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Developmental Toxicity of Triphenyltin Chloride After Administration on Three Consecutive Days During Organogenesis in Rats

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Organotin compounds are a broad group of chemicals that are widely used in agriculture and industry (Piver 1973; WHO 1980). Trisubstituted organotin compounds are the most important class of organotin chemicals. They are very active biologically and are widely used as biocides. The most important groups of triorganotins are triphenyltin (TPT) and tributyltin (TBT) derivatives (WHO 1980). TPTs and TBTs have been used extensively in antifouling products as algaecides and molluscicides. The major use of TPT compounds is in non-systemic fungicides in crop protection (Fent 1996). TPTs are directly introduced into aquatic systems via leaching from antifouling paints and runoff from agriculture fields (Fent 1996). Concentrations of up to 0.2 µg/L of TPT were detected in boat harbors (Fent and Hunn 1991), and 1.5 µg/L of TPT was detected in a ditch adjacent to a potato field after routine spraying with TPT fungicides (Stab et al. 1993). The contamination of fish and other marine organisms by TPTs is widespread. It is reported that the bioconcentration factors of TPT were 2,090 in the carp kidney (Tsuda et al. 1987) and in the range of 80,000-440,000 in the crab hepatopancreas (Kannan et al. 1995) and that fish and other marine products purchased in retail stores in Japan had concentrations of TPT of 0.03 to 1.3 mg/kg (Ishizaka et al. 1989). These observations indicate that TPT bioconcentrates in the food chain and that humans can be exposed to TPT via fish and other marine products.

There are several reports of the adverse effects of TPT on offspring following maternal exposure. Winek et al. (1978) reported that rats given triphenyltin hydroxide (TPTH) at 20 mglkg on days 1-7 of pregnancy did not produce pups nor did they exhibit any resorption sites, that only two of the six rats given TPTH at 15 mg/kg on days 8-14 of pregnancy produced viable pups, and that four of the six rats given TPTH at 15 mg/kg on days 14-20 of pregnancy produced viable pups. Recently, we noted that the pregnancy rate was significantly decreased after administration of triphenyltin chloride (TPTCl) on days 0-3 of pregnancy at 4.7 mg/kg and above and on days 4-6 of pregnancy at 12.5 mg/kg and above in rats (Ema et al. 1997). A transient change in spontaneous locomotor activity and conditioned learning and increased mortality during lactation were observed in rat pups of dams given triphenyltin acetate (TPTA) at 6 mg/kg on days 6-14 of pregnancy (Lehotzky et al. 1982). Oral administration of TPTA on days 6-15 of pregnancy at 15 mg/kg (Giavini et al. 1980) and on days 7-17 of pregnancy at 9 mg/kg and above (Noda et al. 1991) and TPTH on days 6-15 of pregnancy at 13 mg/kg (Chernoff et al. 1990) caused a significant decrease in maternal body weight gain and a significant increase in postimplantation loss, but not fetal malformations, in rats. The previous studies evaluating the developmental toxicity of TPT were designed for the screening of general developmental toxicity and with prolonged administration accompanied by confounding factors. Shorter periods of treatment with a chemical provide more information on developmental toxicity because of allowing to raise the dose, reducing maternal and conceptus general toxicity, such as lethality manifestation, and producing specific types of effects (Wilson 1973; Schardein 1976). Therefore, the present study was conducted to further evaluate the adverse

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effects of TPTCI on the embryo-fetal development after a shorter duration of treatment during organogenesis.

MATERIALS AND METHODS

Wistar rats (Std: Wistar-KY, Japan SLC, Inc., Hamamatsu, Japan) were used throughout this study. Animals were maintained in an air-conditioned room at 24 ± 1 °C, with a relative humidity of 55 ± 5%, under a controlled 12-hr light/dark cycle. The rats were reared on a basal diet (F-1; Funabashi Farm Co., Funabashi, Japan) and tap water ad libitum. Virgin female rats, weighing 212-268 g, were mated overnight with male rats. The day when sperm were detected in the vaginal smear was considered to be day 0 of pregnancy. The pregnant rats were distributed on a random basis into 13 groups and housed individually. The pregnant rats were dosed once daily by gastric intubation with TPTCI (98% pure, Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) at 3.1, 6.3, 9.4 or 12.5 mg/kg on days 7-9 or at 6.3, 9.4 or 12.5 mg/kg on days 10-12 or days 13-15 of pregnancy. The dosage levels were determined based on the results of our previous study (Ema et al. 1997). TPTCI was dissolved in olive oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The volume of each dose was adjusted to 5 mL/kg of body weight. The control rats received olive oil only. All TPTCI solutions were prepared fresh daily.

