

0 to 3 at 16.3 mg/kg and higher and on days 4 to 7 at 65.1 mg/kg caused a decrease in pregnancy rate and an increase in preimplantation embryonic loss. TBTCI on days 4 to 7 of pregnancy caused a significant increase in the incidence of postimplantation loss at 16.3 mg/kg and higher. The results show that the manifestation of adverse effects of TBTCI varies with gestational stage at the time of maternal exposure, and that TBTCI during the preimplantation period causes implantation failure, while TBTCI during the periimplantation period adversely affects the viability of implanted embryos.

Female Wistar rats were given TBTCI by gavage on days 0 to 3 or on days 4 to 7 of pseudopregnancy, and the effects of TBTCI on the uterus, as a cause of implantation failure, were evaluated according to the same procedures described above. After administration of TBTCI on days 0 to 3 of pseudopregnancy, a decrease in the uterine weight was detected at 16.3 mg/kg and higher (Harazono and Ema 2000). Decreased levels of serum progesterone occurred on day 9 at 16.3 mg/kg and higher and on day 4 at 8.1 mg/kg and higher, and increased levels of serum estradiol at 32.5 mg/kg were observed after administration on days 0 to 3. Following administration of TBTCI on days 4 to 7 of pseudopregnancy, uterine weight and serum progesterone levels on day 9 decreased at 16.3 mg/kg and higher. The doses that induced decreases in uterine weight and serum progesterone levels in pseudopregnant rats are consistent with those that induced pre- and postimplantation loss in pregnant rats. These results indicate that TBTCI suppresses uterine decidualization correlated with a reduction in serum progesterone levels, and suggest that the decline in uterine decidualization and serum progesterone levels participate in the induction of implantation failure induced by TBTCI.

TBT compound is reported to be metabolized to di- and MBT derivatives, and DBT was metabolized to MBT in rats (Fish et al. 1976, Kimmel et al. 1977, Ishizaka et al. 1989, Iwai et al. 1981). The adverse effects of dibutyltin dichloride (DBTCI) on the implantation and maintenance of pregnancy, and the role of DBT in the reproductive toxicity of TBT were evaluated after maternal exposure during the pre- or periimplantation period (Ema and Harazono 2000). Female Wistar rats were given DBTCI by gastric intubation at 3.8, 7.6, or 15.2 mg/kg on days 0 to 3 or on days 4 to 7 of pregnancy. The pair-feeding study was also performed. After administration of DBTCI on days 0 to 3, the pregnancy rate in the 7.6 mg/kg group was lower than in the control group, and that in the 15.2 mg/kg group was lower than in the control and pair-fed groups. The incidence of postimplantation embryonic loss in the groups given DBTCI on days 4 to 7 at 7.6 and 15.2 mg/kg was higher than in the control and pair-fed groups. Early embryonic loss was considered to be due to the effects of DBTCI, not to maternal malnutrition from reduced feed consumption, and the lowest dose of DBTCI inducing early embryonic loss was conservatively estimated at 7.6 mg (25 μ mol)/kg. An increase in the incidence of implantation failure was observed after administration of TBTCI, the parent compound of DBTCI, at 16.3 mg (50 μ mol)/kg and higher on days 0 to 3 and on days 4 to 7 of pregnancy,

respectively. The doses of DBTCI that caused early embryonic loss were lower than those of TBTCl (Harazono et al. 1998b). Thus, it is likely that DBTCI and/or its metabolites can be considered the agents responsible for early embryonic loss induced by TBTCl. Suppression of uterine decidualization accompanied by reduced levels of serum progesterone was found in pseudopregnant rats given DBTCI at doses that caused implantation failure (Harazono and Ema 2003), and administration of progesterone protected, at least in part, against the DBTCI-induced implantation failure (Ema et al. 2003). These results suggest that the decline in progesterone levels is a primary mechanism for the implantation failure due to DBTCI. Administration of butyltin trichloride (MBTCI) on days 0 to 3 or on days 4 to 7 of pregnancy did not cause pre- or postimplantation loss, even at 903 mg (equivalent to 3200 μmol)/kg in Wistar rats (Ema and Harazono 2001). It is unlikely that MBTCI and/or metabolites are actively involved in the early embryonic loss due to butyltins. The dose levels of DBTCI that suppressed the DCR were lower than the effective doses of TBTCl on a molar base. The similarity of effects and equivalent or greater effectiveness of DBTCI may suggest that DBTCI participates in the inhibition of DCR and in the decrease in serum progesterone levels associated with TBTCl. Although increased levels of serum estradiol on day 9 of pseudopregnancy was observed in rats given TBTCl on days 0 to 3 (Harazono and Ema 2000), administration of DBTCI did not affect serum estradiol levels. Thus, the mechanisms of TBTCl and DBTCI adversely affecting ovarian function might be different. Further studies are needed to determine the effects of TBTCl and DBTCI on the maternal endocrine system, including ovarian function.

Summary of Reproductive Toxicity of Butyltin Compounds

In a rat two-generation reproductive toxicity study, TBTCl affected the male and female reproductive system. TBTCl caused decreases in weight of the testis, epididymis, and ventral prostate, and spermatid and sperm counts in male offspring. The serum estradiol levels decreased in male offspring, but serum levels of luteinizing hormone and testosterone did not decrease. Total number and average body weight of pups, and live birth index decreased. Delayed vaginal opening and impaired estrous cyclicity were found in female offspring. The AGD increased even at 0.4 mg/kg in female offspring. TBTCl during early pregnancy caused implantation failure in rats. Implantation failure due to TBTCl may be mediated via the suppression of uterine decidualization and correlated with the reduction in serum progesterone levels. Implantation failure was also observed following administration of DBTCI, at lower doses than TBTCl, during early pregnancy. Suppression of uterine decidualization, accompanied by reduced levels of serum progesterone, was also observed in pseudopregnant rats given DBTCI at doses that induced implantation failure. Administration of progesterone protected, at least in part, against the DBTCI-induced implantation failure. Administration of MBTCI during early pregnancy did not cause pre- or postimplantation

loss even at 903 mg/kg. These results suggest that DBT may be responsible for the TBT-induced implantation failure, and that the decrease in serum progesterone levels may be a primary factor in implantation failure due to butyltins.

