and stained with 10% ammonium sulfide to determine the total number of implantations. The living fetuses were examined for their sex and external malformations, and were then weighed. Maternal thymus and brain weights, and the gravid uteri weights (only in the study II) were also recorded. Half of the living fetuses in each litter were fixed with 95% ethanol and processed for staining of the skeleton by the alizarin red S dye method.[26] These preparations were examined for skeletal abnormalities. The other half of each litter was fixed in Bouin's solution and examined for visceral abnormalities according to the method of Wilson.[27]

Statistical Analysis —— Data on the number of dams with living fetuses and with total resorption and the number of malformed fetuses were analyzed by Fisher's exact test. Other data were analyzed by Dunnett's multiple comparison method[28] in a parametric or non-parametric manner. The litter was used as the statistical unit for calculation of fetal values.

RESULTS

Study I

Oral treatment of pregnant rats with DMTC during the organogenetic phase of gestation (days 7–17 of gestation) caused reduction in the maternal body weight gain in a dose dependent manner. Signifi-

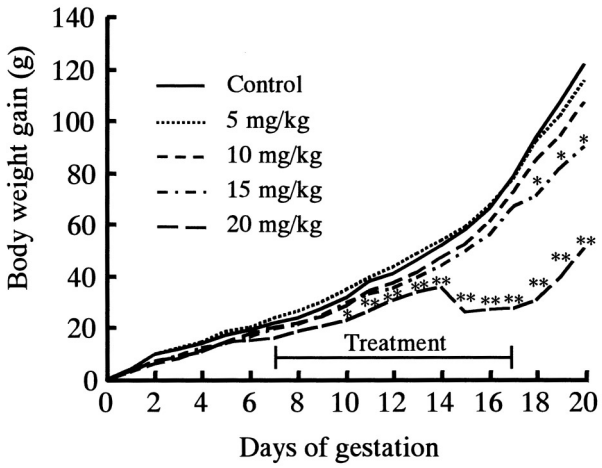

Fig. 1. Body Weight Gain of Pregnant Rats Treated Orally with DMTC on Days 7–17 of Gestation

*Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

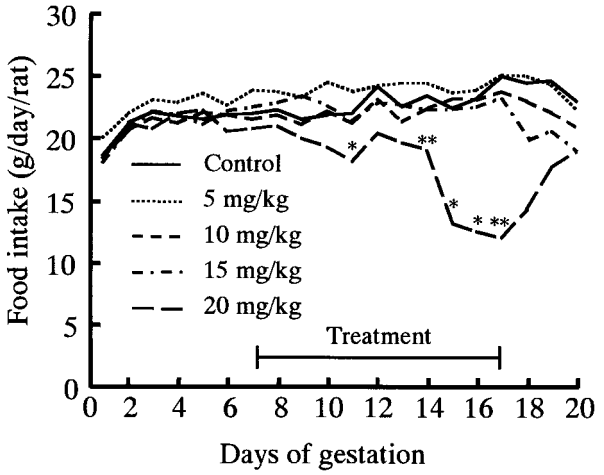

Fig. 2. Food Intake of Pregnant Rats Treated Orally with DMTC on Days 7–17 of Gestation

*Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

Table 1. Effects of DMTC on Maternal Thymus and Brain Weights on Day 20 of Gestation

	Saline	DMTC (mg/kg)			
	2 ml/kg	5	10	15	20
Body wt. (g)	333 ±26.7	334 ±21.9	321 ±21.0	315 ± 9.2	252 ±41.1**
Thymus wt. (mg)	196 ±37.5	190 ±32.6	168 ±26.8	151 ±13.6*	45 ±21.7**
Brain wt. (g)	1.75± 0.02	1.72± 0.05	1.71± 0.07	1.76± 0.04	1.68± 0.04

Pregnant rats were treated orally with DMTC on days 7–17 of gestation. These weights were recorded on day 20 of gestation. Values are mean ± S.D. *Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

cantly reduced body weight gain was observed in pregnant rats treated with DMTC at 15 or 20 mg/kg/day in late gestation (Fig. 1). Maternal food intake was significantly reduced by DMTC-treatment at 20 mg/kg/day, but no other statistically significant differences in maternal food intake were present (Fig. 2).