Maternal body weight and food consumption were recorded daily. The pregnant rats were sacrificed by ether overdose on day 20 of pregnancy. The peritoneal cavity was opened and the number of live and dead fetuses and resorptions were recorded. The live fetuses were sexed, weighed and inspected for external malformations and malformations within the oral cavity. Approximately one-half of the live fetuses in each litter were randomly selected and fixed in alcohol, stained with alizarin red S (Kawamura et al. 1990) and examined for skeletal malformations. The remaining live fetuses in each litter were fixed in Bouin's solution, sectioned with a razor blade and examined for internal malformations (Wilson 1965). Statistical analysis of the offspring data was carried out using the litter as a sample unit. Analysis of variance and Dunnett's multiple comparison test, Kruskal-Wallis test and Mann-Whitney test or Fisher's exact test were used as appropriate.

RESULTS AND DISCUSSION

The maternal and reproductive findings after administration of TPTCI on days 7-9 of pregnancy are presented in Table 1. The pregnant rat given TPTCI had reddish staining of the facial fur, piloerection and/or diarrhea, and the occurrence of these clinical signs was dose-related. The body weight gains and food consumption on days 7-10 at 3.1 mg/kg and higher and on days 10-20 at 6.3 mg/kg and higher were significantly decreased. The adjusted weight gain, which indicated the net weight gain of pregnant rats, at 6.3 mg/kg and higher was significantly lowered. Totally resorbed litters were found in five of the 10 pregnant rats, in 10 of the 13 pregnant rats and eight of the 11 pregnant rats at 6.3, 9.4 and 12.5 mg/kg, respectively. A significant increase in the number of resorptions and dead fetuses and incidence of postimplantation losses and a significant decrease in the number of live fetuses were noted at 6.3 mg/kg and higher. There were no significant differences in the sex ratio and body weight of live fetuses between the TPTCI-treated and control groups. Table 2 shows the results of morphological examinations of fetuses of rats given TPTCI on days 7-9 of pregnancy. External examination revealed three fetuses with anury and anal atresia at 6.3 mg/kg. A few fetuses with deformities of the vertebral column and/or ribs were found in all groups, except for the 12.5 mg/kg group. Dilatation of the renal pelvis occurred in one fetus at 9.4 mg/kg and three fetuses at 12.5 mg/kg. However, no significant differences between the TPTCI-treated and control groups were found in the incidence of fetuses with external, skeletal and internal malformations and with skeletal variations and in the degree of ossification.

The maternal and reproductive findings after administration of TPTCI on days 10-12 of pregnancy are presented in Table 3. The pregnant rats given TPTCI showed reddish

Table 1. Maternal and reproductive findings in rats given TPTCl on days 7-9 of pregnancy

