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Evaluation of Malnutrition as a Cause of Tributyltin-Induced Pregnancy Failure in Rats

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Organotin compounds are a broad-group of chemicals widely used in agriculture and industry (Piver 1973; WHO 1980; Maguire 1991; Fait et al. 1994). Scientific interest has focused on tributyltin (TBT) because it is brought directly into the environment by its use as an antifouling agent in paints. The ubiquitous presence of TBT has been shown in the aquatic environment (Maguire et al.1986; Maguire 1991). Food chain bioamplification of TBT has been demonstrated in oysters, *Crassostrea gigas* (Waldock and Thain 1983), mud crabs, *Rhithropanopeus harrisii* (Evans and Laughlin 1984) marine mussels, *Mytilus edulis* (Laughlin et al. 1986) and chinook salmon, *Oncorhyncus tshawytscha* (Short and Thrower 1986). The possible exposure of humans to TBT has aroused concern about the toxic potential of TBT.

The adverse effects of triorganotin compounds on reproduction have been reported. TBT is known to introduce imposex (imposition of male sex characters onto the female) in dog-whelk, *Nucella lapillus* (Gibbs and Bryan 1986). Triphenyltin acetate and triphenyltin chloride produced degenerative changes in the ovarian tissue of rats, indicating decreased ovulation potential and fertility (Newton and Hays 1968). Winek et al. (1978) reported that triphenyltin hydroxide exhibited antifertility effects in female rats after oral administration on days 1-7 of pregnancy. We have previously reported that tributyltin chloride (TBTCI) on days 0-7 of pregnancy or on days 0-4 of pregnancy produce a significant and dose-dependent reduction in fertility in rats (Harazono et al. 1996, 1998). The antifertility effect, however, was found at doses that also produced maternal toxicity such as reductions in body weight gain and feed consumption. The present study was conducted to examine whether pregnancy failure observed in our previous study (Harazono et al. 1996) was the result of the direct effect of TBTCI on the reproductive system or because of malnutrition from reduced feed consumption.

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MATERIALS AND METHODS

Wistar rats (Jcl:Wistar, Clea Co., Ltd., Tokyo, 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-h 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 of about 12 weeks of age were mated overnight with male rats from the same supplier. The day when sperm was detected in the vaginal smear was considered to be day 0 of pregnancy. The pregnant rats were distributed on a random basis into three groups (control, feed-restricted and TBTCI -treated groups) and housed individually.

The sperm-positive female rats were dosed once daily by gastric intubation with TBTCI (96% pure, Aldrich Chemical Co., Inc., Milwaukee, WI, USA) in olive oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at a dose of 16.3 mg/kg (50 μmol/kg) on day 0 through day 7 of pregnancy. Dose level was determined based on the previous study in which administration of TBTCI at 16.3 mg/kg on day 0 through 7 of pregnancy produced a significant and marked increase in pregnancy failure (Harazono et al. 1996). The volume of each dose was adjusted to 5 ml/kg body weight based on daily body weight. The rats in the control and feed-restricted groups received an equivalent amount of olive oil. TBTCI solutions were prepared fresh daily. The feed-restricted pregnant rats were given an amount of feed equal to that consumed by nonpregnant females in the TBTCI-treated group from day 0 through day 8 of pregnancy, i.e. the rats were given 4 g diet on day 0, 1 g diet on day 1.0 g diet on day 2 through day 7 and 8 g diet on day 8, and were fed ad libitum from day 9 through day 20 of pregnancy. Maternal body weight, feed consumption and evidence of clinical signs of toxicity were recorded daily. The females were over anesthetized with diethyl ether on day 20 of pregnancy. The peritoneal cavity and uterus were opened, and the numbers of live and dead fetuses and resorptions were recorded. The uteri were placed in 2% sodium hydroxide for confirmation of the dam's pregnancy status (Yamada et al. 1985). The live fetuses removed from the uterus were sexed and weighed.

The initial body weight, body weight gain, adjusted weight gain, feed consumption, and the number of corpora lutea, implantations and live fetuses were evaluated by analysis of variance and Dunnett's multiple comparison test. Incidence of pre- and post-implantation loss were analyzed using Kruskal- Wallis test and Mann-Whitney test. Pregnancy rate and

sex ratio of live fetuses were analyzed using Fisher's exact test. The 0.05 level of probability was used as the criterion for significance.