Oral administration of DMTC at 20 mg/kg/day resulted in the death of two pregnant rats, one died on day 18 of gestation and another died on day 19 of gestation. These deaths were considered to be DMTC treatment related because the animals exhibited severe clinical signs of toxicity (piloerection, ataxia, perinasal and periocular staining, vaginal bleeding, tremor and convulsion) for about four days prior to their deaths. No gross pathological changes were noted upon necropsy in the organs of the dead dams. No other mortalities were observed in either the control or DMTC-treatment groups.

Perinasal and periocular staining, piloerection and ataxia were observed in all the pregnant rats administered DMTC at 20 mg/kg/day mainly after the day 15 of gestation. In addition to these clinical signs of toxicity, vaginal bleeding, tremor and convulsion were observed in three pregnant animals other than two dead pregnant rats in the late stage of gestation. No clinical signs of toxicity were observed in the other groups. Maternal thymus weights were reduced in a dose–dependent manner with significance at 15 and 20 mg/kg/day on day 20 of gestation. Maternal brain weight was unaffected in any group (Table 1).

Total resorption was observed in one of eight living pregnant rats at 20 mg/kg/day. This same rat also exhibited vaginal bleeding, tremor and convulsion in the late stage of gestation. Mean body weight in living fetuses of both sexes decreased in a dose–dependent manner with significance at 15 and 20 mg/kg/day. No significant differences were noted in the number of corpora lutea, implants and living fetuses, and the incidence of post-implantation loss and sex ratio (Table 2).

The incidence of external malformations increased in fetuses from dams exposed to DMTC at

Table 2. Effects of DMTC on Pregnant Rats and Their Fetuses

	Saline	DMTC (mg/kg)			
	2 ml/kg	5	10	15	20
No. of females inseminated	10	10	10	10	10
No. of pregnant females	10	10	10	9	10
No. of dams with living fetuses	10	10	10	9	7
No. of dams with total resorption	0	0	0	0	1
No. of dead dams	0	0	0	0	2
No. of corpora lutea[a)]	15.8±1.32	15.5±1.18	15.9±1.91	16.0±1.73	15.9±2.47
No. of implants[a)]	15.4±1.43	14.4±0.84	15.1±1.37	14.0±2.06	14.8±2.12
Incidence of postimplantation loss %	4.69	4.12	6.86	2.96	19.35
Early stage	2.63	4.12	6.86	2.96	6.86
Late stage	2.05	0.00	0.00	0.00	12.49
No. of living fetuses[a)]	14.6±1.90	13.8±1.03	14.1±1.79	13.7±2.06	11.9±5.22
Sex ratio (M/F)	70/76	69/69	79/62	68/55	46/49
Body weight of living fetuses[a,b)] g					
Male	3.5±0.20	3.4±0.22	3.2±0.25	2.9±0.16*	2.2±0.46**
Female	3.3±0.16	3.2±0.20	3.2±0.27	2.8±0.15*	2.1±0.41**

Pregnant rats were treated orally with DMTC on days 7–17 of gestation. Cesarean sections were performed on day 20 of gestation. The litter was used as the statistical unit for calculation of fetal values. *a*) Values are mean ± S.D. *b*) These values represent means of litter means within each group. *Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

20 mg/kg/day from days 7–17 of gestation. There were 21 fetuses with cleft palate from five of seven pregnant rats with living fetuses on day 20 of gestation. In addition to cleft palate, one fetus was associated with general edema and pes varus and one with general edema (Table 3). There were two fetuses with omphalocele from one dam exposed to DMTC at 15/mg/day, but the incidence was not statistically significant. No other external malformations were observed in either the control or DMTC-treatment groups.

No significant difference was observed in the incidence of skeletal malformations and skeletal variations (Table 3). No significant difference was observed in the incidence of visceral malformations, however the number of visceral variation, dilation of the renal pelvis, was significantly increased at 20 mg/kg/day (Table 3).