	TPTCl (mg/kg)				
	0 (control)	3.1	6.3	9.4	12.5
No. of pregnant rats	10	10	10	13	11
No. of dead pregnant rats	0	0	0	0	0
Initial body weight (g)[a]	241 ± 8	236 ± 13	240 ± 16	233 ± 12	236 ± 9
Weight gain during pregnancy (g)[a]					
Days 0-7	24 ± 8	26 ± 5	22 ± 6	24 ± 6	24 ± 5
Days 7-10	12 ± 2	4 ± 7†	-8 ± 3†	-16 ± 5†	-18 ± 6†
Days 10-20	85 ± 11	91 ± 8	46 ± 42*	35 ± 32†	40 ± 35†
Adjusted weight gain[b]	46 ± 8	48 ± 8	23 ± 9†	27 ± 9†	26 ± 8†
Food consumption during pregnancy (g)[a]					
Days 0-7	128 ± 18	136 ± 11	126 ± 12	122 ± 12	124 ± 12
Days 7-10	50 ± 7	33 ± 7†	13 ± 10†	12 ± 4†	13 ± 6†
Days 10-20	218 ± 14	236 ± 18	183 ± 34*	183 ± 28*	186 ± 35*
No. of implantations[a]	15.0 ± 1.1	14.5 ± 0.8	14.3 ± 1.6	13.3 ± 2.1	14.8 ± 1.5
No. of litters totally resorbed	0	0	5*	10†	8†
No. of resorptions and dead fetuses[a]	1.3 ± 0.9	1.8 ± 1.2	7.8 ± 6.3†	11.1 ± 4.2†	11.5 ± 4.8†
Postimplantation loss (%)[c]	8.8	12.4	56.8†	84.3†	80.0†
No. of live fetuses[a]	13.7 ± 1.5	12.7 ± 1.3	6.5 ± 7.0†	2.2 ± 4.3†	3.3 ± 5.7†
Sex ratio (male / female)	67/ 70	74 / 53	34 / 31	14 / 15	17 / 19
Body weight of live fetuses (g)[a]					
Male	4.0 ± 0.2	4.2 ± 0.2	3.9 ± 0.1	4.1 ± 0.1	3.7 ± 0.3
Female	3.8 ± 0.3	3.9 ± 0.3	3.7 ± 0.1	3.9 ± 0.1	3.7 ± 0.1

[a] Values are given as mean ± SD. [b] Adjusted weight gain refers to maternal weight gain excluding the gravid uterus.
[c] (No. of resorptions and dead fetuses / no. of implantations) x 100. *,† Significantly different from the control, $P < 0.05$ and $P < 0.01$, respectively.

Table 2. Morphological examinations of fetuses of rats given TPTCl on days 7-9 of pregnancy

	TPTCL (mg/kg)				
	0 (control)	3.1	6.3	9.4	12.5
External examination					
No. of fetuses (litters) examined	137 (10)	127 (10)	65 (5)	29 (3)	36 (3)
No. of fetuses (litters) with malformations	0	0	3 (2)	0	0
Anury and anal atresia	0	0	3 (2)	0	0
Skeletal examination					
No. of fetuses (litters) examined	71 (10)	66 (10)	34 (5)	16 (3)	19 (3)
No. of fetuses (litters) with malformations	1 (1)	2 (2)	2 (1)	1 (1)	0
Fusion of cervical vertebral arches	1 (1)	1 (1)	0	0	0
Absence of thoracic vertebral arches	0	0 1	(1) 1	(1) 0	
Absence of thoracic vertebral bodies	0	0	0	1 (1)	0
Absence of lumbar vertebral arches	0	1 (1)	1 (1)	0	0
Absence of lumbar vertebral bodies	0	0	1 (1)	0	0
Fusion of ribs	0	0	1 (1)	1 (1)	0
No. of fetuses (litters)with variations	10 (6)	8 (7)	4 (3)	4 (3)	4 (3)
Splitting of thoracic vertebral bodies	1 (1)	2 (2)	1 (1)	2 (1)	0
Splitting of lumbar vertebral bodies	0	1 (1)	0	0	0
Asymmetry of sternebrae	8 (6)	6 (6)	3 (2)	2 (2)	3 (3)
Splitting of sternebrae	3 (2)	1 (1)	0	1 (1)	1 (1)
Degree of ossification[a]					
No. of ossification centers of caudal vertebrae	5.2 ± 0.3	5.2 ± 0.5	5.3 ± 0.6	5.6 ± 0.1	5.2 ± 0.7
No. of sternebrae	5.8 ± 0.2	5.8 ± 0.1	5.7 ± 0.2	5.8 ± 0	5.7 ± 0.1
Internal examination					
No. of fetuses (litters) examined	66 (10)	61 (10)	31 (5)	13 (3)	17 (3)
No. of fetuses (litters) with malformations	0	0	0	1 (1)	3 (2)
Dilatation of renal pelvis	0	0	0	1 (1)	3 (2)