Developmental Toxicity of Butyltin Compounds

In Vivo Developmental Toxic Effects of Butyltin Compounds

Studies on developmental toxicity of butyltins are shown in Table 3.4. Several studies concerning the developmental toxicity of TBTO have been conducted in mice and rats. Davis et al. (1987) reported that an increased incidence of resorptions, reduced fetal weight, and an increased incidence of cleft palate were accompanied by a marked decrease in maternal weight gain after administration of TBTO by gavage to NMRI mice on days 6 to 15 of pregnancy. TBTO at 11.7 mg/kg was the lowest dose resulting in reduced maternal weight with no indication of decreases in litter size and fetal weight. At 35 mg/kg, the incidence of resorptions was 59% and fetal weight was markedly lowered. Doses lower than 11.7 mg/kg did not cause clear-cut teratogenic effects, and the incidences of cleft palate were 7% at 11.7 mg/kg and 48% at 35 mg/kg. They concluded that cleft palate might be a nonspecific toxic effect and not a teratogenic effect of TBTO. Swiss albino mice received TBTO by gavage at on days 6 to 15 of pregnancy (Baroncelli et al. 1990, 1995). In the prenatal study, a decrease in maternal weight gain and fetal weight were found, along with high embryoletality, but no increased incidence of fetal malformations were found at 40 mg/kg (Baroncelli et al. 1990). In the postnatal study, reduced litter size and pup weight at 20 mg/kg and higher, increased percentage of dams that had not built a nest at 10 mg/kg and higher, and decreased maternal weight gain and increased number of early or late deliveries at 5 mg/kg were detected. No malformations in pups were observed (Broncelli et al. 1995). Nonspecific alterations of hematological parameters and thymus or spleen weights were noted in dams and offspring of Swiss mice after administration of TBTO by gavage at 5, 10, or 20 mg/kg on days 6 to 15 of pregnancy (Karrer et al. 1995). A high incidence of cleft palate (11.4 percent) was found at 27 mg/kg in Han:NMRI mice given TBTO by gavage on days 6 to 17 of pregnancy (Faqi et al. 1997). At this dose, two fetuses exhibited a bent radius, eight fetuses were observed with a short mandible, and five fetuses showed a fusion of the occipital bones with their basal parts. In this study, no signs of toxicity in maternal and fetal mice were detected up to the dose of 13.5 mg/kg. Long Evans rats were given TBTO by gavage at 2.5, 5, 10, 12, or 16 mg/kg on days 6 to 20 of pregnancy, allowed to give birth, and pups were examined (Crofton et al. 1989). Maternal body weight gain, and pup litter size, weight, and viability on PNDs 1 and 3 were decreased at 10 mg and higher. A 3% incidence of cleft palate was detected at 12 mg/kg. There were no pups born with malformations at 10 mg/kg and lower. Vaginal opening was delayed in females exposed to 10 mg/kg. Motor activity was decreased on PND 14 at all doses. Adult brain weight

Table 3.4 Developmental Toxicity of Butyltin Compounds

Compounds	Animals	Dose	Days of Administration	Route	Reproductive and Developmental Effects		Author(s)
					Route	Developmental Effects	
TBTO	NMRI mouse	11.7-35 mg/kg	Days 6-15 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt., cleft palate		Davis et al. (1987)
TBTO	Swiss mouse	40 mg/kg	Days 6-15 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt.		Baroncelli et al. (1990)
TBTO	Swiss mouse	10-30 mg/kg	Days 6-15 of pregnancy	Gavage	Decreased litter size, decreased pup wt., changed length of gestation, decreased percentage of dams exhibiting nest-building		Baroncelli et al. (1995)
TBTO	Swiss mouse	5-20 mg/kg	Days 6-15 of pregnancy	Gavage	Nonspecific effects on hematological parameters		Karrer et al. (1995)
TBTO	Ha:NMRI mouse	27 mg/kg	Days 6-17 of pregnancy	Gavage	Decreased fetal wt., cleft palate, skeletal malformations		Faqi et al. (1997)
TBTO	Long Evans rat	2.5-16 mg/kg	Days 6-20 of pregnancy	Gavage	Decreased litter size and pup wt., cleft palate, decreased postnatal wt. gain, delayed vaginal opening, decreased brain wt., transient decrease in motor activity		Crofton et al. (1989)
TBTO	THA rat	5-10 mg/kg	Days 6-20 of pregnancy	Gavage	Postnatal death, disruption of learning acquisition		Miyake et al. (1990)
TBTA	Wistar rat	16 mg/kg	Days 7-17 of pregnancy	Gavage	Postimplantation loss, cleft palate, decreased fetal wt.		Noda et al. (1991b)
TBTCI	Wistar rat	5-25 mg/kg	Days 7-15 of pregnancy	Gavage	Postimplantation loss, delayed ossification		Itami et al. (1990)
TBTCI	Wistar rat	25-50 mg/kg	Days 7-9 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt.		Ema et al. (1995a)

Table 3.4 Developmental Toxicity of Butyltin Compounds (continued)

Compounds	Animals	Dose	Days of Administration	Route	Reproductive and Developmental Effects	Author(s)
TBTCI	Wistar rat	50-100 mg/kg	Days 10-12 of pregnancy	Gavage	Effects as above, cleft palate	Ema et al. (1997b)
		25-100 mg/kg	Days 13-15 of pregnancy	Gavage	Decreased fetal wt., cleft palate	
		100-200 mg/kg	One day during days 7-15 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt., cleft palate after po on day 8, 11, 12, 13, or 14	
TBTCI	SD rat	0.25-20 mg/kg	Days 0-19 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt., increased male AGD, delayed ossification, decreased levels of serum thyroxine and triiodothyronine	Adeeko et al. (2003)
TBTCI	SD rat	2.5-10 mg/kg	Days 8-19 of pregnancy		Decreased levels of serum thyroxine	Cooke et al. (2004)
		0.025-2.5 mg/kg	From day 8 of pregnancy until adulthood	Gavage	Decreased wt of liver, spleen and thymus, reduced serum levels of creatinine, triglyceride, amylase and thyroxine, change in growth profiles	
TBTCI	SD rat	0.25-2.5 mg/kg	From day 8 of pregnancy until adulthood	Gavage	Thymus atrophy, increased no. of natural killer cells, increased levels of IgM and IgG, increased no. of immature T lymphocytes, decreased levels of IgG2a	Tryphonas et al. (2004)