Study II

Oral gavage administration of DMTC at 40 mg/kg/day to pregnant rats on days 10–12 of gestation, caused significant reductions of maternal body weight gain on days 13, 16 and 17 of gestation and reduction of food intake on the consecutive days of gestation after the day 12 of gestation. No significant reduction in maternal body weight gain was observed in other treatment period groups, in spite of the reduced food intake caused by the treatment (data not shown). There were no significant differences in general behavior among the groups including controls.

Maternal thymus weights and adjusted body weight gain were reduced significantly in the group treated with DMTC at 20 mg/kg/day on days 10–12 of gestation and in every treatment period groups at 40 mg/kg/day. Gravid uterus and maternal brain weights were unaffected at either dose level in any treatment period group (Table 4). Total resorption was observed in one of 10 dams in the group treated with DMTC at 40 mg/kg/day on days 7–9 of gestation (Table 5). In this group, mean fetal body weight of the females was reduced. No significant differences were noted in the number of corpora lutea, implants and living fetuses and incidence of postimplantation loss, sex ratio and male fetal body weight at either dose level in any treatment period group (data not shown).

The incidence of external, skeletal and visceral malformations did not significantly increase at either dose in any treatment period group. No cleft palate were found in any fetus treated with DMTC at 20 or 40 mg/kg/day on two to three consecutive days at one of four different treatment periods of gestation (Table 5). The numbers of fetuses with skeletal variation and visceral variation increased significantly at 40 mg/kg/day in some treatment periods. Numbers of fetuses with cervical ribs increased in the groups treated with DMTC on days 7–9 or 13–15 of gestation. Numbers of fetuses with split-

Table 3. External, Visceral and Skeletal Observations of Fetuses from Dams Treated Orally with DMTC during Days 7–17 of Gestation

	Saline	DMTC (mg/kg)			
	2 ml/kg	5	10	15	20
External observations					
No. of fetuses examined	146	138	141	123	95
Incidence of fetuses with malformations %	0	0	0	2.5(1)	22.5(5)**
No. of fetuses with malformations	0	0	0	2(1)	21(5)**
Cleft palate	0	0	0	0	21(5)**
General edema	0	0	0	0	2(2)
Pes varus	0	0	0	0	1(1)
Omphalocele	0	0	0	2(1)	0
Skeletal observations					
No. of fetuses examined	78	73	79	64	50
Incidence of fetuses with malformations %	0	0	3.3(1)	0	0
No. of fetuses with malformations	0	0	2(1)	0	0
Fused ribs	0	0	1(1)	0	0
Fused cervical vertebral arches	0	0	2(1)	0	0
No. of fetuses with variations					
Cervical ribs	2(2)	3(3)	3(3)	6(5)	3(3)
Splitting of 1st cervical vertebral arches	0	1(1)	0	1(1)	0
Rudimentary lumbar ribs	6(1)	0	2(1)	0	1(1)
Splitting of ossification centers of thoracic vertebral bodies	0	0	2(1)	1(1)	4(4)
Visceral observations					
No. of fetuses examined	68	65	62	59	45
Incidence of fetuses with malformations %	0	0	4.8(3)	1.6(1)	2.0(1)
No. of fetuses with malformations	0	0	3(3)	1(1)	1(1)
Ventricular septal defect	0	0	1(1)	1(1)	1(1)
Dilatation of lateral ventricle	0	0	2(2)	0	0
No. of fetuses with variations					
Dilatation of the renal pelvis	0	0	1(1)	4(4)	5(4)*
Thymic remnant in the neck	0	0	2(1)	2(2)	1(1)
Kinked ureter	0	0	1(1)	1(1)	0

Cesarean sections were performed on day 20 of gestation. ( ) No. of conceived mothers with case. *Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