[a] Values are given as mean ± S. D.

staining of the facial fur, piloerection and/or diarrhea, and the incidence of these clinical signs was dose -related. The body weight gains and food consumption on days 10-13 at 6.3 mg/kg and higher and on days 13-20 at 12.5 mg/kg were significantly decreased. The adjusted weight gain at 6.3 and 12.5 mg/kg was significantly lowered. Totally resorbed litters were found in one of the 10 pregnant rats at 12.5 mg/kg. A significant increase in the number of resorptions and dead fetuses and incidence of postimplantation losses and a significant decrease in the number of live fetuses were noted at 9.4 and 12.5 mg/kg. No significant difference in the sex ratio of live fetuses was observed between the TPTCI-treated and control groups. A significant decrease in body weight of male and female fetuses was noted at 12.5 mg/kg. The results of morphological examinations of fetuses of rats given TPTCI on days 10-12 of pregnancy are presented in Table 4. Although one fetus with fusion of the cervical vertebral arches was observed at 6.3 mg/kg, no fetuses with external and internal malformations were found in any of the other groups. No significant differences in the incidence of fetuses with external, skeletal and internal malformations and with skeletal variations and in the degree of ossification were found between the TPTCI-treated and control groups.

Table 3. Maternal and reproductive findings in rats given TPTCI on days 10-12 of pregnancy

	TPTCI (mg/kg)			
	0 (control)	6.3	9.4	12.5
No. of pregnant rats	10	10	10	10
No. of dead pregnant rats	0	0	0	0
Initial body weight (g)[a]	237 ± 8	239 ± 16	236 ± 10	232 ± 10
Weight gain during pregnancy (g)[a]				
Days 0-10	37 ± 2	40 ± 5	47 ± 7†	39 ± 5
Days 10-13	13 ± 4	-5 ± 6†	-8 ± 7†	-16 ± 6†
Days 13-20	69 ± 12	69 ± 16	60 ± 12	41 ± 19†
Adjusted weight gain[b]	49 ± 5	33 ± 12†	46 ± 10	31 ± 9†
Food consumption during pregnancy (g)[a]				
Days 0-10	185 ± 27	214 ± 38	204 ± 14	190 ± 13
Days 10-13	52 ± 6	22 ± 7†	21 ± 6†	12 ± 3†
Days 13-20	164 ± 10	154 ± 11	172 ± 11	141 ± 20†
No. of implantations[a]	13.6 ± 2.3	15.0 ± 0.8	14.2 ± 1.1	13.8 ± 1.8
No. of litters totally resorbed	0	0	0	1
No. of resorptions and dead fetuses[a]	1.6 ± 1.2	2.5 ± 2.5	5.4 ± 3.1†	8.8 ± 3.0†
Postimplantation loss (%)[c]	11.5	16.9	39.7†	65.0†
No. of live fetuses[a]	12.0 ± 2.0	12.5 ± 2.9	8.6 ± 3.4*	5.0 ± 3.8†
Sex ratio of live fetuses (male / female)	59 / 61	57 / 68	37 / 49	24 / 26
Body weight of live fetuses (g)[a]				
Male	4.2 ± 0.2	3.9 ± 0.1	4.0 ± 0.3	3.3 ± 0.7†
Female	3.9 ± 0.2	3.7 ± 0.2	3.8 ± 0.3	3.5 ± 0.3†

[a] Values are given as mean ± SD. [b] Adjusted weight gain refers to maternal weight gain excluding the gravid uterus. [c] (No. of resorption and dead fetuses / no. of implantations) x 100.
*,† Significantly different from the control, $P < 0.05$ and $P < 0.01$, respectively.