RESULTS AND DISCUSSION

No female died during the experiment. Rats given TBTCI showed lethargic and had chromodacryorrhea around the nose and eyes, and diarrhea. Body weight on days O-8 in the feed-restricted group was similar to that in the TBTCI-treated group (Fig. 1). Maternal body weight gain on days 0-8 in the TBTCI-treated and feed-restricted groups was significantly lower than the control group (Table 1). Maternal body weight gain on days 8-20 in the TBTCI-treated group was significantly lower than that in the control and feed-restricted groups. The adjusted weight gain, which indicates the net weight gain, in the TBTCI-treated group was similar to that in the feed-restricted group, and significantly lower than the control group. Feed consumption on days 0-8 in the TBTCI-treated group was significantly lower than that in the control group. A significantly increased food consumption on days 8-20 in the feed-restricted group was found when compared to the control and the TBTCI-treated groups.

All inseminated females in the control group became pregnant (Table 2). Pregnancy failure, which was evidenced by absence of implantation sites, was found in 3 of the 15 inseminated females in the feed-restricted group and 11 of the 13 inseminated females in the TBTCI-treated group. The rate of pregnancy failure in the TBTCI-treated group was significantly higher than that in the control and feed-restricted groups, while that in the feed-restricted group was not significantly different from the control. Total resorption of all implantations was not found in any female. The incidence of post-implantation loss in the feed-restricted group was significantly higher than that in the control group. The numbers of implantations and live fetuses per litter and body weight of male and female fetuses in the feed-restricted group were significantly lower than the control values. The numbers of corpora lutea, implantations, live fetuses and pre- and post-implantation loss in the TBTCI-treated group are comparable to control values. There was no significant difference in the incidence of pre-implantation loss in the pregnant rats among all groups. The body weights of male and female fetuses in the TBTCI-treated group were significantly lighter than those in the control and feed-restricted groups. It may indicate that TBTCI during early pregnancy also affects embryo-fetal growth. The sex ratio of live fetuses was comparable across all groups.

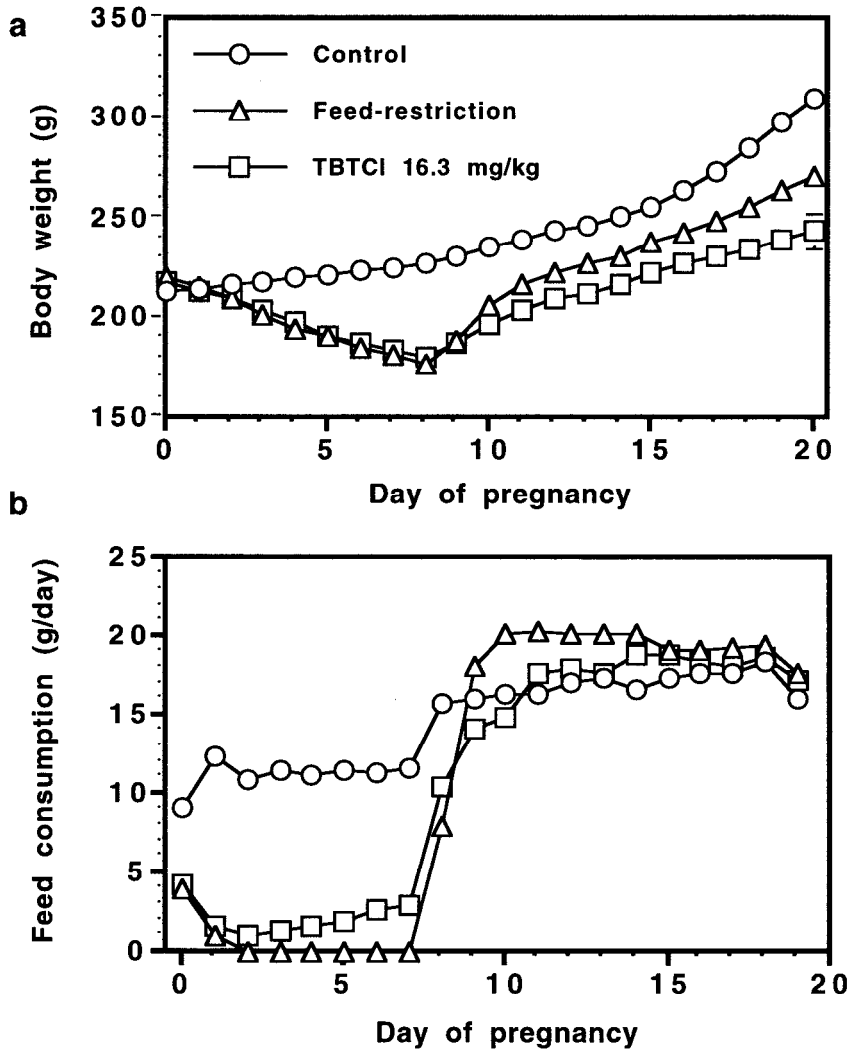

Figure 1. Body weight (a) and feed consumption during pregnancy in rats fed ad libitum (Control or TBTCl) or restricted amounts of lab chow.