Table 4. Effects of DMTC Exposed to Pregnant Rats on Two or Three Consecutive Days at One of Four Different Treatment Periods of Gestation, on Maternal Brain, Thymus, Gravid Uterus Weights and Adjusted Body Weight Gain at the Day 20 of Gestation

	Saline	DMTC (mg/kg)									
	days 10–12	days 7–9		days 10–12		days 13–15			days 16–17		
	2 ml/kg	20	40	20	40	20	40		40		
Body wt. (g)	338 ±19.7	330 ±23.2	330 ±47.4	325 ±23.1	312 ±22.7	331 ±12.7	325 ±14.8		340 ±23.4		
Brain wt. (g)	1.79± 0.08	1.79± 0.04	1.80± 0.05	1.79± 0.04	1.76± 0.06	1.75± 0.06	1.79± 0.05		1.80± 0.05		
Thymus wt. (mg)	253 ±45.1	220 ±22.7	208 ±37.6*	207 ±39.9*	190 ±36.9**	217 ±15.3	182 ±32.7**		172 ±45.1**		
Body weight gain (g)	112 ±21.6	110 ±12.5	110 ±13.1	107 ±18.0	95 ±17.8	111 ± 9.0	103 ±12.4		102 ±22.6		
Gravid uterus wt. (g)	65 ±20.0	69 ±10.2	75 ±11.7	72 ±11.1	66 ±19.7	69 ± 4.5	71 ± 8.6		71 ±15.6		
Adjusted body weight gain[a)] (g)	47 ± 7.0	40 ± 7.4	35 ± 6.0**	35 ± 9.6*	30 ± 9.2**	42 ± 6.5	33 ± 6.6**		31 ±13.3**		

Pregnant rats were sacrificed on day 20 of gestation. Values are the mean ± S.D. of 8–11 animals per group. *a*) (Body weight gain from day 0 to 20 of gestation) − (gravid uterus weight). *Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

Table 5. External, Skeletal and Visceral Observations of Fetuses from Dams Treated Orally with DMTC on Two or Three Consecutive Days at One of Four Different Treatment Periods of Gestation

	Saline	DMTC (mg/kg)						
	days 10–12	days 7–9		days 10–12		days 13–15		days 16–17
	2 ml/kg	20	40	20	40	20	40	40
No. of pregnant females	10	10	10	10	11	9	8	10
No. of dams with living fetuses	10	10	9	10	11	9	8	10
No. of dams with total resorption	0	0	1	0	0	0	0	0
No. of dead dams	0	0	0	0	0	0	0	0
External observations								
No. of fetuses examined	127	136	139	141	145	118	113	142
Incidence of fetuses with malformations %	0.0	0.0	0.6(1)	0.0	0.0	0.0	0.9(1)	0.0
No. of fetuses with malformations	0	0	1(1)	0	0	0	1(1)	0
Peaked mandible	0	0	1(1)	0	0	0	0	0
Vestigial tail	0	0	0	0	0	0	1(1)	0
Skeletal observations								
No. of fetuses examined	69	72	73	74	79	64	61	76
Incidence of fetuses with malformations %	0.0	0.0	2.6(2)	0.0	0.0	0.0	1.6(1)	0.0
No. of fetuses with malformations	0	0	2(2)	0	0	0	1(1)	0
Absence of thoracic vertebral arches	0	0	1(1)	0	0	0	0	0
Fused mandible	0	0	1(1)	0	0	0	0	0
Agenesis of the sacro and coccygeal vertebrae	0	0	0	0	0	0	1(1)	0
No. of fetuses with variations								
Cervical rib	0	0	5(4)*	1(1)	2(2)	1(1)	4(4)*	2(2)
Splitting of 1st cervical vertebral arches	0	0	6(4)*	0	1(1)	0	1(1)	0
Variation in number of lumbar vertebrae	0	0	0	0	0	0	1(1)	0
Rudimentary lumbar rib	1(1)	2(2)	4(2)	4(3)	3(1)	2(2)	5(3)	2(2)
Visceral observations								
No. of fetuses examined	58	64	66	67	66	54	52	66
Incidence of fetuses with malformations %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. of fetuses with variations								
Thymic remnant in the neck	2(1)	1(1)	6(3)	1(1)	7(5)	1(1)	1(1)	0
Dilatation of renal pelvis	1(1)	2(1)	5(4)	3(3)	1(1)	1(1)	1(1)	4(2)
Kinked ureter	0	4(2)	3(2)	2(1)	1(1)	0	2(2)	6(3)*