The maternal and reproductive findings after administration of TPTCI on days 13-15 of pregnancy are presented in Table 5. The pregnant rats given TPTCI had reddish staining of the facial fur, piloerection and/or diarrhea, and the incidence of these clinical signs was directly related to dose. The body weight gains on days 13-15 at 6.3 mg/kg and higher and on days 16-20 at 12.5 mg/kg were significantly decreased. The adjusted weight gain at 6.3 mg/kg and higher was significantly lowered. Food consumption on days 13-15 at 6.3 mg/kg and higher and on days 16-20 at 9.4 and 12.5 mg/kg was significantly decreased. Totally resorbed litters were found in two of the 12 pregnant rats at 12.5 mg/kg. Significant increases in the number of resorptions and dead fetuses at 12.5 mg/kg and in the incidence of postimplantation losses at 9.4 and 12.5 mg/kg were found.

Table 4. Morphological examinations of fetuses of rats given TPTCl on days 10-12 or days 13-15 of pregnancy

Days of treatment	Days 10-12				Days 13-15			
TPTCl (mg/kg)	0 (control)	6.3	9.4	12.5	0 (control)	6.3	9.4	12.5
External examination								
No. of fetuses (litters) examined	120 (10)	125 (10)	86 (10)	50 (9)	126 (10)	123 (10)	115 (11)	95 (10)
No. of fetuses (litters) with malformations	0	0	0	0	0	4 (2)	3 (2)	0
Cleft palate	0	0	0	0	0	4 (2)	3 (2)	0
Skeletal examination								
No. of fetuses (litters) examined	62 (10)	65 (10)	45 (10)	29 (9)	64 (10)	65 (10)	59 (11)	51 (10)
No. of fetuses (litters) with malformations	0	1 (1)	0	0	0	1 (1)	0	0
Absence of cervical vertebral arches	0	0	0	0	0	1 (1)	0	0
Fusion of cervical vertebral arches	0	1 (1)	0	0	0	0	0	0
No. of fetuses (litters) with variations	9 (7)	8 (6)	4 (3)	3 (3)	3 (3)	8 (6)	10 (7)	15 (8)
Splitting of thoracic vertebral bodies	1 (1)	1 (1)	0	0	0	0	0	0
Lumbar ribs	0	1 (1)	0	0	0	0	0	0
Sift of lumbrosacral vertebral border	0	0	0	0	0	0	0	1 (1)
Asymmetry of sternebrae	7 (6)	6 (5)	4 (3)	3 (3)	1 (1)	3 (3)	4 (3)	5 (4)
Splitting of sternebrae	2 (2)	2 (2)	1 (1)	0	3 (3)	7 (5)	7 (5)	11 (7)
Degree of ossification[a]								
No. of ossification centers of caudal vertebrae	5.4 ± 0.2	5.6 ± 0.4	5.6 ± 0.5	5.0 ± 0.6	5.4 ± 0.4	5.8 ± 0.8	5.3 ± 0.6	5.3 ± 0.9
No. of sternebrae	5.9 ± 0.2	5.8 ± 0.3	5.7 ± 0.3	5.3 ± 0.9	5.9 ± 0.1	5.7 ± 0.5	5.8 ± 0.3	5.8 ± 0.2
Internal examination								
No. of fetuses (litters) examined	58 (10)	60 (10)	41 (10)	21 (8)	62 (10)	58 (10)	56 (11)	44 (10)
No. of fetuses (litters) with malformations	0	0	0	0	0	0	0	0

[a] Values are given as mean ± S. D.

A significant decrease in the number of live fetuses was noted at 12.5 mg/kg. No significant difference in the sex ratio of live fetuses was observed between the TPTCI-treated and control groups. Significantly lowered body weights of male and female fetuses were found at 9.4 and 12.5 mg/kg. The results of morphological examinations of fetuses of rats given TPTCI on days 13-15 of pregnancy are presented in Table 4. Cleft palate occurred in four fetuses at 6.3 mg/kg and three fetuses at 9.4 mg/kg. Absence of the cervical vertebral arches was observed in one fetus at 6.3 mg/kg. However, no significant differences between the TPTCI-treated and control groups were found in the incidence of fetuses with external, skeletal and internal malformations and with skeletal variations and in the degree of ossification.