TBTCl	SD rats	1-5 mg/kg	Days 6-20 of pregnancy	Gavage	Increased spontaneous activity, retarded acquisition of the radial arm maze task, potentiation of d-amphetamine-induced hyperactivity	Gårdlung et al. (1991)
DBTA	Wistar rats	15 mg/kg	Days 0-19 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt., mamibular dysplasia, ankyloglossia, schistoglossia, skeletal variation	Nada et al. (1988)
DBTA	Wistar rat	5-15 mg/kg	Days 7-17 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt., cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, tail anomaly, deformity of ribs and vertebrae, skeletal variations	Noda et al. (1992a)
MBTCl	Wistar rat	50-400 mg/kg	Days 7-17 of pregnancy	Gavage	No effects	Noda et al. (1992b)
DBTA	Wistar rat	15 mg/kg	Days 7-9 of pregnancy	Gavage	Effects as above	
		22 mg/kg	Day 8 of pregnancy	Gavage	Malformations as above	
DBTA	Wistar rat	10-22 mg/kg	Day 8 of pregnancy	Gavage	Malformations as above	Noda et al. (2001)
DBTCl	Wistar rat	5-10 mg/kg	Days 7-15 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt., cleft jaw, cleft palate, ankyloglossia, omphalocere, tail anomaly, deformity of ribs and vertebrae	Ema et al. (1991)
DBTCl	Wistar rat	20 mg/kg	Days 7-9, 10-12, or 13-15 of pregnancy	Gavage	Decreased fetal wt., postimplantation loss, malformations as above after p.o. on days 7-9	Ema et al. (1992)

Table 3.4 Developmental Toxicity of Butyltin Compounds (continued)

Compounds	Animals	Dose	Days of Administration	Route	Reproductive and Developmental Effects		Author(s)
					Days of Administration	Route	
		20-40 mg/kg	Day 6, 7, 8, or 9 of pregnancy	Gavage	Decreased fetal wt., postimplantation loss after p.o. on day 6, 7, or 8, malformations as above after p.o. on day 7 or 8		
DBTCI	Wistar rat	1-10 mg/kg	Days 6-15 of pregnancy	Gavage	No effects		Farr et al. (2001)
DBTA	Wistar rat	28.1 mg/kg	Day 8 of pregnancy	Gavage	Cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, exencephaly, deformity of ribs and vertebrae		Noda et al. (1993)
DBTCI	Wistar rat	24.3 mg/kg	Day 8 of pregnancy	Gavage	Decreased fetal wt., malformations as above		
DBTM	Wistar rat	27.8 mg/kg	Day 8 of pregnancy	Gavage	Malformations as above		
DBTO	Wistar rat	19.9 mg/kg	Day 8 of pregnancy	Gavage	Malformations as above		
DBTL	Wistar rat	50.0 mg/kg	Day 8 of pregnancy	Gavage	Malformations as above		
3-OHDBTL	Wistar rat	100 mg/kg	Day 8 of pregnancy	Gavage	Decreased fetal wt., peaked mandible		
TeBT	Wistar rat	1832 mg/kg	Days 13-15 of pregnancy	Gavage	Cleft palate		Ema et al. (1996a)
TBTCI	Wistar rat	54-108 mg/kg	Days 13-15 of pregnancy	Gavage	Decreased fetal wt., cleft palate		
DBTCI	Wistar rat	50-100 mg/kg	Days 13-15 of pregnancy	Gavage	Decreased fetal wt.		
TBTCI	Wistar rat	40-80 mg/kg	Days 7-8 of pregnancy	Gavage	Postimplantation loss, decreased fetal wt.		Ema et al. (1995b)
DBTCI	Wistar rat	10-15 mg/kg	Days 7-8 of pregnancy	Gavage	Effects as above, malformations as above		
MBTCI	Wistar rat	1000-1500 mg/kg	Days 7-8 of pregnancy	Gavage	Decreased fetal wt.		

was reduced at 10 mg/kg. THA rats were given TBTO by gavage at 5 or 10 mg/kg on days 6 to 20 of pregnancy and allowed to deliver spontaneously, and pups were examined (Miyake et al. 1990). All pups died by PND 3 at 10 mg/kg. In pups at 5 mg/kg, prenatal TBTO disrupted learning acquisition in the Sidman avoidance test and a reversal test in the water E-maze.

Pregnant Wistar rats were given tributyltin acetate (TBTA) by gavage at 1, 2, 4, 8, or 16 mg/kg on days 7 to 17 of pregnancy (Noda et al. 1991b). An increase in incidences of intrauterine deaths, cleft palate, and low fetal weight were found at 16 mg/kg. This dose level also induced severe reductions in maternal weight gain and food consumption, and TBTA at 4 mg/kg and higher lowered maternal thymus weight. Noda et al. (1991b) concluded that the observed teratogenic effects may not be a specific action of TBTA because their results were similar to those of Davis et al. (1987).