Cesarean sections were performed on day 20 of gestation. ( ) No. of conceived mothers with case.
*Significantly different from control, $p < 0.05$.

ting of first cervical vertebral arches and with kinked ureter increased in the group treated with DMTC on days 7–9 and 16–17 of gestation, respectively (Table 5).

DISCUSSION

In these studies, oral dosing of DMTC resulted in thymic atrophy which was one of the most sensitive criterion for toxicity of di-*n*-butyltin,[23] tri-*n*-butyltin[29] and di-*n*-octyltin[30] but not mono-*n*-butyltin.[23] However, the severity of thymic atrophy caused by DMTC treatment was less than that caused by di-*n*-butyltin. The lowest doses exhibiting thymic atrophy were 15 mg/kg/day (68 μmol/kg/day) for DMTC and 5 mg/kg/day (14 μmol/kg/day) for di-*n*-butyltin.[23]

Signs of neural damage or cerebral edema have been reported in rats treated with trimethyltin or triethyltin compounds, but not in DMTC-treatment rats.[31] In my preliminary study, however, more than seven consecutive treatments of DMTC caused tremor and aggression at a relatively high dose (30 mg/kg/day). Therefore brain weight was recorded in the first study (Study I), but it was unaf-

fected by the treatment with DMTC.

In the Study I, administration of DMTC at 20 mg/kg/day caused cleft palate. However, it could not be concluded that DMTC was teratogenic because the probability remained that the malformation might be caused by severe maternal toxicity at this dose level. Moreover a dose-related pattern in the incidence of affected fetuses was not observed. Noland *et al*.[25] reported that ^{14}C-DMTC administered orally to pregnant rats on day 19 of gestation could be rapidly absorbed from the intestine and transferred across the placenta to the fetus, and then the blood ^{14}C levels declined slowly. In the present study, DMTC was considered to accumulate in pregnant rats, since pregnant animals treated with DMTC at 20 mg/kg/day exhibited severe clinical signs of toxicity in the latter stages of gestation, particularly after day 15 of gestation. In order to reduce maternal toxicity, shorter periods of DMTC-treatment and relative high doses of DMTC were chosen in the Study II. Consequently, treatment with DMTC at 40 mg/kg/day for two or three consecutive days at one of four different periods of gestation caused slight maternal toxicity as indicated by the reductions of the adjusted body weight gain and thymus weights in the Study II. Oral administration of DMTC failed to induce cleft palate at either dose level in any treatment period group in the Study II, although there were 21 affected fetuses out of 95 at 20 mg/kg/day in the Study I. Cleft palate in the Study I was only observed in the dams exhibiting severe clinical signs of toxicity. Thus the cleft palate observed in Study I might be an abnormality that can be induced in rats non-specifically through the maternal toxicity, although the compound is genotoxic[32,33] and the probability nevertheless remained that dimethyltin level in fetus was insufficient to induce the malformation in the Study II. The conclusive evidence in support of the teratogenicity of DMTC is still lacking.

Di-*n*-butyltin, the other disubstituted organotin, exhibits teratogenic effects such as cleft mandible, cleft lower lip, ankloglossia, schistoglossia, exencephaly, fused ribs or fused vertebral arches, at doses of more than 5 mg/kg/day.[23] The critical day of the teratogenicity of di-*n*-butyltin is day eight of gestation.[24] Thus it is highly suggested that the teratogenic effects of DMTC are distinct from those observed for di-*n*-butyltin.

Under the conditions of the Study I, the no-observed-adverse-effect level (NOAEL) for maternal and fetal toxicity is suggested for consideration to be 10 mg/kg/day.

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