Table 5. Maternal and reproductive findings in rats given TPTCI on days 13-15 of pregnancy

	TPTCI (mg/kg)			
	0 (control)	6.3	9.4	12.5
No. of pregnant rats	10	10	11	12
No. of dead pregnant rats	0	0	0	0
Initial body weight (g)[a]	238 ± 10	239 ± 8	246 ± 8	240 ± 14
Weight gain during pregnancy (g)[a]				
Days 0-13	53 ± 9	53 ± 6	53 ± 7	53 ± 8
Days 13-16	21 ± 4	-1 ± 7†	-6 ± 8†	-9 ± 6†
Days 16-20	45 ± 8	39 ± 17	27 ± 13	11 ± 34†
Adjusted weight gain[b]	48 ± 10	19 ± 13†	15 ± 8†	8 ± 23†
Food consumption during pregnancy (g)[a]				
Days 0-13	256 ± 12	254 ± 12	256 ± 15	259 ± 17
Days 13-16	59 ± 5	20 ± 11†	16 ± 5†	17 ± 7†
Days 16-20	85 ± 6	78 ± 18	63 ± 12†	61 ± 20†
No. of implantations[a]	14.2 ± 2.1	14.6 ± 1.1	14.6 ± 1.2	15.1 ± 1.5
No. of litters totally resorbed	0	0	0	2
No. of resorptions and dead fetuses[a]	1.6 ± 1.7	2.2 ± 2.0	4.2 ± 2.4	7.2 ± 4.9†
Postimplantation loss (%)[c]	11.0	16.3	28.0†	44.7†
No. of live fetuses[a]	12.6 ± 2.5	12.3 ± 2.6	10.5 ± 1.9	7.9 ± 5.1†
Sex ratio of live fetuses (male / female)	59 / 67	59 / 64	48 / 67	49 / 46
Body weight of live fetuses (g)[a]				
Male	4.1 ± 0.2	3.9 ± 0.4	3.6 ± 0.4†	3.3 ± 0.4†
Female	4.0 ± 0.1	3.7 ± 0.4	3.4 ± 0.5†	3.2 ± 0.4†

[a] Values are given as mean ± SD. [b] Adjusted weight gain refers to maternal weight gain excluding the gravid uterus. [c] (No. of resorption and dead fetuses / no. of implantations) x 100.
† Significantly different from the control, $P < 0.01$.

The fetuses of the dams exposed to TPTCI during the earlier stages seem to have time to begin gaining weight again because the dam has recovered from the toxic effects of TPTCI and is eating again. The fetuses exposed to TPTCI during the later stages continue to be affected by the decreased food consumption of the dams because it was more recent. The malformations observed in the present study are not thought to be due to the administration of TPTCI, because the incidence of these malformations was very low and these are typical of malformations seen in full-term rat fetuses (Kameyama et al. 1980; Morita et al. 1987). No consistent trend was observed in the incidence of skeletal variations. Skeletal variations are frequently observed in rat fetuses at term (Kimmel and Wilson 1973; Kameyama et al. 1980; Morita et al. 1987). Therefore, the data of the present study do no indicate a teratogenic response and suggest that TPTCI possesses no teratogenic potential in rats. In the present study, TPTCI was found to induce postimplantation embryonic loss, the incidence decreased as organogenesis proceeded and the highest incidence was found in pregnant rats given TPTCI on days 7-9 of pregnancy, the period shortly after implantation. In our previous study (Ema et al., 1997), implantation failure, viz., preimplantation embryonic loss was caused in rats after

administration of TPTCI on days O-3 of pregnancy, the period before implantatio, or days 4-6 of pregnancy, the period when implantation is in progress. The embryos appear to be susceptible to lethal effects of TPTCI during two periods of development: 1) prior to completion of implantation and 2) shortly after implantation. Embryonic loss induced by chemicals may be mediated by a variety of mechanisms. There is the possibility that embryolethal effects may result from direct effects of chemicals on embryos and/or indirect effects perturbing homeostasis in the dams. Normal reproductive function in females involves the appropriate interaction of the central nervous system, ovary, and uterus, and the toxic effects in these sites can affect the embryonic survival. The function of the uterine endometrium is one of the most principle factors for embryonic survival, especially during early pregnancy. Abnormal decidual formation may be involved. Further studies are presently underway in this direction.

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