No live fetuses were obtained in Wistar rats treated with TBTCI at 25 mg/kg by gavage on days 7 to 15 of pregnancy (Itami et al. 1990). Maternal toxicity at 9 mg/kg and higher, and skeletal retardation in fetuses at 5 mg/kg and higher were observed, but fetal malformations were not found. An increase in placental weight was found at 5 mg/kg and higher. To obtain more precise information on the effects of TBTCI on fetal development, Wistar rats were given TBTCI by gavage at relatively high doses during a shorter period, at 25 or 50 mg/kg on days 7 to 9, at 50 or 100 mg/kg on days 10 to 12, or at 25, 50, or 100 mg/kg on days 13 to 15 of pregnancy (Ema et al. 1995a). A decrease in maternal weight gain was observed in all groups regardless the days of administration. An increase in incidence of postimplantation embryonic loss was found in pregnant rats given TBTCI on days 7 to 9 at 25 mg/kg and higher, and on days 10 to 12 at 100 mg/kg, but not in pregnant rats given TBTCI on days 13 to 15 at up to 100 mg/kg. A lower fetal weight was observed in pregnant rats given TBTCI on days 10 to 12 at 50 and 100 mg/kg, and on days 13 to 15 at 100 mg/kg. An increased incidence of fetuses with malformations was detected after administration of TBTCI on days 10 to 12 at 100 mg/kg, and on days 13 to 15 at 25 mg/kg and higher. The most predominant malformation was cleft palate. These results indicate that the manifestation of abnormal development induced by TBTCI varies with developmental stage at the time of administration, and that TBTCI has teratogenic potential with developmental phase specificity. The most susceptible day to the teratogenicity of TBTCI was determined by a single administration on one of the days during organogenesis (Ema et al. 1997b). An increase in incidence of fetuses with external malformations was detected when TBTCI was given on day 8 at 100 and 200 mg/kg, or on days 11, 12, 13, or 14 at 200 mg/kg, and the most pronounced effect was seen after administration on day 13 of pregnancy. Cleft palate was mainly observed after administration of TBTCI. These findings indicate that TBTCI has a biphasic teratogenicity on day 8 and on days 11 to 14 of pregnancy. Pregnant SD rats were gavaged with TBTCI at 0.25, 2.5, 10, or 20 mg/kg on days 0 to 19 of pregnancy, or at 0.25, 2.5, or 10 mg/kg on days 8 to 19 of pregnancy, and pregnancy outcome was assessed (Adeeko et al. 2003). A

reduced maternal weight gain, decrease in pregnancy rate, increase in postimplantation loss, and decrease in fetal weight were found after administration of TBTCI at 20 mg/kg on days 0 to 19. These findings support the previous results (Harazono et al. 1996, 1998a, b) in which TBTCI during early pregnancy at 12.2 mg/kg and higher caused increases in pre- and postimplantation loss. The incidence of fetal malformations was not increased in any TBTCI-treated groups. An increase in normalized AGD of male fetuses was detected at 0.25 mg/kg and higher on days 0 to 19, but not at any dose on days 8 to 19, even at the highest dose level. Hormonally active agents are known to affect mammalian internal and external genitalia when administered during sex differentiation (i.e., the perinatal period) (Schardein 2000). It is reported that days 16 to 17 of pregnancy were the most sensitive for finasteride-induced feminizing effects, including a decrease in AGD in male rat offspring (Clark et al. 1993), and that the period of days 15 to 17 of pregnancy was the most susceptible for dibutyl phthalate-induced decrease in the AGD of male rat offspring (Ema et al. 2000). There are discrepancies in the effects of TBTCI on the AGD between outcomes after exposure on days 0 to 19 and on days 8 to 19 of pregnancy, and between this study and previous reports in which whole-life exposure to TBTCI caused increase in female AGD in rat two-generation reproductive studies (Ogata et al. 2001). *In vitro* studies showed that TPT and TBT had an ability to activate androgen receptor mediated transcription in mammalian cells (Yamabe et al. 2000). TPTCI, TBTCI, and DBTCI caused aromatase inhibition in the human adrenocortical carcinoma cell line (Sanderson et al. 2002). Although TeBT and MBTCI had no effect on either human 5-reductase type 1 or type 2, TBTCI and DBTCI influenced the human 5-reductase isozymes (Doering et al. 2002). DBTCI specifically inhibited brain 5-reductase type 1 with no effect on prostate 5-reductase type 2. TBTCI inhibited both isoenzymes. Doering et al. (2002) noted that the inhibition of the TBTCI inhibited both isoenzymes. Type 2 could potentially disturb normal male physiology. These *in vitro* findings may explain the *in vivo* reproductive and developmental outcomes induced by organotins. Adeeko et al. (2003) also noted that reduced fetal ossification of the sternbrae was found at 10 mg/kg and higher, for which fetal weights at 10 mg/kg were in normal range, and TBTCI at 10 mg/kg and higher during pregnancy decreased maternal circulating thyroid hormone levels and increased the weight of the placenta. They noted that the TBTCI-induced disturbances in maternal thyroid hormone homeostasis could contribute to the reduction in fetal skeletal ossification.

Pregnant SD rats were gavaged with TBTCI at 0.025, 0.25, or 2.5 mg/kg from day 8 of pregnancy until weaning, and offspring were gavaged with the same dose of TBTCI given to their mothers until adulthood (Cooke et al. 2004, Tryphonas et al. 2004). No effects of TBTCI on body weight, food consumption, or histopathological findings in the thyroid, liver, adrenal or colon were observed in maternal rats. No effects of TCBTCI on litter size, sex ratio, postnatal survival rate, or histopathological findings in the liver,

adrenal gland or colon were also found in offspring. Decreased serum levels of creatinine, triglycerides, and magnesium in female offspring and of thyroxine in male offspring were found at 2.5 mg/kg. Decreased weight of the spleen in male offspring and the thymus in female offspring were observed at 0.25 mg/kg. Significant effects on growth profiles in male and female offspring, and decreased liver weights in female offspring were noted even at 0.025 mg/kg (Cooke et al. 2004). Immunotoxic effects of TBTCI were determined in these rat offspring (Tryphonas et al. 2004). Thymus atrophy, an increase in the number of natural killer cells and immunoglobulin M (IgM) levels, a decrease in the IgG2a levels at 2.5 mg/kg, and an increase in the mean percentage immature T lymphocytes and IgG levels at 0.25 mg/kg and higher were observed in offspring. Significant effects were found more frequently at 0.25 mg/kg and higher, and minor effects were observed at 0.025 mg/kg. Tryphonas et al. (2004) concluded that the low levels of TBTCI affected humoral and cell-mediated immunity, and the number and function of cells involved in the host's immunosurveillance mechanisms against tumors and vital infections in rat offspring.

