# PRE- AND POST-IMPLANTATION EMBRYONIC LOSS INDUCED BY DIBUTYLTIN GIVEN TO MICE DURING EARLY PREGNANCY

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**Introduction**

Organotin compounds are chemicals widely used in agriculture and industry. 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 forming of the resin into its final products, as catalysts in the production of polyurethane foams, and as vulcanizing agents for silicone rubbers.[1,2] The identification of dibutyltin (DBT) and tributyltin (TBT) in aquatic marine organisms[3,4] and marine products[5] has been reported. The dietary exposure of Japanese consumers to organotin compounds was reported that daily intake was 1.7 μg/person for TBT, 0.45 μg/person for DBT, 0.09 μg/person for triphenyltin, and 0 μg/person for diphenyltin.[6]

We previously reported that dibutyltin dichloride (DBTCl) by gavage throughout the period of organogenesis resulted in a significant increase in the incidence of fetal malformations in rats[7] and that rat embryos were highly susceptible to the teratogenic effects of DBTCl when administered on day 7 and day 8 of pregnancy.[8] The developmental toxicity studies on butyltins suggest that the teratogenicity of DBT is different from those of tetrabutyltin (TeBT), TBT and monobutyltin (MBT) in its mode of action, because the susceptible period for teratogenicity and types of fetal malformations induced by DBT are different from those induced by TeBT, TBT and MBT.[9,10] Tributyltin chloride (TBTCl)[11-13] and DBTCl[14-15] during early pregnancy produced reproductive failure in rats. Predominant adverse effects on reproduction and development of TBTCl and DBTCl on days 0-3 of pregnancy were decrease in the pregnancy rate and increase in the incidence of pre-implantation embryonic loss, and TBTCl and DBTCl on days 4-7 of pregnancy mainly caused post-implantation embryonic loss.[13-15] The doses of DBTCl that caused early embryonic loss were lower than those of TBTCl.[15] The possibility exists that DBTCl and/or metabolites participate in the induction of early embryonic loss due to TBTCl. Although the reproductive and developmental toxicity of DBTs was extensively investigated in rats[16], we are unaware of any studies in which the adverse effects of DBT on initiation and maintenance of pregnancy have been assessed in mice. Studies in mice would be of great value in evaluating reproductive and developmental toxicity of DBT. The present study was therefore conducted to determine the adverse effects of maternal exposure to DBTCl during early pregnancy on reproduction and development in mice.

**Materials and Methods**

Crj:CD1(ICR) mice were used throughout this study. Female mice were caged with male mice and checked the following morning for signs of successful mating by examining vaginal plugs. The day when vaginal plugs were detected was considered to be day 0 of pregnancy. Successfully mated females were distributed into eight groups of 12 mice each and housed individually. DBTCl was dissolved in olive oil. The female mice were dosed once daily by gastric intubation with DBTCl at 7.6, 15.2 or 30.4 mg/kg on days 0-3 or days 4-7 of pregnancy. The dosage levels were determined based on the results of our previous studies in which increases in the incidence of pre- and post-implantation embryonic loss were caused in female rats gavaged with DBTCl at 7.6 mg/kg and higher on days 0-3 and days 4-7 of pregnancy, respectively.[14,15] The volume of each dose was adjusted to 5 ml/kg of body weight based on daily body weight. The control mice received olive oil only on days 0-3 or days 4-7 of pregnancy. The female mice were euthanized on day 18 of pregnancy, and pregnancy outcome was determined. Using other successfully mated females, blood samples for measurement of serum progesterone levels were collected on day 4 or day 8 of pregnancy, 24 hours after the last administration of DBTCl at 0 or 30.4 mg/kg on days 0-3 or days 4-7 of pregnancy. The statistical analysis of fetuses was carried out using the litter as the experimental unit.

**Results and Discussion**

The earlier administration period, days 0-3 of pregnancy, corresponds to the period before implantation, and the later administration period, days 4-7 of pregnancy, corresponds to the period when implantation is in progress and the period shortly after implantation in mice.[17]