Table 1. Effects of TBTCl on body weight and feed consumption

Group	Control	Feed-restricted	TBTCl 16.3 mg/kg
Initial body weight[a]	214 ± 8	221 ± 10	219 ± 7
Body weight gain (g)[a]			
Days 0-8	15 ± 6	-43 ± 5**	-37 ± 21**
Days 8-20	81 ± 11	93 ± 22	63 ± 12*††
Adjusted body weight gain[a,b]	29 ± 9	21 ± 10	17 ± 13*
Feed consumption (g)[a]			
Days 0-8	90 ± 11	5	17 ± 21**
Days 8-20	202 ± 17	222 ± 18*	204 ± 21†

[a]Values are given as means ± SD.

[b]Adjusted body weight gain refers to maternal body weight gain excluding the uterus.

*,**Significantly different from the control group, $P < 0.05$ and $P < 0.01$, respectively.

†,††Significantly different from the feed-restricted group, $P < 0.05$ and $P < 0.01$, respectively.

Table 2. Effects of TBTCl on reproductive parameters

Group	Control	Feed-restricted	TBTCl 16.3 mg/kg
No. inseminated females	11	15	13
No. nonpregnant	0	3	11**††
% Pregnancy failure[a]	0	20	84.6**††
No. litters	11	12	2
No. litters totally resorbed	0	0	0
No. corpora lutea per litter[b]	15.0 ± 1.3	14.3 ± 1.2	16.0 ± 0.0
No. implantations per litter[b]	14.4 ± 1.3	12.9 ± 1.5*	14.5 ± 0.7
No. pre-implantation loss per litter[b]	0.6 ± 0.9	1.4 ± 1.0	1.5 ± 0.7
Pre-implantation loss per litter (%)[b,c]	4.2 ± 5.8	9.9 ± 7.2	9.4 ± 4.4
No. post-implantation loss per litter[b]	1.5 ± 0.8	6.0 ± 2.6**	0.5 ± 0.7††
Post-implantation loss per litter (%)[b,d]	10.1 ± 6.3	46.5 ± 20.8**	3.4 ± 4.7††
No. live fetuses per litter[b]	12.9 ± 1.8	6.9 ± 3.0*	14.0 ± 0.0††
Sex ratio of live fetuses (male/female)	75 / 67	45 / 38	13 / 15
Body weight of live fetuses (g)[b]			
Male	3.38 ± 0.15	2.97 ± 0.28**	2.02 ± 0.83**††
Female	3.20 ± 0.13	2.64 ± 0.25**	1.83 ± 0.74**††

[a](No. nonpregnant rats /no. inseminated rats) X 100.

[b]Values are given as means ± SD.

[c](No. pre-implantation loss /no. corpora lutea) X 100.

[d](No. post-implantation loss /no. implantations) X 100.

*,**Significantiy different from the control group, $P < 0.05$ and $P < 0.01$, respectively.

††Significantly different from the feed-restricted group $P < 0.01$.

Some reports on the effects of feed-restriction during early pregnancy on female reproduction are available. Reproductive outcome of inseminated female rats that were subjected to 75% feed-restriction on days 0-7 of pregnancy, followed by ad libitum feeding, was similar to that of females fed ad libitum (Berg 1965). Feeding 40% of control feed consumption on days 0-7 did not affect on the implantation process and embryonic survival in rats (Ema et al. 1991). Pregnant rats, which were given a restricted diet and maintained at 70% of control body weight, showed no significant changes in pre- and post-implantation embryonic loss (Chapin et al. 1993). However, the relationship between maternal alteration induced by decreased feed consumption during pregnancy and embryo-fetal development still remains a critical issue in toxicity studies. In order to determine whether reduced body weight gain and feed intake during early pregnancy can cause pregnancy failure, feed-restricted females were given an amount of feed nearly equal to the feed consumption of rats which did not achieve pregnancy in the TBTCI-treated group in this study. A higher incidence of post-implantation loss and reduced number of live fetuses per litter were observed, but no significant increase in the rate of the pregnancy failure was noted in feed-restricted females. It seems that severely reduced feed intake and weight gain during early pregnancy cause postimplantation embryonic loss more than pregnancy failure. Consideration of these findings together suggests that pregnancy failure observed in the TBTCI-treated females was due to the effects of TBTCI but not due to the malnutrition or reduced body weight from reduced feed consumption during early pregnancy.

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