Postnatal behavioral changes in pups of SD rats that received TBTCI prenatally on days 6 to 20 of pregnancy, at doses not toxic to the mother, were also reported (Gårdlund et al. 1991). An increase in spontaneous activity, such as locomotion, rearing, and total activity, retarded acquisition in radial arm maze performance, and potentiation of d-amphetamine-induced hyperactivity were observed at 1 and 5 mg/kg.

The adverse effects of DBT, a major metabolite of TBT, on embryonic/fetal development were assessed after maternal administration during organogenesis. Pregnant Wistar rats were given DBTA by gavage at 1.7, 5, or 15 mg/kg during the whole period, on days 0 to 19, of pregnancy (Noda et al. 1988). At 15 mg/kg, a decrease in body weight gain and thymus weight in dams, and a low body weight and increased number of fetal malformations occurred. Administration of DBTA by gavage during the organogenetic period, on days 7 to 17, of pregnancy at 10 mg/kg and higher also caused increased fetal malformations, such as cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, exencephaly, anury, vestigial tail, and deformity of the ribs and vertebrae (Noda et al. 1992a). Decreases in thymus weight and fetal weight at 10 mg/kg and higher, and decreases in maternal weight gain at 15 mg/kg were observed following administration of DBTA on days 7 to 17 of pregnancy. The most susceptible gestational day to teratogenicity of DBTA in rats was day 8 of pregnancy (Noda et al. 1992b). Occurrences of similar types of fetal malformations after administration of DBTA on day 8 of pregnancy were also reported in other papers (Node et al. 1993, 1994, 2001). Teratogenic effects of DBTCI were also studied in Wistar rats. Female rats were given DBTCI by gavage at 2.5, 5.0, or 7.5 mg/kg on days 7 to 15 of pregnancy (Ema et al. 1991). The incidence of fetal malformations was increased and roughly proportional to the dose of DBTCI administered at 5.0 mg/kg and higher. Cleft jaw, ankyloglossia, omphalocele, anomaly of

the tail, defect of the mandible, deformity of the vertebral column and ribs, and microphthalmia were frequently observed. In this study, decreases in maternal weight gain and food consumption was observed at 7.5 mg/kg and higher. These results indicate that DBTCl produce teratogenic effects in the absence of overt maternal toxicity. However, the thymus weight was not determined. The susceptible gestational days to teratogenicity of DBTCl was determined after administration of relatively high doses of TBTCI on days 7 to 9, on days 10 to 12, or on days 13 to 15 of pregnancy (Ema et al. 1992). An increase in fetal malformations and postimplantation loss was detected after administration of DBTCl at 20 mg/kg on days 7 to 9, but neither was detected on days 10 to 12 nor on days 13 to 15. The data of the study in which pregnant rats were given a single dose of DBTCl by gavage showed that developing offspring were not susceptible to teratogenicity of DBTCl on day 6, and that day 7 was the earliest susceptible period, day 8 was the most susceptible period, and day 9 was no longer a susceptible period with respect to the teratogenicity of DBTCl (Ema et al. 1992). Occurrences of similar types of fetal malformations after administration of DBTCl on day 8 or on days 7 to 8 of pregnancy were also reported in rats (Noda et al. 1993, Ema et al. 1995b). Farr et al. (2001) also reported the developmental toxicity of DBTCl in rats. Wistar rats were administered DBTCl by gavage at 1, 2.5, 5, or 10 mg/kg on days 6 to 15 of pregnancy. Decreases in maternal weight gain, food consumption, and thymus weight, but not developmental indicators, were observed at the highest dose tested, 10 mg/kg. At this dose, four fetuses out of 262 fetuses had malformations, including ankyloglossia, mandible defects, tail anomaly, and deformity of the vertebrae, which were similar types of malformations to those previously reported after administration of DBTA (Noda et al. 1988, 1992a, b, 1993, 1994, 2001) and DBTCl (Ema et al. 1991, 1992, 1995b, Noda et al. 1993). They concluded that a slightly increased, but not statistically significant, number of malformations was associated with the onset of maternal toxicity, and that no increase in developmental defects was induced at dose levels that did not result in maternal toxicity.

The teratogenic effects of five DBTs with different anions, such as DBTA, DBTCl, dibutyltin maleate (DBTM), dibutyltin oxide (DBTO), and dibutyltin dilaurate (DBTL), were determined in Wistar rats given by gavage at 80 μ mol/kg on the most susceptible day for teratogenicity of DBTA and DBTCl (Noda et al. 1993). Although the incidences of fetuses with malformations were different among DBTs, the types of malformations induced by these DBTs are similar to those in the previous studies with DBTA. Noda et al. (1993) suggest the importance of the dibutyl group rather than the anionic group in the production of fetal malformations. They also noted that butyl(3-hydroxybutyl)tin dilaurate (3-OHDBL), one of the main metabolites of DBTCl (Ishizaka et al. 1989), was not responsible for the teratogenicity of DBTCl because of weak potential for production of fetal malformations.

TeBT is metabolized to tri-, di-, and monobutyltin derivatives (Kimmel et al. 1977). The TBT compound is metabolized to di- and monobutyltin