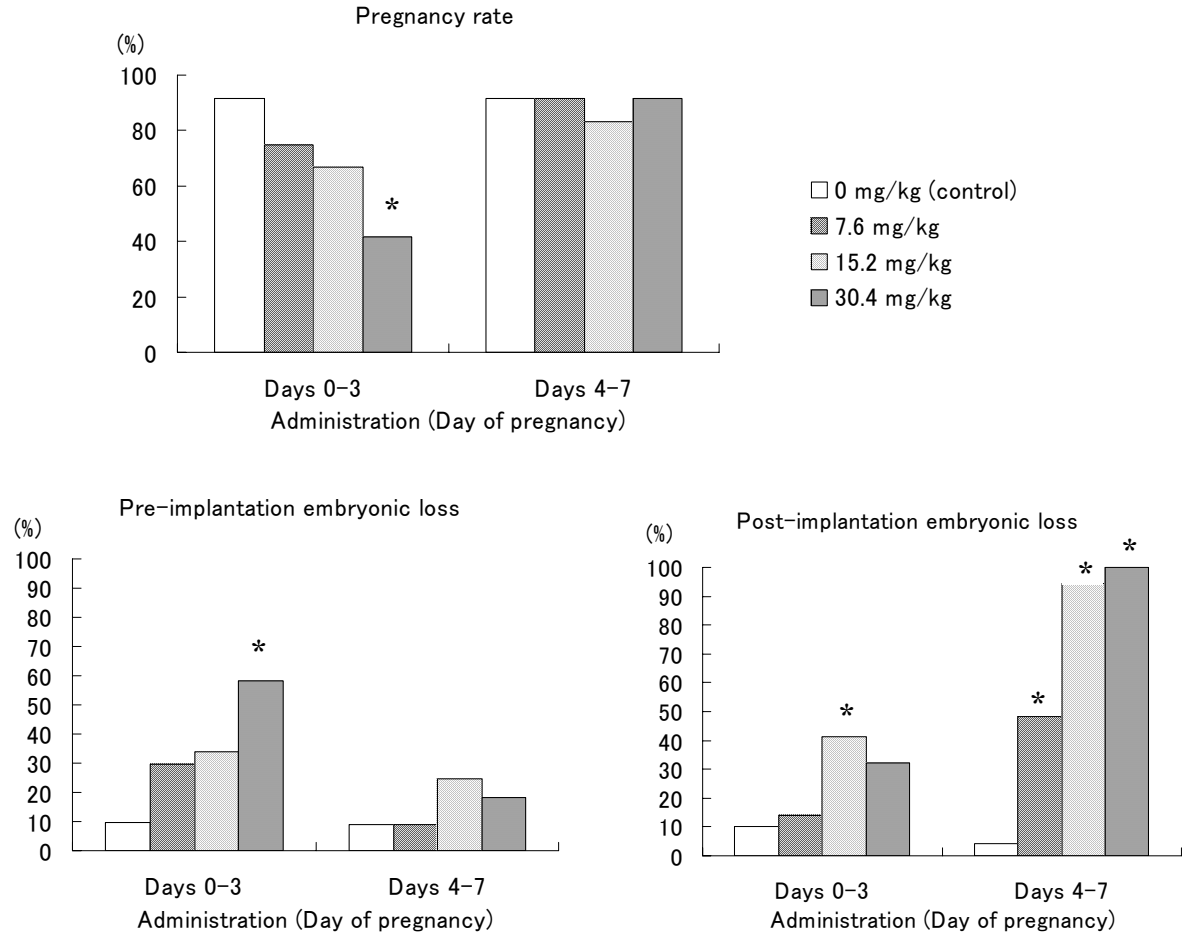


Fig. 1. Pregnancy outcome in mice given DBTCl on days 0-3 or days 4-7 of pregnancy. Pregnancy outcome was determined on day 18 of pregnancy. Pregnancy rate (%) = (No. of pregnant females/no. of females successfully mated) x 100. Pre-implantation embryonic loss (%) = [(No. of corpora lutea – no. of implantations)/no. of corpora lutea] x 100. Post-implantation embryonic loss (%) = (No. of resorptions and dead fetuses/no. of implantations) x 100. Values are given as the mean. *Significantly different from the control group, $p < 0.05$.

Pregnancy outcome in mice given DBTCl on days 0-3 of pregnancy is presented in Fig. 1. The pregnancy rate was significantly decreased and the incidence of pre-implantation embryonic loss was significantly increased in females successfully mated at 30.4 mg/kg. In pregnant females survived until scheduled sacrifice, the incidence of post-implantation embryonic loss was significantly increased at 15.2 mg/kg. A significantly lower fetal weight was found in males at 7.6 mg/kg and in both sexes at 15.2 and 30.4 mg/kg. The most striking adverse effect of DBTCl on reproduction and development was decreased pregnancy rate, complete pre-implantation embryonic loss, when DBTCl was given to female mice on days 0-3 of pregnancy. These findings suggest that

DBTCl adversely affects pre-implantation embryos and also later survival and growth of embryos/fetuses when administered during the pre-implantation period.

Pregnancy outcome in mice given DBTCl on days 4-7 of pregnancy is also presented in Fig. 1. No significant decrease in the pregnancy rate was noted in any DBTCl-treated group. In females successfully mated, the number of implantations was significantly decreased at 15.2 mg/kg. In pregnant females survived until scheduled sacrifice, litters totally resorbed was found in eight of the nine females at 15.2 mg/kg and ten of the ten females at 30.4 mg/kg. At 30.4 mg/kg, no live fetuses were obtained. A significant increase in the number and incidence of post-implantation embryonic loss, and decrease in the number of live fetuses were found in the DBTCl-treated groups. The weights of male and female fetuses were significantly lowered at 15.2 mg/kg. Predominant adverse effect of DBTCl on reproduction and development was litters totally resorbed, complete post-implantation embryonic loss, when DBTCl was given to mice on days 4-7 of pregnancy. These findings suggest that DBTCl has toxic effects on later survival and growth of embryos/fetuses when administered during the peri-implantation period. Considered collectively, the data indicate that DBTCl during early pregnancy adversely affects initiation and maintenance of pregnancy in mice, and the manifestation of adverse effects of DBTCl on reproduction and development varies with the stages of pregnancy at the time of maternal exposure.

Serum progesterone levels in female mice after administration of DBTCl on days 0-3 or days 4-7 of pregnancy are shown in Fig. 2. Significantly reduced levels of serum progesterone were noted in female mice given DBTCl at 30.4 mg/kg on days 0-3 or days 4-7 of pregnancy.

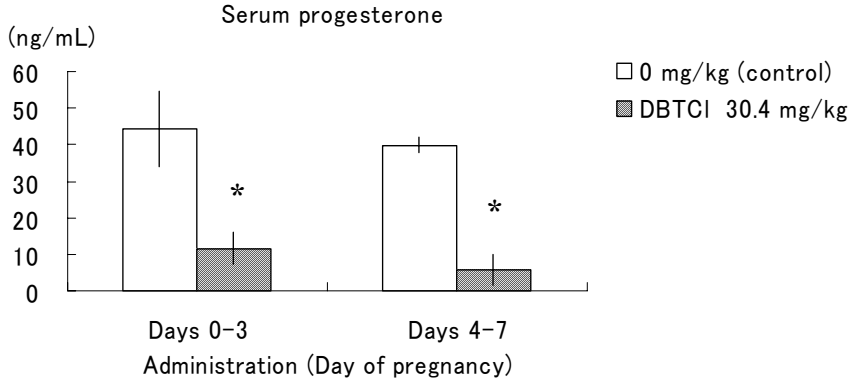


Fig. 2. Serum progesterone levels in female mice given DBTCl on days 0-3 or days 4-7 of pregnancy. Blood samples were collected on day 4 or day 8 of pregnancy, 24 hours after the last administration of DBTCl. Values are given as the mean ± S.E.M. *Significantly different from the control group, $p < 0.05$.

In our previous studies, significant increases in the incidences of pre- and post-implantation embryonic loss in rats were observed after administration of DBTCl on days 0-3 and days 4-7 of pregnancy, respectively.[14, 15] The uterine decidualization was suppressed and serum progesterone levels were reduced in female rats given DBTCl on days 0-3 or days 4-7 of pseudopregnancy.[18] Administration of progesterone reversed the suppression of uterine decidualization in pseudopregnant rats.[18] Based on these findings, we hypothesized that the decline in serum progesterone levels was a primary mechanism for the early embryonic loss due to DBTCl in rats. Furthermore, we showed that lowered reproductive and developmental parameters in pregnant rats given DBTCl were recovered by the administration of progesterone, and the values of reproductive and developmental parameters in pregnant rats given DBTCl in combination with progesterone were comparable to those in the control females and females give progesterone alone.[19] These findings indicated that progesterone protected

against the DBTCl-induced reproductive failure and supported our hypothesis. In the present study, early embryonic loss and decline in serum progesterone levels were detected after administration of DBTCl during early pregnancy in mice. There is a similarity in the effects of DBTCl on reproduction and development and on progesterone levels during early pregnancy in rats and mice, and these suggest that the decline in the serum progesterone levels is also the factor responsible for the early embryonic loss induced by DBTCl in mice.

**Acknowledgements**
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