derivatives, and DBT was metabolized to MBT in rats (Iwai et al. 1981). TeBT, TBTCI, DBTCI, and MBTCI were compared for their developmental toxicity to evaluate these butyltin compounds as potential toxicants in teratogenicity following administration of relatively high doses of butyltins to pregnant rats during the susceptible period to teratogenesis of TBTCI or during the susceptible period to teratogenesis of DBTCI. Pregnant rats were given TeBT, TBTCI, or DBTCI during the period of susceptibility to the teratogenesis of TBTCI, on days 13 to 15 of pregnancy (Ema et al. 1996a). TeBT caused an increased incidence of cleft palate at 1832 mg (5280 μmol)/kg. TBTCI induced a markedly increased incidence of fetuses with cleft palate at 54 mg (165 μmol)/kg and higher, and decreased fetal weight at 108 mg (330 μmol)/kg. Following administration of DBTCI on days 13 to 15 of pregnancy, fetal weight was reduced at 54 mg (165 μmol)/kg and higher, but neither increase in postimplantation loss nor fetuses with malformations was found even at 100 mg (330 μmol)/kg. These results indicate that there are differences in the manifestation and degree of developmental toxicity among TeBT, TBT, and DBT. Pregnant rats received TBTCI, DBTCI, or MBTCI during the period of susceptibility to teratogenesis of DBTCI, on days 7 to 8 of pregnancy (Ema et al. 1995b). TBTCI at 40 and 80 mg/kg caused an increase in postimplantation embryo lethality, but no increase in fetal malformations. DBTCI caused a markedly high incidence of fetal malformations, lower fetal weight, and higher postimplantation embryonic loss at 10 mg/kg and higher. No increase in the incidences of postimplantation loss or malformed fetuses was observed after administration of MBTCI even at 1500 mg/kg. These results indicate that the developmental toxicity of DBTCI is different from that of TBTCI and MBTCI in the level of susceptibility and spectrum of toxicity. A lack of developmental toxicity of MBTCI was also reported by Noda et al. (1992a). MBTCI on days 7 to 17 of pregnancy did not affect maternal body weight and thymus weight, or fetal survival, growth, and morphological development, even at 400 mg/kg in Wistar rats. Their observations support the theory that MBTCI does not participate in the induction of the developmental toxicity of butyltins.

In Vitro Dymorphogenic Effects of Butyltin Compounds

Krowke et al. (1986) evaluated the effects of TBTO on limb differentiation. In the organ culture system using mouse limb buds, TBTO interfered with morphogenetic differentiation at a concentration of 0.03 $\mu\text{g}/\text{mL}$. TBTO affected the differentiation of the paw skeleton and the development of the scapula. They concluded that the effects of TBTO on mouse limb differentiation should be interpreted as a cytotoxic effect rather than a specific dymorphogenic action. Yonemoto et al. (1993) determined the relative teratogenic potencies of TBTO, TBTCI, (3-OH) hydroxybutyl dibutyltin chloride (3-OHHDBTCI), DBTCI, and MBTCI by comparing developmental hazard estimates using rat embryo limb bud cell cultures. The organotin compounds tested, except for MBTCI, were very strong inhibitors of cell differentiation

and cell proliferation. Fifty percent inhibition concentration for cell proliferation (IP50) and for cell differentiation (ID50), and the ratio of the former to the later (P/D ratio) of each compound was determined. Among TBTO, TBTCl, and its metabolites (i.e., 3-OHHDBTCl, DBTCl, and MBTCl), DBTCl showed the lowest ID50 and the highest P/D ratio, therefore the teratogenic potential of DBTCl was considered to be the highest. They noted that the proximate toxicant of DBT teratogenicity is DBT itself, TBT is rather embryolethal than teratogenic. These findings support the results of *in vivo* developmental toxicity studies on butyltins. The embryotoxicity and dysmorphogenic potential of DBTCl were determined for gestation day 8.5 rat embryos, which are highly susceptible to the teratogenic effects of DBTCl when administered to pregnant rats. Markedly decreased incidences in embryos with well-developed vascularization in the body and yolk sac, yolk sac diameter, crown-rump length, and number of somite pairs were found at 30 ng/mL (Ema et al. 1995c). A concentration-dependent decrease in the morphological score and increase in incidence of embryos with anomalies were noted, and the differences were significant for embryos exposed to DBTCl at concentrations of 10 and 30 ng/mL. Open anterior neuropore and craniofacial abnormalities were predominantly observed. These results indicate that DBTCl exerts dysmorphogenic effects on postimplantation embryos *in vitro*. Noda et al. (1994) reported that DBT was detected in rat maternal blood at 100 ng/g, and in embryos at 720 ng/g, at 24 hours after gavage administration of DBTA at 22 mg/kg, teratogenic dose, on day 8 of pregnancy. Their results show that DBT is transferred to embryos, and embryonic levels of DBT exceed those in maternal blood, suggesting that embryos may be able to accumulate DBT. The dysmorphogenic concentrations of DBTCl in embryos cultured from gestation day 8.5 were well within the range of levels detected in maternal blood after the administration of a teratogenic dose of DBT. These findings indicate that teratogenic effects of DBTCl may be due to a direct interference with embryos. The toxic effects of DTBCl were examined in rat embryos during three different stages of organogenesis (i.e., the primitive streak, neural fold, and early forelimb bud stages), using the rat whole embryo culture system (Ema et al. 1996b). Rat embryos were explanted on gestation day 8.5, 9.5, or 11.5 and cultured. Dysmorphogenesis in embryos cultured from gestation day 8.5, 9.5, or 11.5 was observed at concentrations of 10 ng/mL and higher, 50 ng/mL and higher, and 300 ng/mL, respectively. Incomplete turning and craniofacial defects in embryos cultured from gestation day 8.5 and day 9.5, and defects of the forelimb buds and tail in embryos cultured from gestation day 11.5, were frequently observed. These results show that *in vitro* exposure to DBTCl interferes with normal development of embryos during three different stages of organogenesis and that the susceptibility to the embryotoxicity, including dysmorphogenic potential, of DBTCl varies with developmental stage. These findings suggest that the phase specificity for the *in vivo* teratogenesis of DBTCl given to pregnant rats may be attributable to a decline in the susceptibility of embryos to the dysmorphogenesis of DBTCl with advancing development.

Summary of Developmental Toxicity of Butyltin Compounds

Maternal exposure during pregnancy to TBTs, such as TBTO, TBTA, and TBTCI, caused embryonic/fetal deaths and suppression of fetal growth at maternal toxic doses. At severely maternal toxic doses of TBTs, cleft palate was produced in fetuses. Behavioral changes were also reported in postnatal offspring of rats that received TBTs during pregnancy at doses that did not cause overt maternal toxicity. Significant effects on growth profiles in male and female offspring, and decreased liver weights in female offspring were noted after administration of TBTCI by gavage from day 8 of pregnancy until adulthood even at 0.025 mg/kg. Many reports showed that DBT is teratogenic when administered during organogenesis. DBT may increase the incidence of fetal malformations at marginal doses that induced maternal toxicity. Developing embryos were not susceptible to teratogenicity of DBTCI on day 6; day 7 was the earliest susceptible period, day 8 was the most susceptible period, and day 9 was no longer a period of susceptibility to the teratogenicity of DBTCI. There were differences in the manifestation and degree of developmental toxicity among TeBT, TBT, DBT, and MBT. The developmental toxicity studies on butyltins suggest that the teratogenicity of DBT is different from those of TeBT, TBT, and MBT in its mode of action, because the susceptible period for teratogenicity and types of malformations induced by DBT are different from those induced by tetra-, tri-, and mono-substituted organotins. DBTCI exerts dysmorphogenic effects on postimplantation embryos *in vitro*. The dysmorphogenic concentrations of DBTCI in embryos cultured were well within the range of levels detected in maternal blood after the administration of a teratogenic dose of DBT. The phase specificity for the *in vivo* teratogenesis of DBTCI may be attributable to a decline in the susceptibility of embryos to the dysmorphogenesis of DBTCI with advancing development. The findings of *in vivo* and *in vitro* studies suggest that DBT itself is a causative agent in DBT teratogenesis.

Developmental Toxicity of Miscellaneous Organotin Compounds

Table 3.5 presents the developmental toxicity studies on miscellaneous organotin compounds. Behavioral effects were determined in offspring of female SD rats given trimethyltin chloride (TMTCl) in drinking water at a concentration of 0.2, 0.8, or 1.7 mg/L, or monomethyltin trichloride (MMTCl) in drinking water at a concentration of 24.3, 80.9, or 243 mg/L from 12 days before mating, to day 21 of lactation, throughout the mating and pregnancy period (Noland et al. 1982). Only male pups were tested. Learning deficiency was detected in organotin-treated pups. Pups from dams exposed to TMTCl at 1.7 mg/L or MMTCl at 243 mg/L displayed an increased acquisition time in a runway learning test on PND 11. A higher escape time in a swim escape test on PND 21 was also observed in male pups exposed to prenatal MMTCl at 24 and 243 mg/L. In this study, there was no difference between the weights of control and experimental animals in suckling pups and their

Table 3.5 Developmental Toxicity of Miscellaneous Organotin Compounds

Compounds	Animals	Dose	Days of Administration		Route	Reproductive and Developmental Effects		Author(s)
TMTCI	SD rat	1.7 mg/L	14 days before mating to lactation day 21		Drinking water	Learning deficiency in male pups	Noland et al. (1982)	
MMTCI	SD rat	243 mg/L	As above		As above	As above		
TMTCI	SD rat	5-9 mg/kg	Day 7, 12, or 17 of pregnancy		ip	Decreased postnatal wt. gain, decreased no. of pups, degenerative changes in hippocampus	Paule et al. (1986)	
TMTCI	THA rat	5-7 mg/kg	Day 12 of pregnancy		ip	Disruption of learning acquisition	Miyake et al. (1989)	
THTCI	SD rat	5 mg/kg	Day 6-20 of pregnancy		Gavage	Increased spontaneous activity, increased d-amphetamine-stimulate rearing	Gårdlund et al. (1991)	
DMTCI	Wistar rat	15-20 mg/kg	Days 7-17 of pregnancy		Gavage	Decreased fetal wt., cleft palate	Noda (2001)	
		40 mg/kg	Days 7-9 or 13-15 of pregnancy		Gavage	Skeletal variations		
Octyltin stabilizer ZK 30.434 (80% DOTIG and 20% MOTTG)								
	Han:NMRI mouse	20-100 mg/kg	Days 5-16 of pregnancy		Gavage	Postimplantation loss, decreased fetal wt., bent forelimb, cleft palate, exencephaly, skeletal malformations and variations	Faqi et al. (2001)	

dams. Postnatal growth and neuronal alterations were evaluated in pups of SD rats intraperitoneally injected on either day 7, 12, or 17 of pregnancy with a single dose of TMTCl at 5, 7, or 9 mg/kg (Paule et al. 1986). Maternal body weight at term of pregnancy was lower in the TMTCl-treated groups. Prenatal TMTCl decreased pup weight at 7 mg/kg and higher. A decreased number of surviving pups was found only in the group treated TMTCl at 9 mg/kg on day 17 of pregnancy. Generative changes in the hippocampus were more frequently noted in pups exposed to TMTCl on day 12 or 17 than on day 7. Paule et al. (1986) concluded that prenatal exposure to TMTCl causes toxic effects in postnatal offspring, but only in the presence of maternal toxicity. Disruption of learning acquisition was reported in offspring of THA rats intraperitoneally injected with TMTCl at 5 or 7 mg/kg on day 12 of pregnancy (Miyake et al. 1989). No maternal toxicity was found at 5 mg/kg. No effects of TMTCl on body weight, survival, or physical and functional development of pups were detected. In the Sidman avoidance test, the avoidance rate of the TMTCl-treated offspring rats was lower when compared to that of the controls.

Postnatal behavioral changes in pups were determined in rats prenatally administered trihexyltin chloride (THTCl) (Gårdlund et al. 1991). Pregnant SD rats were gavaged THTCl at 5 mg/kg on days 6 to 20 of pregnancy and allowed to litter. An increase in spontaneous activity, including locomotion and total activity, and a marginally increased d-amphetamine-stimulated rearing behavior were observed in postnatal pups at 5 mg/kg. This dose level did not induce maternal toxicity.

Dimethyltin chloride (DMTCl) was given to Wistar rats by gavage at 5, 10, 15, or 20 mg/kg on days 7 to 17 of pregnancy (Noda 2001). At 20 mg/kg, severe clinical signs of toxicity, including death and marked decreases in body weight gain and food consumption in pregnant rats, and incidence of cleft palate in fetuses were observed. Decreases in maternal thymus weight and fetal weight were found at 15 mg/kg and higher. No increase in incidence of fetal malformations was detected following administration of DMTCl on days 7 to 9, on days 10 to 12, on days 13 to 15, or on days 16 to 17 of pregnancy at 20 or 40 mg/kg. Noda (2001) concluded that DMTCl produced fetal malformations at a severely maternal toxic dose.

The octyltin stabilizer ZK 30.434, a mixture of 80% dioctyltin diisooctylthioglycolate and 20% monoctyltin triisooctylthioglycolate (DOTTG/MOTTG) was gavaged to Han:NMRI mice at 20, 30, 45, 67, or 100 mg/kg on days 5 to 16 of pregnancy (Faqi et al. 2001). One death at 100 mg/kg and a decreased thymus weight at 45 and 100 mg/kg were observed in dams. An increase in resorptions and low fetal weight were found at 67 mg/kg and higher. An increase in number of external and skeletal anomalies, such as forelimb bent, cleft palate, exencephaly, clavicle bent, femur bent, and fused ribs, were observed at the highest dose. Incidences of cervical and lumbar ribs were increased at 20 mg/kg and higher. These results indicate that DOTTG/MOTTG is developmentally toxic in mice.

Summary of Developmental Toxicity of Miscellaneous Organotin Compounds

Prenatal and/or postnatal exposure to TMTCI possesses developmental neurotoxic effects in postnatal rat offspring, even at doses that induced no maternal toxicity. The learning deficiency induced by prenatal TMTCI may be due to hippocampal lesions. Prenatal treatment of maternal toxic doses of TMTCI adversely affected survival and growth of offspring. Prenatal treatment of THTCI is also reported to induce behavioral changes in postnatal offspring. An increased number of cleft palates were observed in fetuses of rats given DMTCI during organogenesis at a severely maternal toxic dose. A mixture of DOTG and MOTTG is developmentally toxic and produces fetal malformations in mice.

Conclusions

Many studies on toxic effects of phenyltins and butyltins in aquatic organisms have been conducted. TBT or TPT causes the imposition of male sex organs (termed *imposex*) on female mud snails above the concentration of about 1 ng/L (Sn) in seawater, but DBT or MPT does not induce imposex. The intensity is characterized by a classification system based on the VDS index, and in advanced phases of imposex and sterilization with gross morphological changes would be irreversible. The biochemical mechanism studies suggested that the induction of either neurotropic hormone or androgen titers would lead to imposex induction at extremely low doses of TBT. Also TBT or TPT exposure in early life stages of fish causes altered embryonic development, impaired morphological development, and delayed or inhibited hatching, and induces reduced fecundity and sperm counts as reproductive effects. Such reproductive and developmental defects were also found in other species. The impaired reproduction and subsequent population decline in a variety of aquatic organisms by organotins are important issues in the aquatic ecosystem.

Many reports on reproductive and developmental toxic effects of phenyltins and butyltins in experimental animals have been published. While TPTs caused decreases in male fertility due to degenerative changes in testicular tissue, the female reproductive failure induced by TPTs is more prominent and the harmful effects of TPTs on the ovaries were presented after five days of treatment. TPTCI during early pregnancy caused implantation failure. Implantation failure due to TPTCI might be mediated by the suppression of uterine decidualization and correlated with the reduction in serum progesterone levels. These findings were also shown in rats given DPT, a major metabolite of TPT. Maternal exposure to TPTs during organogenesis caused embryonic/fetal death and suppression of fetal growth at maternal toxic doses. TPTs did not induce an increased number of fetal malformations, even at doses that produced overt maternal toxicity. Behavioral changes were reported in postnatal offspring of maternal rats that

received TPTs during pregnancy at doses that did not cause overt maternal toxicity. In a rat two-generation reproductive toxicity study, TBTCI at relatively low doses affected male and female reproductive systems, including decreased weights of the male reproductive organs, decreased counts of spermatids and sperms, decrease in serum estradiol levels, delayed vaginal opening, impaired estrous cyclicity, and increased female AGD. TBTCI and DBTCI during early pregnancy caused implantation failure in rats. Implantation failure due to TBTCI and DBTCI, at lower doses than TBTCI, may be mediated via the suppression of uterine decidualization and correlated with the reduction in serum progesterone levels. Administration of MBTCI during early pregnancy did not cause pre- or postimplantation loss. Maternal exposure during pregnancy to TBTs caused embryonic/fetal deaths, suppression of fetal growth, and cleft palate at maternal toxic doses. Significant effects on growth profiles and decreased liver weights were reported in offspring of rats given TBTCI by gavage, even at 0.025 mg/kg from day 8 of pregnancy until adulthood. Behavioral changes were also shown in postnatal offspring of rats that received TBTs during pregnancy at doses that did not cause overt maternal toxicity. Many reports demonstrated that DBT derivatives with different anions, such as dichloride, diacetate, maleate, dilaurate, and oxide, are teratogenic when administered during organogenesis in rats. Rat embryos are the most susceptible to teratogenic effects of DBT on day 8 of pregnancy after maternal exposure. The developmental toxicity studies on butyltins suggest that the teratogenic effects of DBT are different from those of TeBT, TBT, and MBT in its mode of action. DBTCI exerts dysmorphogenic effects on postimplantation embryos *in vitro*. The phase specificity for the *in vivo* teratogenic effects of DBTCI may be attributable to a decline in the susceptibility of embryos to the dysmorphogenesis of DBTCI with advancing development. The findings of *in vivo* and *in vitro* studies suggest that DBT itself is a causative agent in DBT teratogenesis. Because the teratogenicity of DTB has been reported in a single species, studies in additional species would be of great value in evaluating developmental toxicity of DBT. As for miscellaneous organotin compounds, several reports on developmental toxicity are published. Prenatal and/or postnatal exposure to TMTCl or THTCI caused behavioral changes in postnatal rat offspring. Behavioral changes in postnatal pups of rats given organotin prenatally and/or postnatally may be a sensitive parameter for reproductive and developmental toxicity. A mixture of DOTTG and MOTTG is developmentally toxic and produces fetal malformations in mice. An increased number of cleft palates was reported in fetuses of rats given DMTCl during organogenesis at severely maternal toxic dose.

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